Methods for Determining the Antioxidant Activity: A Review

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ABSTRACT

Antioxidants are considered as important bioactive compounds on account of many health benefits along with their pivotal role in delaying oxidative rancidity of numerous foods. Consequently, the requirement of a standard assay is very important in order to compare the results of different laboratories and validation of the conclusion. This review article explains the scientific basis of numerous different methods for determining antioxidant activity. Advantages and disadvantages of these methods have been taken into consideration along with either the mechanism or the mode of action for each. Comparative assessment using different antioxidant evaluation methods strongly suggests that not all the adopted methods are highly related and thereby antioxidant capacity should be evaluated by more than one method. Moreover, application of antioxidant assays in food analysis have been also reviewed briefly.

Keywords: ORAC- HORAC- TRAP- TEAC- DPPH- TOSC- PSC- FRAP- ESR- Scavenging activity- reducing power

INTRODUCTION

Antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electron or hydrogen from substances to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions, when the chain reactions occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidative reactions, (Ames *et al*., 1993, Shenoy & Shirwaiker 2002). They do so by being oxidizing themselves. Antioxidants are often reducing agents such as, thiols, ascorbic acid or polyphenols (Sies, 1997). The term antioxidant has been defined in a number of ways like substances that in small quantities are able to prevent or greatly retard the oxidation of easily oxidizable materials, or any substance when present in low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of those substances (Halliwell & Gutteridge 1999).

In food science, it is defined as a substance in foods when present at low concentrations compared to those of an oxidizable substrate significantly decreases or prevents the adverse effects of reactive species such as reactive oxygen and nitrogen species or normal physiological functions in human (Huang *et al.*, 2005).

Antioxidants are responsible for the defense mechanisms of the organism against the pathologies associated to the attack of free radicals. Thus, the intake of plant derived antioxidant is involved in the prevention of degenerative diseases caused oxidative stress such as cancer, Parkinson, Alzehemir or atherosclerosis (Droge, 2002, Lee *et al*., 2004, Valko *et al*., 2004, 2007, Pisochi & Nagulescu 2011).

The first international congress on antioxidant methods was held in Orlando, FL, in June 2004 for the express purpose of dealing with analytical issues relative to assessing antioxidant capacity (AOC) in foods, botanicals, nutraceuticals and other dietary supplements and proposing one or more analytical methods that could be standardized for routine assessment of AOC (Prior *et al*., 2005).

Various antioxidants show substantially varying antioxative effectiveness in different food systems due to different molecular structure. The antioxidants should not impart any off-flavour and off colour. It should be able to get conviently incorporated to food or food systems and should be stable at pH of the food systems and during food processing. Various factors which affect the efficiency of antioxidants include activation energy of antioxidants, redox potential stability of pH and processing and stability, (Sharma & Singh 2013).

Classification of antioxidants

There are different attributes to classify the antioxidants. The first attribute is based on the function (primary and secondary antioxidants). The second attribute is based on enzymatic and non enzymatic antioxidants:

1- Primary antioxidants:-

They are the chain breaking antioxidants which react with lipid radicals and convert them into more stable products. Antioxidants of this group are mainly phenolics, in structure and include the following: Antioxidant minerals, antioxidant vitamins and phytochemicals which include flavonoides,catechins, carotenoids, β-carotene, lycopene, diterpene of, black pepper, thyme, garlic, cumin and their derivatives (Hurrell, 2003).

2- Secondary antioxidants:-

These are phenolic compounds that perform the function of capturing free radicals and stopping the chain reactions. The compounds include: Butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and propyl gallate (PG).

Notwithstanding, according to Ratnam, *et al*. (2006), antioxidants can be divided into two classes namely enzymatic antioxidants and nonenzymatic antioxidants. Some of these antioxidants are endogenously produced which include enzymes, low molecular weight molecules and enzyme cofactors. Among nonenzymatic antioxidants many are obtained from dietary sources. Dietary antioxidants can be classified into various classes of which polyphenols present the largest class. Polyphenols consist of phenolic acids and flavonoids. The other classes of dietary antioxidants include vitamins, carotenoids, organosulfural and minerals. Fig (1) illustrates the classification of antioxidants whereas Fig (2) indicates the broad scope of antioxidants.

