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Plant Extracts as Antioxidant Additives for Food Industry

Ludy C. Pabón-Baquero, Ángela María Otálvaro-Álvarez, Margarita Rosa Rendón Fernández and María Patricia Chaparro-González

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Abstract

Plants have phenolic compounds with antioxidant activity. These compounds are distributed in tissues and cells of plants and their abundance depends on the species, the part of the plant used, maturity stage, light hours, among others. On the other hand, the profile and quantity of phenolic compounds extracted from plant matrices changes depending on the species, cultivar, climate, and other factors. Plant extracts do not present a unique phenolic component, they correspond a mixture and its antioxidant activity will be affected by the concentration of each one and their action depends on this composition. In this chapter, some generalities about the phenolic compounds with antioxidant activity present in plant matrices will be exposed, also the principal methods for their extraction and quantification will be described and this information will be complemented with a review on applications of these compounds in food industry. In that sense, the reader can infer the importance of continue to studying and developing techniques to obtain, extract, and characterize this kind of compounds, also they can identify possible application of them, the most important, they can recognize them as an alternative to replace chemical synthetized antioxidants used in food industry improving the market of natural products.

Keywords: extraction, food additives, natural products, phenolic compounds, vegetal species



1. Introduction

Over the years, benefits have been attributed to fruits and vegetables consumption associated with their content of phenolic compounds with antioxidant activity that may contribute to reduce the risk of cardiovascular diseases and cancer [1]. The antioxidant behavior of phenolic components is related to their ability to chelate metals, inhibit lipoxygenase, and capture free radicals, although sometimes they can also act as promoters of *in vitro* oxidation reactions [2]. In this way, phenolic compounds act as antioxidants by delaying or preventing autooxidation or oxidation and free radicals sequestration, forming more stable compounds that cannot undergo subsequent oxidations, which allows them to protect low density lipoproteins (LDL) of the human body from oxidation [3]. Antioxidant activity of different foods research, such as fruits, vegetables, and plants in general, have indicated the positive effects of this on diseases control related to oxidative stress [4–6].

Phenolic compounds are chemical substances that have an aromatic ring attached to one or more hydroxyl groups, including functional derivatives such as esters, glycosides, among others. They have an acidic behavior, since the oxygen of the hydroxyl group is strongly bounded to aromatic ring, while the oxygen and hydrogen bond is relatively weak, which have allowed the proton dissociation that can be released into the medium, causing a phenolate ion negatively charged [7]. They are secondary metabolites distributed in the plant kingdom, from which more than 8000 compounds have been identified that differ in chemical structures and in activity. Its distribution in tissues and cells varies among different fruit and vegetable species and its abundance depends on the species, the type of crop, the part of the plant used, the type of soil, maturity stage, light hours, fertilization, part of the vegetative cycle, among others [8].

These compounds participate in various functions of plants, such as nutrient absorption, protein synthesis, enzymatic activity, photosynthesis, structural components formation, allelopathy, and defense against adverse environmental factors [9]. They are substances responsible for providing fragrances, colors, and flavors to various plants. As an example, anthocyanins are responsible for the red, blue, and violet color of red fruits such as cherries, blackberries, or currants, while flavones are attributed the yellow color of fruits such as lemon or banana. This type of compounds in addition to giving color also contribute by providing characteristics to taste (astringency) of some foods and affecting the sugar/acid ratio of some fruits, as well as being used as a criterion to determine the overall quality of fresh fruits and derived products of these [10]. Among the polyphenols that are considered important in food, are the acids such as gallic, synaptic, ferulic, caffeic, p-coumaric, and their derivatives; as well as the flavonoids and their glycosides [11]. In that sense, phenolic compounds are usually classified into three groups such as flavonoids, phenolic acids, and polyphenols, the most important being the benzoic acid derivatives for their therapeutic use and specifically the flavonoids for having the greatest chemical diversity with approximately 6000 different structures. The profile and quantity of phenolic compounds extracted from plant matrices changes depending on the species, cultivar, season, climate, and other factors such as the cultivation method. However, the extraction performance depends mainly on the solvent polarity used, the extraction method, the duration of the process, and the quantitative and qualitative distribution of the compounds present [3, 11, 12].

The plant extracts, in their majority do not present a unique phenolic component, but, on the contrary, correspond to the mixture of several of these. In that sense, its antioxidant activity will be affected by the concentration of each one in the analysis matrix [3]. This is how there is no single methodology to know the antioxidant capacity of an extract and, therefore, to obtain more comprehensive and complete information, different methods must be used with different mechanisms of action. In addition, due to the heterogeneity of the analytical conditions used, among which are: wavelength, radical generator, time of analysis and how to express the results, is possible to reach values of antioxidant capacity that are not comparable. In that way, in this chapter some generalities about the phenolic compounds with antioxidant activity present in plant matrices will be exposed, also, the principal methods for their extraction and quantification will be described and this information will be complemented with a review on applications of these compounds in food industry.

2. Classification and properties of phenolic compounds

Phenolic compounds can be classified as extractable and non-extractable, as explained below:

Non-extractable polyphenols: these are compounds with high molecular weight, or polyphenols linked to dietary fiber or proteins that can be found in extraction wastes. Include hydrolysable tannins and condensed tannins with a high number of units in the polymer chain. The hydrolysable tannins are polymeric structures that can derive from gallic acid or its condensation dimer product hexahydroxydiphenic acid. The condensed tannins or proanthocyanidins, on the other hand, are polymeric structures, formed by the union of flavan-3-oles, and can be procyanidins, prodelfinidins, and propelargonidins [13].

Extractable polyphenols: these are low or medium molecular weight compounds that can be extracted with aqueous or aqueous-organic solvents. They are classified according to their chemical structure in: flavonoids, which are much more complex structures, which in turn, are subdivided into flavones (chrysin and rutin); flavonols (quercetin and myricetin); flavanols or catechins (epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate); flavanones (hesperidin and naringenin), anthocyanins (delphinidin, malvidin, and cyanidin) and condensed tannins with a low number of monomers, etc. [13]. On the other hand, non-flavonoid compounds are formed by an aromatic ring substituted by an alcohol in one or more positions and are classified according to the number of carbons they have, within this group can be found: simple phenols, benzoic acids, hydrolysable tannins, acetophenones and phenylacetatic acids, cinnamic acids, coumarins, benzophenones, xanthones, stilbenes, chalcones, lignans, and secoiridoids [14].

