

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

5,300

Open access books available

130,000

International authors and editors

155M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.

For more information visit www.intechopen.com



Recent Advances in Antioxidant Capacity Assays

Andrei Florin Danet

Abstract

This work presents a survey of the important antioxidant capacity/activity assays applied for a diversity of samples including plant extracts, foods, biological material, etc. The published materials are critically discussed, emphasizing the recent findings in the field. New and emergent antioxidant capacity assays, such as nanoparticles-based assay, are also presented. The discussion includes chemical-based methods as well as biochemical and cellular assays. Chemical methods detailed are radical/ROS-based scavenging assays (the trolox equivalent antioxidant capacity (TEAC/ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), oxygen radical absorbance capacity (ORAC) assays, chemiluminescence methods, total radical-trapping antioxidant parameter (TRAP), total oxy radical scavenging capacity (TOSC), and β -carotene bleaching assays), non-radical redox potential-based assays (ferric reducing antioxidant power (FRAP), cupric reducing antioxidant capacity (CUPRAC), nanoparticle-based methods and electrochemical methods), metal chelation capacity and total phenolic content tests. The biochemical-based assays and *in vivo* assays discussed include the oxidation of low density lipoprotein (LDL), the thiobarbituric acid reactive substances (TBARS) and the cellular antioxidant activity (CAA) assays. While a direct link between the antioxidant capacity and health benefits is still a matter of debate, the antioxidant testing methodologies presented in this chapter remain valuable for the high efficiency and cost-effective evaluation of antioxidants, from compound discovery to quality control.

Keywords: antioxidant, total antioxidant capacity, reactive species, phenolic compounds, antioxidant assay, phytochemicals, food analytical method

1. Introduction

Antioxidants are classified in two categories: (1) primary or chain-breaking antioxidants, especially acting by scavenging reactive oxygen species/reactive nitrogen species (ROS/RNS) and (2) secondary or preventive antioxidants, that suppress the oxidation promoters such as metal ions, singlet oxygen, pro-oxidative enzymes and other antioxidants, commonly operating by transition metal ion chelation [1]. An antioxidant may operate directly or indirectly: directly by scavenging ROS/RNS species or by inhibiting their generation, indirectly, e.g., by up-regulating endogenous antioxidant defenses [2, 3]. Antioxidants can be also classified as enzymatic and non-enzymatic antioxidants. In the present review we shall discuss only the non-enzymatic antioxidants. The efficacy of an antioxidant depends on its antioxidant activity and/or its antioxidant capacity.

It should be stated from the very beginning that antioxidant activity and antioxidant capacity are two different terms. The antioxidant activity is linked to rate constant of an antioxidant against a specified free radical, whereas the antioxidant capacity represents the number of moles of a specified free radical, scavenged by an individual antioxidant present in the analyzed mixture [4]. Antioxidant activity is related especially to the reaction kinetics, whereas antioxidant capacity is related to the thermodynamics of the process regarding the oxidative conversion of an antioxidant and is connected with equilibrium constant of the process [5].

The antioxidant assays can target a specific compound (e.g., ascorbic acid, vitamin E, uric acid, etc.) or the total antioxidant capacity (TAC) given by the combined antioxidant capacities of all substances in a sample.

Antioxidant assays include direct and indirect methods. Direct assays are competitive, in which the produced reactive species simultaneously attack a „probe” and the antioxidant. Indirect assays are non-competitive, the redox reactions being simulated using an artificial probe, whose structural changes are measured by different techniques (spectroscopy, electrochemistry, or other methods).

The most common assays for TAC comprise: (i) the measurement of oxygen radical antioxidant capacity (ORAC) using different fluorescent probes [6], (ii) the Trolox equivalent antioxidant capacity based on 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) (TEAC/ABTS) [7], (iii) the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [8], (iv) the ferric reducing antioxidant power (FRAP) test [9], (v) the cupric reducing antioxidant capacity (CUPRAC) assay [10] and (vi) Folin-Ciocalteu's phenol reagent reducing capacity (for the content of total phenolics) [11, 12].

Extensive reviews regarding the methods for assaying antioxidant capacity/activity could be found in literature [1, 5, 6, 13–19] and a book has also been published recently [20] with a focus on the measurement of antioxidant activity and capacity. Several papers have discussed the advantages and disadvantages of different antioxidant assays, with a focus on method selection for specific requirements [14–16, 21, 22].

There are numerous research articles in literature pertaining to the evaluation of antioxidant methodology. However very few discuss the mechanistic steps involved in the respective reactions [23, 24]. In depth evaluation of ORAC, ABTS and DPPH methods were comprehensively presented [25]. Some important antioxidant assays in terms of mechanisms and kinetics of the involved reactions were evaluated [6, 14], while the mechanisms, advantages and disadvantages of different antioxidant assays were also described in [18, 26, 27].

A review of the main methods for monitoring the antioxidant capacity/activity of lipid-containing samples was presented in [28]. In addition, the determination of the antioxidant capacity of lipids via the flow injection analysis (FIA) coupled with chemiluminescence detection was specifically discussed in [29].

The role of antioxidants from a pharmaceutical perspective is presented in [30] and a review of the methodologies for the determination of biological antioxidant capacity *in vitro* is presented in [31].

Compiled information about antioxidants in terms of the chemistry, legislation and their application in foods as preservatives can be found in [32]. The extrapolation of laboratory data relative to the antioxidants' function and their implications on food production and human health, etc. is critically discussed in [33].

Some recent reviews [13, 34, 35] commented on the advance, applications, advantages and disadvantages of total antioxidant capacity assays. The contentions and limitations of some largely used antioxidant assays, hints for suitable assay selection, emerging techniques in antioxidant testing and future perspectives are provided in [5].

An interesting discussion is presented in [36] about the development of several TAC databases of foods, the development of methods for evaluating TAC in the diet, the application of TAC databases in epidemiological studies, the application of TAC

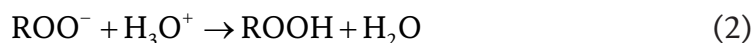
methods to biological fluids and the correlation between consumption of antioxidant rich-foods and the plasma TAC. The advantages and disadvantages of different TAC assays were also summarized.

Unfortunately many studies on TAC have reported disparate results regarding antioxidant capacity measured on the same material in different laboratories even by using the same analytical method, or in a particular laboratory by using different methods. Such discrepancies could be explained by the fact that the employed methods evaluate different things under various conditions, e.g., some measurements are done in homogenous solutions, other in suspensions, some methods evaluate hydrogen atom transfer capacity, other evaluate electron transfer capacity, etc.

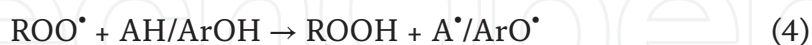
Consequently, developing standardized antioxidant capacity methods might reduce the results spreading. A basic rationale to develop standardized antioxidant capacity methods for food, being provided in [37], which considered three candidates assays for standardization, i.e., ORAC, TEAC/ABTS and Folin Ciocalteu method.

Radicals are usually quenched by two mechanisms [6, 25], i.e., by transferring either an electron (ET) or a hydrogen atom (HAT) to transform the radical to a more stable species, albeit sometimes the mentioned mechanisms may not be well distinguished [37]. Consequently antioxidant capacity measurements may be in large, categorized as electron transfer (ET)- and hydrogen atom transfer, (HAT)-based assays.

In **ET-electron transfer assays**, one or more electrons are transferred to reduce the compounds of interest according to the following reaction schemes:



HAT-hydrogen atom transfer assays involve the transfer of a H atom to the target radical and eventual secondary quenching by radical recombination, as follows:



where AH = any antioxidant with donatable H, ArOH = phenol or polyphenol, M = redox-active metal.

As can be seen from the chemical reactions written above, regardless of the mechanism involved (ET or HAT), antioxidants scavenge ROS/RNS generating the same end products indifferent to mechanism involved, albeit kinetics and influence of system parameters, particularly solvent and pH, and potential for side reactions vary [37]. Moreover, HAT and proton coupled ET reactions may occur concurrently and the main mechanism in a particular system is determined by antioxidant properties and structure, partition coefficient, solvent, etc. [37].

The ET-based methods evaluate an antioxidant's reducing capacity (also of the probe for monitoring the reaction). Mainly HAT-based methods measure competitive reaction kinetics, and the determination is effected taking into account the kinetic curves. HAT-based assays mostly involve a synthetic free radical source, an

oxidizable probe, and an oxidant. An elaborate description of antioxidant mechanisms is well presented in several review papers [6, 13–16, 38].

Antioxidant capacity is expressed as equivalents of a reference antioxidant such as trolox, gallic acid, etc., or antioxidant inhibition against oxidation of the probe (generated by ROS). Oxidation of the probe is determined by different detection techniques, such as: spectrophotometric, fluorimetric, chemiluminescent, EPR, amperometric methods, cyclic voltammetry, etc.

A classification of the methods for the assessment of antioxidant capacity/activity discussed in this work is presented in **Table 1**.

Classifications	Assays	References		
Chemical based assays	Radical/ROS-based scavenging assays	HAT/ET assays	TEAC/ABTS assay	[1, 15, 18, 24, 34]
		(Mixed mode)	DPPH assay	[1, 15, 34]
		HAT assay	ORAC assay	[1, 15, 25, 37]
			Chemiluminescence methods	[29, 37, 39, 40]
			TRAP assay	[13, 15, 41]
			TOSC assay	[15, 42]
			β -Carotene bleaching assay	[15, 34, 43]
	Non-radical redox potential-based assays	ET assay	FRAP assay	[13, 15, 20, 34]
			CUPRAC assay	[21, 35, 44]
			Nanoparticles based assays	[15, 45–47]
			• colorimetric detection, AuNPs- and AgNPs-based assays	[15, 45–47]
			• electrochemical detection, AuNPs-based assays	[15, 45–47]
			• magnetic NPs-based assays	[48]
			Electrochemical methods	[49–51]
			• cyclic voltammetry (CV) based assays	[52, 53]
• differential pulse voltammetry (DPV) based assays	[54]			
• Square wave voltammetry (SWV) based assays	[55]			
• Amperometry, biamperometry-based assays	[56–58]			
Metal chelation capacity	[13, 59]			
Total phenolic content (TPC)	[60, 61]			
Biochemical based assays and <i>in vivo</i> assays		Oxidation of low density lipoproteins (LDL) assay	[18, 62]	
		The thiobarbituric acid reactive substances (TBARS) assay	[18, 63, 64]	
		Cellular antioxidant activity assay	[18, 65, 66]	

Table 1.
Classifications of antioxidant capacity/activity assays.

2. Chemical based assays

2.1 Radical/ROS scavenging assays

2.1.1 Scavenging ability toward stable free radicals ABTS^{•+} and DPPH[•]

2,2'-azino-bis(3-ethylbenzothiazole-6-sulphonate) radical cation, ABTS^{•+} and 2,2-diphenyl-1-picrylhydrazyl, DPPH[•] are colored and stable free radicals that have been largely used to measure antioxidant capacity. DPPH[•] is commercially available, but ABTS^{•+} must be produced from the oxidation of ABTS with chemical reagents such as K₂S₂O₈, MnO₂, etc. ABTS^{•+} is soluble in aqueous and in alcoholic media (λ_{\max} 734 nm), while DPPH[•] is soluble in different organic solvents (λ_{\max} 517 nm, in ethanol). The chemical structures of ABTS^{•+} and DPPH[•] are presented in **Figure 1**.

The trolox equivalent antioxidant capacity (TEAC/ABTS) assay based on the use of ABTS^{•+} radical cation and DPPH[•] radical-based (DPPH) assay are among the most used antioxidant capacity assays.

In TEAC/ABTS assays, the antioxidant capacity is evaluated as the capability of analyzed sample to diminish the color intensity after reacting with the ABTS^{•+} radical. This assay can be employed for lipophilic as well as hydrophilic compounds. The assay is technically simple, being widely applied for screening and habitual determinations. Most often, ABTS^{•+} is produced by oxidation of ABTS with K₂S₂O₈. The reaction of antioxidants with ABTS^{•+} is quite fast. Generally, the measurements are done after a fixed period of time. The TEAC/ABTS assays were recently investigated with regards to their basic chemistry, reaction stoichiometry and the reaction pathways behind the ABTS/potassium persulfate decolorization assay [24].

A recent review [67] of TEAC/ABTS assays gives a comprehensive insight into this approach for evaluating the antioxidant capacity, including different methods of ABTS^{•+} generation, experimental design, and quantification strategies, as well as TEAC value data collection obtained using a diversity of samples. Other recent reviews regarding both ABTS/TEAC and DPPH assays can be found in [1, 5, 18, 34].

A comprehensive critical evaluation of the TEAC/ABTS, DPPH, and oxygen radical absorbance capacity (ORAC) assays, presented in [25] discusses the different methods, the intrinsic mechanisms of reactions, the advantages and disadvantages, the limitations and recommendations for applications of the methods.

The TEAC method has several advantages:

- It allows the assessment of a plethora of synthetic as well as natural antioxidants (phenols, peptides, thiols, indols, flavonoids, aminoacids, carotenoids, tocopherols, vitamin C, etc.).
- It can be applied over a large pH range.

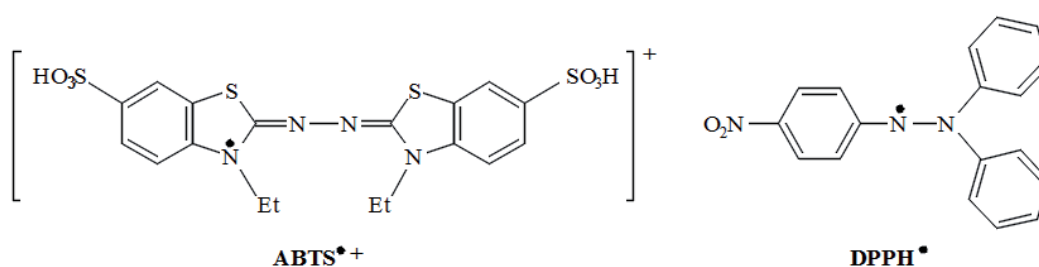


Figure 1.
Chemical structures of 2,2'-azino-bis(3-ethylbenzothiazole-6-sulphonate) radical cation, ABTS^{•+} and 2,2-diphenyl-1-picrylhydrazyl, DPPH[•] radical.