It should be emphasized that there is a great difference between antiradical and antioxidant activity. The antiradical activity characterizes the ability of compounds to react with free radical while antioxidant activity represents the ability to inhibit the process of oxidation. Consequently, all the tested systems using a stable free radical (DPPH, ABTS, etc) give information on the radical scavenging or antioxidant activity, although in many cases this activity doesn't correspond to the antioxidant activity. In order to obtain information about the real antioxidant activity with respect to lipids or food stabilization, it is necessary to carry out the study on the real products, although it may be seen this as a complex problem. According to

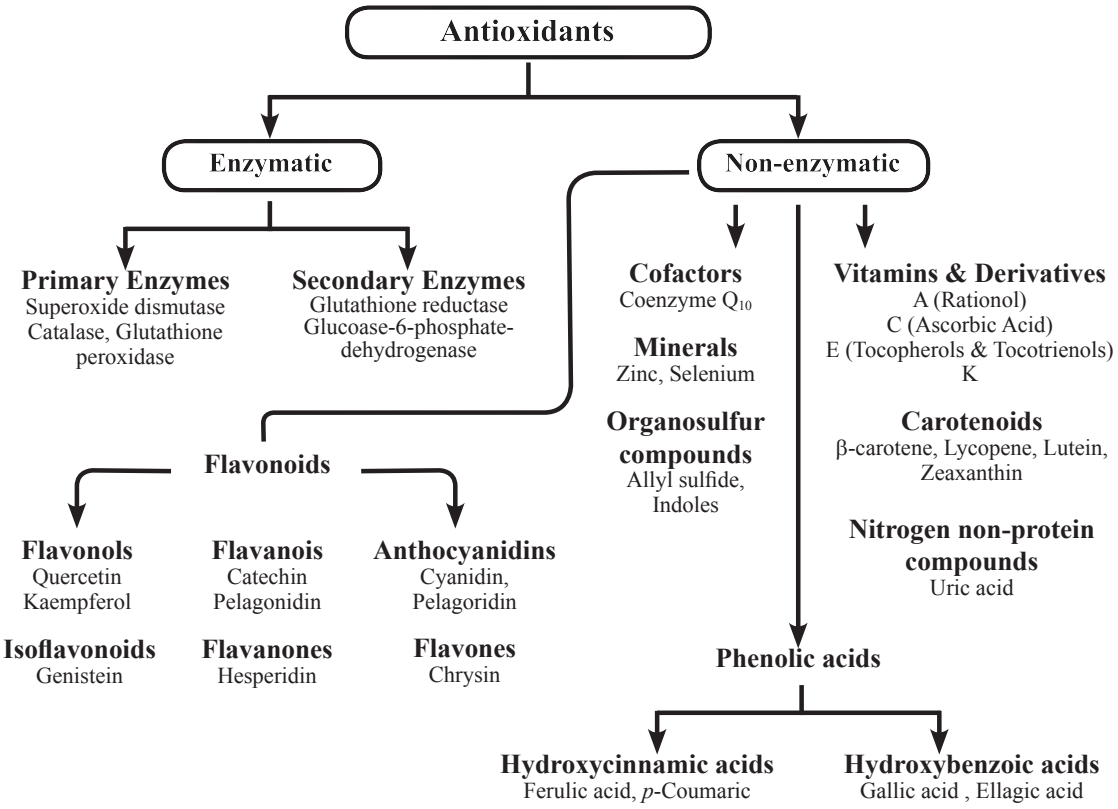

Fig. 1: Classification of antioxidants (Carocho & Ferreira, 2013)

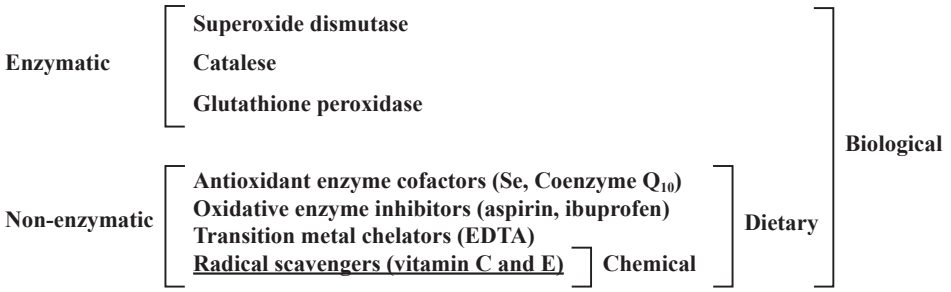

Fig. 2: Broad scope of antioxidants (Huang *et al.*, 2005)

Frankel & Finley (2008), agreement on standardized test methods allows for:-

1- Guidance for appropriate application of assays.
2- Full comparisons of foods or commercial products.
3- A means to control variation within or between products.
4- Provision of quality standards for regulatory issues and health claims.