Showing up next a description of some of the phenolic compounds present in plant species, including some of their properties and possible applications.

Simple phenolic: these are compounds to which antioxidant, antibiotic, antiparasitic, and cytotoxic properties have been attributed. In general, foods such as cereals are found as resorcinol derivatives [15].

Aromatic carboxylic acids and their derivatives: they occur naturally in many plant species [16] and have been linked to immunostimulant, choleretic, anti-inflammatory, analgesic, anti-pyretic, and protective properties of the cardiovascular system [11]. The antioxidant properties of phenolic acids have been associated to their capacity to purify free radicals avoiding lipids peroxidation, inhibiting LDL plasma oxidation, and purifying reactive oxygen, which plays a very important role in the promotion of tumors, carcinogenesis. They also act preventively on diseases of the coronary arteries, by decreasing platelet aggregation [17]. Among phenolic acids, antioxidant properties have been attributed as follows: caffeic acid, chlorogenic acid and its isomers, such as 4-O-caffeyl-quinic [18], being the chlorogenic acid the most abundant in plants and the most active antioxidant in this group. It is also known that benzoic acid plays important roles in plant metabolism by regulating their growth and chemically modifying the rhizosphere forming part of the radical exudates; it also increases the capacity to capture minerals when it accumulates in soil in a great concentration [19, 20]. This acid is also a mediator of stress responses, as it is incorporated into numerous secondary metabolites associated with plant-herbivore or plant-pathogen interactions [21].

Acetophenones and phenylacetic acids: they are used as sunscreens in sun creams and o-hydroxy-benzophenones are used to protect fibers from photodegradation in the textile industry [11].

Phenylpropanoids: these are products that have a specific role in the response to pathogens or protection from ultraviolet rays, through their antioxidant capacity and energy dissipation, as well as their function as structural components of the cell wall [22]. Due to the phytotoxicity of these compounds, they are stored in their glycosylated form in the vacuoles or conjugated with other components of the cell wall [23]. The cinnamic acid derivatives are abundant in nature in free form (coumaric acid and caffeic acid) or esterified with sugars (caffeyl-tartaric acid), quinic acid (chlorogenic acid), etc. While the benzoic acid derivatives are in free form, such as acids (vanillic acid and gallic acid) or aldehydes (vanillin and anisaldehyde).

Coumarins and related: in plants, these compounds can be present in seed cover, fruits, flowers, roots, leaves and stems, but in general, the highest concentrations are found in fruits and flowers. Its function is related to plant defense, given its antimicrobial, antifeedant, protective properties of UV radiation and inhibiting germination. The pharmaceutical interest for coumarins is due to their vitamin properties, their ability to decrease capillary permeability and an increasing in the strength of capillary walls. They present a wide range of physiological effects in animals, ranging from the less complex element of this group known as simple coumarin, which is toxic to mammals, to the last in the scale that is noboviocin, recognized as a commercial antibiotic [17].

Flavonoids and derivatives: these compounds are widely distributed in plants, especially in leaves, flowers and pollen, as well as in woody parts, such as peduncles and barks. It is known that plants that grow in full sun exposition have more flavonoids than those that grow in the shade, which makes interpreting their presence as a mechanism of plant defense to oxidations promoted by UV light. Among its plant functions, antifungal and bactericidal role is highlighted. Flavonoids have an enormous scientific interest due to the protection they exert against chronic diseases such as cancer and cardiovascular diseases [24, 25]. Flavonoids contain a variable number of phenolic hydroxyl groups in their chemical structure, have excellent chelation properties

of iron and other transition metals, which gives them a high antioxidant capacity and therefore, play an essential role in protection against the phenomena of oxidative damage [26, 27].

The growing interest in flavonoids as antioxidants is due to the appreciation of their broad pharmacological activity, since they can bind to biological polymers, such as enzymes, hormone transporters and DNA; chelate transient metal ions, such as Fe²⁺, Cu²⁺ and Zn²⁺, catalyze the transport of electrons and purify free radicals. So, these type of compounds have an interest from a nutritional point of view, since the obtaining and food preparation with a high content of these, supposes a decreasing in the use of antioxidant additives, at the same time as obtaining healthier foods, which can even be considered functional foods [28].

The activity of flavonoids as antioxidants depends on the redox properties of their hydroxyphenolic groups and the structural relationship. For example, it has been determined that chemical criteria that are related to antioxidant capacity of the flavonoids are as follows: (a) the presence of O-dihydroxy structure in the B ring; that gives greater stability to the radical form and participates in electrons delocalization, (b) the double bond in conjunction with 4-oxo function of the C ring and (c) the hydroxyl groups in the three and five positions. Given these concerns, quercetin flavonol is the one that exerts an effective antioxidant function, with a Trolox value of 4.7 mM, which is five times greater than that shown by vitamins E and C and has a water-solubility similar to vitamin E. In addition, the antioxidant function of quercetin shows synergistic effects with vitamin C, since ascorbic acid reduces the quercetin oxidation, in such a way that combined with flavonoid can maintain its antioxidant functions for longer [29]. Another flavonol is kaempferol, a bleaching inhibitor of illuminated chloroplasts, due to the inhibition of the reactions promoted by the triplet of oxygen inside the chloroplast and the protection against thermal autooxidation of palm oils, corn, sunflower, soy, olive, peanut, coconut, butter and margarine [30]. In that sense, polyhydroxylated chalcones, such as butein, have shown considerable antioxidant activity for shortening, being twice as active as quercetin or α -tocoferol. Chalcones with two adjacent hydroxyl groups are almost as effective as butein, however, the presence of additional hydroxyl only slightly increases the inhibitory activity of rancidity, while hydrogenation of the chalcone double bond increases the antioxidant power, is found that pentahydroxydihydrochalcone is 2–3 times more active than the corresponding unsaturated chalcone [31].

Other examples of this type of metabolites are the catechin, epicatechin, gallocatechin, compounds that act as OH' radical sequestration generated in a Fenton system ranged from 100 to 300 times higher than the effects of mannitol, a typical sequestration of the most toxic from all reactive oxygen-ERO species *in vivo* generated [32]. In this, there is a positive correlation between phenolic compounds and antioxidant capacity, in this case, the antioxidant capacity depends on secondary metabolites, especially those of phenolic nature such as flavonoids [33].

