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Antioxidants: Types, Functions and Usage

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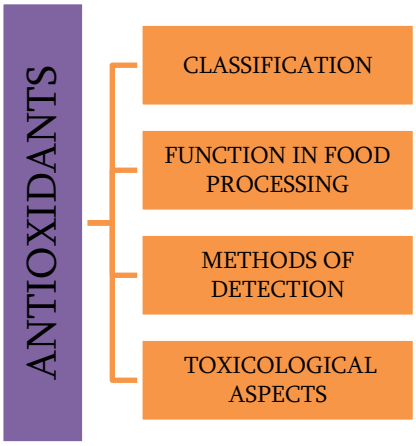
ABSTRACT

Natural antioxidants are becoming more and more well-liked as health difficulties become more widely known. These substances are in charge of lowering oxidative stress in the human body and impeding autoxidation reactions in the food system. The objective of this review is to provide a clear understanding of antioxidants in terms of their classification, function in food processing, and methods of detection. The review also identifies a few commercially accessible antioxidants that are used in food processing.



Keywords: Antioxidants, classification, methods of detection, toxicity

I. INTRODUCTION

1.1 DEFINATION OF ANTIOXIDANTS

Antioxidant means "against oxidation". An antioxidant is any material that, when present in small amounts relative to an oxidizable substrate, markedly inhibits or delays the substrate's oxidation. Antioxidants are essential for both sustaining human health and the quality of food.

II. NEEDS OF ANTIOXIDANTS

Oxidation reaction depending upon site of occurrences presents specific repercussions. If the site of occurrence is food system, thenfood deteriorates. When oxidation occurs in biological cell system, it causes damage or death to the cell.

Depending on where it occurs, an oxidation process can have different effects. Food deteriorates if the

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food system is the site of the occurrence. In biological cell systems, oxidation resultsincelldamageordeath. When fats and oils are present in food, their oxidative degradation causes a rancid flavour and odour, which lowers the food's nutritional value, sensory appeal, and safety. This is brought about by the auto-oxidation of unsaturated fatty acids, which results in the production of primary hydroperoxides and subsequent potentially hazardous chemicals via a free radical chain mechanism (Figure 1).

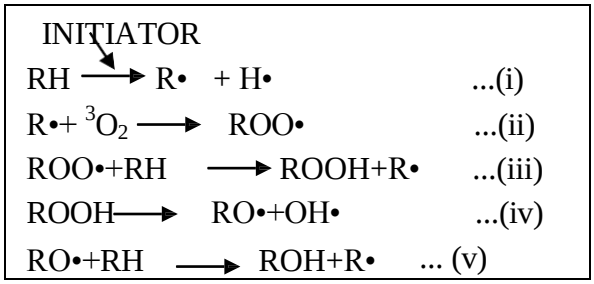

Fig.1: Reactions of Oxidation Process. "R"isanAlkyl Group of Unsaturated Fatty Acid,"H"isaα- methylenic Hydrogen Atom which is Easily Detachable, "RO•' is AlkoxyRadical and 'ROO•' is Peroxy Radical.

Spin is prohibited when oxygen is in its ground triplet state, which has two free electrons in distinct orbitals with the same spin direction, and directly oxidizes unsaturated lipids with the double bond in a singlet state (no unpaired electrons, paired electrons are in the same orbital andhave opposing spin). By taking an electron from the lipid or the oxygen, or by altering the oxygen's electron spin, initiators or catalysts are needed to begin the oxidation of lipids in order to get over this spin barrier (Figure 2).

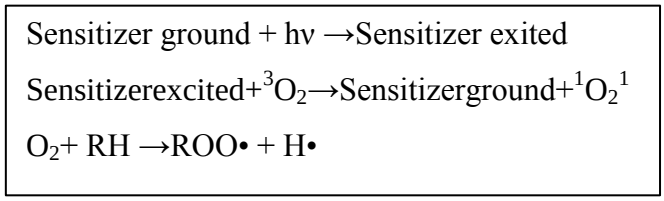

Fig. 2: Formation of Hydroperoxides by Photoxidation of a Lipid with a Sensitizer (hν is Energy in the form of UV Light, Sensitizers That are Naturally Present in Photosensitive Pigments, their DegradationProducts,orPolycyclicAromaticHydrocarbonsCapableofTransferringEnergyFrom Light to Chemical Molecules).