- The solubility of ABTS^{•+} in buffered and organic media enables measurement of both hydrophilic and lipophilic antioxidant activities.
- ABTS is affordable and easy to use.

Disadvantages of TEAC include:

- For some antioxidants different TEAC values may be obtained, depending on the way in which ABTS^{•+} is generated and on the measurement time interval selected.
- ABTS^{•+} (the same is applicable for DPPH[•]) is a metastable radical that does not exist in nature, being a “non-physiological” radical.
- The results of the assays depend on the reaction time. Some antioxidants react very fast and completely while other react slowly or combine a mix of fast and slow reactions [68].
- In the TEAC the molecular size and steric hindrance is an important characteristic. The accessibility of polyphenolics with bulky substituents to the radical cation ABTS is sterically restricted.

The DPPH assay is low-cost and simple and consequently has been largely used in laboratory settings for many applications. The assay is based on measuring the decrease of the absorbance of DPPH[•] radical (at a wavelength of 517 nm) as a result of its reaction with antioxidants from the sample. This method was criticized for lacking standardization in different stages of the analytical process [37].

The criticism regarding DPPH assay is expressed even harder in [25]: “The DPPH reaction has been used as if it is a simplistic chemical “black box” – reagents are mixed and a number is generated, and the chemistry occurring between is ignored.” In fact, antioxidant reactions with DPPH reagent are actually complex and reaction curves show multiple reactivity patterns [69]. DPPH reactions are very sensitive to the reaction medium, such as: water and solvent, pH, light exposure, dissolved oxygen, pH, etc. [69, 70].

The disadvantages of DPPH assay consist of the following:

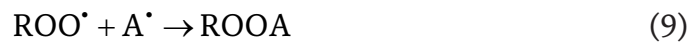
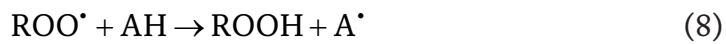
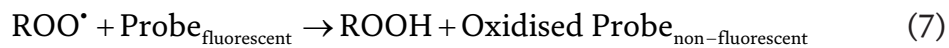
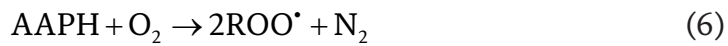
- The evaluation of antioxidant capacity by the change in DPPH[•] absorbance has to be carefully evaluated since the absorbance of DPPH[•] after reaction with an analyzed sample may be diminished by some other factors (pH, O₂, light, type of solvent, etc.).
- Fixed-time assays may undervalue the radical scavenging capacities of slow-reacting antioxidants.
- Since the ionization of phenols – and consequently the reaction rates – are highly influenced by solvent composition and pH, the DPPH assay is not adequate to ranking antioxidant compounds and natural extracts.

In essence, the significant shortcomings of both TEAC/ABTS and DPPH assays are related to the intricacy of the mechanisms of reaction with antioxidants, the big influence of the experimental conditions on the obtained results, and the important difference between DPPH[•] and ABTS^{•+} chemical structures and those of free radicals existing in biological systems.

2.1.2 Oxygen radical absorbance capacity (ORAC) assay

The ORAC method determines the radical chain breaking capacity of antioxidants by measuring the blocking-up of peroxy radical generated oxidation. The peroxy radical reacts with a probe (usually fluorescent) to form a non-fluorescent product, and the process can be monitored with a good sensitivity by fluorescence. Antioxidant capacity is determined by measuring rate and amount of product generated over time. Competition between reaction of probe and antioxidants with the ROO^\bullet radical (or other ROS/RNS) constitute the premise of the assay.

Peroxy radicals (ROO^\bullet) are the main free radicals that act in lipid oxidation in biological environment under physiological circumstances and in foods. For this reason, ORAC assay could be considered to have a biological concern as a reference for antioxidant efficacy. Commonly, 2,2'-azo bis(2-methylpropionamide) hydrochloride (AAPH) is employed as ROO^\bullet source that generates peroxy radical at a known rate at incubation in aqueous media. The reactions involved in ORAC assay are as follows:



The antioxidant capacity is measured by a diminished rate and through the quantity of product generated over time. A set of fluorescence decay curves can be obtained with or without antioxidants. The difference in the area under the curves (AUC) between the curves recorded in the presence and in the absence of the oxidant is considered to be a marker of the peroxy radical scavenging capacity. Usually trolox (a standard antioxidant) is employed as reference and the obtained ORAC

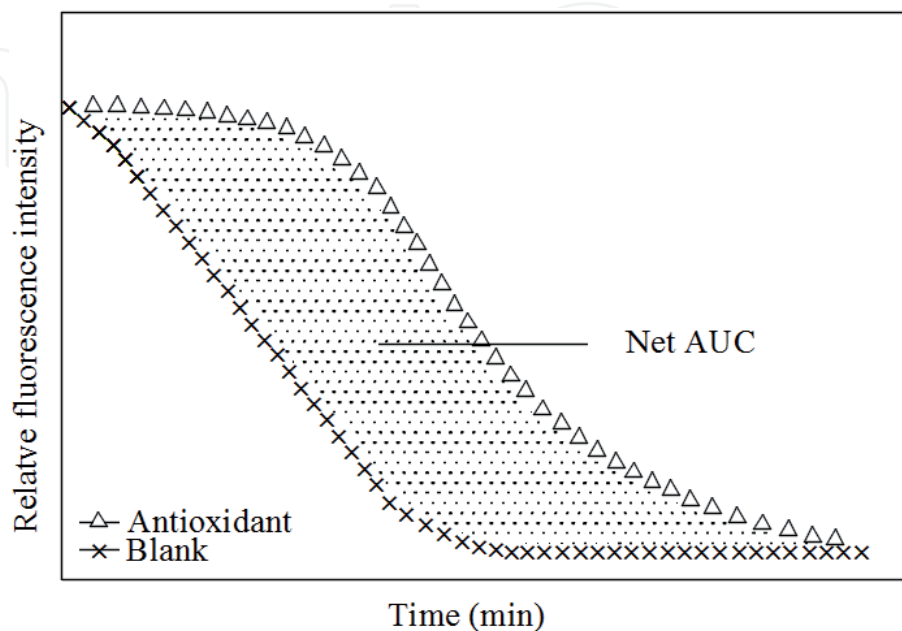


Figure 2.
 ORAC antioxidant capacity of a sample expressed as the net AUC.

values are provided as trolox equivalents of the tested antioxidants. Data are shown as micromoles of trolox equivalents (TE) per liter or per gram of sample (μmol of TE/L or μmol of TE/g). The ORAC antioxidant capacity of a sample shown as the net area under the curve (AUC) is presented in **Figure 2**.

ORAC assay is a HAT-based method because it measures the capacity of hydrogen atom donating ability of antioxidants. β -phycoerythrin (β -PE), a protein obtained from *Porphyridium cruentum*, was employed as the fluorescent probe in the first studies. However, the use of β -PE in antioxidant assays has several shortcomings and can cause false ORAC values. The currently preferred fluorescent probes are fluorescein and dichlorofluorescein diacetate [37], as they are more stable and less reactive. Nevertheless, fluorescein may undergo undesired fluorescence quenching and side reactions [71] and other fluorescent probes have been suggested in consequence.

In order to measure both hydrophilic as well as lipophilic antioxidants the initial ORAC assay was modified using a solution of 50% acetone/50% water (v/v) and 7% randomly methylated β -cyclodextrin as a solubility enhancer of the antioxidants [72, 73].

The ORAC method has the utility to be a simple and standardized assay, however, secondary reactions can occur, affecting the reported results. For example, it was reported that antioxidant-metal reactions could result in a smaller concentration of antioxidants and hence to a depreciation of the ORAC value [74].

The ORAC method can be readily automated and it is perhaps the most largely recognized of all the antioxidant methods.

2.1.3 Chemiluminescence methods

The fundamental chemistry of chemiluminescence measurements of antioxidants is based on the reaction of ROS/RNS species with special reagents to generate species in an excited state that light up (chemiluminescence). The chemical compounds that react with the initiating reactive species diminish the light generation. Hence, generally, chemiluminescence measurements for antioxidant capacity assay are based on competitive reactions. By changing the oxidant initiator (e.g., O_2^\bullet , HO^\bullet , ROO^\bullet , ONOO^- , HOCl , $^1\text{O}_2$, etc.) it is possible to measure the capacity of quenching of different ROS/RNS by an antioxidant [37]. Chemiluminescence is a highly sensitive analytical method. The detection limit is very low, below that of most chemical methods. The mainly used chemiluminescence reagents are luminol [37, 75–79], lucigenin [39], pholasin (a bioluminescent protein) [80] and peroxyoxalate [81]. Luminol is the main commonly employed aqueous chemiluminescent reagent. Luminol reacts with an oxidizing agent, hydrogen peroxide (in presence of a catalyst) to yield 3-aminophthalate in an excited electronic state, which emits light. Antioxidants can quench the produced ROS (by hydrogen peroxide) and diminish hydrogen peroxide-induced chemiluminescence.

Chemiluminescence method has been automated in flow-based assays, e.g., flow injection analysis (FIA) [29, 76, 79, 82], sequential injection analysis (SIA) [83, 84], multi-syringe FIA (MS-FIA) and multi commutation.

A review on antioxidant assays with chemiluminescence detection is presented in [40] and other more general reviews of antioxidant assays including methods with chemiluminescence detection are presented in [1, 16, 17].

The methods for the determination of lipid hydroperoxides and of the antioxidant capacity of lipids by using flow injection analysis with chemiluminescence reagents are reviewed in [29].

The TAC of some *Rosmarinus officinalis* L. (rosemary) extracts was measured by an in batch analytical method based on Co(II)-ethylendiaminetetraacetic acid

(EDTA)-induced luminol-hydrogen peroxide chemiluminescence (luminol/Co(II) EDTA/H₂O₂) [75]. The method allows for TAC determination in the range 10⁻⁵–2.5 10⁻³ moles L⁻¹ of gallic acid equivalent. The same in batch method was applied for the TAC determination of fruit juices and noncarbonated soft drinks [77] and fruit seeds extracts [85].

The luminol/Co(II)EDTA/H₂O₂ system with chemiluminescence detection was used also in a flow injection analysis (FIA) method for the total antioxidant capacity determination of wines [79] and culinary and medicinal plants extracts [76].

Amperometric TAC measurements of several plant extracts using an electrochemical gold nanozyme-sensor based on the enzyme-like catalytic activity of gold nanoparticles [58] were associated with those obtained from a chemiluminescence method reported in [75]. A good correlation has been found between the two methods (Pearson's correlation coefficient of 0.958).

A new microfluidic chemiluminescence method for fast determination of the TAC of apple and pomegranate juices and honey samples was reported in [86]. The method is based on the NaHCO₃-H₂O₂-Co²⁺ chemiluminescence reaction.

A chemiluminescence-sensing platform for the determination of natural antioxidants and imaging of their tissue distribution is reported in [87]. The chemiluminescence radiation is emitted upon the redox reaction of antioxidants (e.g., L-ascorbic acid) with quinones (e.g., menadione), in the presence of luminol.

Different chemiluminescent system that allow the evaluation of both hydrophilic and lipophilic antioxidants by using the same method were reported. Thus, lucigenin–hydrogen peroxide chemiluminescence in 2-propanol has been proposed to measure the activity of both hydrophilic and lipophilic antioxidants [88].

A peroxyoxalate–hydrogen peroxide–imidazol–fluorophore system was applied in the evaluation of antioxidants in olive oils and honey samples. The system relies on a furan dicarboxylate derivative as fluorophore [81].

2.1.4 Other radical/ROS scavenging assays

Total radical-trapping antioxidant parameter (TRAP) assay. This method generally measures the antioxidant's capability to interfere with the reaction between ROO[•] (usually generated from AAPH) and a probe. It is relatively complex and laborious to perform [39 [37]. An early review of TRAP assay is presented in [89].

A TRAP assay for measuring total plasma antioxidant capacity used R-phycoerythrin (red protein pigments from the cells of red algae) as a fluorescent probe and AAPH, as ROO[•] radical generator [41]. Fluorescence quenching was measured in absence and in presence of the analyzed antioxidant samples. The quantification of antioxidants is based on the duration of the lag phase.

Initiators for ROO[•] radicals have been produced selectively by azides, enzymes (e.g., horseradish peroxidase) [90], or H₂O₂-hemin [91], etc. Some of the probes used in TRAP assays include fluorescein, dichlorofluorescein diacetate [92], R-phycoerythrin [93] and luminol [90].

It was reported that an important limitation of the TRAP assay is the use of the lag phase for determination of antioxidant capacity because not all antioxidants have a clear lag phase [94].

Total oxy radical scavenging capacity (TOSC) assay. The assay is based on the determination of antioxidants particularly toward three strong oxidants ([•]OH, ROO[•], and ONOO⁻) [15, 42]. In TOSC assay the oxidation of α -keto- γ -methiolbutyric acid (KMBA) to ethylene by ROS and ethylene formation was determined by head space gas chromatography relative to a reference reaction. The antioxidants compete with KMBA for ROS and the formation of ethylene is inhibited.

The most important drawback of this assay is the long reaction time (hundreds of minutes) and the necessity of several chromatographic analyses for each experiment [21].

β -Carotene bleaching assay. This assay employs an aqueous emulsion of linoleic acid and β -carotene, which is discolored under the influence of the radicals generated through the spontaneous oxidation of the fatty acid, owing to exposure to dissolved O_2 , promoted by thermal induction. The measurements are done typically at 50 °C. Quantification is based on varying the rate at which β -carotene absorbance decays (at a wavelength of about 470–490 nm) in the presence of increasing concentrations of the antioxidant or prooxidant under evaluation. The decolorization is due to the breaking of π -conjugation by the addition reaction of radicals into a C=C bond of β -carotene [34]. The antioxidant capacity/activity is calculated in terms of % inhibition with regard to the reference.

