Chapter 4

Phenolic Antioxidant Capacity: A Review of the State of the Art

Rubén San Miguel-Chávez

Additional information is available at the end of the chapter

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Abstract

There are many evidences pointing to oxidative stress as the promoter of the development of many degenerative diseases such as cancer, cardiovascular diseases, and neurodegeneration. It has been suggested that a diet rich in antioxidants would be beneficial to human health. To determine the antioxidant capacity of the different sources of antioxidants, they have different chemical methods used, in vitro cells, laboratory animals, and recently nanoparticles. This chapter provides an account of the main antioxidant evaluation methods applied to phenolic compounds, recounting their advantages and disadvantages, as well as a reflection on the parameters that should always care to obtain reproducible results.

Keywords: antioxidant capacity, phenolics, free radicals, standardized methods

1. Introduction

Phenolic compounds, or polyphenols, are a wide group of metabolites that originate from the secondary metabolism of plants. They contain one or more hydroxyl groups attached to a benzene ring and have an important role in the defense against plant pathogens and abiotic stressors [1].

This is one of the largest groups given its high chemical diversity. The basis of their structure is precisely a phenol group, that is, a hydroxyl attached to an aromatic ring [2] (**Figure 1**). Phenolic compounds are a chemically heterogeneous group, with the following chemical properties: some compounds are water soluble, some are soluble in organic solvents, some are found as glycosides, and some others are large insoluble polymers. Another characteristic is that this is a chemical group with high antioxidant activity (AOA) [3].

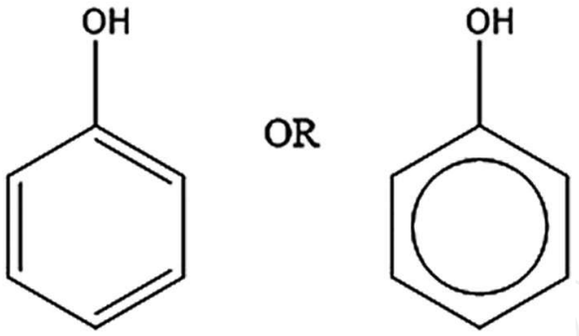

Figure 1. Phenol group, the basic structure of phenolic compounds.

2. Antioxidants

The term "antioxidant" is more important every day in modern society, since it is being associated with a whole series of benefits to human health. Despite that, antioxidants have applications not only in health but also in fields such as the chemical industry, where they are used as additives in the manufacture of rubbers and plastics to delay damage by the action of oxygen [4]. Also, in the food industry adding these antioxidant molecules results in prolonged shelf life, as in the case of fats, which become rancid due to the action of reactive oxygen and nitrogen species [5]. In the widest sense, an antioxidant molecule can be defined as a substance capable of preventing or delaying the oxidation of other molecules, such as lipids, proteins, or nucleic acids [6]. In the preceding definition, it is understood that the antioxidant molecule per se can perform such activity, as is the case of molecules as big as proteins and enzymes, or smaller molecules including vitamins, carotenoids, and phenolic compounds, of which flavonoids have an important role. However, authors like [6] mention that a more updated definition of what an antioxidant is would have to include not only the molecules that scavenge or reduce an oxidizing chemical compound but also those that act as chemical signals that induce the synthesis of enzymes related to the antioxidant mechanism of the cell.

Such oxidation can be carried out by two types of chemical reactive species: free radicals and other molecules that, without being radicals, due to their reactive nature can induce oxidation in molecules as the ones already mentioned.

3. Free radicals and reactive oxygen species (ROS) and their reaction mechanisms

It is widely known that in the atom, the electrons are ordered in their energy orbitals, with an even number of them in the last, most external level. This distribution gives the atom stability and a low possibility of reaction with a nearby atom. However, under certain conditions, the last level of energy can lose its stability by losing or gaining an electron. When this happens, the last orbital shows an unpaired electron, making the atom a free radical. This characteristic results in

a drastic increment of its ability to react with other atoms and/or molecules present nearby; in the cell environment, these molecules include lipids, proteins, and nucleic acids. When chemical interaction between free radicals and the aforementioned molecules occurs, changes in the structural properties of macromolecules can result, which eventually affect their function [7].

It is important to mention also the reactive oxygen species (ROS). This term includes all those reactive molecules, free radicals or not, that center their reactivity on an atom of oxygen. In spite of the delimitation that the presence of oxygen [8] gives, this title also includes chemical species with chemical reactivity centered on or derived from atoms that are different from oxygen. Such is the case of species that contain nitrogen or chlorine, atoms that are responsible for their chemical reactivity [9] (**Table 1**).

Free radicals	Nonradical reactive species
Superoxide O_2^-	Hydrogen peroxide H_2O_2
Hydroxyl HO^-	Hydroperoxide ROOH
Alkoxy RO^-	Hypochlorite ClO^-
Peroxy-OOH	Singlet oxygen $^1O_2$
Nitric oxide NO	Ozone O_3
Nitric dioxide NO_2	Peroxynitrite NO O_2^-

Modified from Ref. [5].

Table 1. Examples of free radicals and reactive oxygen, nitrogen, and chlorine species.