Tannins: antioxidant activity study of condensed tannins *in vitro* and *in vivo* shows that they are effective scavengers of free radicals and they inhibit the tissues oxidation better than vitamin C, vitamin E and β -carotene. *In vitro* conditions has been shown that condensed tannins have a preference for neutralizing the hydroxyl free radical (*OH), as well as it has been demonstrated that they have the capacity to act as noncompetitive inhibitors of the enzyme xanthine oxidase, one of the biggest generators of free radicals in cellular metabolism [34]. The biological properties of tannins are linked to their capacity to form complexes with macromolecules,

particularly with proteins (digestive proteins, fungal or viral enzymes). This explains the problems that its presence can cause in industrial processes associated with food and beverages (cloudiness of beer) production or in agriculture (formation of humic acid, which decreases soybean nutritional value) [15]. Due to tannins affinity with proteins, they can be used as follows: antidiarrheals, in products for skin protection and mucous membranes, vasoconstrictors, skin regenerators, antiseptics, antibacterials and antifungals. Additionally, they act as scavengers of free radicals and inhibitors of superoxides formation, enzymatic inhibitors of 5-lipoxygenase, angiotensin-converting enzyme (ACE), elastase and protein kinase C.

Xanthones, benzophenones and stilbenes: xanthones and benzophenones are present in roots and exotic fruits, while stilbenes have been found in different types of fruits and foods. Its major representative is resveratrol (3,5,4'- trihydroxystilbene), which occurs in grapes, red fruits and peanuts. This compound has an anticancer effect and inhibits reactions that increase the risk of coronary heart disease [35]. Additionally, it has been found to be useful in treatment of various chronic diseases, such as inflammation, arthritis, cardiovascular diseases and delaying aging, while xanthones have shown antiprotozoal activity including activity against Leishmania [15].

Lignans and lignins: these are the most abundant organic substances in plants, after cellulose. These are part of the lignin of the cell wall and participate in plant growth. They also belong to the group of phytoestrogens acting as antioxidants, decreasing the effects of free radicals. There are simple lignans and cyclic lignans that fight against the damaging effects of free radicals and whose compounds like enterodiol and enterolactone have anticancer potential that mimics the functions of human hormones, in addition to inhibiting the growth of breast and prostate tumors [36].

Phenolic diterpenes: this type of compounds are widely known to be potent inhibitors of the linoleic acid oxidation. Its mechanism of action is related to metals chelation by the central beta-diketone group [37].

It can also be included in this review, ubiquinol, which is a product of the ubiquinone (vitamin Q) reduction, a potent antioxidant *in vivo* conditions of low oxygen concentration, such as those found in many cellular environments, also inhibits the peroxidation of arachidonic acid in emulsion, which have hemoglobin as an initiator and prevents lipids photooxidation in mitochondria. The observed reactivity of ubiquinol with the free radical diphenyl-picryl-hydrazyl suggests that is an antioxidant that breaks the chain reaction and probably reacts *in vivo* with peroxy radicals [38].

In **Table 1** some of these compounds isolated from different plant matrices are presented [39–41].

2.1. Extraction and quantification of phenolic compounds

Among the techniques used in phenolic compounds extraction are as follows: maceration, digestion, decoction, infusion, percolation, soxhlet, countercurrent extraction, ultrasound-assisted extraction, microwave extraction, extraction with supercritical fluids, extraction with hydrofluorocarbon solvents, micro-extraction in solid phase, partition, chromatography, hydrolytic maceration followed by distillation, hydro-distillation, micro-distillation,

| Group | Number of carbons | Plant matrix | Compound or type |
|--------------------------------------|-------------------|---------------------------------------|----------------------------------|
| Simple phenolic | C6 | Banana | Transcinnamic acid |
| Benzoic acid | C6-C1 | Strawberry | p-Hydroxybenzoic |
| Acetophenones and phenylacetic acids | C6-C2 | Blueberry | |
| Phenylpropanoids | C6-C3 | Grapes | |
| Coumarins | C6-C3 | Tangerine, orange, lemon | Caffeine acid, scopolin |
| Flavonoids | C6-C3-C6 | Cherry, orange, grape, soybeans | Isoflavones, quercetin, cyanidin |
| | | Tangerine, orange | Flavanones |
| | | Parsley, celery, oreganum | Flavones |
| | | Apple, pear | Flavonols |
| | | Grapes | Flavanols |
| Benzophenones and stilbenes | C6-C1-C6 | Grapes | Resveratrol |
| Xanthones | C6-C2-C6 | Mangosteen, mango | Mangiferine |
| Lignans and lignins | (C6-C3)n | Passion fruit, lime, fruits with bone | Daidzein |

Table 1. Classification of phenolic compounds.

molecular distillation, thermo-micro-distillation, among others. In these methods, the solvents used depend on the hydrophilic or lipophilic character of the compounds of interest, thus, for the lipophilic extracts preparation, hexane, diethyl ether and methylene chloride can be used, while for the extraction of hydrophilic compounds, methanol has been used, ethanol, mixtures of ethanol/water, acetone/water and methanol/water and extraction in acidic medium (pH = 2) with methanol/water, followed by acetone/water and water/acetonitrile in acidic medium [14].

Showing up next applications of some solvents according to the polarity:

- Water: extraction of anthocyanins, lectins (carbohydrates bound to proteins), polypeptides, saponins, starch, terpenoids and tannins.
- Methanol: extraction of antoncianins, lactones, phenols, polyphenols, saponins, tannins, terpenoids and xanthoxillins.
- Ethanol: extraction of alkaloids, flavonols, polyphenols, polyacetylenes, terpenoids, steroids and tannins.
- Acetone: extraction of alkaloids, coumarins, fatty acids and terpenoids.
- Chloroform: extraction of flavonoids and terpenoids.
- Ether: to extract flavonols and phenols [42].

To minimize the generation of organic waste from extractions and reduce the environmental impact caused by the disposal of toxic solvents used in some extractions, in recent years, the development of sustainable extraction methods has been studied, such as those in which they use acidified water and ethanol as solvents, in addition to the developments in the area of extraction with sub-critical water [14].

