International Journal of Advances in Engineering and Management (IJAEM)
Volume 3, Issue 7 July 2021, pp: 2065-2076 www.ijaem.net ISSN: 2395-5252

Antioxidant detection, estimation, and evaluation in food systems

[1]*Sheffali Chauhan, [2] Rashmi, and [3]Anil Kumar Verma

[1,2]*MScScholar, Department of Food Science and Technology, College of Horticulture & Forestry-Neri, Hamirpur,Himachal Pradesh, India-177001.*

[3] *Assistant Professor, Department of Food Science and Technology, College of Horticulture & Forestry-Neri, Hamirpur, Himachal Pradesh, India-177001.*

Submitted: 01-07-2021 Revised: 13-07-2021 Accepted: 16-07-2021

ABSTRACT: Antioxidants are chemicals used to improve shelf life and maintain the quality of fats and oils and foods containing lipids through the suppression of their unsaturated components' responses to oxidation. These chemicals may be present, added purposefully, or produced during treatment. Small amounts of antioxidants are required to participate or interfere with the cascade of lipid autoxidation through different mechanisms. They should not give the meal any unwanted characteristics, be stable, and be non-toxic.It is widely known that antioxidants in foods delay oxidative rancidity produced by the oxidation process and therefore protect the fat, oil, and soluble substances such as vitamins, carotenoids, and other nutrients. Moreover, unwanted change resulting from oxidation in foods such as discoloration, browning, and 'scald' in meat and animal products,etc., is delayed. Care in the preparation of food and processing of antioxidant packaging materials minimizes such degradation. The rancid or spoiled foods do not make antioxidants appetizing, neither do they repress hydrolytic rancidity, a catalyst of enzymes.The use of antioxidants in foodstuffs is subject to national legislation or international norms. While a lot of natural and synthetic compounds have antioxidants, only a few have been accepted by the Joint FAOWHO Expert Committee on Food Additives (JECFA) and the Scientific Food Committee of the European Community as "generally recognized as safe" (GRAS) for use in food products by the international bodies (SCF).

KEYWORDS: Antioxidants;standardized methods; antioxidant capacity; foods, dietary supplements; nutraceuticals; ORAC; TRAP; DPPH.

I. INTRODUCTION:

[1, 2]Antioxidant is a chemical that prevents other molecules from oxidating. Oxidation is a chemical process that transfers electrons or hydrogen to an oxidizing agent from substances. Oxidation reactions can create free radicals. Those radicals can in turn initiate chain reactions, which can harm or kill the cell if the chain reactions take place in a cell. These chain reactions are ended by antioxidants by eliminating free radical intermediates and inhibiting other oxidative responses.[3]The word antioxidant is defined in a variety of ways, such as chemicals in tiny amounts which, when present at low concentrations relative to those of an oxidized substrate, can considerably delay or prevent the oxidation of readily oxidizable materials, or any substance.[4]In food science, it is described as a material in food, if the detrimental effects of reactive species, such as reactive oxygen and nitrogen species or normal physiological processes, in human beings diminish considerably compared to the oxidizable substrate at low concentrations. [5-9]The systems for the body's defense against the diseases of free radicals are accountable for antioxidants. The consumption of plant-derived Antioxidants thus helps to avoid oxidative such as cancer, Parkinson's, Alzheimer and atherosclerosis from degenerative illnesses.

[10]In June 2004, the inaugural International Conference on Antioxidant Methods was conducted in Orlando, F.L., for the express aim of analyzing the analysis questions about antioxidant capacity evaluating (AOC) in foodstuffs, botanical agents, nutraceuticals, and other dietary supplements.[11]Various antioxidants have significantly variable antioxidant efficacy due to different chemical structures in different dietary systems. No off-flavor and color should be imparted by antioxidants. It should be suitable for food or food systems and stable at pH in food systems and food processing. Different variables affecting antioxidant effectiveness include antioxidant energy activation, pH, and processing redox stability and stability potential.

International Journal of Advances in Engineering and Management (IJAEM)
Volume 3, Issue 7 July 2021, pp: 2065-2076 www.ijaem.net ISSN: 2395-5252

The distinction between antiradical action and antioxidant activity should be underlined. The anti-radical activity describes the capacity of substances to respond to radical and antioxidant activity to prevent the oxidation process. Therefore, the radical scavenges or antioxidant activity of all studied systems utilizing a steady free radical (DPPH, ABST, etc.) is not covered in this action, even if in many situations the antioxidant activity is not equated.[12] The agreement on standardized test techniques provides:

A. Guide to proper testing.
B. Full food or commercial product comparisons.
C. A technique of controlling variance within or among items.
D. The establishment of regulatory quality standards and health claims.

II. CLASSIFICATION OF ANTIOXIDANTS

The classification of the antioxidants has distinct properties. The first characteristic is function-based (primary and secondary antioxidants). The second characteristic is based on enzymes and non-enzymes, based onthe mode of action, antioxidants can be classified into two main groups, namely, hydrogen atom transfer (HAT) and single electron transfer (SET) assays:

1. Primary antioxidants

[13]The antioxidants are the chain-breaking agents that react with lipid radicals and make them more stable. This category of antioxidants is largely structured in phenolics and includes antioxidant minerals (flavonoid, catechin, carotenoid, β-carotene, lycopene, and diterpene), black pepper, thyme, garlic, cumin, and its derivatives, and other antioxidant vitamins.