Since only minute amounts of catalysts are required, many conditions that seem to be uncatalysed or spontaneous are instead caused by contaminants or other factors that have gone unnoticed or unanalysed. It is accurate to state that several catalysts and initiators are constantly at work in the majority of foods, biological systems, and lab studies.

Primary hydroperoxides are therefore produced when lipids are exposed to initiators such as light, metals, singlet oxygen, sensitizers (such as chlorophyll, hemoproteins, and riboflavin), or preformed hydroperoxide breakdown products.Hydroperoxides are another by-product of oxidation catalysed by lipoxygenase [1]. Antioxidants must be added in order to prevent oxidative damage.

In human body, about 5% of the inhaled oxygen is converted into reactive oxygen species which encompasses the hydroxyl radical,thesuperoxideanionradical,hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage.

As a defense mechanism against reactive oxygen species, addition of antioxidants is required to food system. In human body, however, a variety of components, both endogenous (body's immune system) and exogenousin origin,function interactivelyand synergistically. As part of a healthy lifestyle and a well-balanced, wholesome diet, antioxidant supplementation is now being recognized as an important means of improving free radical protection.

III. CLASSIFICATION OF OXIDENTS

Broadly, there are five major types of antioxidants [2] as described below:

- Primary antioxidants or chain breaking antioxidants are those compounds, mainly phenolic substances that terminate the free radical chains in lipid oxidation and function as hydrogen and electron donors.

In addition, primary antioxidants chelate transition metals acting as catalyst in lipid oxidation.

- Oxygen scavengers are those substances which react with oxygen and can thus removeitinaclosed system, e.g.,ascorbic acid (vitamin C).
- Secondary antioxidants are those compounds which function by decomposingthelipidhydroperoxidesinto stable end products.
- Enzymatic antioxidants are those enzymes which function either by removing dissolved or head space oxygen, e.g., glucose oxidase, or by removing highly oxidative species, e.g., super oxide dismutase.
- Chelating agents are synergisticsubstances which greatly enhance the action of phenolic antioxidants. Most of these synergists exhibit little or no antioxidant activity like citric acid, amino acid, and phospholipids such as cephalin.

IV. MECAHNISM OF ACTIONS

4.1. Primary Antioxidants

Primary antioxidants work in three main ways to curb the oxidation reaction.

4.1.1. Chain Breakers or Free Radical Interceptors

Mostoftheprimaryantioxidantsthatactas chain breakers or free radical interceptors are monoorpolyhydroxyphenolswithvarious ringsubstitutions.Asprimaryantioxidants (AH),theyworkonhydrogenatomtransfer mechanism.Inthis,theantioxidant,reactswithhighlyreactiveradicallipidandperoxy radicals (ROO•) and transfer one hydrogen atom to radical to form stable organic lipid derivatives and antioxidant radicals (A•) that are more stable and less readily available to participate in propagation reactions. Primary antioxidants have higher affinities for peroxy radicals than lipids and react predominantly with peroxy radicals (Figure 3) [3].

$$R^{\bullet} + AH \rightarrow RH + A^{\bullet} \quad \text{----} \quad \text{(vi)}$$

$$ROO^{\bullet} + AH \rightarrow ROOH + A^{\bullet} \quad \text{---} \quad \text{(vii)}$$

$$ROO^{\bullet} + A^{\bullet} \rightarrow ROOA \quad \text{---} \quad \text{(viii)}$$

$$RO^{\bullet} + AH \rightarrow ROH + AO^{\bullet} \quad \text{----} \quad \text{(ix)}$$

$$RO^{\bullet} + A^{\bullet} \rightarrow ROH + R^{\bullet} \quad \text{---} \quad \text{(x)}$$

$$A^{\bullet} + A^{\bullet} \rightarrow AA \quad \text{----} \quad \text{(xi)}$$

Fig.3:MechanismofPrimaryAntioxidantActivity(AHisanAntioxidantMolecule).

4.1.2. Single Electron Transfer Mechanism

In single electron transfer mechanism (Figure 4), an electron is donated to free radical to form energetically stable anion, while the antioxidant forms a cation radical which is also a less reactive species.

$$R^{\bullet} + AH \xrightarrow[\text{highest occupied orbital}]{\text{electron abstraction from the}} R^{-} + AH^{+}$$

Fig. 4: Single Electron Transfer Mechanism of Antioxidant (AH).

4.1.3. Metal Chelation

Third mechanism is metal chelation (Figure 5), as transition metals act as catalyst and also proxidants in oxidation reaction. The antioxidant forms a stable complex by ligating metal ions.