4. The presence of free radicals and reactive oxygen species in living organisms

In mammals, the reactive chemical species generated are nitric oxide (NO^-). This free radical is a product of the enzymatic action mediated by the nitric oxide synthase located in the cytosol of the cell; it is continuously produced by vascular endothelial cells [10].

Regarding the endogenous generation of ROS, it is part of the normal working of the aerobic organism. Under normal physiological conditions, animal tissues produce significant amounts of ROS. Among the most produced ROS, the free radical superoxide (O_2^-) prevails [11]. This radical is produced through the electron transport chain in the mitochondrion (during the interaction between oxygen molecules and complexes I and III) [12]. It is necessary to remember that the electron transport chain is a series of reactions oriented to producing, between the matrix and the intermembrane space, a proton gradient that is used by the cell to synthetize ATP from ADP. During the functioning of such chain, from 1 to 3% of oxygen that enters the mitochondria is transformed to superoxide (O_2^-), that is, it gains an electron. Despite that, and thanks to the presence in the mitochondrion of the superoxide dismutase, the levels of O_2^- diminish,

becoming oxygen and hydrogen peroxide. Hydrogen peroxide too is quickly reduced to water inside the mitochondrion by the action of the enzyme glutathione peroxidase, and the hydrogen peroxide that is not reduced exits the mitochondrion to be eventually reduced by another class of peroxidases present in the cytoplasm and by catalase in the peroxisomes [12].

5. Methods for the evaluation of antioxidant activity

In the modern world, many scientists around the globe attribute the origin of many diseases to oxidative stress; there is much evidence to support this theory [13, 14]. For this reason many nutritionists recommend the consumption of at least a minimum of foods such as fruits, vegetables, some drinks like grape wine, and spices and also food supplements from natural and synthetic origin containing antioxidants to help keep an individual healthy [15].

The antioxidant molecules present in these foods, drinks, and supplements, among which phenols are included, have been characterized as antioxidants by means of several methods and under different experimental conditions. Despite that, sometimes the results from the same molecule may vary when different methods are used [5]. This can be understood in two ways: on the one hand, inside living systems there are multiple radicals and reactive chemical species, as well as mechanisms involved in oxidative stress; on the other hand, when an in vitro method is used, it is important to take into account the chemical nature of other molecules being tested to employ the most adequate assay in order to get results that are closest to reality. For these reasons, there is no simple and universal method to characterize the antioxidant chemical abilities of all molecules [7]. Each proposed method will always have advantages and disadvantages, which need to be taken into account in terms of complexity, required facilities and equipment, the chemical mechanism that it tests, the quantification method, and its relevance in biological systems.

Several methods have been proposed to evaluate the antioxidant activity (AOA) of a molecule, which can be classified in several ways. In this chapter, they will be divided according to their reaction mechanism.

Antioxidants can deactivate radicals basically in two ways: (a) by a single-electron transfer (SET) and (b) by a hydrogen atom transfer (HAT). In the first case, the method will evaluate the capacity of the possible antioxidant to transfer an electron and reduce certain compound, including carbonyls, metals, and radicals [4, 7]. In the second case (HAT), the capacity of an antioxidant to scavenge free radicals by proton donation is measured (**Figure 2**).

In the case of HAT, several inconveniences can arise during the evaluation, since the presence of reducing agents, including metals, can generate errors by an apparent reactivity [5]. Also, the result can be affected in SET by the presence of contaminating metals, and given that the SET reaction is normally very slow and requires quite a long time to finish, secondary reactions can occur, which may contribute to a high variability and poor repeatability of the results [5].

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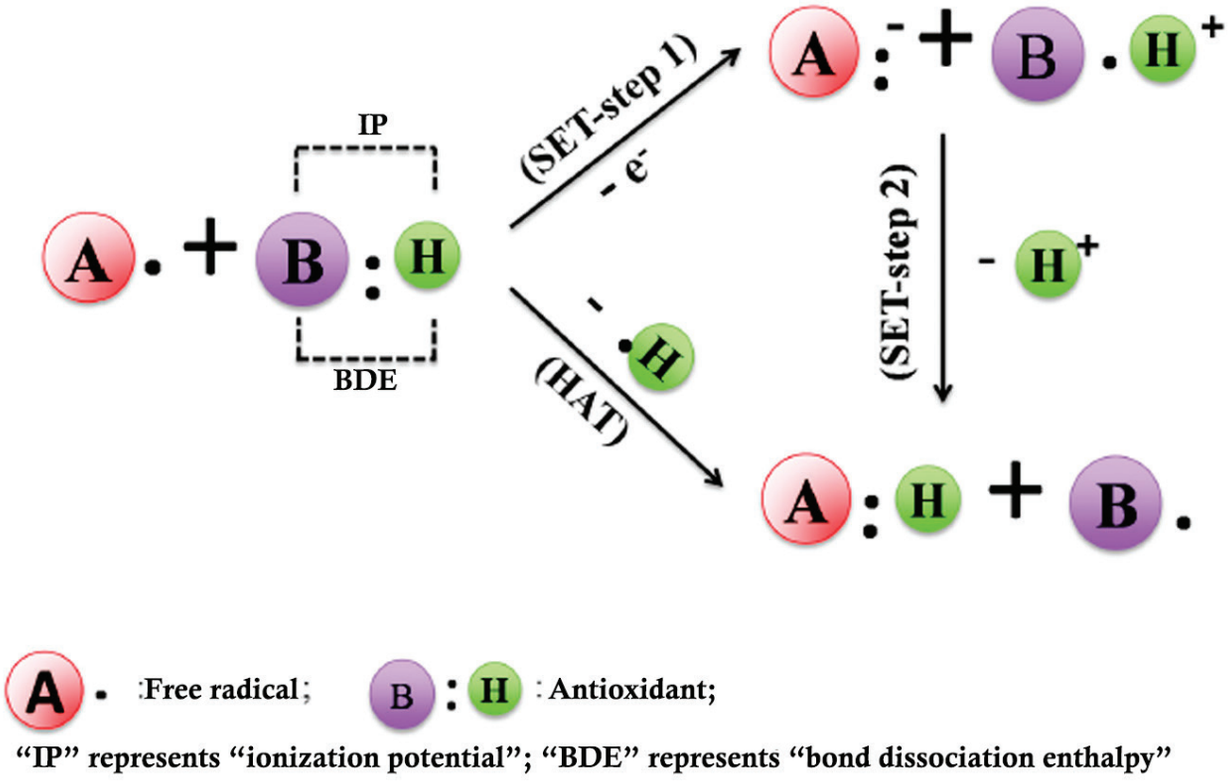