2. Secondary antioxidants

These are phenolic compounds to capture free radicals and halt the processes in the chain.The compounds include Butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG).Nonetheless, antioxidants may be split into two categories, namely enzyme antioxidants, and non-enzymatic antioxidants, according to Ratnam, et al. (2006) [14]. Some of these antioxidants, such as enzymes, low molecular weight compounds, and cofactors, are endogenously generated. Many are derived from food sources among non-enzymatic antioxidants. Dietary antioxidants may be divided into many groups, the biggest class being polyphenols. Phenolic acids and flavonoids are composed of polyphenols. Vitamins, carotenoids, organosulfur, and minerals are further types of dietary antioxidants.

III. ANTIOXIDANTS IN FOOD PROCESSING

A. Synthetic Antioxidants

The manufacture of synthetic antioxidants as pure material is possible as such or in combinations in a specific composition with other pure chemicals. Therefore, the application is very straightforward without any major changes to the recipe and circumstances of treatment. Among the kinds synthesized, butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butyl hydroquinone are the most often employed antioxidants for food storage (TBHQ). Depending on the food and processing and storage conditions, antioxidant efficacy varies [15]. In high-temperature therapy, antioxidants may decrease their efficacy [16-18]. Kim and Pratt [19], where tetra-butylbenzoquinone (TBBQ) was recognized as the primary and significant oxidation product, were described as TBHQ decombination products at the frying temperature of a modeling system. This interconversion serves a crucial function in preventing the preservation of food by antioxidants. Volatilization at high temperatures can also cause antioxidants to be lost and the volatile products that arise may decrease the stability of the oil after thermal treatment.Higher polarity compounds are lower than other compounds. As a result, BHT displayed the largest volatility due to its low polarity [20]. Interaction between antioxidants may cause a negative or positive synergy during high-temperature therapy depending on the kind and concentration of each component in the combination. Food manufacturing requires antioxidants that withstand high temperatures in baking, chilling, or frying and preserve final products.

B. Nature Identical Antioxidant

More and more worry about the safety of chemical additions in foods has been shown to substitute natural antioxidants with synthetic antioxidants. Natural antioxidants, most commonly utilized, are not precisely natural but equivalent. Its structure is identical to that of natural chemicals, but it is synthesized. They can be given like other synthetic antioxidants in a relatively pure condition and are thus very simply applied as desired. In this category, there are antioxidants such as tocopherol, ascorbic acid, citric acid, and βcarotene [21].

C. Natural Antioxidant

Natural antioxidants, generally from varied sources, are derived from a combination of numerous components. The combination that

International Journal of Advances in Engineering and Management (IJAEM)
Volume 3, Issue 7 July 2021, pp: 2065-2076 www.ijaem.net ISSN: 2395-5252

contains active and other chemicals that may be inert or of minimal activity depends on the type of plants, agrotechnology, the circumstances of the climate, the degree of maturity, and many more elements. Its composition should be determined on each lot, and the preparation process or application should, if required, be adjusted to reflect analysis results and the quantity added to food items.

D. Food Extracts as Antioxidant

Tocopherols are the most often used natural antioxidants on the market. Inedible oils and grains are naturally found in 0,02 to 0,2% by weight [22]. The highest concentration of tocopherols, α, β, µl, and the µl type, at 50 to 500 mg/kg depending on the nature of the meals should be maintained at α-tocopherol.Lecithin is extracted from crude oil during the degumming of crude edible oils. Lecithin or its concentrations can potentially be utilized as an antioxidant food additive[21].

E. Spice and Herbal Extracts as Antioxidants

An antioxidant is rich in spices and crops. They not just increase the flavor but, for their antioxidant properties, they increase the shelf-life of many foods in their original shape. They do not have to be declared as antioxidants if they are applied to food. They are fragrant and pungent, though; their direct usage as antioxidants is thus restricted to meals that are normally seasoned. This promotes extraction as a precondition for widespread application. Apply commercially natural antioxidants to spices such as clove, ginger, garlic, mace, and nutmeg, and Labiatae herbs like rosemary, salad, thyme, and oregano. For foods that include fat, oregano is proven to be the most effective. Rosemary and sage antioxidants have been patented with edible vegetable oil [23].

Labex™, a commercial rosemary-sage oleoresin antioxidant fraction, exhibits outstanding food preservation performance For Labex™ antioxidants, the use levels are suitably low, and the natural scent and taste of the basic food items are therefore unchanged. Labex® has also been shown to inhibit oxidation of the carotenoid pigments to protect paprika oleoresin colors after prolonged heating. It is GRAS and is suitable for all food applications at any level. A study of the antioxidant activity for a range of Labiatae plants' herbal extracts indicates that they are at least as efficient as the BHA and BHT (1:1) synthetic antioxidant combination at levels between 200 and 300 ppm. Cal-Pfizer also sells Rosemary Deodorized™.

Spice oleoresin's commercial antioxidants, such as the Nestle Spicer Extract AR™, usually come in fine powder form. It is suggested for their usage at levels between 200 and 1000 mg/kg of the final product to be stabilized, depending on their active ingredient content [23].