Too many analytical methods result in inconsistent inappropriate application and interpretation of assays (Prior *et al.*, 2005). Therefore, a variety of *in-vitro* chemical methods are being used to determine the antioxidant activity of products and ingredients.

On the basis of the chemical reactions involved, major antioxidant capacity assays can be roughly divided into two categories as mentioned by Huang *et al.* (2005) as shown in Fig. (2).

A number of assays have been developed for the detection of both general and specific antioxidant action. Of these, oxygen radical absorbance capacity (ORAC), and total radical-trapping antioxidant parameter (TRAP) (and some of its variants) meet the most requirements for screening assays (Prior *et al*., 2005).

Mode of action of antioxidants: On the basis of mode of action, antioxidants can be classified into two main groups, namely, hydrogen atom transfer (HAT) and single electron transfer (SET) assays:

Hydrogen Atom Transfer (HAT) based assays: The HAT-based assays measure the capability of an antioxidant to quench free radicals (generally, peroxyl radicals considered to be biologically more relevant) by H-atom donation. The HAT mechanisms of antioxidant action in which the hydrogen atom (H) of a phenol (Ar–OH) is transfered to a ROO• radical can be summarized by the reaction ROO• + AH/ArOH → ROOH + A•/ArO•

Where the aryloxy radical (ArO•) formed from the reaction of antioxidant phenol with peroxyl radical is stabilized by resonance. The AH and ArOH species denote the protected biomolecules and phenolic antioxidants, respectively. Effective phenolic antioxidants need to react faster than biomolecules with free radicals to protect the latter from oxidation. Since in HAT-based antioxidant assays, both the fluorescent probe and antioxidants react with ROO•, the antioxidant activity can be determined from competition kinetics by measuring the fluorescence decay curve of the probe in the absence and presence of antioxidants, integrating the area under these curves, and finding the difference between them.

Single Electron Transfer (SET) based assays: In most SET-based assays, the antioxidant action is simulated with a suitable redox-potential probe, namely, the antioxidants react with a fluorescent or coloured probe (oxidising agent) instead of peroxyl radicals. Spectrophotometric SET-based assays measure the capacity of an antioxidant in the reduction of an oxidant, which changes colour when reduced. The degree of colour change (either an increase or decrease of absorbance of the probe at a given wavelength) is correlated to the concentration of antioxidants in the sample 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)/ Trolox- equivalent antioxidant capacity (TEAC) and [2,2-di (4-tert-octylphenyl)-1 -picrylhydrazyl (DPPH)] are decolourisation assays, whereas in Folin total phenols assay, ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) there is an increase in absorbance at a prespecified wavelength as the antioxidant reacts with the chromogenic reagent [i.e., in the latter two methods, the lower valencies of iron and copper, namely, Fe(II) and Cu(I), form charge transfer complexes with the corresponding ligands, respectively]. There is no visible chromophore in the Ce4+-reducing antioxidant capacity assay developed recently by as the re-

maining Ce (IV) in dilute sulfuric acid solution after polyphenol oxidation under carefully controlled conditions was measured at 320 nm (i.e., in the UV region of the electromagnetic spectrum) Table (1) shows HAT and SET methods.