Figure 2. Reaction mechanisms of single-electron transfer (SET) and hydrogen atom transfer (HAT) [16]. Both mechanisms almost always occur together in all samples, with the balance determined by antioxidant structure and pH.

There are several methods reported in the literature to determine the antioxidant activity of polyphenols; however, in this chapter only the most common methods related to antioxidant activity of phenols will be discussed.

5.1. Methods based on the HAT reaction mechanism

5.1.1. Method with phycoerythrin

The substrates that are employed in this assay are two proteins found in several species of red algae: β-phycoerythrin and R- phycoerythrin [17, 18]. Their most important trait, relevant to the assay, is that they are fluorescent; this fluorescence diminishes on contact with peroxyl radicals, product of heat decomposition of 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), and the decrease is proportional to the amount of those radicals. But, when molecules of an antioxidant are added, the loss of fluorescence is decreased. However, phycoerythrin presented serious disadvantages identified in [19], such as the variability in the quality of this fluorescent protein varied from one extraction to another; it is also photoblanched under plate-reader conditions, and finally this protein interacts with polyphenols due to the nonspecific protein binding and loses fluorescence even without added radical generator [4]. For this reason, another fluorescent molecule that did not have such disadvantages being the fluorescein (3′,6′-dihydroxyspiro[isobenzofuran-1[3H],9′[9H]

thioxanthene]-3-one), a nonprotein synthetic molecule, was the most appropriate [19]. Using this method, the automated oxygen radical absorbance capacity (ORAC) of several products, including fruit juices and nectars, has been reported using Trolox as standard; these results have been published as equivalents of such standard [20]. The results involve both the time that the inhibition of the oxidation lasts and the concentration of the substrate that can be inhibited.

5.1.2. Comments about this method

When this method is used to evaluate phenolic acids, they show a low activity against peroxyl radicals compared with some flavonoids, which have several hydroxyl groups. Despite this appreciation, even flavonoids in form of glycogens can show ORAC activity. This shows that several factors are involved in the antioxidant activity of the molecules, such as their propensity to donate hydrogens or oxygens, which is directly related to the reduction of their potential [20].

5.1.3. Total radical-trapping antioxidant parameter (TRAP)

This method evaluates the capacity of antioxidant compounds to block the potential reaction between peroxyl radicals originated from the 2,2′-azobis(2-amidinopropane) (ABAP) and the R-phycoerythrin by measuring its fluorescence or from the 2,2′-azobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) by measuring its absorbance [21, 22]. Using this method, the antioxidant capacity is determined as the time needed for all the antioxidant to be consumed, by the increment of the time needed for the oxidized products to appear when the antioxidants are added or also as the percentage of reduction of the oxidation reaction. The values of this test are generally expressed as the increment of the time needed for the oxidized products to appear or the reaction time of the antioxidant, compared with the times of Trolox [23].

5.1.4. Comments about this method

When one wishes to compare the results obtained in different laboratories, there is the problem that the time allowed for the reaction to occur is different. This problem can be overcome by reducing the time of observation when testing a specific antioxidant and also by using more adequate equipment. It is necessary to mention that this assay has also been criticized for using nonphysiological oxidative stress (water-soluble peroxyl radicals) [24].

5.2. Methods based on the SET reaction mechanism

5.2.1. FRAP method

The initials FRAP stand for "ferric reducing antioxidant power method." This method is based on the reductive capacity of the iron that is part of the compound Fe $(TPTZ)^{3+}$ [25]. When this compound is reduced to Fe $(TPTZ)^{2+}$, a blue color appears, and its absorbance can be measured at 593 nm. The medium of the reaction is acid, and the results can be expressed

in the form of Fe^{2+} equivalents, or as in other methods, by using a standard compound. This method was initially developed to be used in plasma, but nowadays it is employed in other liquids such as fruit juices and pulps and other foods.