IV. ANTIOXIDANTS IN HUMAN HEALTH

1. Superoxide dismutase, glutathione peroxidase, and glutathione reductase, for example, are antioxidant enzymes that catalyze free radical quenching processes.
2. Proteins that bind metals such as ferritin, lactoferrin, albumin, and ceruloplasmincan catalyze oxidative processes and sequester free iron and copper.
3. Nutrients from ascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), carotenoids, and other molecules of low molecular weight, such as glutathione and lipoic acid.
4. A vast array of plant foods is made up of several additional antioxidant phytonutrients. In terms of bioavailability and tissue healing mechanism, oligomeric proanthocyanidin in the extracts of grape seed demonstrated by clinical studies has shown fifty times more potent than vitamin C [24].

V. DETECTION AND EVALUATION OF ANTIOXIDANT ACTIVITY

[10]Several tests for general or particular antioxidant activity have been developed. ORAC, and the total parameter TRAP (and certain variations) for radical oxygen absorbance, fulfill the most demanded of testing tests. Too many analytical techniques lead to inadequate use and interpretation of tests. Therefore, the antioxidant activities of goods and substances are determined using several in-vitro chemical techniques. The substantial antioxidant capacity tests might be approximately split into two groups, as Huang et al(2005)[14] said, based on the chemical processes concerned.The antioxidants can be evaluated by in vitro methods:

International Journal of Advances in Engineering and Management (IJAEM)
Volume 3, Issue 7 July 2021, pp: 2065-2076 www.ijaem.net ISSN: 2395-5252

1. Assays involving hydrogen atom transfer reactions **ROO• + AH à ROOH + A•** **ROO• + LH à 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
2. Assays by electron-transfer reaction **M(n) + e (from AH) à AH• + M (n – 1)**	▪ TEAC (Trolox equivalent antioxidant capacity) ▪ FRAP (ferric ion reducing antioxidant parameter)
3. Other assays	▪ DPPH (diphenyl-1-picrylhydrazyl) Copper (II) reduction capacity Total phenols assay by Follin-Ciocalteu reagent ▪ TOSC (total oxidant scavenging capacity) Inhibition of Briggs-Rauscher oscillation reactionChemiluminescence Electrochemiluminescenc

1. Hydrogen Atom Transfer (HAT) based assays:

The HAT-based tests evaluate the capacity of an antioxidant to quench radicals free (usually physiologically more important peroxyl radicals) via H-atom donating.

The ROO$^{\bullet}$ + AH/ArOH = ROOH + A$^{\bullet}$/ArO)

This reaction summarizes HAT processes of the antioxidant effect in which a phenol's hydrogen atom (H) is transferred to a radical ROO$^{\bullet}$.The radical aryloxy (ArO$^{\bullet}$) generated by antioxidant phenol with peroxyl-radical resonance is stabilized. The protected biomolecules and phenolic antioxidants are shown by the AH and ArOH species. To protect the latter from oxidation, effective phenolic antioxidants must react more quickly than biomolecules with free radicals.As the fluorescents, as well as antioxidants, react with ROO$^{\bullet}$ in HAT-based antioxidants testing can measure the fluorescence decline curves of the sample when antioxidants are present and lack, integrate the area under these curves and identify the difference. The antioxidant activity is determined by the competition kinetic.

A. **Oxygen Radical Absorbance Capacity method (ORAC):**

[25]In addition to a fluorescent molecule, such as β-phycoerythrin, or fluorescein and heated, a free generator such as the azo-inducer creates peroxyl free radicals, which damage the fluorescent molecule and result in loss of fluorescence. Curves with fluoresce intensity vs time are recorded and the area of antioxidant (±)-6-hydroxy-2,5,7,8-tetramethyl chromane-2-carboxylic acid, water-soluble vitamin E analog trademarked by Hoffman-LaRoche as a Trolox ™ is calculated with and without the addition of an antioxidant.

B. **Hydroxyl Radical Antioxidant Capacity (HORAC) assay:**

[25, 26]This approach uses Fenton-like reactions to evaluate the metal-chelating activity of antioxidants. This technique employs a complex Co (II) and thereby assesses its protection against hydroxyl radical production. The Fenton mixture (producing hydroxyl radicals) is then added to the sample for analysis. It measures the first fluorescence, following which each minute of the measurements will be taken. The standard curve generates gallic acid solutions. The HORAC test measures directly the antioxidant ability against the breaking hydroxyl radicals of the hydrophilic chain.

C. **Total Radical-Trapping Antioxidant Parameter (TRAP) method:**

[25]In the course of a controlled peroxidation process, the fluorescent decline of R-phycoerythrin is measured with a luminescence spectrometer. In comparison with Trolox, TRAP levels are computed from the duration of the antioxidant lagstage.

2. Single Electron Transfer (SET) based assays:

The antioxidant activity in many SET-based tests isreplicated with an appropriate redox-potential sonde, namely that antioxidants are used instead of peroxyl radicals in the reaction with a fluorescent or colored (oxidizing agent). Spectrophotometric SET-based tests assess the antioxidant capability in reducing the oxidant, which when decreased alters the color.The level of change in colors (whether increased or decreased absorption of the sample at a certain wavelength) is

International Journal of Advances in Engineering and Management (IJAEM)
Volume 3, Issue 7 July 2021, pp: 2065-2076 www.ijaem.net ISSN: 2395-5252

associated with the antioxidant content in the sample. The discoloration tests include 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 decolorization assays, whereas,in Folin TA, FRAP and CUPRAK, there is an increase in absorption at a prespecified wavelength, while antioxidant reactions are made by chromogens [i.e. the latter two methods, Fe(II) and Cu(I), which are lower valences in iron and copper, and the corresponding load complexes have corresponding transfers of formal loads.There is no visibility of chromophores in the Ce^{4+} capability test which was recently established since the residual Ce(IV) was measured under carefully regulated circumstances at 320 nm in diluted sulfuric acid solution following polyphenol oxidation (i.e., in the UV region of the electromagnetic spectrum).