An investigation of the experimental conditions that influence β -carotene bleaching assay is presented in [43] and in [95]. The β -carotene bleaching assay can screen both lipophilic and hydrophilic samples. It is sensitive to temperature, oxygen, pH and solvent effects and is time-consuming (an assay last hundreds of minutes).

2.2 Non-radical redox potential-based assays

2.2.1 Ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) assays

Ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) assays were reviewed in several recent papers [13, 14, 20, 21, 34, 35].

FRAP assay is based on antioxidants to reduce the ferric 2,4,6-tripyridyl-s-triazine complex $[Fe^{3+} - (TPTZ)_2]^{3+}$ to the blue colored ferrous complex, $[Fe^{2+} - (TPTZ)_2]^{2+}$ in acidic medium (pH 3.6). Measuring the increase in absorption at 593 nm monitors this reduction. The antioxidant capacity is expressed as $\mu M Fe^{2+}$ equivalents or as a standard antioxidant equivalents. The FRAP assay is conducted at acidic pH 3.6 in order to prevent iron precipitation.

The reaction detects compounds with redox potentials lower than 0.7 V so FRAP is an adequate screen for the capacity to maintain redox status in cells or tissues. FRAP cannot measure compounds that act by radical quenching (H transfer), specifically bio-thiols (such as glutathione) and proteins [96]. For this reason the method is rather inadequate to measure the antioxidant capacity of intracellular fluids and human plasma/serum [97, 98].

Because the redox potential of $[Fe^{3+} - (TPTZ)_2]^{3+}$ is similar to $ABTS^{*+}$ potential (0.68 V), similar compounds react in both the FRAP and TEAC assays. The FRAP mechanism is totally electron transfer and not mixed ET and HAT, and so in association with other antioxidant methods can be very useful in differentiating preponderant mechanisms with different antioxidants [37].

FRAP really determine only the reducing capacity based upon the ferric ion, which is not relevant to antioxidant capacity physiologically and mechanistically. However, in contrast to other assays of TAC, the FRAP method is simple, fast, inexpensive and robust and does not necessitate special equipment.

Cupric reducing antioxidant capacity (CUPRAC) assay. The method measures the reducing power of antioxidants to convert cupric (Cu^{2+}) to cuprous (Cu^+) ion. The copper reducing ability is measured by complexation of Cu^+ with bathocuproine (2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline) or neocuproine (2,9-dimethyl-1,10-phenanthroline) the corresponding complexes having absorption maximum at 490 nm and 450 nm, respectively [99]. **Figure 3** presents the cupric reducing antioxidant capacity (CUPRAC) reaction mechanism.

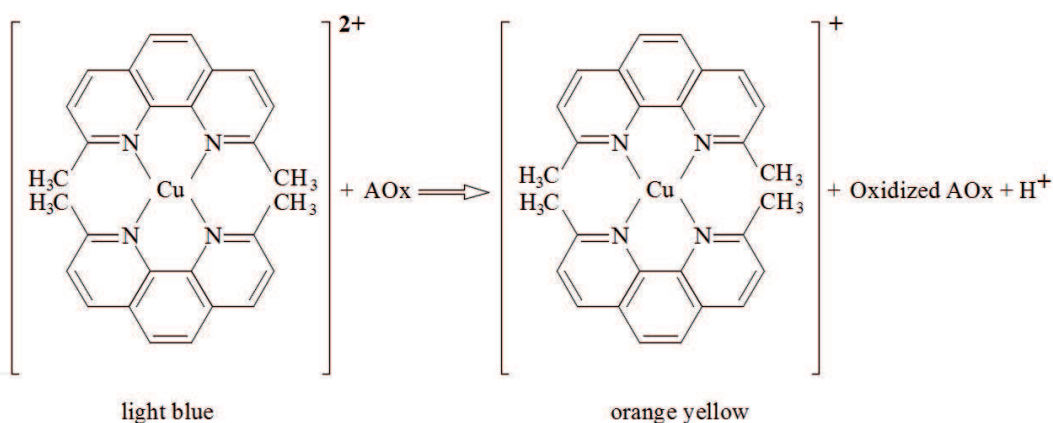


Figure 3. Cupric reducing antioxidant capacity (CUPRAC) reaction mechanism.

FRAP and CUPRAC have comparable values with TEAC values (with some exceptions) since similar redox potential probes are employed in the assay. The original CUPRAC assay has been modified in order to allow analysis of different samples, e.g., acetone/water medium containing methyl- β -cyclodextrin has been employed for simultaneous assay of hydrophilic and lipophilic antioxidants [100].

In another modified CUPRAC assay, an optical sensor containing immobilized CUPRAC reagent Cu^{2+} -neocuproine complex onto a perfluorosulfonate cation-exchange polymer (Nafion) membrane matrix was developed. The measurements of absorbance were done at 450 nm [101].

CUPRAC assay is more selective due to its lower redox potential than that of redox couples like $\text{Ce}^{4+}/\text{Ce}^{3+}$ and $\text{Fe}^{3+}/\text{Fe}^{2+}$ [102]. The CUPRAC assay have been discussed in a comprehensive review in [44].

2.2.2 Nanoparticles based assays

For the determination of antioxidants, nanoparticles (NPs) can be employed as electrochemical or colorimetric probes, components in chemical and biological detection systems, and for radical generation. Several reviews regarding TAC determination by using NPs can be found in literature [18, 45–47, 103].

Chemical reduction-based nanotechnological assays of colorimetric TAC measurements make use of the generation or growth of noble metal nanoparticles (AuNPs, AgNPs, etc.) upon reaction of Au^{3+} or Ag^+ salts with antioxidant. The strong visible light absorption at a specific wavelength results from the surface plasmon resonance absorption of metal nanoparticles.

In a pioneering work, reported in [104] the antioxidant capacity of several phenolic acids was determined from the formation and growth of gold nanoparticles (AuNPs). The same experimental approach was employed in [105] to evaluate the antioxidant capacity of chrysanthemum extracts and tea beverages.

A comparison of a AgNPs-based method for TAC assays in different rapeseed varieties with those of several spectrophotometric methods (total phenolic with Folin–Ciocalteu reagent, FRAP and DPPH assays) was performed in [106]. A significant correlation (r : 0.59–0.91) was found between the spectrophotometric methods and the nanoparticle-based assay.

Another interesting alternative, an optoelectronic tongue based on an array of gold and silver nano-particles for analysis of a diversity of natural, synthetic and biological antioxidants is described in [107].

A portable nanoparticle based-assay for rapid and sensitive measurement of food antioxidants was proposed in [108] based on the use of immobilized ceria

(cerium oxide) nanoparticles. Due to the reversible oxidation state of cerium Ce^{3+}/Ce^{4+} on the NPs surface, nanoceria is capable of changing redox states and surface properties after interaction with antioxidants.

Furthermore, a novel chemical sensing array, based on metal oxide nanoparticles (i. e., cerium oxide, titanyl oxalate, TiO_2 , Fe_2O_3 , ZrO_2 , ZnO and SiO_2) immobilized onto cellulose, was described as a portable and cheap paper-based colorimetric assay for polyphenol detection and field evaluation of antioxidant containing samples [109].

Last but not least, a novel method was proposed in [110] for evaluating the composition of mixtures of natural polyphenolic compounds by using an array of nano-oxides sensors and by chemometric analysis of the experimental data.

Some spectrometric and electrochemical nanomaterial-based assays for antioxidant assessment are presented in **Table 2**.

The nanoparticle-based assays to evaluate antioxidant capacity of natural products embody a novel and promising domain melding nanoscience with food and health research [18, 47].

	Antioxidant	Nano-material	Detection principle	Real samples	Reference
Spectro-metric	Total polyphenols in fat-rich samples	AuNPs	Detection of polyphenols in organic medium without extraction, by AuNPs formation at 540 nm	Chocolate, olive oil	[111]
	Polyphenols in food	AuNPs	Detection of polyphenol-mediated AuNPs formation from extracts via LSPR* by UV- visible spectroscopy at 540 nm	Tea, apple, pear, wine, honey	[112]
	Polyphenols	AuNPs	Au reduction, mild conditions, LSPR* detection	Fruit extracts	[113]
	Polyphenols	AgNPs	AgNPs seed-growth, LSPR* detection	Fruit juices, olive oils	[114]
	Polyphenols	AgNPs	AgNPs seed-growth, LSPR* detection	Ginger	[115]
	Total catechins evaluation	RhNPs	RhNPs LSPR* shifting	Teas	[116]
	Polyphenols	CdTe QDs**	CdTe QDs** fluorescence quenching inhibition	Teas	[117]
	Polyphenols	Graphene QDs	Graphene QDs fluorescence quenching	Olive oil extracts	[118]
	Phenolic acids	AuNPs on paper	Reduction of gold ions to AuNPs on paper sensors and measurement of the resultant color intensity	Tea, red wine	[119]

	Antioxidant	Nano-material	Detection principle	Real samples	Reference
	Flavonoids	Fluorescent gold nanocluster	Fluorescence quenching of gold nanoclusters imbedded into the cavity of bovine serum albumin tertiary structure	Serum, plasma, pharmaceutical analysis	[120]
Electro-chemical	<i>tert</i> -butylhydroquinone (TBHQ) and butylated hydroxyanisole (BHA)	Nano-carbon black	Measurements in the presence of the cationic surfactant CPB*** by square wave voltammetry using a carbon black paste electrode	Food samples and biodiesel	[121]
	Gallic acid	NiAl ₂ O ₄ glassy carbon nano-composite	Cyclic voltammetry and amperometry with a NiAl ₂ O ₄ glassy carbon working electrode	Food samples	[122]
	Flavonoids (myricetin and rutin)	Single-walled carbon nanohorns	Based on host-guest supramolecular recognition concept	Human serum	[123]
	Antioxidant capacity	Multi-walled carbon nanotubes	Chronocoulometry at glassy carbon electrode modified with multi-walled carbon nanotubes	Red/white wine	[124]
	Antioxidant capacity (o-diphenols)	Cerium (IV)oxide NPs	Polyphenols oxidation at quinones, quinones reduction at screen printed carbon electrode-CeO ₂ (IV) NPs	Red/white wine	[125]

*Laser surface plasmon resonance.

**Quantum dots.

***Cetylpyridinium bromide.

Table 2.
 Spectrometric and electrochemical nanomaterial-based assays for antioxidant assessment.

2.2.3 Electrochemical methods

Electrochemical techniques emerged as an alternative strategy for a quick, precise, and cost-effective determination of the TAC of different samples, e.g., foods and beverages, plant extracts, etc. They circumvent some of the drawbacks of spectrophotometric methods such as long analyses and sample preparation time, the use of expensive reagent and undefined reaction time. These methods also enable the quantification of the antioxidant compounds, with very good sensitivity [126, 127] and sometimes, they permit determinations in the presence of compounds that interfere in other methods, such as the case of ascorbic acid in juice [128].

Electrochemical methods for antioxidant capacity/activity evaluation have been reviewed in [129] and more recently in [14, 49–51, 130]. The most commonly used electrochemical techniques for antioxidant assays in different samples are

cyclic voltammetry, differential pulse voltammetry, square wave voltammetry and amperometry.

Cyclic voltammetry (CV) [131]. The half-wave potential ($E_{1/2}$) of the registered cyclic voltammogram indicates a specific constituent in the analyzed sample (its ability to donate electrons) whereas the maximum current intensity indicates the concentration of a constituent. Antioxidants with similar structures have similar electron donating abilities and therefore similar half-wave potentials in cyclic voltammetry. Thus, when present in mixtures, they contribute globally to the observed features of the sample cyclic voltammogram.

Cyclic voltammetry has been widely used for evaluating the TAC of low-molecular weight antioxidants present in biological fluids, animal plasma, plants and fruits [52].

In [53] the results obtained for the TAC determination of 10 different fruit tea infusions using spectrophotometric methods (TEAC/ABTS, FRAP, DPPH and Folin–Ciocalteu's reagent total phenolic content) and by applying the CV method were reported comparatively.

In addition, CV has been used to measure the antioxidant capacity of a diversity of samples such as different winemaking by-products (pomace, skins, seeds, and stems) [132], propolis [133], edible oils [134] and berry fruits [135], among others.

Differential pulse voltammetry (DPV) has been applied for TAC assay of white and red wines [54] by using gallic acid as reference. The elaborated method is based on gallic acid electro-oxidation at carbon nanotubes-modified carbon paste electrode, at 350 mV (vs. Ag/AgCl) in 0.1 M phosphate buffer solution (pH = 2.50). The method enabled a reliable evaluation of the TAC for red and white wine samples, when glucose and ascorbic acid do not interfere.

Square wave voltammetry (SWV) has been used to analyze catechins in green and black teas [55] obtaining a detection limit of 40 nM for epigallocatechin gallate in green teas.

A databank of the content of antioxidants in food products was created based on amperometric measurements [56]. The antioxidants were quantified in 1140 food products, beverages, etc.

Amperometric, CV and DPV measurements using an electrochemical gold nanozyme-sensor [58] (based on the enzyme-like catalytic activity of gold nanoparticles), were used to evaluate the TAC of several plant extracts. The results of the amperometric measurements were compared to those from a chemiluminescence method for TAC assays [75] and a good correlation was found.

Biamperometric determinations are based on the reaction of the analyte with a redox pair such as I_2/I^- , Fe^{3+}/Fe^{2+} , $DPPH^*/DPPH$, $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$. $DPPH^*/DPPH$ biamperometry was used in the analysis of fruit juices for the determination of their TAC, using two identical Pt electrodes [57] and for tea, wine and coffee using glassy carbon electrodes [136].

Analytical characteristics of some electrochemical methods applied for the determination of antioxidants or total antioxidant capacity are presented in **Table 3**.

Electrochemical measurements of antioxidant capacity are redox-based methods with many advantages over conventional chemical assays since they are rapid and simple and do not require special chemical reagents or complicated sample preparation. Thus, they allow analysis of colored samples that do not permit direct evaluation by spectrophotometric techniques (e.g., wine and fruit juice) [150]. Electrochemical techniques allow also a large number of experimental parameters to be easily controlled and to register important information from a sample (e.g., the half-wave potential, the voltammetric charge, peak current intensity, etc.) that helps characterize different compounds from a sample [53]. These methods can be used to evaluate samples of whatever lipophilicity or hydrophilicity [151].