5.2.2. Comments about this method

The most criticized part of this method is that it assumes that the maximum reaction time is between 4 and 6 min [5], but in the case of phenolic compounds including acids such as caffeic, tannic, and ferulic, or quercetin, the reaction can last for more than an hour.

Also, according to [20] since this method is used in complex liquid mixtures such as fruit nectars, all the molecules that are part of that fluid take part in the reduction of Fe^{3+}; therefore, it is not possible to determine the individual participation of the different components from the mix of antioxidants in the total antioxidant activity.

5.2.3. Copper reduction assay

This assay is based on the reduction of Cu(II) to Cu(I). In this reaction, all the antioxidant molecules from a sample are involved. There are basically two methods that use copper. The first of them is the assay Bioxytech AOP-490, where the molecule bathocuproine (2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline) forms a complex 2:1 with copper (Cu(I)), and a chromophore is formed, with a maximum of absorbance at 490 nm [21]. The amount of bathocuproine that contains Cu(I), product of the reaction of Cu(II), causes a variation in the absorbance of the color complex at 450 nm. Similarly, the assay with CUPRAC uses neocuproine (2,9-dimethyl-1,10-phenanthroline), and the complex with Cu(I) will be the one that presents coloration at 450 nm. Uric acid is used in the standard curve, and, therefore, the results will be expressed as equivalents of that acid [5].

5.2.4. Comments about this method

The advantage of copper over iron is that every class of antioxidant, including thiols, will be detected with very little interference from free reactive radicals, and the kinetic of the reaction when using copper is faster compared with iron (FRAP) [5]. An inconvenience that can arise is that the phenanthroline is not water miscible, and, therefore, it must be mixed with organic solvents such as 95% methanol [26].

5.2.5. Trolox equivalent antioxidant capacity/ABTS radical cation decolorization assay

By using a spectrophotometer in this assay, the loss of color can be measured when an antioxidant is added to the chromophore ABTS. + (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)). When the antioxidant molecule reduces ABTS. to ABTS, the solution diminishes its blue-green coloration, tending to be colorless. [27]. The usual way to prepare and use ABTS is by adding 80 mg of manganese dioxide to a stock solution of 5 mM ABTS prepared in a buffer of 17 mM Na/K at pH 7. As an antioxidant standard, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) is used. The standard curve is made with at least six concentrations, ranging from 0 to 350 μM. The samples to be tested are diluted in the buffer of Na/K pH

7 and then mixed with 200 μL of the ABTS solution in a 96-well plate. The absorbance at 750 nm is recorded. Trolox equivalent antioxidant capacity (TEAC) values are calculated from the standard curve with Trolox, and the results are expressed in Trolox equivalents (mM) [27].

5.2.6. Comments about this method

Currently, this method is widely used in many laboratories around the world due to its simplicity of use [28–30]. Thus, the antioxidant activity of a vast range of compounds has been reported using this method. The assays can be carried out in media with both organic and inorganic solvents without affecting ABTS activity. It can be carried out in plates with wells, greatly reducing the use of reagents and making this method environmentally friendly. However, this radical is not naturally found in living organisms, and, thus, the results may not be considered representative of those that take place in living organisms. Finally, regarding the thermodynamic properties of phenols, one compound can reduce ABTS only if its redox potential is lower than that of ABTS; the potential of phenols is lower, so they can reduce ABTS, and it can be used as an antioxidant test for these molecules [5].

5.2.7. 2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay

DPPH is a nitrogenous organic radical with a delocalized electron, and this characteristic gives it a purple coloration, with a maximum absorbance at 515 nm [31]. The assay is based on the measurement of the capacity of antioxidants to reduce DPPH [32]. Such reduction can be measured by the decolorization of the purple color in its absorbance. This assay was first reported by [33], and according to [34], to carry it out, it is necessary to dilute 200 μL of the sample in methanol and mix it with 2 mL of 0.5 mM DPPH. After 30 min, the absorbance is measured at 515 nm in a spectrophotometer. The percentage of DPPH radical scavenging is calculated with the expression:

$$\% \text{ inhibition of DPPH radical} = ((Abr - Aar)/Abr) \times 100 \quad (1)$$

where Abr is the absorbance before the reaction and Aar is the absorbance after the reaction occurred.

5.2.8. Comments about this method

As ABTS, DPPH is also widely used in many laboratories in the world due to its simplicity and the ease to carry it out. However, the fact that the absorbance is read at 515 nm can cause interferences with compounds that absorb at the same wavelength, which may complicate the interpretation of the results [5]. On the other hand, other chemical characteristics of DPPH make understanding the results more difficult. This assay is not competitive because DPPH is a radical and an oxidant at the same time, so decolorization of the reactive can be attributed both to the reaction of the radical and to a reduction of the steric accessibility, with the latter being determinant for the reaction. Thus, small molecules, with better accessibility to the site of the radical, would seem to have better antioxidant capacity. In contrast, larger molecules with much faster reaction times with DPPH than some smaller molecules could apparently

show lower antioxidant capacity. For these reasons, the results with DPPH must be interpreted with caution.