A. **Trolox Equivalent Antioxidant Capacity (TEAC) method:**

[4]In theory, this technique, similar to ORAC, employs a diode-array spectrometer to determine color loss when the blue-green chromophore ABTS•+ is supplemented by antioxidant, 2.2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid). The antioxidant lowers and decolorizes ABTS•+ to ABTS. The radical ABTS•+ has no stable presence in the human body

B. **The DPPH method**

In this test, the capacity of antioxidants, other radicals not typically found in biological systems, is measured by the spectrophotometer to decrease 2,2- diphenylpicrylhydrazyl (DPPH). For antioxidant testing DPPH, many procedures were used, and the findings of various laboratories were changed. In addition to sensitivity to light, pH, and solubility for the chemical, Sharma, and Bhat[27] have given the methods followed by different employees with incongruous findings and proposed a standard process within the spectral sensitivity range.

C. **Ferric Reducing/Antioxidant Power (FRAP) method**:

28]This means that antioxidants are capable of reducing ferric iron. It is based on the decrease of the ferrous iron complex and the ferrous form at low pH, 2,3,5-triphenyl-1,3,4-triaza-2-acioniacyclopenta-1,4-diene chloride (TPTZ). This decrease is tracked using a diode array spectrophotometer to measure the change in absorbance at 593 nm.

Hydrogen Atom Transfer methods (HAT)	**Single Electron Transfer methods (SET)**
1) Oxygen radical absorbance capacity (ORAC)	1) Trolox equivalent antioxidant capacity (TEAC) decolorization
2) Lipid peroxidation inhibition capacity (LPIC)	2) Ferric reducing antioxidant power (FRAP)
3) Total radical trapping antioxidant parameter (TRAP)	3) DPPH free radical scavenging
4) Inhibited oxygen uptake (IOC)	4) Copper(II) reduction capacity
5) Crocin bleaching nitric oxide radical inhibition activity	5) Total phenols by Folin-Ciocalteu
6) Hydroxyl radical scavenging activity by p-NDA (p-butrisidunethyl aniline)	6) N, N-dimethyl-p-phenylenediamine (DMPD)
7) Scavenging of H_2O_2, radicals	
8) ABTS radical scavenging	
9) Scavenging of super oxide radical formation by alkaline (SASA)	Source: [12]

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

3. Other assays

A. Total Oxyradical Scavenging Capacity (TOSC) method:

[27]It is based on the reaction of peroxyl radicals and methiolbutyric acid (KMBA) oxidized to ethylene as a reaction between this technique. The addition of antioxidants to peroxyl radicals competes with KMBA, decreasing the formation of ethylene, typically assessed by gas chromatography. The Ion-flow tube spectrometric

International Journal of Advances in Engineering and Management (IJAEM)
Volume 3, Issue 7 July 2021, pp: 2065-2076 www.ijaem.net ISSN: 2395-5252

selected (SIFT-MS) test is based on TOSC (Syft Technologies Ltd.).

B. The Electron Spinning Resonance method (ESR)

It involves the capture by diamagnetic ESR silent compound (spin trap) via an additional double-bond spin trap to provide a more stable radical product (in the experimental system, utilizing chemical reaction, thermal decomposition, and photochemical excitement) (spin adduct). Spin adducts are paramagnetic and have ESRs with hyperfine divisive constants and g-value of the free radical type traps by Li et al (1988) [29].[30]The room temperature degradation of an ESR signal was observed for DPPH standard solution mixes and red tea made out of several water sorts: mineral water, osmosis reverse water, tap water, and reverse osmotic water from the ceramic composite filter. In a solution made with tap water filtered using a specific ceramic reverse osmotic filter, the greatest decay rate for DPPH-free radicals may be seen. Thus, the filtered tap water may be concluded to be effective in "free radical neutralization".

VI. ANTIOXIDANT ACTIVITY MEASUREMENT

[9]The many analytical procedures for assessing antioxidant capacity are divided into three separate cities: spectrometry, electrochemical testing, and chromatography.

Antioxidant capacity assay	Principle of the method	End-product determination
A) Spectrometry		
a. DPPH	Organic radical antioxidant reaction	Colorimetry
b. ABTS	Organic radical antioxidant reaction	Colorimetry
c. FRAP	Fe (III) complex antioxidant reaction	Colorimetry
d. PFRAP	Reduction of potassium ferricyanide by antioxidants and consequent reaction of Fe^{3+} potassium ferrocyanide	Colorimetry
e. CUPRAC	Cu (II) antioxidant decrease to Cu(I).	Colorimetry
f. ORAC	Antioxidant response with AAPH (2,2'-azobis-2-amidino-propane) generated peroxyl-radicals	Loss of fluorescence of fluorescein
g. HORAC	Fenton-like system based on Co(II) antioxidants to quench OH radicals.	Loss of fluorescence of fluorescein
h. TRAP	Antioxidant ability to scavenge radicals produced by the breakdown of AAPH	Chemiluminescence quenching
i. Fluorimetry	Light emission by a substance with different wavelengths, which absorbs light or other electromagnetic light	Recording of fluorescence excitation/ emission spectra