Electrochemical method	Electrode	Antioxidants	LOD/Linear range	Real samples	References
Cyclic voltammetry	Iridium-containing carbon (Ir-C)	Caffeic acid	0.0–25.0 mg L ⁻¹	Wine	[137]
	Graphite Carbon microspheres Carbon nanotubes	Vanillic acid	2.85 μM 3.82 μM 4.13 μM/ 10–400 μM	Artificial wine solutions	[138]
	Glassy carbon	Curcumin	4.1 × 10 ⁻⁶ M	Spices	[139]
	Carbon ink chemically modified electrode containing [Cu(neocuproine) ₂] (NO ₃) ₂	Trolox Gallic acid Ascorbic acid	2.51 × 10 ⁻⁵ M - -	Teas	[140]
	Glassy carbon electrode	TAC*	2–80 μmol L ⁻¹ trolox	Berry fruits	[135]
	Glassy carbon disc electrode	(+)-catechin as standard	0.0078 to 1 mM	Food grade oenological tannins	[141]
Differential pulse voltammetry	Carbon paste platinum	Ascorbic acid	0.02 mM/0.07–20 mM; 0.087 mM/ 0.31–20 mM	Fruit juices and wines	[142]
	Carbon nanotubes modified carbon paste	TAC* (vs gallic acid)	3.0 × 10 ⁻⁷ M/ 5.0 × 10 ⁻⁷ - 5.0 × 10 ⁻⁵ M	Red and white wines	[54]
	Dropping mercury	Gallic acid	0.3 μM/ 1.0–50 μM	Fruit juices	[143]
	Glassy carbon electrode surface activated by <i>in situ</i> chemical oxidation	Tertiary butyl hydroquinone	67 nM/ 1.0 μM – 1.1 mM	Jatropha biodiesel	[144]
Square wave voltammetry	4-[(4-decyloxyphenyl)- ethynyl]-1- methylpyridinium iodide modified glassy carbon	Total phenolic compounds (vs caffeic acid)	9.0 × 10 ⁻⁷ molL ⁻¹ / 9.9 × 10 ⁻⁷ - 3.8 × 10 ⁻⁵ molL ⁻¹	Total polyphenol content of Yerba mate extracts	[145]

Electrochemical method	Electrode	Antioxidants	LOD/Linear range	Real samples	References
	Screen printed electrode modified with CeNPs. (CeNPs/C/SPE)	Gallic acid Caffeic acid Quercetin <i>t</i> -resveratrol	7.0 μM 10.0 μM 9.0 μM 8.0 μM	White/red wines	[146]
Amperometry	Biosensor based on peroxidase-modified carbon paste	<i>t</i> -resveratrol Caffeic acid	0.023 mg L^{-1} / 0.05–52 mg L^{-1} 0.020 mg L^{-1} / 0.06–69 mg L^{-1}	Wine	[147]
Amperometry (flow injection)	Carbon nanotube modified-glassy carbon electrode	Gallic acid Catechin Quercetin Caffeic acid Trolox TAC* (vs trolox)	0.04 μM 0.02 μM 0.03 μM 0.08 μM 0.04 μM	Thai vegetables/herbs	[148]
Amperometry (flow injection)	Glassy carbon/carbon nanotubes/polyethyleneimine electrode	Caffeic acid, gallic acid Ferulic acid <i>p</i> -coumaric acid	< 0.1 μM / 10^{-7} – 10^{-4} M	Wines	[149]

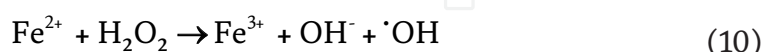
*Total antioxidant capacity.

Table 3.
Analytical characteristics of some electrochemical methods applied for the determination of antioxidants or total antioxidant capacity.

A disadvantage of the electrochemical methods of antioxidant capacity determination in complex media is the difficulty to analyze the macromolecules with antioxidant properties.

2.3 Metal chelating assay

Metal chelation capacity is evaluated by measuring the chelating effect of antioxidants for metal ions. Fe^{2+} ions are known to enhance lipid peroxidation through Fenton reaction and also by decomposing lipid hydroperoxides into peroxy and alkoxy radicals, which are more reactive. By Fenton reaction (written below), the ferrous ions produce $\cdot\text{OH}$ radicals, which are highly reactive, and contribute appreciably to oxidative stress. The resulting hydroxy radicals cause damage to proteins, carbohydrates, cellular lipids and nucleic acids leading to cellular damage.



Numerous metal ions such as Cu^+ , Ti^{3+} , Cr^{2+} , and Co^{2+} and their complexes in their lower oxidation states react with H_2O_2 in a similar manner as Fe^{2+} , and the mixtures of these metal ions with H_2O_2 were named “Fenton-like” reagents [96].

Metal chelation capability could be used as an indicator of antioxidant capacity. Chelating agents stabilizing the oxidized form of the metal ions are effective as secondary antioxidants.

Commonly, metal chelation capacity is evaluated by determining the chelating effect of antioxidants for ferrous ion [59]. The evaluation of the metal-chelating activity of an antioxidant is based on the absorbance measurement of Fe^{2+} -ferrozine complex in presence and in absence of the analyzed sample. The decrease in absorbance of the solution after the introduction of test sample is related to the metal chelation capacity of the sample. The measurements are performed spectrophotometrically at 562 nm [13]. Ethylenediaminetetraacetic acid (EDTA) is generally used as a standard metal chelator. Metal chelation capability of different samples is expressed as EDTA equivalents.

In [38] the results obtained at the determination of metal chelation capacity for a number of antioxidants and extracts are presented. A study regarding the standardization of the experimental protocols to evaluate the capability to chelate Fe^{2+} (employing ferrozine as chromogenic reagent) and Cu^{2+} (employing pyrocatechol violet as the chromogen agent) is presented in [152]. This study used 96-well microplates and analyzed Brazilian coffees ($n = 20$).

2.4 Total phenolic content (TPC)

Total phenolic content (TPC) or Folin–Ciocalteu reducing (FCR) assay is an important parameter of total antioxidant capacity (TAC) and largely employed for evaluation of a diversity of samples. The TPC assay has been used for a long period as a measure of total phenolic content in natural products [37]. In this method, TPC values are evaluated as equivalents of gallic acid or another phenolic compound, e.g., caffeic acid, catechin, ferrulic acid, etc.

The Folin Ciocalteu reagent contains phosphomolybdic/phosphotungstic acid complexes, with added lithium sulfate and bromine, in strong basic medium (5–10% aqueous Na_2CO_3 , pH 10–12) to generate the phenolate anion [153]. The TPC method is based on the measurement of the blue-colored chromophore ($\lambda_{\text{max}} = 620\text{--}765$ nm) generated as a result of reduction of Folin Ciocalteu reagent with phenols from the sample [154]. The reduction site is considered the molybdenum centre in the complex (Mo^{6+} ion is reduced to Mo^{5+} by phenols).

TPC assay is operationally simple, reproducible and convenient for evaluation of total phenolic for a variety of samples because the reagent is commercially available.

However many non-phenolic compounds e.g., ascorbic acid, aromatic amines, sulfur dioxide, some metal ions (Cu^+ and Fe^{2+}), etc. can interfere by reducing Folin Ciocalteu reagent. Several methodologies have been studied to increase the selectivity of the TPC method for total phenolic determinations in plant extracts [60].

A critical review of the methods for the assays of TPC in food matrices is presented in [61]. The review focuses on the most used methods to measure by UV-Vis spectrometry the TPC, *o*-diphenols, flavonoids, flavonols, anthocyanins, and tannins. Examples of application of TPC assay for winemaking byproducts (seeds, skins, stems, and pomace) and in Venezuelan propolis are given in [155, 156], respectively.

The TPC assay is still widely employed. However solid phase extraction (SPE) was considered as clean-up step in only a few cases. When SPE was employed, the SPE-FCR assay presented excellent reproducibility [157].

TPC assays are low-cost, simple, do not require expensive equipment and they are used largely to evaluate a big diversity of samples.

3. Biochemical-based assays and *in vivo* assays

3.1 Oxidation of low density lipoprotein (LDL) assay

A review of this assay alongside other assays measuring lipid oxidation can be found in [62]. The oxidation of LDL generated by ROS/RNS was studied long ago. ROS play an very important role in the initiation, propagation and termination reactions of the LDL lipid peroxidation. The lipid peroxidation processes could be followed by different methods, e.g., UV spectrophotometry and/or chemiluminescence techniques. As an initiator of LDL oxidation is commonly employed cupric sulfate. By using a spectrophotometric methods the formation of diene conjugates at 234 nm is measured. By using a chemiluminescence methods the emitted radiation is measured as a result of the formation of oxidative products. By mixing a cupric sulfate solution with LDL sample, the kinetic profiles correspond to the occurrence of a lag phase owing to the existence of endogenous antioxidants such as coenzyme Q and vitamin E in the LDL particle. Following the lag time, the peroxidation of lipids is measured as an growth of the analytical signal (absorbance or chemiluminescence intensity) that finally, after minutes or hours, hit a plateau. By adding an antioxidant to the reaction mixture the lag time is enhanced. The antioxidant capacity is evaluated by measuring of the lag time. The most important advantage of this method is the employment of a biological significant target.

3.2 The thiobarbituric acid reactive substances (TBARS) assay

Two review dedicated exclusively to TBARS assays are presented in [63, 64]. Important aspects of the TBARS assay such as state-of-the-art of the method, determination in physiological systems, assays in food systems and the employment of TBARS in antioxidant evaluation studies are presented in [63].

The thiobarbituric reactive substances (TBARS) assay is frequently used to evaluate lipid peroxidation. The method is based on the reaction of malondialdehyde (MDA) generated as an advanced product of unsaturated lipid degradation under the influence of ROS/RNS, with thiobarbituric acid (TBA) under acidic conditions and at high temperature (100 °C) [158]. It is obtained a characteristic colored product $[\text{MDA}-(\text{TBA})_2]$ which is measured spectrophotometrically at

532 nm. MDA is a marker of oxidative stress. It is formed from polyunsaturated fatty acids (PUFA) with at least three double bonds in their molecule. This method is not a selective assay for lipid peroxidation products because TBA reacts with a diversity of aldehydes, not only those generated in the lipid peroxidation process [14]. The lack of specificity of the method is emphasized by the designation: thiobarbituric acid reactive substances (TBARS). MDA formation is the most largely employed method for lipid peroxidation evaluation. The method was significantly enhanced by coupling with HPLC. Several food components such as sugar degradation products, proteins and Maillard browning products affect the measurements. The thiobarbituric acid reactive substances (TBARS) method is widely employed to evaluate antioxidant activity and lipid oxidation in a diversity of samples.

3.3 Cellular antioxidant activity (CAA) assay

Cellular-based antioxidant activity assays (CAA) are performed within the cell medium and are presumed to be biologically more appropriate than the respective chemical assays owing to their better representation of the physico-chemical characteristics of the medium [159]. At the cellular level the antioxidant outcome is not confined only to reactive species scavenging, but imply also gene expression, modulation of redox cell signaling and upregulation of detoxifying or antioxidant enzymes. Moreover, in order to assay antioxidant capacity/activity it is very important to take into consideration some features regarding the bioavailability of an antioxidant such as the uptake, the partitioning in membranes and the metabolism. CAA assay is very useful for the evaluation of a new antioxidant because the change of the redox state at the cellular level (caused by the antioxidant) is strongly influenced by the different cell components.

The principle of CAA is presented in **Figure 4**. The cell-permeable non-polar 2,7-dichlorofluorescein diacetate (DCFH-DA) is used as a fluorescence probe. Within the cells this molecule is deacetylated by cellular esterases generating a polar molecule, 2,7-dichlorofluorescein (DCFH) which is captured in the cells. Afterwards, peroxy radicals produced inside the cells from 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) which cross easily the cellular membrane oxidize DCFH to form dichlorofluorescein (DCF) which is fluorescent. The fluorescence intensity generated within the cells is related with the extent of oxidation. The molecules with antioxidant properties scavenge peroxy radicals and will decrease the generation of fluorescence. Consequently, the antioxidant activity of a sample can be evaluated by assessing the decrease in the cellular fluorescence.

Several reviews were published regarding this topic [18, 65, 66]. Based on the CAA concept introduced in [160], CAA was used to determine the antioxidant capacity of dietary supplements, foods and phytochemicals in cell cultures [159]. In this study, human hepatocarcinoma HepG2 cells were loaded with the redox sensor DCFH which is oxidized to fluorescent DCF by the ROO^\bullet resulted from the thermal decomposition of AAPH. Antioxidants diminish the fluorescent radiation emitted by DCF. CAA is expressed as μmoles of quercetin equivalents per $100 \mu\text{mol}$ of tested pure compound or per 100 g product (vegetables, fruits, etc.). Several cell sorts have been employed for the CAA assay beyond HepG2, e.g., Caco-2 matured differentiated intestinal cells [161], human gastric adenocarcinoma cell line AGS [162], etc.

Cellular oxidative stress can also be elicited by exposing cell cultures to H_2O_2 (in the mM range) and then measuring fluorimetrically the oxidation of the probe (DCFH) [163].