Table 1: The HAT and ET methods used to evaluate antioxidant activity

S. No	Name of the method
I	**Hydrogen Atom Transfer methods (HAT)**
1)	Oxygen radical absorbance capacity (ORAC)
2)	Lipid peroxidation inhibition capacity (LPIC)
3)	Total radical trapping antioxidant parameter (TRAP)
4)	Inhibited oxygen uptake (IOC)
5)	Crocin bleaching nitric oxide radical inhibition activity
6)	Hydroxyl radical scavenging activity by p-NDA (p-butrisidunethyl aniline)
7)	Scavenging of H_2O_2, radicals
8)	ABTS radical scavenging
9)	Scavenging of super oxide radical formation by alkaline (SASA)
II	**Electron Transfer methods (ET)**
1)	Trolox equivalent antioxidant capacity (TEAC) decolourization
2)	Ferric reducing antioxidant power (FRAP)
3)	DPPH free radical scavenging
4)	Copper(II) reduction capacity
5)	Total phenols by Folin-Ciocalteu
6)	N,N-dimethyl-p-phenylenediamine (DMPD)

Source: Frankel & Finley (2008).

The antioxidants can be evaluated by *in vitro* methods (Table 2).

Measuring the antioxidant activity

The various analytical methods for evaluation of the antioxidant capacity fall into three distinct categories namely, spectrometry, electrochemical assays and chromatography (Pisogchi & Negutescu, 2011) as shown in Table (3).

Table (4) shows a list of the most important assays to screen antioxidant activity.

It is worth to mention that numerous techniques are currently applied to antioxidant assays. Table (5) shows the most common assays in this respect.

The ABTS, DPPH, FRAP and ORAC assays gave comparable results for the antioxidant activity measured in methanolic extracts. The FRAP technique showed high reproducibility, was simple, rapidly performed and showed the highest correlations with both ascorbic acid and total phenolics, therefore, it would be an appropriate technique for determining antioxidants in fruit extract. Antioxidant activity measured in methanol extract may also be estimated indirectly (Thaipong *et al*., 2006) by using ascorbic acid or total phenolics since they showed high correlations with all assays.

The proposed screening methods using online HPLC-DPPH seems to be useful for the detection of antioxidant because of its highly sensitive and ease of handling. The method is advantageous for the sensitive determination of individual antioxidants in complex mixtures with sample operation.

Table 2: *In vitro* antioxidant capacity assays

assays involving hydrogen atom transfer reactions ROO• + AH → ROOH + A• ROO• + LH → ROOH + L•	ORAC (oxygen radical absorbance capacity) TRAP (total radical trapping antioxidant parameter) Carbon bleaching assay IOU (inhibited oxygen uptake) Inhibition of linoleic acid oxidation Inhibition of LDL oxidation
Assays by electron-transfer reaction M(n) + e (from AH) → AH• + M (n – 1)	TEAC (Trolox equivalent antioxidant capacity) FRAP (ferric ion reducing antioxidant parameter)
Other assays	DPPH (diphenyl-1-picrythydrazyl) Copper (II) reduction capacity Total phenols assay by Follin-Ciocalteu reagent
	TOSC (total oxidant scavenging capacity) Inhibition of Briggs-Rauscher oscillation reaction Chemiluminescence Electrochemiluminescence

Table 3: Categories of antioxidant capacity assays

Antioxidant capacity assay	Principle of the method	End-product determination
Spectrometry		
DPPH	Antioxidant reaction with an organic radical	Colorimetry
ABTS	Antioxidant reaction with an organic cation radical	Colorimetry
FRAP	Antioxidant reaction with a Fe(III) complex	Colorimetry
PFRAP	Potassium ferricyanide reduction by antioxidants and subsequent reaction of potassium ferrocyanide with Fe^{3+}	Colorimetry
CUPRAC	Cu (II) reduction to Cu (I) by antioxidants	Colorimetry
ORAC	Antioxidant reaction with peroxyl radicals, induced by AAPH (2,2'-azobis-2-amidino-propane)	Loss of fluorescence of fluorescein
HORAC	Antioxidant capacity to quench OH radicals generated by a Co(II) based Fenton-like system	Loss of fluorescence of fluorescein
TRAP	Antioxidant capacity to scavenge luminol-derived radicals, generated from AAPH decomposition	Chemiluminescence quenching
Fluorimetry	Emission of light by a substance that has absorbed light or other electromagnetic radiation of a different wavelength	Recording of fluorescence excitation/ emission spectra
Electrochemical Techniques		
Cyclic voltammetry	The potential of a working electrode is linearly varied from an initial value to a final value and back, and the respective current intensity is recorded	Measurement of the intensity of the cathodic/ anodic peak
Amperometry	The potential of the working electrode is set at a fixed value with respect to a reference electrode	Measurement of the intensity of the current generated by the oxidation/ reduction of an electroactive analyte
Biamperometry	The reaction of the analyte (antioxidant) with the oxidized form of a reversible indicating redox couple	Measurement of the current flowing between two identical working electrodes, at a small potential difference and immersed in a solution containing the analysed sample and a reversible redox couple
Chromatography		
Gas chromatography	Separation of the compounds in a mixture is based on the repartition between a liquid stationary phase and a gas mobile phase	Flame ionisation or thermal conductivity detection
High performance liquid chromatography	Separation of the compounds in a mixture is based on the repartition between a solid stationary phase and a liquid mobile phase with different polarities, at high flow rate and pressure of the mobile phase	UV-VIS (e.g. diode array) detection, fluorescence, mass spectrometry or electrochemical detection