International Journal of Advances in Engineering and Management (IJAEM)
Volume 3, Issue 7 July 2021, pp: 2065-2076 www.ijaem.net ISSN: 2395-5252

B) Electrochemical Techniques		
a. Cyclic voltammetry	There is a linear variation in the potential of a working electrode between a starting and a final value and the current intensity is recorded.	Measure the cathodic/ anodic peak intensity
b. Amperometry	The work electrode's voltage is fixed concerning a reference electrode	Intensity measurement of the current generated by an electroactive analysis oxidation/reduction
c. Biamperometry	The analysis (antioxidant) reaction with the oxidated form of a reversible redox pair	Measuring the current flow between two identical working electrodes at a tiny variation of voltage and submerged in a solution that contains the sample and a reversible redox pair
C) Chromatography		
a. Gas chromatography	The division between the liquid stationery and the gas mobile phase in a combination is based	Flame ionization or detection of thermal conductivity
b. High-performance liquid chromatography	The compounds are separated by a mixture based on the division between solid stationery and a liquid phase, with distinct polarity, high flux rate, and mobile phase pressure.	Detection of UV-VIS, fluorescence, mass spectrometry, or electrochemical detection e.g., diode array

Table 2: Categories of antioxidant capacity assays

Comparable findings for antioxidant activity assessed in methanol extracts were provided for the tests ABTS, DPPH, FRAP, and ORAC. It was easy and completed quickly and had the strongest correlations to ascorbic acid and total phenolics, so it is a suitable approach for evaluating antioxidants in fruit extract. The technique of FRAP demonstrated high replicability. [31] Methanol extract antioxidant activity may also be evaluated using ascorbic acid or overall phenolics indirectly, since they have shown excellent correlations with any testing.Due to their very sensitivity and easy handling, the suggested HPLC-DPPH testing techniques appear to be helpful for antioxidant detection. The approach is favorable when individual antioxidants are sensitively determined in complicated blends with sample operation.[32]For quantitative analyses of antioxidants, the above approach has been used. A linear effect on the level of antioxidants was detected in the negative peak region. Each chemical is indicated by an increase in its antioxidant activity in the peak region following an increased concentration following the post-column reaction. UV absorption is nevertheless more sensitive and hence more suitable for quantifying individual compounds. In addition to UV-detection quantifying, radical scavenging may be assessed of one single component and its contribution to the total activity of the antioxidant combination can be determined.

VII. MOST IMPORTANT ASSAYS TO SCREEN ANTIOXIDANT ACTIVITY

Assay	Mechanism	Reference
ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)		[28] [33]
DPPH (2,2-diphenyl-1-picrylhydrazyl)	Scavenging activity	

International Journal of Advances in Engineering and Management (IJAEM)
Volume 3, Issue 7 July 2021, pp: 2065-2076 www.ijaem.net ISSN: 2395-5252

		[28] [34] [33]
HO• scavenging activity		[4]
H_2O, scavenging activity		
O_2 –• scavenging activity		
Peroxynitrite (ONOO-) scavenging capacity		
ESR (electron spin resonance spectrometry)	Free radical's quantification	[28]
Spin trapping	Alkoxyl and peroxyl radical's quantification	[35]
FRAP (ferric reducing antioxidant power)	Reducing power	[28] [4] [36] [33]
Conjugated diene	Lipid peroxidation inhibition	[33]
FOX (ferrous oxidation-xylenol)		
FTC (ferric thiocyanate)		
GSHPx (glutathione peroxidase)		[35]
Heme degradation of peroxides		
Iodine liberation		
TBARS (thiobarbituric reactive substances)		[35] [33]
TEAC assay (Trolox Equiv. antioxidant capacity)	Antioxidant activity	[4]
Total oxidant potential using Cu (II) as an oxidant		
TRAP (total radical-trapping antioxidant parameter)		[28]
ACA (aldehyde/carboxylic acid)	Slow oxidation phenomena	[33]

Source: [42]

VIII. MOST IMPORTANT TECHNIQUES USED FOR ANTIOXIDANTS ANALYSIS

Technique	**Compounds**	**Reference**
Antibody techniques	Individual aldehydes (HPLC)	[35]
Fluorescence assay	Total aldehydes	
FolinCiocalteuspretrophotometric assay	Total phenolics	[4]
Gas chromatography (GC)	Lipid peroxides Aldehydes Tocopherols Sterols Phenolic acids Flavonoids	[37] [35] [54] [33]
High-performance liquid chromatography (HPLC)	Flavonoids Tocopherols Aldehydes Phenolic acids	[38] [39] [40] [41] [33]
Light emission	Excited-state carbonyls and singlet O2•	[35]

Source: [42]

International Journal of Advances in Engineering and Management (IJAEM)
Volume 3, Issue 7 July 2021, pp: 2065-2076 www.ijaem.net ISSN: 2395-5252

IX. THE PRECISION OF VARIOUS ANTIOXIDANT ACTIVITY ASSESSMENTS:

[10]No antioxidant (AOC) test will reflect the "total antioxidant ability" of a specific sample. It is worth mentioning. In terms of the entire abilities of antioxidants, both lipophilic and hydrophilic must be reflected and differentiated between hydrogen transfer (radical quenching) and electron transfer at least for physiological activities (radical reduction). In addition, testing for the efficacy of reactive oxygen species such as $O2^{\bullet-}$, $HO^{\bullet}$, and $ONOO^{-}$ is necessary to completely reveal a comprehensive profile of antioxidant capability.[42]To date many antioxidants activity tests have been carried out, each with its unique objective inside the matrix and they all have advantages and limitations. There is no unambiguous technique, and the best answer is to employ several approaches rather than a one-dimensional approach.