Saccharomyces cerevisiae cells were employed in a CAA assay to measure antioxidant capacity of different types of products in living systems [164]. Pretreatment of

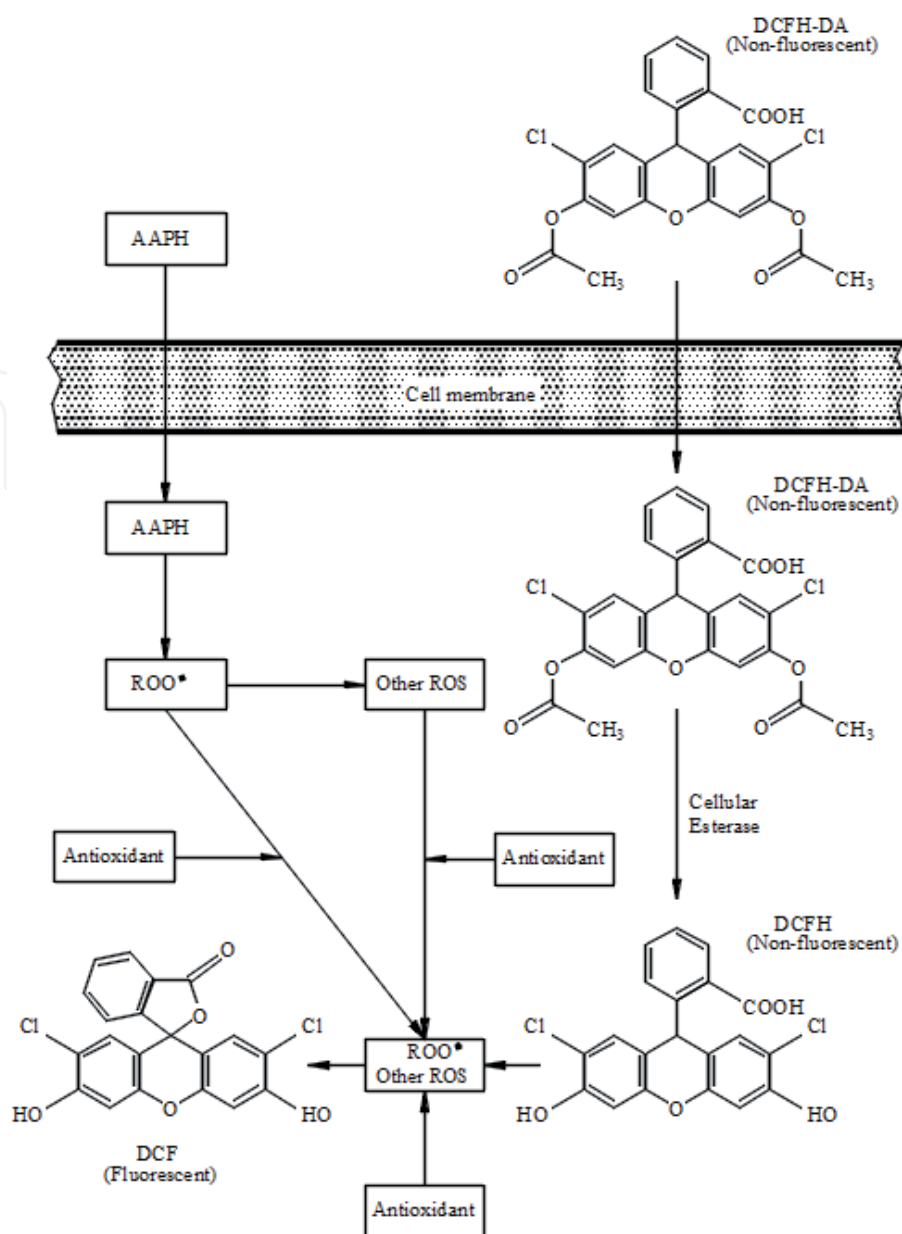


Figure 4.

Schematic presentation of cellular antioxidant activity assay. DCFH-DA, _ dichlorofluorescin diacetate; DCFH, 2',7'-dichlorofluorescin; DCF, dichlorofluorescein; AAPH, 2, 2'-azo bis(2-amidinopropane) dihydrochloride.

the cells with different flavonoids [164] or mixtures of polyphenols [165] partially diminished the damage generated by H_2O_2 .

S. cerevisiae as a model organism system for the antioxidant activity assessment of dietary natural products is reviewed in [65].

An investigation of antioxidant activities of 44 types of dark teas using the DPPH, ABTS, FRAP assays, and CAA assay (by using HepG2 cells) is reported in [166]. Correlation analysis indicated that there was a significant positive correlation between the levels of epigallocatechin gallate and the antioxidant activities evaluated using the ABTS and FRAP assays.

The CAA assay is an adequate and very good technique to measure the performance of antioxidants against oxidative stress. In this manner it is evaluated the capacity of a compound or a mixture of compounds to exercise an antioxidant response at the cellular level and to reduce intracellular oxidative stress, not just its capability as a reducing agent or its ROS/RNS scavenging ability. The CAA methodology is closer to a biological approach, and an antioxidant is regarded as a compound useful to modulate the redox state of the cell.

It is indicated to evaluate the antioxidant capacity of a sample by employing several chemical methods and CAA assays. The antioxidant capacities evaluated by CAA assays are not related well with their chemical values because the two types of methods are affected by very distinct factors.

4. Conclusions

Many studies were published concerning the antioxidant capacity of different products. However, with all these research efforts, a direct link between a food TAC value and health benefits was not found [12].

Taking into account the vast material published in the literature the following conclusion regarding the antioxidant assays can be drawn [14, 15]:

- The expression “total antioxidant capacity” (TAC) correspond to the cooperant effect of antioxidants existing in a sample (cumulative and maybe synergistic/antagonistic). It is a more adequate term to express the total antioxidant capability of a sample than the summation of individual antioxidant constituents.
- It is a stringent need to standardize the TAC assays and to formulate the results of measurements as equivalents of a standard material so that to enable relevant comparison between different methods and different samples [18].
- Most methods developed for TAC evaluation are not based on well detailed investigations of the chemical system involved in measurements (antioxidants interactions, pH, effect of solvents, kinetics, etc.) [16, 19].
- Many *in vitro* antioxidant methods are accomplished at pH values far from physiological pH and cannot have much sense for *in vivo* determinations of antioxidant effect.
- It is very useful to add a cellular-based assay to assess the analyzed sample capability to generate a cellular antioxidant response, in addition to its ability as a good scavenger of ROS/RNS [18].
- Potential mutual action of antioxidants (i.e., synergistic or antagonistic effects) or prooxidant actions of antioxidants (e.g., under the influence of the composition of the medium) should be taken into account [16, 167].
- For testing natural compounds it is necessary to employ several *in vitro* chemical-based assay that measures various facets of the reactivity of the antioxidants toward ROS/RNS [18]. Including a CAA assay is highly recommended [20].

Taking into account our evaluation regarding the state-of-the-art in the field of antioxidant capacity/activity assays we consider that the assessment regarding this subject expressed in [25] is correct, namely:” Twenty five years of antioxidant screening have NOT resolved issues of assay chemistry, standardization, and reporting; provided significant insight into chemical mechanisms and factors controlling antioxidant action; clearly connected *in vitro* assay chemistry to *in vivo* actions; established rate constants for reaction of antioxidants with radicals that are relevant in foods and biological tissues;...”

The *in vitro* antioxidant assays and the determination of total phenolic content employing colorimetric methods are not only used for the evaluation of potential beneficial effects of different products. There are also used for the quality control of natural products and foods [166, 168] where the antioxidant capacity of commercial samples, evaluated by *in vitro* assays, can be collated against reference materials. Hence, trends can be very valuable for comparing samples from the same materials. In food technology, *in vitro* antioxidant methods and TPC assay may be useful to assess, e.g., the antioxidant actions of herbal extracts on lipid-rich foods, the effects of processing steps on the stability of phenolic compounds from herbal extracts [168] employed to counteract lipid oxidation, or to obtain more antioxidant compounds from raw materials. In the area of active packaging, radical scavenging assays can contribute to assessing efficiency of antioxidant packaging formulations [169]. The *in vitro* methodologies for antioxidant and TPC assays are applied in routine quality control programs by food companies in many countries [170, 171]. The methodologies for antioxidant and TPC assays can be considered valuable high-throughput, low cost tools used to evaluate and find antioxidant sources and for quality control of foods and natural products.

Acknowledgements

This work was supported by a grant of the Romanian Ministry of Education and Research, CCCDI - UEFISCDI, project number PN-III-P2-2.1-PED-2019-3945, contract 362 PED, within PNCDI III.

IntechOpen

Author details

Andrei Florin Danet

Faculty of Chemistry, University of Bucharest, Bucharest, Romania

*Address all correspondence to: andrei.danet@g.unibuc.ro

IntechOpen

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Shahidi F, Zhong Y. Measurement of antioxidant activity. *Journal of Functional Foods*. 2015;18:757-781. DOI: 10.1016/j.jff.2015.01.047
- [2] Halliwell B. Antioxidant characterization - methodology and mechanism. *Biochemical Pharmacology*. 1995;49(10):1341-1348. DOI: 10.1016/0006-2952(95)00088-h
- [3] Halliwell B, Murcia MA, Chirico S, Aruoma OI. Free-radicals and antioxidants in food and in vivo -what they do and how they work *Critical Reviews in Food Science and Nutrition*. 1995;35(1-2):7-20. DOI: 10.1080/10408399509527682
- [4] Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B, Weil JA. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry*. 2004;84(4):551-562. DOI: 10.1016/s0308-8146(03)00278-4
- [5] Apak R. Current Issues in Antioxidant Measurement. *Journal of Agricultural and Food Chemistry*. 2019;67(33):9187-9202. DOI: 10.1021/acs.jafc.9b03657
- [6] Huang DJ, Ou BX, Prior RL. The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*. 2005;53(6):1841-1856. DOI: 10.1021/jf030723c
- [7] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*. 1999;26(9-10):1231-1237. DOI: 10.1016/s0891-5849(98)00315-3
- [8] Brand-Williams W, Cuvelier ME, Berset C. Use of free-radical method to evaluate antioxidant activity *Food Science and Technology-Lebensmittel-Wissenschaft & Technologie*. 1995;28(1):25-30. DOI: 10.1016/j.jff.2015.01.047
- [9] Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*. 1996;239(1):70-76. DOI: 10.1006/abio.1996.0292
- [10] Apak R, Guclu K, Ozyurek M, Celik SE. Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchimica Acta*. 2008;160(4):413-419. DOI: 10.1007/s00604-007-0777-0
- [11] Berker KI, Olgun FAO, Ozyurt D, Demirata B, Apak R. Modified Folin-Ciocalteu Antioxidant Capacity Assay for Measuring Lipophilic Antioxidants. *Journal of Agricultural and Food Chemistry*. 2013;61(20):4783-4791. DOI: 10.1021/jf400249k
- [12] Pellegrini N, Vitaglione P, Granato D, Fogliano V. Twenty-five years of total antioxidant capacity measurement of foods and biological fluids: merits and limitations. *Journal of the Science of Food and Agriculture*. 2020;100(14):5064-5078. DOI: 10.1002/jsfa.9550
- [13] Gulcin I. Antioxidants and antioxidant methods: an updated overview. *Archives of Toxicology*. 2020;94(3):651-715. DOI: 10.1007/s00204-020-02689-3
- [14] Apak R, Ozyurek M, Guclu K, Capanoglu E. Antioxidant Activity/ Capacity Measurement. 1. Classification, Physicochemical Principles, Mechanisms, and Electron Transfer (ET)-Based Assays. *Journal of Agricultural and Food Chemistry*. 2016;64(5):997-1027. DOI: 10.1021/acs.jafc.5b04739

- [15] Apak R, Ozyurek M, Guclu K, Capanoglu E. Antioxidant Activity/Capacity Measurement. 2. Hydrogen Atom Transfer (HAT)-Based, Mixed-Mode (Electron Transfer (ET)/HAT), and Lipid Peroxidation Assays. *Journal of Agricultural and Food Chemistry*. 2016;64(5):1028-1045. DOI: 10.1021/acs.jafc.5b04743
- [16] Apak R, Ozyurek M, Guclu K, Capanoglu E. Antioxidant Activity/Capacity Measurement. 3. Reactive Oxygen and Nitrogen Species (ROS/RNS) Scavenging Assays, Oxidative Stress Biomarkers, and Chromatographic/Chemometric Assays. *Journal of Agricultural and Food Chemistry*. 2016;64(5):1046-1070. DOI: 10.1021/acs.jafc.5b04744
- [17] Bunaciu AA, Danet AF, Fleschin S, Aboul-Enein HY. Recent Applications for in Vitro Antioxidant Activity Assay. *Critical Reviews in Analytical Chemistry*. 2016;46(5):389-399. DOI: 10.1080/10408347.2015.1101369
- [18] Lopez-Alarcon C, Denicola A. Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays. *Analytica Chimica Acta*. 2013;763:1-10. DOI: 10.1016/j.aca.2012.11.051
- [19] Niki E. Assessment of Antioxidant Capacity in vitro and in vivo. *Free Radical Biology and Medicine*. 2010;49(4):503-515. DOI: 10.1016/j.freeradbiomed.2010.04.016
- [20] Apak R, Capanoglu E, Shahidi F, editors. *Measurement of Antioxidant Activity & Capacity: Recent Trends and Applications*. 1st ed. Chichester: Wiley; 2018. DOI: 10.1002/9781119135388
- [21] Amorati R, Valgimigli L. Advantages and limitations of common testing methods for antioxidants. *Free Radical Research*. 2015;49(5):633-649. DOI: 10.3109/10715762.2014.996146
- [22] Amorati R, Valgimigli L. Methods To Measure the Antioxidant Activity of Phytochemicals and Plant Extracts. *Journal of Agricultural and Food Chemistry*. 2018;66(13):3324-3329. DOI: 10.1021/acs.jafc.8b01079
- [23] Shivakumar A, Kumar MSY. Critical Review on the Analytical Mechanistic Steps in the Evaluation of Antioxidant Activity. *Critical Reviews in Analytical Chemistry*. 2018;48(3):214-236. DOI: 10.1080/10408347.2017.1400423
- [24] Ilyasov IR, Beloborodov VL, Selivanova IA, Terekhov RP. ABTS/PP Decolorization Assay of Antioxidant Capacity Reaction Pathways. *International Journal of Molecular Sciences*. 2020;21(3):article number 1131. DOI: 10.3390/ijms21031131
- [25] Schaich KM, Tian X, Xie J. Hurdles and pitfalls in measuring antioxidant efficacy: A critical evaluation of ABTS, DPPH, and ORAC assays. *Journal of Functional Foods*. 2015;14:111-125. DOI: 10.1016/j.jff.2015.01.043
- [26] Karadag A, Ozcelik B, Saner S. Review of Methods to Determine Antioxidant Capacities. *Food Analytical Methods*. 2009;2(1):41-60. DOI: 10.1007/s12161-008-9067-7
- [27] Moon JK, Shibamoto T. Antioxidant Assays for Plant and Food Components. *Journal of Agricultural and Food Chemistry*. 2009;57(5):1655-1666. DOI: 10.1021/jf803537k
- [28] Antolovich M, Prenzler PD, Patsalides E, McDonald S, Robards K. Methods for testing antioxidant activity. *Analyst*. 2002;127:183-198. DOI: 10.1039/b009171p
- [29] Danet AF, Badea-Doni M. Determination of Lipid Oxidation by Chemiluminescence Reagents. In: RuizCapillas C, Nollet LML, editors. *Flow Injection Analysis of Food Additives*. Boca Raton: CRC Press; 2016. p. 623-638. DOI: 10.1201/b19644