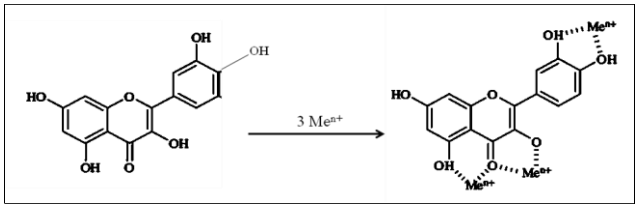

Fig.5: TransitionMetalChelationMechanismof Antioxidant.

4.2. Antioxidants other than primary antioxidants

Antioxidants other than primary antioxidants do not convert free radicals into stable molecules. They act as chelators for pro-oxidantorcatalystmetalions,provideH toprimaryantioxidants,decompose hydroperoxide to non-radical species, deactivate singlet oxygen, absorb ultraviolet radiation, or act as oxygen scavengers. They often enhance the antioxidant activity of primary antioxidants. Table 1 provides examplesofsomeofthesecompounds.

Table1: Antioxidants with Mode of Action Excluding Free Radicals.

ModeofActivity	Antioxidants
Metalchelation	Cirtic, Malic, Succinic and Tartaric acids Ethylenediaminetetraacetic acid,Phosphates
Oxygenscavengingandreducingagents	Ascorbicacid,Ascorbylpalmitate,Erythorbic acid, Sodium erythorbate, Sulfites
Singletoxygenquenching	Carotenoids(β-Carotene,LycopeneandLutein)

V. ANTIOXIDENTS IN FOOD PROCESSING

5.1. Synthetic antioxidants

Synthetic antioxidants are produced as pure substance, and therefore, can be applied as such or in mixtures with other pure substances in definite composition. Application is thus relatively easy, requiring no substantial modifications of the recipe and processing conditions. Among the synthetic types, the most frequently used antioxidants to preserve food are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ).

The effectiveness of antioxidants varies depending on the food and conditions of processing and storage [4]. Antioxidants may lose their effectiveness during high-temperature treatment [5–7]. Decomposition products of TBHQ under frying temperatures in a model system were characterized by Kim and Pratt [8], where tert-butylbenzoquinone (TBBQ) was identified as the primary and major oxidation product. Such inter- conversion plays an important role in antioxidant effectiveness in food preservation. Antioxidants may even be lost through volatilization at high temperature, and the resulting volatiles may impair the stability of oils during thermal processing. Compounds with higher polarity have lower volatility. Consequently, due to its low polarity, BHT showed the highest volatility [9]. Dependingon the type and concentration of individual components in mixture, interaction between antioxidants could lead to a negative or positive synergism during the high-temperature treatment. Food processing operations require antioxidants that survive high temperatures experienced during baking, cooling or frying and provide protection to finished products.

5.2. Nature identical Antioxidants

Increasing concern over the safety of chemical additives in foods has given rise to an interest inreplacingsyntheticantioxidantswithnatural antioxidants. Most widely used natural antioxidants are not exactly purely natural, but nature identical. Their structure is the same as that of natural substances, but they areprepared by synthesis. They are supplied in a relatively pure state like other synthetic antioxidantsandsocanbeaddedveryeasilyin the amount desired. Antioxidants like tocopherol, ascorbic acid, citric acid and β- carotene belong in this group [10].

5.3. Botanical/ Natural Antioxidants

Natural antioxidants are extracted, usually in a mixture of several compounds, from variable sources. The composition of the mixture containing active substance(s) and other compounds, which may be inactive or possessing negligible activities, depend on

the plant variety, agro-technology, climatic conditions, degree of ripeness, and many other factors. Their composition should be determined in every batch, and if necessary,the procedure of their preparation or application, and the amount added to food products should be adapted according to analytical results.Some natural sources which have long been known to exhibit antioxidant properties are shown in Figure6.