2.2. Methodologies for the quantification of phenolic compounds

Depending on equipment availability and the needs of the measurement in terms of costs, precision and speed, quantification techniques can range from simple spectrophotometric analysis to complex chromatographic analysis [43]. Among the spectrophotometric techniques for quantification of phenolic compounds are the Folin-Ciocalteau method, which determines the ability of polyphenols to reduce Mo(VI) to Mo(V), by using the Folin-Ciocalteau reagent (mixed of phosphotungstic acid and phosphomolybdic acid). As a result of such reaction, a color change from yellow to blue is observed [3, 44]. The results of quantification of polyphenols obtained by this methodology, can be expressed in equivalents of gallic acid, catechin, colorgenic, caffeic, protocatecuic, vanillinic or ferulic acid, making it difficult to establish comparisons among different determinations [13]. This methodology can be applied on a routine basis, due to the speed with determinations, which can be made at relatively low costs of these in comparison with other techniques [43]. However, it has been observed that the amount of phenols can be overestimated, since other compounds than phenols (sugars, organic acids, iron (II), nitrogenous compounds and other inorganic substances such as sulfates, phosphates and chlorides) can present interferences due to their reactions with reagent or at the wavelength used (close to 730 nm) in the determination [8, 13]. An example of the methodology is that proposed by García, for the quantification of phenolic compounds in plant extracts [8].

Another spectrophotometric technique is the one used for the quantification of total flavonoids where the ortho-dihydroxylated, meta-hydroxylated and para-hydroxylated flavonoids are chelated by reaction with AlCl₃ [45]. However, since plant extracts may vary in their turbidity, is possible that in some samples there are alterations in the results [46]. For anthocyanins measurement, a colorimetric method based on the color change obtained as a result of pH variation can also be used. Taking into account that anthocyanins have a red, violet or purple coloring at acidic pH, while at basic pH, they are green or blue [47]. An example of its application was carried out by Gajula et al., for flavonoids and anthocyanins quantification in a plant extract (*Ocimum basilicum* L.) [48].

Apart from the spectrophotometric techniques are the chromatographic techniques for phenolic compounds quantification and identification. From these, the simplest technique is the column chromatography based on sample separation as it passes through a column packed with silica that allows compounds separation and their subsequent quantification through the use of a detector used at different wavelengths. Each type of compound is quantified by the preparation of a calibration curve and the results are expressed in terms of concentration or total amount of specific compounds in the analyzed extracts. One of the advantages of this technique when is used for separation and determination of phenolic compounds, is that does not require the previous sample derivatization [49]. Some examples of the application of this type of techniques are reported by Stefenona et al., Sánchez et al., and Martínez [14, 45, 50].

As a complement to chromatographic techniques and to improve the compounds identification found in the extracts, mass spectrometry is currently used, in the analysis of flavonoids extracted from plants [51]. This have allowed to know significant information about the structure of the present compounds even when sample quantities analyzed are very small or correspond to mixtures. With respect to flavonoids characterization applying mass spectrometry, the information obtained can be as follows: (1) the remains of aglycones, (2) types of carbohydrates (mono, di, tri, or tetrasaccharides and hexoses or pentoses) present, (3) the stereochemical assignment of terminal carbohydrate units, (4) the sequence of the glycan part, (5) the inter-glycosidic linkages and (6) points where the substituents bind to the aglycone [52]. Given that there are cases in which mass spectrometry and analysis through high performance liquid chromatography (HPLC) in the UV-Vis spectrum are not sufficient to identify the compounds present in a sample, is necessary to use gas chromatography (GC) coupled to mass spectrometry to achieve more accurate results. However, in the case of gas chromatography for the analysis of non-volatile and thermolabile compounds is necessary to previously carry out their conversion into volatile and thermotolerant chemical derivatives. In this sense, silylation of these compounds has been proposed, prior to GC quantification [49].

2.3. Methodologies for the quantification of antioxidant activity

The measurements of the antioxidant activity can be carried out based on the information you want to obtain:

- Direct determination: a radical is used as a quantification factor (since it produces an analytical signal). In this sense, the addition of the antioxidant, before or after the generation of the radical, causes a decreasing in the signal (ABTS*+ or DPPH methods), which is proportional to the antioxidant activity of the sample.
- Indirect determination: the presence of free radicals causes the loss or appearance of a reagent and therefore, in the presence of an antioxidant, an increasing or decreasing in the signal is caused (ORAC and FRAP methods) proportional to the antioxidant activity of the sample.

In that way, it is necessary to mention the differences between the free radical stabilizing activity or antiradicalaria (indirect methods) and the antioxidant activity (direct methods), the first being completely determined by the reactivity of an antioxidant against free radicals, characterized by reaction speed, while the second measures the ability to retard oxidative processes [53]. In this sense, the results of the antioxidant capacity measurement obtained by each of the methods do not always coincide, even among methods based on the same redox mechanism, there may be variations. Therefore, it is recommended that an assessment of the antioxidant capacity be carried out using more than one analytical technique and comparisons among results only be made when the same method has been used and samples have been obtained with the same solvents [8, 13].

In general, it has been suggested to combine FRAP and ABTS techniques [54]. This is because the use of the FRAP technique in combination with others such as ABTS and DPPH, allows

to evaluate different interactions of the antioxidant compounds, expanding the knowledge about them, which is relevant in the exploration of the antioxidant properties of nutraceutical products from natural sources or simply from some products included in the diet, such as fruits and vegetables [55]. When two techniques are used, as mentioned above, is generally sought that through one of them, is possible to determine the antioxidant activity based on transfer reactions of one electron (SET) and on the other, this same property is determined based on a transfer reaction of a hydrogen atom (HAT for its acronym in English) between an antioxidant and a free radical, allowing to evaluate the two mechanisms to extend the spectrum of the results obtained [8]. In **Figure 1**, the HAT and SET mechanisms are showed.

Table 2 summarizes the principal methods to quantify the antioxidant activity [13].