- [30] Ratnam DV, Ankola DD, Bhardwaj V, Sahana DK, Kumar MNVR. Role of antioxidants in prophylaxis and therapy: A pharmaceutical perspective. *Journal of Controlled Release*. 2006;113(3):189-207. DOI: 10.1016/j.jconrel.2006.04.015
- [31] MacDonald-Wicks LK, Wood LG, Garg ML. Methodology for the determination of biological antioxidant capacity in vitro: a review. *Journal of the Science of Food and Agriculture*. 2006;86(13):2046-2056. DOI: 10.1002/jsfa.2603
- [32] Carocho M, Morales P, Ferreira ICFR. Antioxidants: Reviewing the chemistry, food applications, legislation and role as preservatives. *Trends in Food Science & Technology*. 2018;71:107-120. DOI: 10.1016/j.tifs.2017.11.008
- [33] Munialo CD, Naumovski N, Sergi D, Stewart D, Mellor DD. Critical evaluation of the extrapolation of data relative to antioxidant function from the laboratory and their implications on food production and human health: a review. *International Journal of Food Science and Technology*. 2019;54(5):1448-1459. DOI: 10.1111/ijfs.14135
- [34] Sadeer NB, Montesano D, Albrizio S, Zengin G, Mahomoodally MF. The Versatility of Antioxidant Assays in Food Science and Safety-Chemistry, Applications, Strengths, and Limitations. *Antioxidants*. 2020;9(8):article number 709. DOI: 10.3390/antiox9080709
- [35] Ivanova A, Gerasimova E, Gazizullina E. Study of Antioxidant Properties of Agents from the Perspective of Their Action Mechanisms. *Molecules*. 2020;25(18):article number 4251. DOI: 10.3390/molecules25184251
- [36] Pellegrini N, Del Rio D, Colombi B, Bianchi M, Brighenti F. Application of the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation assay to a flow injection system for the evaluation of antioxidant activity of some pure compounds and beverages. *Journal of Agricultural and Food Chemistry*. 2003;51(1):260-264. DOI: 10.1021/jf020657z
- [37] Prior RL, Wu XL, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*. 2005;53(10):4290-4302. DOI: 10.1021/jf0502698
- [38] Shahidi F, Zhong Y. Lipid oxidation and improving the oxidative stability. *Chemical Society Reviews*. 2010;39(11):4067-4079. DOI: 10.1039/b922183m
- [39] Christodouleas D, Papadopoulou K, Calokerinos AC. Determination of Total Antioxidant Activity of Edible Oils as well as Their Aqueous and Organic Extracts by Chemiluminescence. *Food Analytical Methods*. 2011;4(4):475-484. DOI: 10.1007/s12161-010-9189-6
- [40] Hirayama O, Takagi M, Hukumoto K, Katoh S. Evaluation of antioxidant activity by chemiluminescence. *Analytical Biochemistry*. 1997;247(2):237-241. DOI: 10.1006/abio.1997.2053
- [41] Ghiselli A, Serafini M, Maiani G, Azzini E, Ferroluzzi A. A FLUORESCENCE-BASED METHOD FOR MEASURING TOTAL PLASMA ANTIOXIDANT CAPABILITY. *Free Radical Biology and Medicine*. 1995;18(1):29-36. DOI: 10.1016/0891-5849(94)00102-p
- [42] Senthilmohan ST, Davis BM, Wilson PF, McEwan MJ. Improved peroxy radical scavenging TOSC assay to quantify antioxidant capacity using SIFT-MS. *Redox Report*.

- 2009;14(5):197-204. DOI: 10.1179/13510009x12525712409571
- [43] Prieto MA, Rodriguez-Amado I, Vazquez JA, Murado MA. beta-Carotene Assay Revisited. Application To Characterize and Quantify Antioxidant and Prooxidant Activities in a Microplate. *Journal of Agricultural and Food Chemistry*. 2012;60(36):8983-8993. DOI: 10.1021/jf302218g
- [44] Ozyurek M, Guclu K, Tutem E, Baskan KS, Ercag E, Celik SE, et al. A comprehensive review of CUPRAC methodology. *Analytical Methods*. 2011;3(11):2439-2453. DOI: 10.1039/c1ay05320e
- [45] Alarcon-Angeles G, Alvarez-Romero GA, Merkoci A. Emerging Nanomaterials for Analytical Detection. *Biosensors for Sustainable Food - New Opportunities and Technical Challenges*, Comprehensive Analytical Chemistry. 74. Amsterdam: Elsevier; 2016. p. 195-246. DOI:10.1016/bs.coac.2016.03.022
- [46] Della Pelle F, Compagnone D. Nanomaterial-Based Sensing and Biosensing of Phenolic Compounds and Related Antioxidant Capacity in Food. *Sensors*. 2018;18(2):article number 462. DOI: 10.3390/s18020462
- [47] Vasilescu A, Sharpe E, Andreescu S. Nanoparticle-Based Technologies for the Detection of Food Antioxidants. *Current Analytical Chemistry*. 2012;8(4):495-505. DOI: 10.2174/157341112803216780
- [48] Yu ZY, Li HJ, Lu JH, Zhang XM, Liu NK, Zhang X. Hydrothermal synthesis of Fe₂O₃/graphene nanocomposite for selective determination of ascorbic acid in the presence of uric acid. *Electrochimica Acta*. 2015;158:264-270. DOI: 10.1016/j.electacta.2015.01.131
- [49] Sochor J, Dobes J, Krystofova O, Ruttkay-Nedecky B, Babula P, Pohanka M, et al. Electrochemistry as a Tool for Studying Antioxidant Properties. *International Journal of Electrochemical Science*. 2013;8(6):8464-8489. DOI:
- [50] Pisoschi AM, Cimpeanu C, Predoi G. Electrochemical Methods for Total Antioxidant Capacity and its Main Contributors Determination: A review. *Open Chemistry*. 2015;13(1):824-856. DOI: 10.1515/chem-2015-0099
- [51] Hoyos-Arbelaez J, Vazquez M, Contreras-Calderon J. Electrochemical methods as a tool for determining the antioxidant capacity of food and beverages: A review. *Food Chemistry*. 2017;221:1371-1381. DOI: 10.1016/j.foodchem.2016.11.017
- [52] Chevion S, Roberts MA, Chevion M. The use of cyclic voltammetry for the evaluation of antioxidant capacity. *Free Radical Biology and Medicine*. 2000;28(6):860-870. DOI: 10.1016/s0891-5849(00)00178-7
- [53] Piljac-Zegarac J, Valek L, Stipcevic T, Martinez S. Electrochemical determination of antioxidant capacity of fruit tea infusions. *Food Chemistry*. 2010;121(3):820-825. DOI: 10.1016/j.foodchem.2009.12.090
- [54] Souza LP, Calegari F, Zarbin AJG, Marcolino-Junior LH, Bergamini MF. Voltammetric Determination of the Antioxidant Capacity in Wine Samples Using a Carbon Nanotube Modified Electrode. *Journal of Agricultural and Food Chemistry*. 2011;59(14):7620-7625. DOI: 10.1021/jf2005589
- [55] Novak I, Seruga M, Komorsky-Lovric S. Characterisation of catechins in green and black teas using square-wave voltammetry and RP-HPLC-ECD. *Food Chemistry*. 2010;122(4):1283-1289. DOI: 10.1016/j.foodchem.2010.03.084
- [56] Yashin YI, Nemzer BV, Ryzhnev VY, Yashin AY, Chernousova NI, Fedina PA.

Creation of a Databank for Content of Antioxidants in Food Products by an Amperometric Method. *Molecules*. 2010;15(10):7450-7466. DOI: 10.3390/molecules15107450

[57] Pisoschi A, Cheregi M, Danet A. Total Antioxidant Capacity of Some Commercial Fruit Juices: Electrochemical and Spectrophotometrical Approaches. *Molecules*. 2009;14(1):480-493. DOI: 10.3390/molecules14010480

[58] David M, Serban A, Radulescu C, Danet AF, Florescu M. Bioelectrochemical evaluation of plant extracts and gold nanozyme-based sensors for total antioxidant capacity determination. *Bioelectrochemistry*. 2019;129:124-134. DOI: 10.1016/j.bioelechem.2019.05.011

[59] Gulcin I, Sat IG, Beydemir S, Kufrevioglu OI. Evaluation of the in vitro antioxidant properties of broccoli extracts (*Brassica oleracea* L.). *Italian Journal of Food Science*. 2004;16(1):17-30. DOI:

[60] Carlos Sanchez-Rangel J, Benavides J, Basilio Heredia J, Cisneros-Zevallos L, Jacobo-Velazquez DA. The Folin-Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination. *Analytical Methods*. 2013;5(21):5990-5999. DOI: 10.1039/c3ay41125g

[61] Granato D, Santos JS, Maciel LG, Nunes DS. Chemical perspective and criticism on selected analytical methods used to estimate the total content of phenolic compounds in food matrices. *Trac-Trends in Analytical Chemistry*. 2016;80:266-279. DOI: 10.1016/j.trac.2016.03.010

[62] Barriuso B, Astiasaran I, Ansorena D. A review of analytical methods measuring lipid oxidation status in foods: a challenging task.

European Food Research and Technology. 2013;236(1):1-15. DOI: 10.1007/s00217-012-1866-9

[63] Ghani MA, Barril C, Bedgood DR, Jr., Prenzler PD. Measurement of antioxidant activity with the thiobarbituric acid reactive substances assay. *Food Chemistry*. 2017;230:195-207. DOI: 10.1016/j.foodchem.2017.02.127

[64] Guillen-Sans R, Guzman-Chozas M. The thiobarbituric acid (TBA) reaction in foods: A review. *Critical Reviews in Food Science and Nutrition*. 1998;38(4):315-330. DOI: 10.1080/10408699891274228

[65] Meng D, Zhang P, Li S, Ho C-T, Zhao H. Antioxidant activity evaluation of dietary phytochemicals using *Saccharomyces cerevisiae* as a model. *Journal of Functional Foods*. 2017;38:36-44. DOI: 10.1016/j.jff.2017.08.041

[66] Yang C, Shahidi F, Tsao R. Biomarkers of oxidative stress and cellular-based assays of indirect antioxidant measurement. In: Apak R, Capanoglu E, Shahidi F, editors. *Measurement of Antioxidant Activity & Capacity: Recent Trends and Applications*. Chichester: Wiley; 2018. p. 165-186. DOI:10.1002/9781119135388.ch9

[67] Cano A, Arnao MB. ABTS/TEAC (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)/Trolox (R)-Equivalent Antioxidant Capacity) radical scavenging mixed-mode assay. In: Apak R, Capanoglu E, Shahidi F, editors. *Measurement of Antioxidant Activity & Capacity: Recent Trends and Applications*. Chichester: Wiley; 2018. p. 117-139. DOI:10.1002/9781119135388.ch7

[68] Zheng L, Zhao MM, Xiao CQ, Zhao QZ, Su GW. Practical problems when using ABTS assay to assess the radical-scavenging activity of

- peptides: Importance of controlling reaction pH and time. *Food Chemistry*. 2016;192:288-294. DOI: 10.1016/j.foodchem.2015.07.015
- [69] Xie J, Schaich KM. Re-evaluation of the 2,2-Diphenyl-1-picrylhydrazyl Free Radical (DPPH) Assay for Antioxidant Activity. *Journal of Agricultural and Food Chemistry*. 2014;62(19):4251-4260. DOI: 10.1021/jf500180u
- [70] Ozcelik B, Lee JH, Min DB. Effects of light, oxygen, and pH on the absorbance of 2,2-diphenyl-1-picrylhydrazyl. *Journal of Food Science*. 2003;68(2):487-490. DOI: 10.1111/j.1365-2621.2003.tb05699.x
- [71] Apak R, Gorinstein S, Bohm V, Schaich KM, Ozyurek M, Guclu K. Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC Technical Report). *Pure and Applied Chemistry*. 2013;85(5):957-998. DOI: 10.1351/pac-rep-12-07-15
- [72] Huang DJ, Ou BX, Hampsch-Woodill M, Flanagan JA, Deemer EK. Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated beta-cyclodextrin as the solubility enhancer. *Journal of Agricultural and Food Chemistry*. 2002;50(7):1815-1821. DOI: 10.1021/jf0113732
- [73] Wu XL, Gu LW, Holden J, Haytowitz DB, Gebhardt SE, Beecher G, et al. Development of a database for total antioxidant capacity in foods: a preliminary study. *Journal of Food Composition and Analysis*. 2004;17(3-4):407-422. DOI: 10.1016/j.jfca.2004.03.001
- [74] Nkhili E, Brat P. Reexamination of the ORAC assay: effect of metal ions. *Analytical and Bioanalytical Chemistry*. 2011;400(5):1451-1458. DOI: 10.1007/s00216-011-4884-8
- [75] Popa C, Farcasanu I, Jipa S, Zaharescu T, Danet A. Chemiluminescence Determination of the Total Antioxidant Capacity of Rosemary Extract. *Revista De Chimie*. 2012;63(7):715-719. DOI:
- [76] Popa C-V, Lungu L, Savoiu M, Bradu C, Dinoiu V, Danet AF. Total antioxidant activity and phenols and flavonoids content of several plant extracts. *International Journal of Food Properties*. 2012;15(3):691-701. DOI: 10.1080/10942912.2010.498545
- [77] Popa CV, Danet AF, Jipa S, Zaharescu T. Determination of Total Antioxidant Capacity of Some Fruit Juices and Noncarbonated Soft Drinks by a FIA-CL Method. *Revista De Chimie*. 2012;63(10):978-983. DOI:
- [78] Lungu L, Popa C, Savoiu M, Danet A, Dinoiu V. Antioxidant Activity of Brassica Oleracea L., Allium Cepa L. and Beta Vulgaris L. Extracts. *Revista De Chimie*. 2010;61(10):911-914. DOI:
- [79] Popa C, Danet A, Jipa S, Zaharescu T. Determination of Total Antioxidant Activity of Wines Using a Flow Injection Method with Chemiluminescence Detection. *Revista De Chimie*. 2010;61(1):11-16. DOI:
- [80] Weingerl V, Strlic M, Kocar D. Evaluation of chemiluminometric method for determination of polyphenols in wine *Analytical Letters*. 2011;44(7):1310-1322. DOI: 10.1080/00032719.2010.512674
- [81] Zargoosh K, Ghayeb Y, Aeineh N, Qandalee M. Evaluation of Antioxidant Capacity of Hydrophilic and Hydrophobic Antioxidants Using Peroxyoxalate Chemiluminescence Reaction of the Novel Furandicarboxylate Derivative. *Food Analytical Methods*. 2014;7(2):283-290. DOI: 10.1007/s12161-013-9625-5
- [82] Giokas DL, Vlessidis AG, Evmiridis NP. On-line selective

detection of antioxidants free-radical scavenging activity based on Co(II)/EDTA-induced luminol chemiluminescence by flow injection analysis. *Analytica Chimica Acta*. 2007;589(1):59-65. DOI: 10.1016/j.aca.2007.02.041