[43]Total spice extract phenol concentration is a linear correlation with the antioxidant activity assessed by oxygen depletion and not the ESR trapping test (free radicals scavenging effects). Extracts of the spices examined included components containing at least two distinct antioxidant processes.Cizet al.,(2010) [25] were investigating different techniques of checking and comparing the antioxidant capabilities of 22 plants. A study was carried out with the total peroxyl parameter of TRAP, radical oxygen absorption (ORAC), and hydroxyl radical warning capacity (HORAC) methods. The data indicated a strong correlation between ORAC, TRAP, and HORAC levels and polyphenol content. There was also a high connection between the above approaches. However, ORAC was shown to be the most sensitive approach for measuring the breaking up of the chain of antioxidants.While TRAP, ORAC, and HORAC have been goodly correlated, it is advised that many antioxidant tests be used to better understand the principles of antioxidant capabilities.

[44] Recent research has examined the capabilities of antioxidants and major reducing components in 110 fruits and vegetables consumed in China. The study sought to screen fruits and vegetables as highly antioxidant and provide practical recommendations in terms of public diet. Four tests have been employed, namely: DPPH, FRAP, ABTS, and TRP, on the assessment of antioxidant capacity. In total phenolic content, antioxidant capabilities were more correlated with FRAP and TRP tests than with the DPPH or ABTS assay. The antioxidant potential in most fruits and vegetables was due to phenolics and flavonoids rather than vitamin C.

X. TOXICOLOGICAL ASPECTS

However, the abusive and negligent administration may result in toxicological consequences in antioxidants that are popular to reduce oxidative stress and associated illnesses. The delusive safety of natural antioxidants with toxicity risks and a wide range of adverse effects was highlighted by researchers [45]. Conceptually, after certain concentrations any antioxidant functions as a prooxidant. The influence of the antioxidant on the dose, prooxidant activity, secondary effects, bioavailability, and interaction with other nutrients is required to be certain of the antioxidant's function.

Different studies have depicted the pros and cons of antioxidants. Jakeman and Maxwell [46] found that vitamin C supplementation before the exercise resulted in a faster recovery of muscle strength, however, Urso and Clarkson [47] reported that antioxidant supplements could have a negative effect on recovery from muscle-damaging exercise. The pro-oxidant effects of vitamins C and E have also been reported [45]. As pro-oxidants, these vitamins create transition metal ions.

Vitamin E supplements have shown a pro-oxidant impact, resulting in deadly myocardial infarctions [45, 48] and inhibiting S-transferases Gluteathione P 1-1 (GST P1-1)[49]. In addition, high vitamin E levels have been recorded to aggravate blood coagulation impairment [50]. Bast and Haenen[45] discovered some extremely lethal poisonous vitamin E metabolites. However, a substantial nutraceutical effect is also found to possess a further metabolite 2,7,8-trimethyl-(β-carboxyethyl)-6-hydroxychroman [45,51]. Substantial injury to the gastrointestinal tract is the efficient inhibitor of the cyclooxygenase enzyme (COX-1) [45].

β-carotene has been found to work as a moderate oxidative stress antioxidant while stimulating lipid peroxidation under high-stress conditions[52]. Carcinogenesis is also facilitated by the persistent oxidized metabolites of β-carotene. Dihydrolipoic acid, a lipoic acid metabolism, may also work as a pro-oxidant, similar to the vitamins C and E [45]. Coffee acid can also function as a pro-oxidant in thermal treatment, a widely used antioxidant. Indeed, during the early phases of caffeic acid breakdown extremely reactive cations were produced influencing both the oxidative state and the system's reaction course[53].

Detailed toxical research and particular requirements for excluding toxicological consequences are connected to the factual list of repercussions associated with abuse with antioxidants. The general population should be educated fairly about the toxicological and positive effects of antioxidants.

XI. PROSPECT AND CONCLUSIONS

The protection of antioxidants in food items against oxidative degradation and the body against oxidative stress-medical processes is increasingly intriguing to researchers and medical practitioners. Efficient research on natural antioxidant sources and the development of novel antioxidant compounds requires accurate methodologies for the assessment of antioxidant activity.Conventional techniques for antioxidant activity assessment are still required, with particular methodological protocols complicated and time-consuming. The working pH is one of the major antioxidant selection criteria. Acidic, neutral, or alkaline testing (FRAP) or Folin-Ciocalteu (CUPRAC) are performed. Furthermore, the antioxidant test must apply to both hydrophilic and lipophilic antioxidants. While both hydrophilic and lipophilic antioxidants may be measuring ABTS and CUPRAC tests, certain techniques measure only hydrophilic antioxidants (FRAP and Folin-Ciocalteu) while other methods only apply to hydrophobic systems (DPPH). In addition, the background color of the food matrix may cause changes in the absorbance which, compared to color formation reactions (FRAP,CUPRAC), have a more unfavorable effect in the event of discoloration reactions (ABTS, DPPH).