Regarding the expression of results of antioxidant capacity, several methods (FRAP, ABTS and ORAC) express the results in μ mol Trolox/g of sample on dry or wet basis (Trolox is a water-soluble analog of vitamin E). Likewise, these results can be expressed in terms of vitamin C and E. In summary, a suitable method for the quantification of antioxidant activity

Single Electron Transfer (SET) OH +R +R +R-H

Figure 1. Mechanisms of antioxidant reacting with free radical: single electron transfer (SET) and hydrogen atom transfer (HAT).

Hydrogen AtomTransfer (HAT)

| Principle | Method |
|---|--|
| Metal reduction | Ferric ion-reducing antioxidant power (FRAP) |
| Peroxyl radical absorption capacity | Oxygen radical absorbance capacity (ORAC) |
| | Total radical-trapping antioxidant parameter (TRAP) |
| Hydroxyl radical absorption capacity | Deoxyribose assay |
| Capacity for radicals absorption generated from certain organic molecules | 2,2'-azinobis acid (3-ethylbenzothiazolin)-6-sulfonic (ABTS), radical 2,2 diphenyl-1-picrilhydrazil (DPPH) |
| Quantification of products generated during the lipid peroxidation | Tiobarbituric acid reactive species (TBARs), oxidation of LDLs |

Table 2. Principles of the most common methods used to quantify in vitro antioxidant activity.

should consider the electron transfer and hydrogen atoms reaction, establish the oxidation substrate, ensure that the substrate and how to induce oxidation, became relevant in terms of oxidative damage, be simple, have a mechanism and a defined endpoint, use available and affordable instrumentation, be reproducible, be adaptable to measure hydrophilic and lipophilic antioxidants, use different sources of free radicals with relevant biological characteristics and be adaptable for routine large-scale analyzes [13].

This is increasingly important, since is known that no single method reflects the total antioxidant capacity of a sample, that is, its ability to act as an antioxidant of lipophilic and hydrophilic compounds through specific mechanisms, in addition to its reactivity against different species [56]. In addition, is known that the antioxidant activity of a sample is not only given by the sum of the antioxidant capacities of the components present in it, but also depends on the synergistic and inhibitory effects that may exist among compounds and the microenvironment in which this one is [57].

2.3.1. Principal methods for in vitro antioxidant activity quantification

In **Table 3**, the advantage and disadvantages of in *vitro* antioxidant activity determination methods will be presented.

2.3.2. Principal methods for in vivo antioxidant activity quantification

There are different techniques to perform these measurements among which are as follows [68].

Techniques based on lipid oxidation:

- Malondialdehyde (MDA): a breakdown of lipid hydroperoxides generated from polyunsaturated fatty acids generates different aldehydes, such as MDA, which is also produced in food and can be absorbed in the gastrointestinal tract.
- Exhaled alkanes: in this technique the exhalation of volatile hydrocarbons, mainly ethane and pentane, derived from the oxidation of polyunsaturated fatty acids, is measured. The principal advantage of this method is that is a non-invasive procedure.

| Method | Advantages | Disadvantages | Refs. |
|--|--|---|-------------------------|
| ABTS (2,2'-Azinobis acid (3-ethylbenzothiazolin)-6-sulfonic) | This method has a high sensitivity, is practical and fast. Is used to evaluate the antioxidant potential in plants, food and drink. Additionally, is a stable and soluble radical both in aqueous and organic media, allowing the evaluation of hydrophilic and lipophilic antioxidants; with the advantage that its spectrum has maximum absorbance at 414, 654, 754 and 815 nm in alcoholic medium, which represents a greater availability of wavelengths to carry out determinations, avoiding color interference. Correlations have been observed among ABTS values and the polyphenol content determined by the Folin-Ciocalteau method, especially when measurements are carried out at the beginning of the reaction (after 2 min) than when data came from measurements at longer times (15 min), which could be because in the first part of the reaction are the polyphenols that react, while in the second part are other metabolites derived. | In this case, the radical must be previously generated and reaction kinetics with some antioxidants can be slow, causing the determination of the endpoint to be carried out arbitrarily. This has led to differences in the literature regarding the time taken to carry out the determinations (between 1 and 7 min) affecting the antioxidant activity values, since the TEAC determination is dependent on the incubation time, as well as the relationship between the amount of sample and ABTS* concentration. It has also been found that the type of solvent used in antioxidants extraction from the sample influences the results obtained. In addition, as this technique is based on mechanisms of hydrogen atom transfer, the reactivity patterns and the reaction mechanism are difficult to interpret when the chemical structure of the evaluated antioxidant is unknown. | [3, 13, 53, 55, 57–59] |
| FRAP (Ferric ion-reducing antioxidant power) | Method based on the power that an antioxidant substance has to reduce Fe³+ to Fe². In this sense, 2,4,6-tripyridyl-s-triazine (TPTZ) colorless ferric compound used in the test is reduced to a colored ferrous complex. Among its advantages is that the redox potential of Fe³-TPTZ is comparable with that of the ABTS allowing to analyze similar compounds with both methods, although the reaction conditions are different. This technique, having as a base mechanism the metal reduction used as chain reaction propagator of lipid peroxidation through breakdown of hydroperoxides to alkoxyl radicals, allows its use to correlate the antioxidant activity with the ability of the compounds to act as prooxidants, in addition to its ferric ion-reducing capacity. | These types of techniques are quite sensitive to ascorbic acid and uric acid, compounds that could reduce the ferric ion to ferrous ion and react with the latter to generate new free radicals. Also, being based on an electron transfer mechanism, this technique does not allow detecting compounds that act by the hydrogen transfer mechanism. In addition, any compound with a redox potential lower than 0.7 V could reduce Fe(III), overestimating the value obtained for the activity. | [3, 13, 53, 55, 58, 60] |