5.2.9. Folin-Ciocalteu AOC method or total phenolic assay

This is a classic method for the detection of total phenols in the laboratory [35]. However, during color development an oxidation-reduction reaction takes place; due to that, this method has been proposed as a method for the detection of antioxidant activity, particularly in phenols [36]. It was originally proposed in 1927, using molybdotungstate as a reagent for phenol reduction, with which a colorized compound was obtained; this compound was read at wavelengths between 745 and 750 nm [36].

This simple, sensitive and precise method, when the reaction occurred in an acid medium, it was much too slow. For this reason [37] improved the method by substituting the molybdotungstate for molybdotungstophosphoric heteropolyanion, which reduces phenols more specifically, and the colorized product is read at 765 nm. The experimental conditions proposed in [37] consist in mixing 1 mL of the sample diluted in at least 60 mL of water and 5 mL of the Folin-Ciocalteu reagent; afterward, the mix is agitated, and 15 mL of Na_2CO_3 is added, mixed, and diluted to 100 mL with water; finally, it is incubated for 2h at 24°C, and the absorbance is measured at the indicated wavelength. Despite the simplicity of the instructions, several recent articles report variations in the incubation period, the concentrations of the reagents, and especially the interchange of gallic acid for some other standards, among which can be cited acids like tannic, chlorogenic, caffeic, vanillic, and ferulic, among others. This can lead to variations in the results, sometimes of several orders of magnitude [38].

5.2.10. Comments about this method

The advantage of the method is that it can be used with practically any plant sample, but only when the aforementioned conditions are controlled [38]. There is also a list of molecules, both inorganic (hydrazine, iron ammonium sulfate, manganese sulfate, sodium cyanide, sodium sulfite, and xanthine, among others) and organic that include amino acids such as adenine, sugars such as fructose, proteins, and fatty acids. If said interferences are controlled, then it is possible to consider the results of this method to report the antioxidant activity of the samples.

5.3. Assessment of the antioxidant capacity in cell culture

The methods used to assess antioxidant capacity that have been mentioned so far are carried out mainly in vitro, using only one oxidizing chemical species and a single reagent to show oxidant and antioxidant activity by means of color development, fluorescence, etc. However, it is necessary to know the real role of antioxidant molecules in living systems [39]. Cell culture is being used to address that issue; in them, chemical molecules that cause oxidation per se can interact with each other, as well as with molecules that do not cause oxidation by themselves, but that generate a disturbance in the redox balance of the cell, provoking in the end oxidative stress [40]. One of the advantages of this system is its ease of handling, compared with laboratory animals, which are harder to obtain and handle given the strict regulations for their ethical management. In this type of assays, it is important to verify the

final concentration accumulated in the cell that is a product of their addition in the culture medium and/or its direct addition to cells, since not all antioxidants are incorporated into the cell in the same way, as it is cited by [40]. An example could be tocopherol and tocotrienol, because in several studies it has been reported that apparently, tocotrienols have a higher antioxidant activity compared with tocopherols; however, if the concentration of these antioxidants is adjusted, both compounds have the same antioxidant capacity. The apparent disparity in antioxidant activity is due to the fact that tocotrienols can enter the cell more easily given their short chain compared with tocopherols.

5.4. Nanotechnology-aided assays

With the advent of the nanotechnology in the decades of 1980–1990 [41], the technology that is being developed has also proposed the use of a method that uses nanoparticles to evaluate antioxidant activity as well. Scampicchio proposed, in one of the first works in the field [42], the assessment of some phenolic compounds by the generation and growth of gold nanoparticles (AuNPs) from a gold solution (AuIII) in solution ($HAuCl_4$) by the generation of a sharp plasmon absorption band at 555 nm. The optical properties of the AuNPs correlate well with the reduction potential of phenolic acids, something that can be determined by voltimetric measurements, and this method is proposed to evaluate antioxidant capacity of pure compounds or their mixtures [6].

When [43] conducted an experiment where they characterized the kinetic of AuNP generation at an absorbance of 540 nm and described a sigmoid curve as a function of the concentration of polyphenols, they proposed the following equation:

$$A_{540} = A_{max}/1 + e - KAuNPs(X - XC_{50}) \quad (2)$$

XC_{50} = the concentration of polyphenols that give half of the maximum plasmon resonance absorption; KAuNPs = the number of AuNPs produced by concentration unit of polyphenols.

The authors proposed that KAuNPs be used as a parameter to estimate antioxidant activity. This method has already been employed in the determination of antioxidant activity of several products including honey [44], wine [45], tea [46], apples [47], etc. For their part, [48] also used silver nanoparticles for the same goal. The method, whose name is silver nanoparticle antioxidant capacity (SNPAC), uses Trolox as its standard. The rationale of this method is that polyphenols are able to reduce Ag^+ ions in the presence of citrate-stabilized silver seeds; the intensity of the plasmon, visible at 423 nm, is evaluated, and thus antioxidant capacity [49] is quantitatively assessed. With respect to polyphenols, this method is more robust and repeatable than assays that use a direct reduction of metallic ions by antioxidants. As the gold method, this method has already been employed in several fruit juices and teas [50]. Some of its main advantages are good linearity with the concentration of the sample (polyphenols) and the lack of interference by molecules present in the samples such as reducing sugars, fruit acids, or amino acids.