[83] Fassoula E, Economou A, Calokerinos A. Development and validation of a sequential-injection method with chemiluminescence detection for the high throughput assay of the total antioxidant capacity of wines. *Talanta*. 2011;85(3):1412-1418. DOI: 10.1016/j.talanta.2011.06.037

[84] Chladkova G, Kunovska K, Chocholous P, Polasek M, Sklenarova H. Automatic screening of antioxidants based on the evaluation of kinetics of suppression of chemiluminescence in a luminol-hydrogen peroxide system using a sequential injection analysis setup with a flow-batch detection cell. *Analytical Methods*. 2019;11(19):2531-2536. DOI: 10.1039/c9ay00160c

[85] Popa C-V, Cristea N-I, Farcasanu I-C, Danet AF. Total Antioxidant Capacity of Some Fruit Seeds Extracts. *Revista De Chimie*. 2013;64(12):1377-1380.

[86] Iranifam M, Al Lawati HAJ. Monitoring the antioxidant capacity in honey and fruit juices using a microfluidic device with a NaHCO₃-H₂O₂-Co²⁺ chemiluminescence reaction. *Food Chemistry*. 2019;297:article number 124930. DOI: 10.1016/j.foodchem.2019.05.204

[87] Kishikawa N, El-Maghrabey M, Nagamune Y, Nagai K, Ohyama K, Kuroda N. A Smart Advanced Chemiluminescence-Sensing Platform for Determination and Imaging of the Tissue Distribution of Natural Antioxidants. *Analytical Chemistry*. 2020;92(10):6984-6992. DOI: 10.1021/acs.analchem.0c00044

[88] Christodouleas D, Fotakis C, Papadopoulou K, Yannakopoulou E, Calokerinos AC. Development and validation of a chemiluminogenic method for the evaluation of antioxidant activity of hydrophilic and hydrophobic antioxidants. *Analytica Chimica Acta*. 2009;652(1-2):295-302. DOI: 10.1016/j.aca.2009.08.012

[89] Ghiselli A, Serafini M, Natella F, Scaccini C. Total antioxidant capacity as a tool to assess redox status: Critical view and experimental data. *Free Radical Biology and Medicine*. 2000;29(11):1106-1114. DOI: 10.1016/s0891-5849(00)00394-4

[90] Whitehead TP, Thorpe GHG, Maxwell SRJ. Enhanced chemiluminescence assay for antioxidant capacity in biological-fluids *Analytica Chimica Acta*. 1992;266(2):265-277. DOI: 10.1016/0003-2670(92)85052-8

[91] Bastos E, Romoff P, Eckert C, Baader W. Evaluation of antiradical capacity by H₂O₂-hemin-induced luminol chemiluminescence. *Journal of Agricultural and Food Chemistry*. 2003;51(25):7481-7488. DOI: 10.1021/jf0345189

[92] Valkonen M, Kuusi T. Spectrophotometric assay for total peroxy radical-trapping antioxidant potential in human serum. *J Lipid Res*. 1997;38(4):823-833. DOI:

[93] Delange RJ, Glazer AN. Phycoerythrin fluorescence-based assay for peroxy-radicals - a screen for biologically relevant protective agents. *Analytical Biochemistry*. 1989;177(2):300-306. DOI: 10.1016/0003-2697(89)90056-0

[94] Somogyi A, Rosta K, Pusztai P, Tulassay Z, Nagy G. Antioxidant measurements. *Physiological Measurement*. 2007;28(4):R41-R55. DOI: 10.1088/0967-3334/28/4/r01

- [95] Lage MAP, Garcia MAM, Alvarez JAV, Anders Y, Curran TP. A new microplate procedure for simultaneous assessment of lipophilic and hydrophilic antioxidants and pro-oxidants, using crocin and beta-carotene bleaching methods in a single combined assay: Tea extracts as a case study. *Food Research International*. 2013;53(2):836-846. DOI: 10.1016/j.foodres.2012.11.026
- [96] Ou BX, Huang DJ, Hampsch-Woodill M, Flanagan JA, Deemer EK. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. *Journal of Agricultural and Food Chemistry*. 2002;50(11):3122-3128. DOI: 10.1021/jf0116606
- [97] Cekic SD, Kara N, Tutem E, Baskan KS, Apak R. Protein-incorporated serum total antioxidant capacity measurement by a modified CUPRAC (cupric reducing antioxidant capacity) method *Analytical Letters*. 2012;45(7):754-763. DOI: 10.1080/00032719.2011.653901
- [98] Bean H, Radu F, De E, Schuler C, Leggett RE, Levin RM. Comparative evaluation of antioxidant reactivity within obstructed and control rabbit urinary bladder tissue using FRAP and CUPRAC assays. *Molecular and Cellular Biochemistry*. 2009;323(1-2):139-142. DOI: 10.1007/s11010-008-9972-5
- [99] Apak R, Guclu K, Ozyurek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry*. 2004;52(26):7970-7981. DOI: 10.1021/jf048741x
- [100] Ozyurek M, Bektasoglu B, Guclu K, Gungor N, Apak R. Simultaneous total antioxidant capacity assay of lipophilic and hydrophilic antioxidants in the same acetone-water solution containing 2% methyl-beta-cyclodextrin using the cupric reducing antioxidant capacity (CUPRAC) method. *Analytica Chimica Acta*. 2008;630(1):28-39. DOI: 10.1016/j.aca.2008.09.057
- [101] Bener M, Ozyurek M, Guclu K, Apak R. Development of a Low-Cost Optical Sensor for Cupric Reducing Antioxidant Capacity Measurement of Food Extracts. *Analytical Chemistry*. 2010;82(10):4252-4258. DOI: 10.1021/ac100646k
- [102] Apak R, Guclu K, Demirata B, Ozyurek M, Celik SE, Bektasoglu B, et al. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Molecules*. 2007;12(7):1496-1547. DOI: 10.3390/12071496
- [103] Vilela D, Cristina Gonzalez M, Escarpa A. Nanoparticles as analytical tools for in-vitro antioxidant-capacity assessment and beyond. *Trac-Trends in Analytical Chemistry*. 2015;64:1-16. DOI: 10.1016/j.trac.2014.07.017
- [104] Scampicchio M, Wang J, Blasco AJ, Arribas AS, Mannino S, Escarpa A. Nanoparticle-based assays of antioxidant activity. *Analytical Chemistry*. 2006;78(6):2060-2063. DOI: 10.1021/ac052007a
- [105] Liu Q, Liu H, Yuan Z, Wei D, Ye Y. Evaluation of antioxidant activity of chrysanthemum extracts and tea beverages by gold nanoparticles-based assay. *Colloids and Surfaces B-Biointerfaces*. 2012;92:348-352. DOI: 10.1016/j.colsurfb.2011.12.007
- [106] Szydłowska-Czerniak A, Tulodziecka A. Comparison of a silver nanoparticle-based method and the modified spectrophotometric methods for assessing antioxidant capacity of rapeseed varieties. *Food Chemistry*.

- 2013;141(3):1865-1871. DOI: 10.1016/j.foodchem.2013.04.111
- [107] Bordbar MM, Hemmateenejad B, Tashkhourian J, Nami-Ana SF. An optoelectronic tongue based on an array of gold and silver nanoparticles for analysis of natural, synthetic and biological antioxidants. *Microchimica Acta*. 2018;185(10):article number 493. DOI: 10.1007/s00604-018-3021-1
- [108] Sharpe E, Frasco T, Andreescu D, Andreescu S. Portable ceria nanoparticle-based assay for rapid detection of food antioxidants (NanoCerac). *Analyst*. 2013;138(1):249-262. DOI: 10.1039/c2an36205h
- [109] Sharpe E, Bradley R, Frasco T, Jayathilaka D, Marsh A, Andreescu S. Metal oxide based multisensor array and portable database for field analysis of antioxidants. *Sensors and Actuators B-Chemical*. 2014;193:552-562. DOI: 10.1016/j.snb.2013.11.088
- [110] Popa CV, Vasilescu A, Litescu SC, Albu C, Danet AF. Metal Nano-Oxide based Colorimetric Sensor Array for the Determination of Plant Polyphenols with Antioxidant Properties. *Analytical Letters*. 2020;53(4):627-645. DOI: 10.1080/00032719.2019.1662430
- [111] Della Pelle F, Cristina Gonzalez M, Sergi M, Del Carlo M, Compagnone D, Escarpa A. Gold Nanoparticles-based Extraction-Free Colorimetric Assay in Organic Media: An Optical Index for Determination of Total Polyphenols in Fat-Rich Samples. *Analytical Chemistry*. 2015;87(13):6905-6911. DOI: 10.1021/acs.analchem.5b01489
- [112] Vilela D, Cristina Gonzalez M, Escarpa A. Gold-nanosphere formation using food sample endogenous polyphenols for in-vitro assessment of antioxidant capacity. *Analytical and Bioanalytical Chemistry*. 2012;404(2):341-349. DOI: 10.1007/s00216-012-6084-6
- [113] Vilela D, Castaneda R, Cristina Gonzalez M, Mendoza S, Escarpa A. Fast and reliable determination of antioxidant capacity based on the formation of gold nanoparticles. *Microchimica Acta*. 2015;182(1-2):105-111. DOI: 10.1007/s00604-014-1306-6
- [114] Ozyurek M, Gungor N, Baki S, Guclu K, Apak R. Development of a Silver Nanoparticle-Based Method for the Antioxidant Capacity Measurement of Polyphenols. *Analytical Chemistry*. 2012;84(18):8052-8059. DOI: 10.1021/ac301925b
- [115] Teerasong S, Jinnarak A, Chaneam S, Wilairat P, Nacapricha D. Poly(vinyl alcohol) capped silver nanoparticles for antioxidant assay based on seed-mediated nanoparticle growth. *Talanta*. 2017;170:193-198. DOI: 10.1016/j.talanta.2017.04.009
- [116] Gatselou V, Christodouleas DC, Kouloumpis A, Gournis D, Giokas DL. Determination of phenolic compounds using spectral and color transitions of rhodium nanoparticles. *Analytica Chimica Acta*. 2016;932:80-87. DOI: 10.1016/j.aca.2016.05.029
- [117] Hemmateenejad B, Shamsipur M, Khosousi T, Shanehsaz M, Firuzi O. Antioxidant activity assay based on the inhibition of oxidation and photobleaching of L-cysteine-capped CdTe quantum dots. *Analyst*. 2012;137(17):4029-4036. DOI: 10.1039/c2an35588d
- [118] Benitez-Martinez S, Valcarcel M. Graphene quantum dots as sensor for phenols in olive oil. *Sensors and Actuators B-Chemical*. 2014;197:350-357. DOI: 10.1016/j.snb.2014.03.008
- [119] Choleva TG, Kappi FA, Giokas DL, Vlessidis AG. Paper-based assay of antioxidant activity using analyte-mediated on-paper nucleation of gold nanoparticles as colorimetric probes.