| Method | Advantages | Disadvantages | Refs. |
|--|--|--|--------------------------|
| DPPH (Radical 2,2 diphenyl-1-picrilhidrazil) | In this method, DPPH* reacts with the sample in such a way that the determination is carried out indirectly by monitoring the decreasing in absorbance at 515 nm. A modification of this method, introduce kinetic parameters like EC ₅₀ (effective concentration of the antioxidant necessary to reduce by 50% the initial amount of radical), tEC ₅₀ (time necessary to reduce by 50% the initial amount of radical with the given antioxidant concentration) and the antiradical efficiency (AE) calculated based on the aforementioned parameters, which also have allowed to establish relationships between the concentration of phenolic compounds and their antioxidant activity. These kinetic parameters are determined as follows: | It is known that many substances have an absorption spectrum that overlaps with the only maximum absorption that the radical presents interfering with the measurement. Additionally, its application is restricted to the fact that only allows measuring the activity of lipophilic compound | [13, 53, 55, 57, 58, 60] |
| | AE = 1/(EC ₅₀ * t _{EC50}) At higher AE, better the performance of the antioxidant even at low concentrations and in short times. Among the advantages of this method are the stability of the organic radical, which does not have to be generated <i>in situ</i> and its simplicity by not requiring sophisticated equipment. | | |
| ORAC (Oxygen radical absorbance capacity) | It is based on the ability of antioxidant compounds to block free radicals by hydrogen atom donation. In this method, the AAPH (2,2'-Azobis-(2-aminopropane)-dihydrochloride) artificial radical, oxidizes fluorescein in such way that fluorescein loses its fluorescence. Among its advantages is that it is easy to follow when using a colored or fluorescent probe. In addition, the use of a protein as a substrate prevents the substrate itself from generation of free radicals due to its oxidation. Likewise, the method is used to determine the antioxidant capacity of aqueous and hydrophobic samples, varying the source of radicals and the solvent. On the other hand, based on a mechanism of hydrogen atoms transfer, in this method, the reactions are of approximately 30 min to guarantee the reaction stabilization, | It is found that the fluorescein solution must be prepared daily, the reaction kinetics may vary depending on the antioxidant concentration, the temperature and the presence of metals and reducing agents. In addition, the results may be affected by the solvent used in the extraction and by non-antioxidant compounds present in plant foods, such as some amino acids and uronic acids, which become interferences, providing overestimated results. | [3, 13, 53, 58, 61] |

independent of pH and solvent.

maximum of absorbance at 505 nm. The experimental procedure is rapid, economical, sensitive and reproducible in the quantification of the antioxidant activity of hydrophilic compounds and

in some cases lipophilic.

| Method | Advantages | Disadvantages | Refs. |
|---|--|--|----------|
| TOSC (Total oxyradical scavenging capacity) | It is based on the oxidation of alpha-keto-gamma-methylbutyric acid to ethylene by the action of hydroxyl, peroxyl and peroxynitrite radicals generated from 2,2-azobis amidinopropane (ABAP). The TOSC value is found by results comparison obtained for the sample with those of a pattern. As advantages of this methodology application is its availability for the total capacity measurement of oxiradical elimination in biological tissues and the possibility of used it to discriminate different oxyradicals indicating the functions of these species or their metabolic pathways. | In reaction kinetics, there is no dose-response relationship between the amount of antioxidant and the percentage of inhibition, preventing comparisons among results. | [13] |
| CUPRAC (Cupric ion-reducing antioxidant capacity) | It is a method to assess the concentration and antioxidant capacity in biological samples, in samples from the food industry and in cosmetics. In addition, this method measures the total antioxidant capacity of a sample. This design is based on Cu(II) to Cu(I) reduction by the combined action of all antioxidants (reducing agents) in a sample. The CUPRAC assay uses a related compound neocuproin (2,9-dimethyl-1,10-phenanthroline), the Cu(I) complex which absorbs at 450 nm. | Slowly reacting antioxidants required an incubation at 50 °C for 20 min for color development. Certain compounds also needed incubation after acid hydrolysis for color development. | [64] |
| Oxidation method of LDLs (Low density lipoproteins) | In this method, the oxidation of LDLs isolated from different individuals is induced by different elements and compounds such as Cu ²⁺ or AAPH and subsequently, the absorbance at 234 nm is measured, which is absorbed by the conjugated dienes generated during the oxidation process of LDLs. | I some cases the duration of the determination is too long. | [13, 53] |
| Deoxyribose assay | This method measures the ability of an antioxidant or mixture of antioxidants to capture the hydroxyl radical. Is based on reaction of 2-deoxyribose (DR) when is oxidized in the presence of hydroxyl radical generated by the Fenton reagent in such way that malondialdehyde (MDA) is produced. MDA is mixed with 2-thiobarbituric acid (TBA) in acidic medium which allows the development of a pink color chromogen that can be measured at an absorbance of 532 nm. Higher absorbance values would indicate higher levels of OH* radicals [13, 65]. As an advantage, this assay has been widely used to determine the antioxidant activity of foods and medicines. | There may be a strong alteration of the result when working with ethanolic extracts due to the interference caused by alcohol on the measurement. | [65, 66] |

| Method | Advantages | Disadvantages | Refs. |
|--------------------------|--|--|----------|
| XO | It is an enzyme dehydrogenase that | Modifications to this method | [13, 67] |
| (Xanthine oxidase assay) | catalyzes the oxidation of hypoxanthine or xanthine to uric acid, by transferring an electron to the nicotinamide-adenine-dinucleotide (NAD). During the XO reoxidation, molecular oxygen acts as an electron acceptor producing radical superoxide and hydrogen peroxide. | have also been developed in which the reaction is not monitored through determining changes in absorbance at 295 nm, but through HPLC or where what is determined is the evolution in uric acid formation and it can increase the cost and complexity of the method. | |

Table 3. Principal methods for in vitro antioxidant activity quantification.

• Ex vitro oxidation of LDLs: this method consists of subjecting the LDLs isolated from a particular subject to the same oxidation process, in order to observe their basal level of antioxidants or the effects of an antioxidant supplementation.