Source: Pisoschi & Negulescu (2011).

Table 4: A list of the most important assays to screen antioxidant activity

Assay	Mechanism	Reference
ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)	Scavenging activity	Antolovich *et al.* (2000) Moon & Shibamoto (2009)
DPPH (2,2-diphenyl-1-picrylhydrazyl)	Scavenging activity	Antolovich *et al.* (2000) Amarowicz *et al.* (2004) Moon & Shibamoto (2009)
HO• scavenging activity	Scavenging activity	Huang *et al.* (2005)
H_2O, scavenging activity	Scavenging activity	Huang *et al.* (2005)
O_2^{-}• scavenging activity	Scavenging activity	Huang *et al.* (2005)
Peroxynitrite ($ONOO^-$) scavenging capacity	Scavenging activity	Huang *et al.* (2005)
ESR (electron spin resonance spectrometry)	Free radicals quantification	Antolovich *et al.* (2000)
Spin trapping	Alkoxyl and peroxyl radicals quantification	Gutteridge (1995)
FRAP (ferric reducing antioxidant power)	Reducing power	Antolovich *et al.* (2000) Huang *et al.* (2005) Berker *et al.* (2007) Moon & Shibamoto (2009)
Conjugated diene	Lipid peroxidation inhibition	Moon & Shibamoto (2009)
FOX (ferrous oxidation-xylenol)	Lipid peroxidation inhibition	Moon & Shibamoto (2009)
FTC (ferric thiocyanate)	Lipid peroxidation inhibition	Moon & Shibamoto (2009)
GSHPx (glutathione peroxidase)	Lipid peroxidation inhibition	Gutteridge (1995)
Heme degradation of peroxides	Lipid peroxidation inhibition	Gutteridge (1995)
Iodine liberation	Lipid peroxidation inhibition	Gutteridge (1995)
TBARS (thiobarbituric reactive substances)	Lipid peroxidation inhibition	Gutteridge (1995) Moon & Shibamoto (2009)
TEAC assay (Trolox equiv. antioxidant capacity)	Antioxidant activity	Huang *et al.* (2005)
Total oxidant potential using Cu (II) as an oxidant	Antioxidant activity	Huang *et al.* (2005)
TRAP (total radical-trapping antioxidant parameter)	Antioxidant activity	Antolovich *et al.* (2000)
ACA (aldehyde/carboxylic acid)	Slow oxidation phenomena	Moon & Shibamoto (2009)

Source: Carocho & Ferreira (2013).

The aforementioned method was applied for quantitative analysis of the antioxidants. A linear dependence of negative peak area on concentration of the antioxidants was observed. The antioxidant activity of each substance is reflected by the increase of the peak area after the post column reaction with increased concentration. However, UV absorption is more sensitive and therefore better suited for the quantification of single substances. The general benefit of the method is that beside quantification by UV detection, the radical scavenging of a single substances can be measured and its contribution to the overall activity of a mixture of antioxidants can be calculated (Bandoniene & Murkovic, 2002).

Notwithstanding, the determination of antioxidant and antioxidant capacity by biosensors has been reviewed (Mello & Kubota, 2007).