In this field of investigation, then, there is great potential to create innovative analytical techniques for the determination of the antioxidant capacity of substances, in particular for food items. The creation and application of electrochemical biosensors in the study of process kinetics, for example, maybe of significant importance.A large number of biological reorganization components, such as enzymes, aptamers, DNA/RNA, and whole cells are important to electrochemical biological sensors for antioxidant testing. For studying antioxidants in complicated samples, the benefits of biosensors include mobility, rapid measurement, and the use of a minimal number of samples.

REFERENCES

[1]. Ames, B.N, Shigenag, M.K and Hagen, T.M, 1993. “Oxidants, antioxidants and the degenerative diseases of aging. Processing of the National Academic Science, 90: 7915-22

[2]. Shenoy, K., andShirwaiker, A. 2002. Anti-inflammatory and free radical scavenging studies of Hyptissnravolens. Indian drug, 39: 574-577.

[3]. Halliwell, B. R.,andGutteridge, J.M.C. 1999. Free Radicals in Biology and Medicine. 3rd ed, Oxford Univ. press. Oxford U.K. Huang. D., Boxin, O.U. and Prior P.L., 2005. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry, 55: 1841-1856.

[4]. Huang, D., Boxin, O.U., and Prior. P.L., 2005. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry, 55: 1841-1856.

[5]. Droge, W. 2002. Free radicals in the physiological control of cell function. Physiological Reviews, 82: 47-95.

[6]. Lee, J., Koo, N. and Min, D.B. 2004. Reactive oxygen species, aging, and antioxidative nutraceuticals. Comprehensive Reviews in Food Science and Food Safety, 3: 21-33.

[7]. Valko, M., Izakovic, M., Mazur, M., Rhodes, C.J., and Telser, J. 2004. Role of oxygen radicals in DNA damage and cancer incidence. Molecular Cell Biochemistry, 266: 37-56.

[8]. Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M. and Teiser, J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. International Journal of Biochemistry and Cell Biology, 39: 44-84.

[9]. Pisochi, A.M and Negulescu, G.P., 2011. Methods for total antioxidants activity determination. A. Review, Biochemistry, and Analytical Biochemistry, 1: 1-10.

[10]. Prior, R.L, Wu. X. and Schaich. K., 2005. Standardized methods for the determination of antioxidant capacity and phenolics of foods and dietary supplements. Journal of Agricultural and Food Chemistry, 53: 4290-4302.

[11]. Sharma, P.J. and Singh, R.P., 2013. Evaluation of antioxidant activity in foods with special References to TEAC method. American Journal of Food Technology, 8: 83-101.

[12]. Frankel, E.N. and Finley, J.W. 2008. How to standardized the multiplicity of methods to evaluate natural antioxidants. Journal of Agricultural and Food Chemistry, 56: 4901-4908.

[13]. Hurrel, R., 2003. Influence of vegetable protein source on trace elements and mineral bioavailability. Journal of Nutrition, 133: 29735- 29775.
[14]. Ratnam, K.V., Ankola, D.D, Bahrdwai, J.K.V, Sahana, D. K. and Kavar M.N.V, 2006. A review: Role of antioxidant in prophylaxis and therapy. A pharmaceutical perspective. Journal of Controlled Release, 11: 189-207.
[15]. Coppen, P.P., 1989. Rancidity in Foods. London: Elseiver Appl Sci.
[16]. Augustin, M.A., S.K. Berry., 1983. J. Am Oil Chem Soc.; 60: 1520–1523p.
[17]. Dobarganes, M.C. , Perez-Camino, M.C., Gutierrez-Rosales F., 1986. Emulsified Oils and Fats. 37: 262–266p.
[18]. Tyagi, V.K., Vasishtha., 1996. J. Am Oil Chem Soc.; 73: 499–506p.
[19]. Kim, C.M., Pratt., D.E., 1990. J. Food Sci.; 55: 853p.
[20]. Freeman, I.P., Padley, F.H. , Sheppard, W.L., 1973. J. Am Oil Chem Soc.; 50: 101p.
[21]. Pokorny, J., Korczak, J., 2001.Preparation of Natural Antioxidant. In: Antioxidants in Food: Practical Applications, ed. Pokorny, J, Yanishlieva, N, and Gordon, M. England: Woodhead Publishing Ltd. Abington.
[22]. Schuler, P., 1990. Natural Antioxidants Exploited Commercially, In Food Antioxidants, ed. Hudson, B. J. F. England: Elsevier Applied Science. 1990.
[23]. Mukhopadhyay, M., 2000. Natural Extracts Using Supercritical Carbon Dioxide. CRC Press LLC.
[24]. Antioxidant-Free Radical Scavenger [updated on 2013 March 1; cited on 2013 march 20]. Available on http://www.optimalhealthsystems.com/pdf /OHSAntioxidants.pdf.
[25]. Ciz, M., Cizova, H., Denev, P., Kratchanova, M., Slavov, A. and Lojek, A. 2010. Different methods for control and comparison of the antioxidant properties of vegetables. Food Control, 21: 518-523.
[26]. Bailey, H.R., Zacks, J.M., Hambrick, D.Z., Zacks, R.T., Head, D., Kurby, C.A. and Sargent, J.Q. 2013. Medial temporal lobe volume predicts eldres' everyday memory. Physiological Science, 24: 1113-1122.
[27]. Sharma, O. P., and Bhat, T.K. 2009. DPPH antioxidant assay revisited. Food Chemistry, 113: 1202-1205.
[28]. Antolovich, M., Prenzler, P.D., Patsalides, E., McDonald, S., and Robards, K. 2002. Methods for testing antioxidant activity. Analyst, 127: 183-198.
[29]. Li, A.S.W., Ceurmming, K.B, Roethiling, H.P. Beneltner, G.R. and Chignell, C.F., 1988. A spin trapping database implemented on IBM pc/At. Journal of Magnetic Resonance, 79: 140-142.
[30]. Zhu, Q.Y., Zhang, A., Tsang. D., Thang, D., Huang, Y. and Chen, Z. Y. 1997. Stability of green tea catechins. Journal of Agricultural and Food Chemistry, 45: 4624-4628.
[31]. Thaipong. K, Boonprakob. U, Crosby. K, Zeval-41 Alex. J. Fd. Sci. and Technol. Vol. 11, No. 1, pp. 31-42, 2014 los, I.C. and Biyne, D.H. 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from Guava fruit extract. Journal of Food Composition and Analysis, 19: 669-675
[32]. Bandoniene, D. and Murkovic, M. 2002. On-line HPLC-DPPH screening method for the evaluation of Radical scavenging phenols extracted from apple Malusdomedice L. Journal of Agriculture and Food Chemistry, 50: 2482-2487.
[33]. Moon, J. and Schibamoto, T. 2009. Antioxidant assays for plant and food components.Journal of Agricultural and Food Chemistry, 57: 1655-1666.
[34]. Amarowicz, R., Pegg, R.B., Rahimi-Moghaddam, P., Bart, B., and Weil, J.A. 2004. Free radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chemistry, 84: 551-562.
[35]. Gutteridge, J.M.C. 1995. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clinical Chemistry, 41: 1819-1828.
[36]. Berker, K.I., Güclü, K., Tor, I., and Apak, R. 2007. Comparative evaluation of Fe (III) reducing power-based antioxidant capacity assays in the presence of phenanthroline, batho-phenanthroline, tripyridyltriazine (FRAP), and ferricyanide reagents. Talanta, 72: 1157-1165
[37]. Slover, H.T., Thompson, R.H.J.R. and Merola, G.V. 1983. Determination of tocopherols and sterols by capillary gas chromatography. Journal of the American Oil Chemist's Society, 60: 1524-1528.
[38]. Carpenter, A.P. 1979. Determination of tocopherols in vegetable oils. Journal of the American Oil Chemists Society, 56: 668-671.
[39]. Merken, H. M., and Beecher, G.R. 2000. Measurement of food flavonoids by high-