6. Conclusions

Throughout the development of different methods to determine antioxidant activity of several molecules, polyphenols among them, one can observe an evolution, which goes from the involvement of the antioxidant with its substrate, its exposition to several antioxidants, and a medium that shows the changes that take place during the reaction, to more sophisticated methods where the kinetic of the formation of nanoparticles show the presence or absence of antioxidant activity.

In the light of so many methods, a question arises: Why is not there a universal method to evaluate the antioxidant activity of any molecule? The answer may not be simple, but it would have to do with the goals that are sought; they may be the simple detection of antioxidant activity in a sample, or they may include the comparison of antioxidants with each other and the understanding of the process inside a living system such as cells in culture or more complex systems as laboratory animals and of course the human body.

Many authors recommend that certain steps be considered to select and report the results of antioxidant activity (**Table 2**). These include carrying out the assay in a systematic way, always respecting the conditions established for its development: controlling the source of the sample that is subject to experimentation regarding its origin, management during its collection and transport to the laboratory, and always controlling the standard used to compare the results of the sample. By adhering to these steps, variations in the results obtained by different laboratories can be reduced when the antioxidant activities from samples of the same origin are compared.

Method	Required equipment	Biological relevance	Mechanism	End point	References
Fluorescein	Sophisticated	Medium	?	Fixed time	[19]
TRAP	Sophisticated	High	HAT	Lag phase	[21, 22]
FRAP	Medium	Low	SET	Time varies	[25]
Copper reduction	Medium	Low	SET	Time	[26]
TEAC/ABTS	Simple	Low	SET	Time	[27]
DPPH	Simple	Low	SET	IC_{50}	[31, 32]
Folin-Ciocalteu AOC	Simple	Medium	SET	IC_{50}	[36]
Cell culture	Medium	High	?	?	[39]
Nanotechnology	Sophisticated	High	?	?	[42, 43]

Modified from Ref. [5].

Table 2. Relevant characteristics of the methods to evaluate the antioxidant capacity of phenolic compounds.

In the same way, the selection of one of the many available methods must be based on the following criteria, to optimize the results: (a) select a relevant source for the sample that is

abundant enough to repeat the results; (b) select a method that is as simple as possible, taking into account the facilities, the equipment, and the reagents that are available; (c) choose a method where reaction times are clear, as well as the type of mechanisms that will be carried out; (d) select an assay with good repeatability; (e) consider if the antioxidants are of hydrophilic or lipophilic nature; and (f) take into account the nature of the oxidants.

Many of the proposed assays that have been traditionally used to give a value to a molecule or group of molecules, in the case of crude extracts, are complex, since several factors can participate. For example, particle size has an effect over the determination of its activity, the pH, and the medium of dissolution. This can lead us to declare that simple tests that need little equipment are not always the most adequate. This is the case of DPPH; since it is a test that requires only a methanol, DPPH, glassware, and spectrophotometer, it could give the best results when assigning an antioxidant value to a molecule.

Another important aspect that could be a source of confusion in the literature is the interchangeable use of the terms activity and antioxidant capacity. During the compilation of articles for the preparation of this chapter, it was noted that several authors titled his research as antioxidant activity and reported figures rather denote the ability of extracts or molecules for antioxidation, reporting them in units of a standard as can be equivalent of Trolox or per unit of time. So that being strict about definitions, if the antioxidant activity of a sample is reported, it should simply describe whether or not presented such a phenomenon, something similar to what is done when colorful preliminary tests are done to detect the presence or no major group of secondary metabolites. As suggested be careful in handling of such terms and keep in mind that in the case of the antioxidant activity refers to whether or not an oxidation retardant of a substrate regardless of the magnitude. On the other hand, it indicates how much capacity has antioxidation an extract or molecule to a substrate, in this case being reported in% inhibition, IC_{50}, XC_{50}, Trolox equivalents, etc., units that can be compared to know which sample is more suitable to prevent oxidation of said substrate.

The rise all over the world of the number of people with chronic-degenerative diseases caused by oxidative stress and a lack of antioxidants in the diet is more and more alarming, especially if the high cost that this implies for the health sector of any country is considered, due to the need to treat everyday more cases of cancer, diabetes, arteriosclerosis, hypertension, etc. Finding new sources of antioxidants, especially from natural sources, is paramount, and comparing them with the traditional molecules is also important; therefore, it is desirable to employ assays that show if an extract or molecule has or not antioxidant activity and that are able to quantify their antioxidant capacity.

Author details

Rubén San Miguel-Chávez

Address all correspondence to: sanmi@colpos.mx

Colegio de Postgraduados, Campus Montecillo, Texcoco, Estado de México, Mexico

References

[1] Daglia M. Polyphenols as antimicrobial agents. Current Opinion in Biotechnology. 2012;**23**:174–181. DOI: 10.1016/j.copbio.2011.08.007.

[2] Vermerris W, Nicholson R. Families of phenolic compounds and means of classification. In: Vermerris W, Nicholson R, editors. Phenolic compound biochemistry. The Netherlands: Springer; 2006. pp. 1–34. DOI: 10.1007/978-1-4020-5164-7_4.