Analytica Chimica Acta. 2015;860:61-69. DOI: 10.1016/j.aca.2014.12.025

[120] Peng J, Su Y, Huang F-Q, Zuo Q, Yang L, Li J, et al. A simple and rapid fluorescent approach for flavonoids sensor based on gold nanoclusters. *Journal of Colloid and Interface Science*. 2019;539:175-183. DOI: 10.1016/j.jcis.2018.12.042

[121] Angelis PN, Mendonca JdC, da Rocha LR, Capelari TB, Prete MC, Segatelli MG, et al. Feasibility of a Nano-Carbon Black Paste Electrode for Simultaneous Voltammetric Determination of Antioxidants in Food Samples and Biodiesel in the Presence of Surfactant. *Electroanalysis*. 2020;32(6):1198-1207. DOI: 10.1002/elan.201900479

[122] Sivakumar M, Pandi K, Chen S-M, Yadav S, Chen T-W, Veeramani V. Highly Sensitive Detection of Gallic Acid in Food Samples by Using Robust NiAl₂O₄ Nanocomposite Materials. *Journal of the Electrochemical Society*. 2019;166(2):B29-B34. DOI: 10.1149/2.0121902jes

[123] Ran X, Yang L, Zhang J, Deng G, Li Y, Xie X, et al. Highly sensitive electrochemical sensor based on beta-cyclodextrine-gold@3, 4, 9, 10-perylene tetracarboxylic acid functionalized single-walled carbon nanohorns for simultaneous determination of myricetin and rutin. *Analytica Chimica Acta*. 2015;892:85-94. DOI: 10.1016/j.aca.2015.08.046

[124] Ziyatdinova G, Kozlova E, Budnikov H. Chronocoulometry of wine on multi-walled carbon nanotube modified electrode: Antioxidant capacity assay. *Food Chemistry*. 2016;196:405-410. DOI: 10.1016/j.foodchem.2015.09.075

[125] Andrei V, Sharpe E, Vasilescu A, Andreescu S. A single use electrochemical sensor based on biomimetic nanoceria for the

detection of wine antioxidants. *Talanta*. 2016;156:112-118. DOI: 10.1016/j.talanta.2016.04.067

[126] Ceballos C, Fernandez H. Synthetic antioxidants in edible oils by square-wave voltammetry on ultramicroelectrodes. *Journal of the American Oil Chemists Society*. 2000;77(7):731-735. DOI: 10.1007/s11746-000-0118-1

[127] Medeiros RA, Rocha-Filho RC, Fatibello-Filho O. Simultaneous voltammetric determination of phenolic antioxidants in food using a boron-doped diamond electrode. *Food Chemistry*. 2010;123(3):886-891. DOI: 10.1016/j.foodchem.2010.05.010

[128] Sousa WR, da Rocha C, Cardoso CL, Silva DHS, Zanoni MVB. Determination of the relative contribution of phenolic antioxidants in orange juice by voltammetric methods. *Journal of Food Composition and Analysis*. 2004;17(5):619-633. DOI: 10.1016/j.jfca.2003.09.013

[129] Blasco AJ, Crevillen AG, Gonzalez MC, Escarpa A. Direct electrochemical sensing and detection of natural antioxidants and antioxidant capacity in vitro systems. *Electroanalysis*. 2007;19(22):2275-2286. DOI: 10.1002/elan.200704004

[130] Ivanova AV, Gerasimova EL, Brainina KZ. Potentiometric Study of Antioxidant Activity: Development and Prospects. *Critical Reviews in Analytical Chemistry*. 2015;45(4):311-322. DOI: 10.1080/10408347.2014.910443

[131] Wang J. *Analytical electrochemistry*. 2nd ed. Chichester: Wiley-WCH; 2001. 203 p. DOI: 10.1021/ed078p457.2

[132] Jara-PalaciosMJ, Escudero-GileteML, Hernandez-Hierro JM, Heredia FJ, Hernanz D. Cyclic voltammetry to evaluate the antioxidant potential in winemaking by-products. *Talanta*. 2017;165:211-215. DOI: 10.1016/j.talanta.2016.12.058

- [133] Masek A, Chrzescijanska E, Latos M, Kosmalka A. Electrochemical and Spectrophotometric Characterization of the Propolis Antioxidants Properties. *International Journal of Electrochemical Science*. 2019;14(2):1231-1247. DOI: 10.20964/2019.02.66
- [134] Gulaboski R, Mirceski V, Mitrev S. Development of a rapid and simple voltammetric method to determine total antioxidative capacity of edible oils. *Food Chemistry*. 2013;138(1):116-121. DOI: 10.1016/j.foodchem.2012.10.050
- [135] Nikolic MD, Pavlovic AN, Mitic SS, Tomic SB, Mitic MN, Kalicanin BM, et al. Use of cyclic voltammetry to determine the antioxidant capacity of berry fruits: correlation with spectrophotometric assays. *European Journal of Horticultural Science*. 2019;84(3):152-160. DOI: 10.17660/eJHS.2019/84.3.5
- [136] Milardovic S, Ivekovic D, Ruwenjak V, Grabaric BS. Use of DPPH center dot vertical bar DPPH redox couple for biamperometric determination of antioxidant activity. *Electroanalysis*. 2005;17(20):1847-1853. DOI: 10.1002/elan.200503312
- [137] Photinon K, Chalermchart Y, Khanongnuch C, Wang S-H, Liu C-C. A Thick-film Sensor as a Novel Device for Determination of Polyphenols and Their Antioxidant Capacity in White Wine. *Sensors*. 2010;10(3):1670-1678. DOI: 10.3390/s100301670
- [138] Apetrei C, Mirela Apetrei I, Antonio De Saja J, Luz Rodriguez-Mendez M. Carbon Paste Electrodes Made from Different Carbonaceous Materials: Application in the Study of Antioxidants. *Sensors*. 2011;11(2):1328-1344. DOI: 10.3390/s110201328
- [139] Ziyatdinova GK, Nizamova AM, Budnikov HC. Voltammetric determination of curcumin in spices. *Journal of Analytical Chemistry*. 2012;67(6):591-594. DOI: 10.1134/s1061934812040132
- [140] Cardenas A, Frontana C. Evaluation of a carbon ink chemically modified electrode incorporating a copper-neocuproine complex for the quantification of antioxidants. *Sensors and Actuators B-Chemical*. 2020;313:article number 128070. DOI: 10.1016/j.snb.2020.128070
- [141] Ricci A, Parpinello GP, Teslic N, Kilmartin PA, Versari A. Suitability of the Cyclic Voltammetry Measurements and DPPH center dot Spectrophotometric Assay to Determine the Antioxidant Capacity of Food-Grade Oenological Tannins. *Molecules*. 2019;24(16):article number 2925. DOI: 10.3390/molecules24162925
- [142] Pisoschi AM, Pop A, Negulescu GP, Pisoschi A. Determination of Ascorbic Acid Content of Some Fruit Juices and Wine by Voltammetry Performed at Pt and Carbon Paste Electrodes. *Molecules*. 2011;16(2):1349-1365. DOI: 10.3390/molecules16021349
- [143] Yilmaz UT, Kekillioglu A, Mert R. Determination of Gallic acid by differential pulse polarography: Application to fruit juices. *Journal of Analytical Chemistry*. 2013;68(12):1064-1069. DOI: 10.1134/s1061934813120113
- [144] Wang Z, Yang F, Zheng H, Qin X, Luo J, Li Y, et al. Voltammetric determination of TBHQ at a glassy carbon electrode surface activated by in situ chemical oxidation. *Analyst*. 2014;139(14):3622-3628. DOI: 10.1039/c4an00325j
- [145] Silva TR, Westphal E, Gallardo H, Vieira IC. Ionic Organic Film Sensor for Determination of Phenolic Compounds. *Electroanalysis*. 2014;26(8):1801-1809. DOI: 10.1002/elan.201400197
- [146] Tortolini C, Bollella P, Zumpano R, Favero G, Mazzei F, Antiochia R. Metal Oxide Nanoparticle

Based Electrochemical Sensor for Total Antioxidant Capacity (TAC) Detection in Wine Samples. *Biosensors-Basel*. 2018;8(4): article number 108. DOI: 10.3390/bios8040108

[147] Marcelo Granero A, Fernandez H, Agostini E, Alicia Zon M. An amperometric biosensor based on peroxidases from *Brassica napus* for the determination of the total polyphenolic content in wine and tea samples. *Talanta*. 2010;83(1):249-255. DOI: 10.1016/j.talanta.2010.09.016

[148] Amatongchai M, Laosing S, Chailapakul O, Nacapricha D. Simple flow injection for screening of total antioxidant capacity by amperometric detection of DPPH radical on carbon nanotube modified-glassy carbon electrode. *Talanta*. 2012;97:267-272. DOI: 10.1016/j.talanta.2012.04.029

[149] Sanchez Arribas A, Martinez-Fernandez M, Moreno M, Bermejo E, Zapardiel A, Chicharro M. Analysis of total polyphenols in wines by FIA with highly stable amperometric detection using carbon nanotube-modified electrodes. *Food Chemistry*. 2013;136(3-4):1183-1192. DOI: 10.1016/j.foodchem.2012.09.027

[150] Lino FMA, de Sa LZ, Torres IMS, Rocha ML, Dinis TCP, Ghedini PC, et al. Voltammetric and spectrometric determination of antioxidant capacity of selected wines. *Electrochimica Acta*. 2014;128:25-31. DOI: 10.1016/j.electacta.2013.08.109

[151] Ragubeer N, Beukes DR, Limson JL. Critical assessment of voltammetry for rapid screening of antioxidants in marine algae. *Food Chemistry*. 2010;121(1):227-232. DOI: 10.1016/j.foodchem.2009.11.076

[152] Santos JS, Alvarenga Brizola VR, Granato D. High-throughput assay comparison and standardization for metal chelating capacity screening: A proposal and application. *Food*

Chemistry. 2017;214:515-522. DOI: 10.1016/j.foodchem.2016.07.091

[153] Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Oxidants and Antioxidants, Pt A*. 1999;299:152-178. DOI:

[154] Magalhaes LM, Segundo MA, Reis S, Lima J. Methodological aspects about in vitro evaluation of antioxidant properties. *Analytica Chimica Acta*. 2008;613(1):1-19. DOI: 10.1016/j.aca.2008.02.047

[155] Jara-Palacios MJ, Goncalves S, Heredia FJ, Hernanz D, Romano A. Extraction of Antioxidants from Winemaking Byproducts: Effect of the Solvent on Phenolic Composition, Antioxidant and Anti-Cholinesterase Activities, and Electrochemical Behaviour. *Antioxidants*. 2020;9(8):article number 675. DOI: 10.3390/antiox9080675

[156] Mohtar LG, Messina GA, Bertolino FA, Pereira SV, Raba J, Nazareno MA. Comparative study of different methodologies for the determination the antioxidant activity of Venezuelan propolis. *Microchemical Journal*. 2020;158:article number 105244. DOI: 10.1016/j.microc.2020.105244

[157] Pico J, Pismag RY, Laudouze M, Martinez MM. Systematic evaluation of the Folin-Ciocalteu and Fast Blue BB reactions during the analysis of total phenolics in legumes, nuts and plant seeds. *Food & Function*. 2020;11(11):9868-9880. DOI: 10.1039/d0fo01857k

[158] Gao Y, Guo X, Liu Y, Zhang M, Zhang R, Abbasi AM, et al. Comparative assessment of phytochemical profile, antioxidant capacity and anti-proliferative activity in different varieties of brown rice (*Oryza sativa* L.). *Lwt-Food Science and Technology*.

2018;96:19-25. DOI: 10.1016/j.lwt.2018.05.002

[159] Wolfe KL, Liu RH. Cellular antioxidant activity (CAA) assay for assessing antioxidants, foods, and dietary supplements. *Journal of Agricultural and Food Chemistry*. 2007;55(22):8896-8907. DOI: 10.1021/jf0715166

[160] Wang H, Joseph JA. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. *Free Radical Biology and Medicine*. 1999;27(5-6):612-616. DOI: 10.1016/s0891-5849(99)00107-0

[161] Sessa M, Tsao R, Liu RH, Ferrari G, Donsi F. Evaluation of the Stability and Antioxidant Activity of Nanoencapsulated Resveratrol during in Vitro Digestion. *Journal of Agricultural and Food Chemistry*. 2011;59(23):12352-12360. DOI: 10.1021/jf2031346

[162] Kellett ME, Greenspan P, Pegg RB. Modification of the cellular antioxidant activity (CAA) assay to study phenolic antioxidants in a Caco-2 cell line. *Food Chemistry*. 2018;244:359-363. DOI: 10.1016/j.foodchem.2017.10.035

[163] Guo R, Guo X, Li T, Fu X, Liu RH. Comparative assessment of phytochemical profiles, antioxidant and antiproliferative activities of Sea buckthorn (*Hippophae rhamnoides* L.) berries. *Food Chemistry*. 2017;221:997-1003. DOI: 10.1016/j.foodchem.2016.11.063

[164] Dani C, Bonatto D, Salvador M, Pereira MD, Henriques JAP, Eleutherio E. Antioxidant protection of resveratrol and catechin in *Saccharomyces cerevisiae*. *Journal of Agricultural and Food Chemistry*. 2008;56(11):4268-4272. DOI: 10.1021/jf800752s

[165] Baroni MV, Naranjo RDD, Garcia-Ferreira C, Otaiza S, Wunderlin DA. How good antioxidant is the red wine? Comparison of some in vitro and in vivo methods to assess the

antioxidant capacity of Argentinean red wines. *Lwt-Food Science and Technology*. 2012;47(1):1-7. DOI: 10.1016/j.lwt.2012.01.015

[166] Lv H-p, Zhang Y, Shi J, Lin Z. Phytochemical profiles and antioxidant activities of Chinese dark teas obtained by different processing technologies. *Food Research International*. 2017;100:486-493. DOI: 10.1016/j.foodres.2016.10.024

[167] Capanoglu E, Beekwilder J, Boyacioglu D, De Vos RCH, Hall RD. The Effect of Industrial Food Processing on Potentially Health-Beneficial Tomato Antioxidants. *Critical Reviews in Food Science and Nutrition*. 2010;50(10):919-930. DOI: 10.1080/10408390903001503

[168] Granato D, Shahidi F, Wrolstad R, Kilmartin P, Melton LD, Hidalgo FJ, et al. Antioxidant activity, total phenolics and flavonoids contents: Should we ban in vitro screening methods? *Food Chemistry*. 2018;264:471-475. DOI: 10.1016/j.foodchem.2018.04.012

[169] Nand AV, Swift S, Uy B, Kilmartin PA. Evaluation of antioxidant and antimicrobial properties of biocompatible low density polyethylene/polyaniline blends. *Journal of Food Engineering*. 2013;116(2):422-429. DOI: 10.1016/j.jfoodeng.2012.11.023

[170] Ferreira FS, Sampaio GR, Keller LM, Sawaya ACHF, Chavez DWH, Torres EAFS, et al. Impact of Air Frying on Cholesterol and Fatty Acids Oxidation in Sardines: Protective Effects of Aromatic Herbs. *Journal of Food Science*. 2017;82(12):2823-2831. DOI: 10.1111/1750-3841.13967

[171] Yi B, Kim M-J, Lee J. Antioxidant Properties of Astaxanthin in Oil-in-Water Emulsions with Differently-Charged Emulsifiers Under Chlorophyll Photosensitization. *Journal of Food Science*. 2018;83(3):589-596. DOI: 10.1111/1750-3841.14005