Techniques based on protein oxidation:

Within these techniques, three biomarkers have been used: 2-aminoadipic-semialdehyde (AAS), gamma-glutamic semialdehyde (2-GGS) product of the lysine and proline oxidation, respectively (supposed as the major products of protein oxidation), and nitrosine, oxidized derivative of proteins that can be generated by action of peroxynitrite or peroxidase.

| Matrix | Folin ciocalteau | DPPH | ABTS | FRAP | DMPD | ORAC | VCEAC | CUPRAC | HPLC | Refs. |
|--|---------------------|------|------|------|------|------|-------|--------|------|-------|
| Pineapple, soursop, sweetsop, Artocarpus jackfruit, murici, papaya, mangaba, sapodilla, ciruela, umbu and tamarind | x | х | х | П | | | х | | | [58] |
| Broccoli, kale, cabbage and carrot. | x | x | | | | | | | | [69] |
| Carrot, green pepper and lettuce. | x | x | | | | | | | | [70] |
| Ulva species (Ulva clathrata (Roth), Ulva linza Linnaeus, Ulva flexuosa Wulfen and Ulva intestinalis Linnaeus | X | X | | | | | | | | [71] |
| Kale, carrot, cabbage and barcoli | Х | X | | | | | | | | [72] |
| Edible fungi, Marasmius oreades, Lactarius deliciosus and Macrolepiota procera | х | x | | | | | | х | | [64] |

| Matrix | Folin ciocalteau | DPPH | ABTS | FRAP | DMPD | ORAC | VCEAC | CUPRAC | HPLC | Refs. |
|---|---------------------------|------|------|-------------|------|------|-------|--------|--------|-----------------------------|
| Marine algae, Halimeda opuntia and halimeda monile | Х | х | | | | | | | х | [73] |
| Medicinal plants | X | | X | | | | | | | [74] |
| Honey | $\mathbf{x} = \mathbf{x}$ | | x | | | | | | | [75] |
| Rosa damascena Mill. | x | x 7 | | | | | | | | [76] |
| Fruits such as: acai, banana, star fruit, purple prune, curuba, peach, strawberry, passion fruit, guava, apple guava, kiwi, lulo, tangerine, tommy mango, red apple and Colombian vegetables such as: garlic, white bulb onion, red bulb onion, cauliflower, spinach, capira potato, etc. | x | | | | | X | | | | [77] |
| Fruits such as: blackberry, passion fruit, anana, granadilla, guava and feijoa | x | х | | | | x | | | X | [78] |
| Guava | | X | x | | | | | | | [79] |
| Lulo | | X | X | | | | | | | [80] |
| Opuntia fruit Gulupa Campomanesia lineatifolia Ruiz & Pav. | | -x | G | x x x | x | | | | x x | [81] [82] [83, 84] |

Table 4. Analytical methods for the quantification of antioxidant capacity and total polyphenols.

3. Quantification of antioxidant activity and phenolic compounds in plant extracts

In Table 4, a compilation of information associated with the analytical methods and the conditions used for the quantification of antioxidant capacity and total polyphenols in plant extracts, is presented.

4. Application of plant extracts with antioxidant activity in food processing

In **Table 5**, a list of some applications of plant extracts in food processing will be presented, highlighting its use in conservation processes improvement and shelf life extension.

Other applications can be found in the works developed in food matrices that include meats, oils, fruits, vegetables and cereal products [106].

| Antioxidant | Application | Concentration | Results | Refs. |
|-------------|--|---|---|-------|
| Achiote | Biodegradable container for palm oil | Proportion of the natural additive (0.25, 0.5 and 1%, respectively) | During the storage period (45 days), a decreasing in phenolic content (17.8–36.2%) was observed in the additive used to make the packaging and a decreasing in the peroxide value of the three evaluated formulations in comparison with controls. The protective effect of natural additive was established, since the one that underwent the oxidation processes was the additive and not the palm oil. The results were directly proportional to the used concentration. The incorporation of this natural additive did not change the mechanical and barrier properties of the containers. | [85] |
| Yerba Mate | Biodegradable container for palm oil | Proportion of the natural additive 20% | During the storage period (45 days) under accelerated oxidation conditions (63% UR/30°C), the total polyphenol losses in the films (40% total loss) correlated with a lower increase in the peroxide value of the packaged product, demonstrating, in such way, instead of the product, the packaging compounds were those that underwent the oxidation processes. The yerba mate extract did not alter the mechanical and barrier properties of the films. | [86] |
| Prune | Conservation of meat products | | Prune extract showed antioxidant properties in products such as irradiated turkey, precooked pork sausage and roasted meat. However, in slices of ham the results were not adequate due to an increasing in cooking loss, values of cutting force and redness. The sensory quality of products derived from meat and poultry treated with prune products presented minimal differences with respect to untreated products. | [87] |
| Grape | Meat conservation | 0.05 and 0.1% | The extract of grape seed has been shown to have an antioxidant potential 20 and 50 times higher than vitamin E and vitamin C, respectively. Numerous studies concluded that is an effective antioxidant for the preservation of raw and cooked pork. Likewise, it has been determined that the use of ActiVin (extract of grape seed) at 1.0% in minced meat, inhibits TBARS values by 92%. Likewise, it was established that low concentrations of grape seed extract, ≤0.2%, do not present adverse effects on sensory characteristics such as color, odor and taste, while concentrations higher than 1% affected the color of the finished products. | [88] |

| Antioxidant | Application | Concentration | Results | Refs. |
|---------------------------------------|--|---|---|----------|
| Bearberry and grape seeds | Additive in raw and cooked pork meat patties | $(80-1000 \mu g/g \text{ of meat}).$ | The antioxidant activity of bearberry have allowed a significant decreasing in lipid oxidation compared to control under refrigeration conditions, the effect being greater in raw meats than in cooked ones. Likewise, it has been reported that bearberry did not generate differences in color, taste, texture and juiciness. | [89] |
| Pomegranate | Additive in cooked chicken burgers | 10 mg of phenolic equivalents of tannic acid/100 g in fresh chicken | By using pomegranate powder in hamburgers preparation and refrigerating them for a period of 15 days, a greater reduction of TBARS values was observed in comparison with BHT control (68%). Likewise, it was determined that pomegranate powder and pomegranate juice powder have little effect on sensory or quality attributes when used in concentrations of 5–20 mg equivalents of phenolic tannic acid/100 g of meat. Similar results have been obtained in raw goat meat, where the reduction was 67%. | [90] |
| Blueberry | Turkey conservation and cooked pork | Blueberry powder juice at 0.32% | It has also been reported that the use of blueberry powder juice at 0.32% in turkey meat and cooked pork meat, have allowed to inhibit the lipid oxidation almost 10 times compared to control. This is attributed to the fact that this fruit has a high concentration of phenolic compounds (158.8 μ mol of total phenols/g of dry weight) and especially of anthocyanins, which can inhibit the oxidation of lipids. | [91, 92] |
| Lotus flower | Conservation of Cantonese Chinese sausage | Extract of lotus seed epicarp 0.1 and 0.2% | The extract delayed the initial oxidation of lipids and the effect was dose dependent, likewise it was determined that inhibited the generation of molecules responsible for unwanted odors. The extract did not have significant inhibitory effects on the enzymatic hydrolysis of lipids. | [93] |
| Potato peel and sugar beet pulp | Oxidation control of sunflower oil and soybean oil | 5, 10, 50, 100 and 200 ppm | The oil conservation tests were carried out under accelerated conditions (72 h at 70°C) evaluating the action of natural extracts and synthetic antioxidants on the change in the peroxide value of the oils. It was observed that the action associated with an increasing in the peroxide index varied as follows: TBHQ> potato peel extract> BHT = sugar beet pulp> BHA. | [94] |
| Grape | Osmo- dehydrated foods | | The study confirmed the potential of osmotic treatments for food development that incorporate functional ingredients such as antioxidants in a successful manner. It was observed that molecular weight of the phenolic compounds limits their penetration during treatment. | [95] |
| Wastes from wine | Oxidation control of frozen raw chicken meat | 60 mg of total phenolic compounds/ (PC)/kg of meat | The protective effect of the extract against lipid oxidation of frozen raw chicken meat stored at freezing conditions (–18°C) was demonstrated. There were some differences in the color of the cooked product and its aroma, however these results were not very different from those obtained using synthetic antioxidants. | [96] |