It is worth to mention that electrochemistry has been applied as analytical tool for studing antioxidant properties (Pisochi & Negulescu, 2011 & Sachor *et al*., 2013

Principles of antioxidant assay methods:

The following are some of the most widely used *in vitro* methods as described by Mermelstein (2008):

Oxygen Radical Absorbance Capacity method (ORAC): When a free-radical generator such as an azo-initiator compound is added to a fluorescent molecule such as β-phicoerythrin or fluorescein and heated, the azo-initiator produces peroxyl free radicals, which damage the fluorescent molecule, resulting in the loss of fluorescence. Curves of fluorescence intensity *vs* time are recorded, and the area under the

Table 5: A list of the most important techniques used for antoxidants analysis

Technique	Compounds	Reference
Antibody techniques	Individual aldehydes (HPLC)	Gutteridge (1995)
Fluorescence assay	Total aldehydes	Gutteridge (1995)
Folin-Ciocalteu spretrophotometric assay	Total phenolics	Huang *et al.* (2005)
Gas chromatography (GC)	Lipid peroxides	Slover *et al.* (1983)
	Aldehydes	Gutteridge (1995)
	Tocopherols	Wu *et al.* (1999)
	Sterols	Moon & Shibamoto (2009)
	Phenolic acids	
	Flavonoids	
High performance liquid chromatography (HPLC)	Flavonoids	Carpenter (1979)
	Tocopherols	Merken & Beecher (2000)
	Aldehydes	Rijke *et al.* (2006)
	Phenolic acids	Stalinkas (2007)
		Moon & Shibamoto (2009)
Light emission	Excited-state carbonyls and singlet O_2•	Gutteridge (1995)

Source: Carocho & Ferreira (2013).

curves with and without addition of an antioxidant is calculated and compared to a standard curve generated using the antioxidant (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, a water-soluble vitamin E analog trademarked by Hoffman- LaRoche as Trolox™ (Ciz *et al.*, 2010).

Hydroxyl Radical Antioxidant Capacity (HORAC) assay: This technique relies on the measurement of the metal-chelating activity of antioxidants, under the conditions of Fenton-like reactions. The method uses a Co(II) complex and hence evaluates the protecting ability against the formation of hydroxyl radical. Fluorescein is incubated with the sample to be analyzed, and then the Fenton mixture (generating hydroxyl radicals) is added. The initial fluorescence is measured, after which the readings are taken every minute after shaking. Gallic acid solutions are used for building the standard curve. The HORAC assay provides a direct measurement of antioxidant capacity against hydrophilic chain breaking hydroxyl radicals (Ciz, *et al*., 2010, Bailey *et al.,* 2013).

Trolox Equivalent Antioxiadant Capacity (TEAC) method: This method, is similar in principle to ORAC, uses a diode-array spectrophotometer to measure the loss of colour when an antioxidant is added to the blue-green chromophore $ABTS^{\bullet+}$, 2,2'-azino-bis (3-ethylbenzthiazoline- 6-sulfonic acid). The antioxidant reduces $ABTS^{\bullet+}$ to ABTS, decolorizing it. The $ABTS^{\bullet+}$ is a stable radical not found in the human body (Huang *et al*., 2005).

Total Radical-Trapping Antioxidant Parameter (TRAP) method: This method uses a luminescence spectrometer to measure the fluorescence decay of R-phycoerythrin during a controlled peroxidation reaction. The TRAP values are calculated from the length of the lag-phase caused by the antioxidant compared to that of Trolox (Ciz *et al*., 2010).

The DPPH method This assay measures by spectrophotometer the ability of antioxidants to reduce 2,2- diphenylpicrylhydrazyl (DPPH), another radical not commonly found in biological systems.

A number of protocols have been followed for DPPH antioxidant assays, resulting in variation in the results of different laboratories. Sharma & Bhat (2009) have presented a perspective of the protocols followed by different workers with incongruity in their results and recommended a standard procedure within the sensitivity range of spectrophotometry, besides sensitivity of DPPH to light, pH and solubility of the compound.

Total Oxyradical Scavenging Capacity (TOSC) method: This method is based on the reaction between peroxyl radicals and □-keto-□-methiolbutyric acid (KMBA), which is oxidized to ethylene. Added antioxidant competes with KMBA

for the peroxyl radicals, reducing the production of ethylene, which is generally measured by gas chromatography. Syft Technologies Ltd. (www.syft.com) has developed a Selected Ion Flow Tube Mass Spectrometric (SIFT-MS) test that is based on TOSC (Sharma & Bhat, 2009).