[3] Rice-Evans C, Miller N, Paganga G. Antioxidant properties of phenolic compounds. Trends in Plant Science. 1997;**2**:152–159. DOI: 10.1016/S1360-1385(97)01018-2.

[4] Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. Journal of Agriculture and Food Chemistry. 2005;**53**:1841–1856. DOI 10.1021/jf030723c.

[5] Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agriculture and Food Chemistry. 2005;**53**:4290–4302. DOI: 10.1021/jf0502698.

[6] López-Alarcón 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.

[7] Wright JS, Johnson ER, DiLabio GA. Predicting the activity of phenolic antioxidants: theoretical method, analysis of substituent effects, and application to major families of antioxidants. Journal of the American Chemistry Society. 2001;**123**:1173–1183. DOI: 10.1021/ja002455u.

[8] Bartosz G. Reactive oxygen species: destroyers or messengers?. Biochemical Pharmacology. 2009;**77**:1303–1315.

[9] Hernández-García D, Wood CD, Castro-Obregon S, Covarrubias L. Reactive oxygen species: a radical role in development?. Free Radical Biology and Medicine. 2010;**49**:130–143.

[10] Jones DP, Go YM. Redox compartmentalization and cellular stress. Diabetes, Obesity and Metabolism. 2010;**2**:116–125. DOI: 10.1111/j.1463-1326.2010.01266.x.

[11] Miwa S, Brand MD. Mitochondrial matrix reactive oxygen species production is very sensitive to mild uncoupling. Biochemical Society Transactions. 2003;**31**:1300–1301. DOI:10.1042/bst0311300.

[12] Koepke JI, Kerry-Ann N, Wood CS, Boucher KK, Terlecky LJ, Walton PA, Terlecky SR. Restoration of peroxisomal catalase import in a model of human cellular aging. Traffic 2007;**8**:1590–1600. DOI: 10.1111/j.1600-0854.2007.00633.x.

[13] Willet WC. Eat, drink and be healthy-the Harvard medical school guide to healthy eating. New York: Simon and Schuster. 2011.

[14] Halliwell B, Gutteridge JMC. Free radicals in Biology and medicine. Fifth ed. Clarendon: Oxford University Press; 2015. 905 p.

[15] Kaur C, Kapoor HC. Antioxidants in fruits and vegetables – the millennium's health. International Journal of Food Science and Technology. 2001;703–725. DOI: 10.1111/j.1365-2621.2001.00513.x.

[16] Liang N, Kitts DD. Antioxidant property of coffee components: assessment of methods that define mechanisms of action. Molecules. 2014;**19**:19180–19208. DOI: 10.3390/molecules191119180.

[17] Wang H, Cao G, Prior L. Total capacity of fruits. Journal of Agricultural and Food Chemistry 1996;**44**:701–705. DOI: 10.1021/jf950579y.

[18] Cao GH, Alessio HM, Cutler RG. Oxygen-radical absorbency capacity assay for antioxidants. Free Radical Biology and Medicine. 1993;**14**:303–311. DOI: 10.1016/0891-5849(93)90027-R.

[19] Ou B, Hampsch-Woodill M, Flanagan J,Deemer EK, Prior RL, Huang D. Novel fluorometric assay for hydroxyl radical prevention capacity using fluorescein as the probe. Journal of Agricultural and Food Chemistry. 2002;**50**:2772–2777. DOI: 10.1021/jf011480w.

[20] Antolovich M, Prenzler PD, Patsalides E, McDonals S, Robards K. Methods for testing antioxidant activity. Analyst. 2002;**127**:183–198. DOI: 10.1039/B009171P

[21] Ghiselle A, Serafini M, Maiane G, Azzini E, Ferro-Luzzi A. A fluorescence-based method for measuring total plasma antioxidant capacity. Free Radical Biology and Medicine. 1995;**18**:29–36. DOI: 10.1016/0891-5849(94)00102-P.

[22] Ghiselle A, Serafini M, Natella F, Scaccini C. Total antioxidant capacity as a tool to assess redox status: critical review and experimental data. Free Radical Biology and Medicine. 2000;**29**:1106–1114. DOI: 10.1016/S0891-5849(00)00394-4.

[23] Meyer AS, Yi OS, Pearson DA, Waterhouse AL, Frankel EN. Inhibition of human low-density lipoprotein oxidation in relation to composition of phenolic antioxidants in grape (*Vitis vinifera*). Journal of Agricultural and Food Chemistry. 1997;**45**:1638–1643. DOI: 10.1021/jf960721a.

[24] Frankel EN, Meyer AS. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. Journal of the Science of Food and Agriculture. 2000;**80**:1925–1941. DOI: 10.1002/1097-0010(200010)80:13<1925::AID-JSFA714>3.0.CO;2-4.

[25] Benzie IFF, Szeto YT. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. Journal of Agricultural and Food Chemistry. 1999;**47**:633–636. DOI: 10.1021/jf9807768.

[26] Apak R, Güçlü K,Özyürek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric iron reducing capability in the presence of neocuproine: CUPRAC method. Journal of Agricultural and Food Chemistry. 2004;**52**:7970–7981. DOI: 10.1021/jf048741x.

[27] Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clinical Science. 1993;**84**:407–412. DOI:10.1042/cs0840407.