| Antioxidant | Application | Concentration | Results | Refs. |
|--|--|---|--|-------|
| Grape extracts | Bread enrichment in antioxidant compounds | Addition of 300 mg, 600 mg and 1 g of extract in 500 g bread | A decreasing in the antioxidant activity of the extract added to the bread was observed due to the thermal process involved in its elaboration. However, the use of the extracts has allowed to enrich the bread in compounds with antioxidant activity. The results showed that the addition of appropriate amounts of the extract contributed to the development of favorable changes in the bread color without altering the sensory properties thereof. | [97] |
| Moringa. | Extension of the shelf life of cookies | Extract in cookie mass 0.5, 1, 2 and 3%, respectively | The extract was used to control the oxidation of fats and oils present in cookies in such way that their shelf life could be prolonged. The extracts showed better results in comparison with those obtained using BHA, this perhaps due to its greater stability during the production process of the product. | [98] |
| Rosemary and Oreganum | Substitution of sodium erythorbate by natural extracts in lamb burgers | Concentration equivalent to 500 ppm of sodium erythorbate | The substitution did not affect the sensory quality of the product and managed to reduce the changes caused by meat deterioration, converting these plant extracts in a healthy alternative for the formulation of meat derivatives. | [99] |
| Natural antioxidants exploited commercially | Oxidation control in meat and meat products | Varies depending on the product and application | The natural antioxidants used in the elaboration of meat products (pork, chicken, goat, and cow) manage to reduce lipids and proteins oxidation in different meat matrices. | [100] |
| Rosemary and clove | Raw chicken conservation stored in refrigeration | Concentrations: 1% rosemary extract, 1% clove extract and mix 0.5% from each extracts. | The chicken was stored for 15 days at 4°C. As a control, the synthetic antioxidant BHT was used. It was demonstrated that the extracts of the studied spices showed a high effectiveness against microbial growth and lipid oxidation, demonstrating their potential as natural antioxidants for raw chicken meat. | [101] |
| Rosemary | Control of thermoxidation of soybean oil | 3000 mg of rosemary extract/kg, a positive control of 50 mg of TBHQ/kg and extract mixture with TBHQ, were evaluated. | The assay included the addition of the antioxidant to the oil and its heating at 180°C for 20 h. Time during which determinations of oxidative stability, total polar compounds, tocopherol content and fatty acid profile, were carried out. The addition of the extract increased the oxidative stability and resulted in a low formation of polar compounds and a high retention of tocopherols. In the treatment with rosemary extract, a high amount of polyunsaturated fatty acids was observed after 20 h. No synergies were observed between the rosemary extract and TBHQ in terms of preventing the oil oxidation. | [102] |
| Betula pendula | Oxidative stability of meat empanadas. | 0.1 and 0.3% w/w. | A reduction of lipid oxidation was observed in the studied samples, which was followed by color changes and metmyoglobin concentration. The extract demonstrated ability to delay the lipids degradation present in the muscle of the meat. | [103] |

| Antioxidant | Application | Concentration | Results | Refs. |
|------------------------------|---|---|--|-------|
| Mango shell extract | Stability of pork meatballs stored at 4°C. | 0.05, 0.10, 0.15 and 0.20% (v/w) | During storage, the amount of phenolic compounds added to the product decreased slightly, while the lipid oxidation increased slightly. Mango shell extracts showed greater efficiency in the control of lipid oxidation against other extracts such as santol and rambutan. It was also determined that the treatments that included mango shell extract presented lower rancidity than control (without addition of antioxidants) after 10 days of storage. Mango extract applied at 0.2% (v/w) in pork meatballs showed the greatest effectiveness against lipid oxidation. | [104] |
| Campomanesia lineatifolia | Enzymatic browning control of potato | Potato immersion in a extract solution of 0.5, 1 and 1.5%, respectively | A decreasing in the speed change of the color parameter "L", corresponding to the brightness, had achieved by extract addition. The potatoes treated with the extract, presented a lower darkening than potatoes that were not treated during storage at 5°C and 55% of relative humidity. | [105] |

Table 5. Applications of plant extracts with antioxidant activity in food processing.

5. Conclusions

The compiled information in this work demonstrates the need to continue developing techniques for the extraction of phenolic compounds with antioxidant activity from plant species in order to improve the obtained yields, have greater control over the extracts composition and their mechanism of action, to facilitate their implementation in food industry, where they can find great acceptance due to their natural character and their properties to lessen the impact of diseases attributed to the oxidation processes proper to human organism.

Author details

Ludy C. Pabón-Baquero*, Ángela María Otálvaro-Álvarez, Margarita Rosa Rendón Fernández and María Patricia Chaparro-González

*Address all correspondence to: lupabon@unisalle.edu.co

Programa de Ingeniería de Alimentos y Departamento de Ciencias Básicas, Universidad de La Salle, Bogotá, DC, Colombia

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