performance liquid chromatography: A Review. Journal of Agricultural and Food Chemistry, 48: 577-59

[40]. Rijke, E., Out, P., Niessen, W.M.A., Aiere, F., Gooijer, C. and Brinkman, U.A.T. 2006. Analytical separation and detection methods for flavonoids. Journal of Chromatographic Analysis, 1112: 31-63.

[41]. Stalinkas, C.D. 2007. Extraction, separation, and detection methods for phenolic acids and flavonoids. Journal of Separation Science, 30: 3268-3295.

[42]. Carocho, M. and Ferreira, C.F.R. 2013. A review on antioxidants, prooxidants, and related controversy. Natural and synthetic compounds, screening and analysis methodologies, and future perspectives. Food and Chemical Toxicology, 51: 15-25.

[43]. Madsen, H.L., Nielsen, B.R., Bertelsen, E. and Skibsted, L.H. 1996. Screening of antioxidative activity of spices. A comparison between assays based on ESR spin trapping and electrochemical measurement of oxygen consumption. Food Chemistry, 57: 331-337.

[44]. Liu, C., Zhao, Y., Li, X., Jia, J. Chen, Y., and Hua, Z. 2014. Antioxidant capacities and main reducing substance contents in 110 fruits and vegetables eaten in China. Food and Nutrition Sciences, 5: 293-307

[45]. Bast, Haenen, G.R.M.M., 2002. Toxicol Pharma.; 11: 251–258p

[46]. Jakeman, P., Maxwell, S, 1993. Eur J. Appl Physiol.; 67: 426–430p.

[47]. Urso, M.L., Clarkson, P.M., 2003. Toxicol.; 189: 41–54p.

[48]. Van-Haaften, R.I.M., Evelo, C.T.A. , Haenen, G.R.M.M., Bast, A., 2001. Biochem Bioph Res Co.; 28: 631p.

[49]. Halliwell, B., 2000. Lancet.; 355: 1179p.

[50]. Kappus, H., 1992. A.T. Diplock. Free Radical Bio Med. 1992; 13: 55p.

[51]. Wechter, W.J., Kantoci,D., Murray, E.D., et al., 1996. Proceedings of National Academy of Sciences USA.; 93: 6002p.

[52]. Bast, Haenen, G.R.M.M., Van den Berg, R., et al., 1998. Int J. Vit. Nutr. Res. 68. 399p.

[53]. Andueza, S., Manzocco, L., Paz de Peña, M., et al., 2009. Food Res Int.; 42: 51–55p.

[54]. Wu, H., Haig, T., Pratley, J., Lemerle, D., and An, M. 1999. Simultaneous determination of phenolicic acids and 2,4-dihydroxy-7-methoxy-1,4 benzoxazine-3-one in wheat (Triticum aestivum L.) by gas chromatography-tandem mass spectrometry. Journal of Chromatography, 864: 315-321.