[28] Stratakosa AC, Delgado-Pandoa G, Lintonb M, Pattersonb MF, Koidisa A. Industrial scale microwave processing of tomato juice using a novel continuous microwave system. Food Chemistry. 2016;**190**:622–628. DOI: 10.1016/j.foodchem.2015.06.015.

[29] Schempp H, Christof S, Mayr U, Treutter D. Phenolic compounds in juices of apple cultivars and their relation to antioxidant activity. Journal of Applied Botany and Food Quality. 2016;**89**:11–20. DOI: 10.5073/JABFQ.2016.089.002.

[30] Islam T, Yu X, Xu B. Phenolic profiles, antioxidant capacities and metal chelating ability of edible mushrooms commonly consumed in China. LWT—Food Science and Technology. 2016;**72**:423–431. DOI: 10.1016/j.lwt.2016.05.005.

[31] Bondet V,Brand-Williams W, Berset C. Kinetics and mechanisms of antioxidant activity using the DPPH. Free radical method. LWT—Food Science and Technology. 1997;**30**:609–615. DOI: 10.1006/fstl.1997.0240.

[32] Molyneux, P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin Journal of Science and Technology. 2004;**26**:211–219.

[33] Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT—Food Science and Technology. 1995;**28**:1:25–30. DOI: 10.1016/S0023-6438(95)80008-5.

[34] Manzocco L, Anese M, Nicoli MC. Antioxidant properties of tea extracts as affected by processing. LWT—Food Science and Technology. 1998;**31**:694–698. DOI: 10.1006/fstl.1998.0491.

[35] Folin O, Denis W. A colorimetric method for the determination of phenols (and phenol derivatives) in urine. The Journal of Biological Chemistry. 1915;**22**:305–308.

[36] Singleton VL, Orthofer R, Lamuela-Raventós RM. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology. 1999;**299**:152–178. DOI: 10.1016/S0076-6879(99)99017-1.

[37] Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture. 1965;**16**:144–158.

[38] Escarpa A, González MC. Approach to the content of total extractable phenolic compounds from different food samples by comparison of chromatographic and spectrophotometric methods. Analytica Chimica Acta. 2001;**427**:1:119–127. DOI: 10.1016/S0003-2670(00)01188-0.

[39] Song W, Derito CM,Liu MK, He X, Dong M, Liu RH. Cellular antioxidant activity of common vegetables. Journal of Agricultural and Food Chemistry. 2010;**58**:6621–6629. DOI: 10.1021/jf9035832.

[40] Niki E. Assessment of antioxidant capacity *in vitro* and *in vivo*. Free Radical Biology and Medicine. 2010;**49**:503–515.

[41] Paull R, Wolfe J, Hébert P, Sinkula M. Investing in nanotechnology. Nature Biotechnology. 2003;**21**:1144–1147. DOI:10.1038/nbt1003-1144.

[42] Scampicchio M, Wang J, Blasco AJ, Sanchez AA, Mannino S, Escarpa A. Nanoparticle-based assays of antioxidant activity. Analytical Chemistry. 2006;**78**:2060–2063. DOI: 10.1021/ac052007a.

[43] Vilela D, González MC, Escarpa A. Sensing colorimetric approaches based on gold and silver nanoparticles aggregation: chemical creativity behind the assay. A review. Analytica Chimica Acta. 2012;**751**:24–43. DOI: 10.1016/j.aca.2012.08.043.

[44] Vilela D, González MC, 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.

[45] Wu Z, Xu E, Li J, Long J, Jiao A, Jin Z, Xu X. Determination of antioxidant capacity of Chinese rice wine and Zhuyeqing Liquor using nanoparticle-based colorimetric methods. Food Analytical Methods. 2016;**1**:1–11. DOI:10.1007/s12161-016-0646-8.

[46] Sharpea E, Huaa F, Schuckersa S, Andreescua S, Bradley R. Effects of brewing conditions on the antioxidant capacity of twenty-four commercial green tea varieties. Food Chemistry. 2016;**192**:380–387. DOI: 10.1016/j.foodchem.2015.07.005.

[47] Pelle FD, Compagnone D, Del Carlo M, Vilela D, González MC, Escarpa A. Development of an optical sensing strategy based on gold nanoparticles formation driven by polyphenols. Application to food samples In: Compagnone D, Baldini F, Di Natale C, Betta G, Siciliano P, editors. Sensors. Switzerland: Sringer International Publishing; 2015. pp. 39-46. DOI: 10.1007/978-3-319-09617-9_8.

[48] Özyürek M, Güngör N, Sefa Baki S, Güçlü K, Apak R. Development of a silver nanoparticle-based method for the antioxidant capacity measurement of polyphenols. Analytical Chemistry. 2012;**84**:8052–8059. DOI: 10.1021/ac301925b.

[49] Dauthal P, Mukhopadhyay M. *In-vitro* free radical scavenging activity of biosynthesized gold and silver nanoparticles using *Prunus armeniaca* (apricot) fruit extract. Journal of Nanoparticle Research. 2013;**15**:1366. DOI: 10.1007/s11051-012-1366-7.

[50] Zhang H, Tsao R. Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. Current Opinion in Food Science. 2016;**8**:33–42. DOI: 10.1016/j.cofs.2016.02.002.