Peroxyl Radical Scavenging Capacity (PSC) method: This method, is also similar to ORAC, is based on the degree of inhibition of dichloro fluorescin oxidation by antioxidants that scavenge peroxyl radicals generated from thermal degradation of 2,2'-azobis (amidinopropane) (Sharma & Bhat, 2009).

Ferric Reducing/Antioxidant Power (FRAP) method: This method measures the ability of antioxidant to reduce ferric iron. It is based on the reduction of the complex of ferric iron and 2,3,5-triphenyl-1,3,4- triaza-2-azoniacyclopenta-1,4-diene chloride (TPTZ) to the ferrous form at low pH. This reduction is monitored by measuring the change in absorption at 593 nm, using a diode-array spectrophotometer (Antovich *et al*., 2002).

Other methods for antioxidant activity

The Electron Spinning Resonance (ESR) method involves trapping of reactive short-lived free radicals, (produced in the experimental system *via* chemical reaction, thermal decomposition, or by photochemical excitation) by a diamagnetic ESR silent compound (spin trap) *via* addition to a spin trap double bond to produce a more stable radical product (spin adduct). Spin adducts are paramagnetic, and have ESR spectra with hyperfine splitting constants and g-value characteristic of the type of free radical trapped (Fig. 3) according to Li *et al*. (1988).

The room temperature decay of the integrated ESR signal obtained for mixtures of a DPPH methanol standard solution and red tea brewed from various kinds of water: mineral water, reverse osmosis water, common tap water and reverse osmotic water with the composite ceramic filter was investigated. One can observe the highest decay rate of the DPPH free radicals in the solution prepared with tap water purified using the special reverse osmotic ceramic filter (Fig 3). Thus, one can conclude that the tap water filtered is efficient in "free radical neutralization" (Zhu *et al*., 1997)

Accuracy of different assays for antioxidant activity:

It is worth to mention that no one antioxidant capacity (AOC) assay will truly reflect the "total antioxidant capacity" of a particular sample. The total antioxidant capacity needs to reflect both lipophilic and hydrophilic capacity, and at least for physiological activity it needs to reflect and differentiate both hydrogen atom transfer (radical quencing) and electron transfer (radical reduction). In addition, to fully elucidate a full profile of antioxidant capacity, tests evaluating effectiveness against various reactive oxygen special reactive nitrogen species

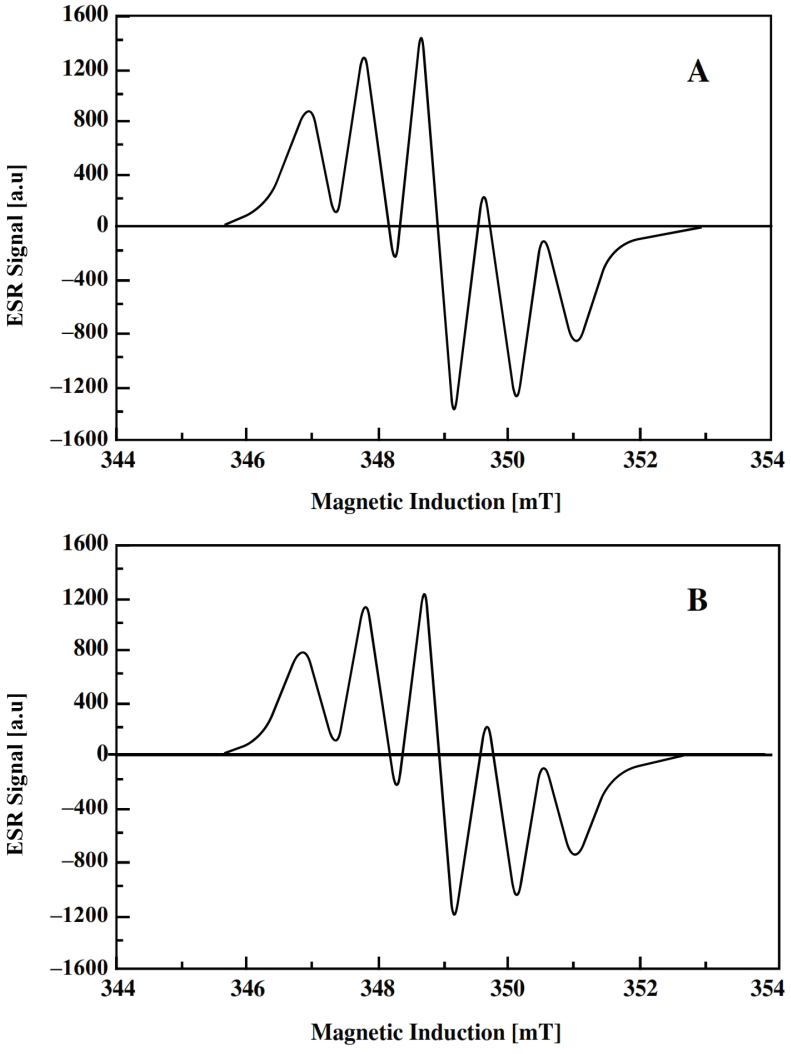

Fig. 3: Relationship between magnetic induction and ESR signal

Source: Li *et al*. (1988). A: at room temperature B: at 40°C.

such as $O_2^{\bullet-}$, $HO^{\bullet}$ and $ONOO^-$ are needed (Prior *et al*., 2005). To date, there are various antioxidant activity assays, each one having their specific target within the matrix and all of them with advantages and disadvantages. There is not one method that can provide unequivocal results and the best solution is to use various methods instead of one-dimension approach (Carocho & Ferreira, 2013).

Total phenol content in the extract of spice correlated linearly with the antioxidant activity as measured by oxygen depletion but not with ESR spin trapping assay (free radicals scavenging effects). It was concluded that extracts of the investigated spices contain components with at least two different antioxidant mechanisms (Madsen *et al*., 1996).

Different methods for control and comparison of the antioxidant properties of 22 vegetable crops were investigated by Ciz *et al*. (2010). The total peroxyl radical-trapping parameter (TRAP), oxygen radical absorbance capacity (ORAC) and hydroxyl radical averting capacity (HORAC) metods were investigated. Data revealed that ORAC, TRAP and HORAC values were well correlated with polyphenol content. A good correlation was found also between the aforementioned methods. Nevertheless, ORAC has been found to be the most sensitive method to measure chain breaking antioxidant activity. Although a good correlation was found between TRAP, ORAC and HORAC, using more than one antioxidant assay is recommended for more detailed understanding the principles of antioxidant properties of samples.

Recently, antioxidant capacities and main reducing substance contents in 110 fruits and vegetables eaten in China were investigated (Liu, *et al*., 2014). The study aimed to screen strongly-antioxidant fruits and vegetables and supply practical diet guidance for the public. Four assays for evaluating antioxidant capacity were applied, namely. DPPH, FRAP, ABTS and TRP. Total phenolic contents showed higher correlation with antioxidant capacity when using FRAP and TRP assays than when using the DPPH or ABTS assay. Phenolics and flavonoides, rather vitamin C, contributed to antioxidant potential in most fruits and vegetables.

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طرق تقدير النشاط المضاد للأكسدة: استعراض مرجعي

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تعتبر مضادات الأكسدة من المركبات المهمة النشطة حيوياً وذلك للعديد من فوائدها الصحية فضلاً عن دورها الحيوي في تأخير عملية التزنخ التأكسدي للعديد من الأغذية، ومن ثم فإن وجود طريقة قياسية أو مرجعية لتقدير النشاط المضاد للأكسدة لهذه المركبات يعتبر من الأهمية بمكان وذلك لكي يتسنى مقارنة النتائج المتحصل عليها من المعامل المختلفة ومن ثم اتساق الاستنتاجات المتحصل عليها من تحليل هذه النتائج.

يشرح هذا الاستعراض المرجعي عدة طرق مختلفة لتقدير النشاط المضاد للأكسدة مع شرح ميكانيكية عمل كل طريقة ومزاياها ومثالبها من منظور الدقة وتشير الدراسات المقارنة للعديد من طرق تقدير النشاط المضاد للأكسدة إلى أن بعض هذه الطرق لا ترتبط ببعضها ارتباطاً عالي المعنوية، ومن ثم فمن الضروري استخدام أكثر من طريقة واحدة لتقدير النشاط المضاد للأكسدة لنفس المادة الغذائية.

وكذلك فقد عنى هذا الاستعراض المرجعي بإعطاء بعض الأمثلة لاستخدام هذه الطرق في مجال تحليل الأغذية بإيجاز.