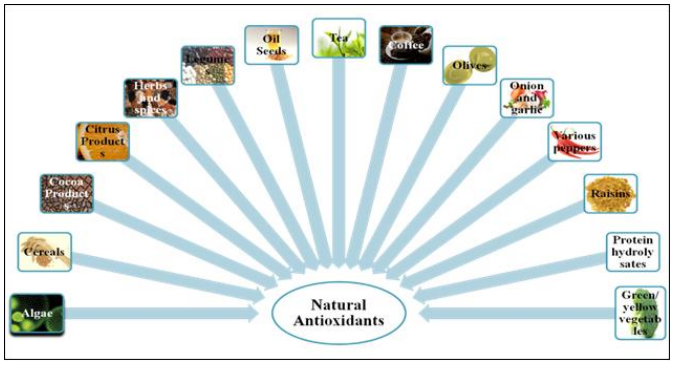

Fig.6: Some Sources of Natural Antioxidants among the Naturally Occurring Antioxidants, only a Few that are Commercially available

5.4. Food Extracts as Antioxidants

The most common natural antioxidants commercially exploited are tocopherols. They arepresentnaturallyin 0.02to0.2%byweight in edible oils and cereals [11]. It is recommended to keep α-tocopherol, the most potent of all tocopherols namely α, β, γ, and δ type, content at a level of 50 to 500 mg/kg of the substrate, depending on the nature of the foods.

Tocopherols present in crude vegetable oilsget concentrated in the deodorizer distillate during the process of deodorization by the steam distillation of crude vegetable oils. The distillate is then separated into aqueous and organic layers. The organic layer containsmost of the tocopherols transferred and is subsequently subjected to vacuum distillation to obtain the tocopherol concentrate. The extract can then be further concentrated by super critical (SC) CO2to obtain individual or mixed tocopherols. For antioxidant applications, extracted tocopherols (usually diluted in a vegetable oil), and synergistic mixtures composed of extracted tocopherols, ascorbyl palmitate, or other antioxidants, synergists, e.g., lecithin, citric acid, and other carriers, are marketed in oily form. Commercially, colorless and odorless mixed tocopherols of 50 and 70% strength, respectively, as Tenox GT-1 and Tenox GT-2 ismarketedbyEastmanChemicals,Kingsport, Tennessee. Similarly, Henkel Corporation, United States, has two commercial products calledCovi-oxT50andT70havingsimilar concentrations of tocopherols. They represent the largest group of commercial natural antioxidants currently being marketed [12].

During the degumming of crude edible oils, lecithin is isolated from crude oil. Lecithin or its concentrates may also be used as a food additive with an antioxidant activity [10].

5.5. Spice and Herbal Extracts as Antioxidants

Spices and herbs are rich in antioxidant. They areusednotonlytoenhanceflavorbutalso the shelf-life of various foods in their natural formfortheirantioxidantcharacteristics.If they are applied to foods, they do not need to bedeclaredasantioxidants.However,these arearomaticandpungent;therefore,their directuseasantioxidantsislimitedtofoods that areusually seasoned.Thispropels extractionasaprerequisiteforgeneraluse.

Spiceslikeclove,ginger,garlic,mace, nutmeg, etc., and labiatae herbs like rosemary, sage,thyme,andoreganoarecurrentlyused forextractingnaturalantioxidants commercially.Oreganoisfoundtobethemost effective forlipid-containingfoods.Theextractionofantioxidantsfromrosemaryand sagewithediblevegetableoilhasbeenpatented [2].

Labex™, a commercial antioxidant oleoresin fraction from rosemary and sage, shows excellent performance in food preservation. The usage levels are sufficiently low for Labex™ antioxidants and accordingly there is

nochangeintheoriginalaromaandflavorofthe base food products. Labex™ has also been found to protect the color of paprika oleoresin during extended heating by preventing the oxidation of carotenoid pigments. It is GRAS and can be used at any level for any food applications. An evaluation of the antioxidant activity for a number of herbal extracts from Labiatae plants reveals that they are fully effective at a level of 200 to 300 ppm and are at least as strong as synthetic antioxidantsBHA + BHT (1:1) mixture. Rosemary Deodorized™ is also marketed by Cal-Pfizer.

Commercial antioxidants from spice oleoresins,e.g.,SpicerExtractAR™marketed by Nestle, are normally in the form of fine powders. Depending on their content of active substances, it is recommended to use them at levels between 200 to 1000 mg/kg of finished product to be stabilized [2].

VI. ANTIOXIDENTS IN HUMAN HEALTHS

Antioxidantsinhumanhealthinclude:

- Nutrient-derivedantioxidantslikeascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), carotenoids, and other low molecular weight compounds such as glutathione and lipoic acid.
- Antioxidant enzymes, e.g., superoxide dismutase, glutathione peroxidase, and glutathione reductase, which catalyze free radical quenching reactions.
- Metal binding proteins, such as ferritin, lactoferrin, albumin, and ceruloplasmin that sequester free iron and copper ions that are capable of catalyzing oxidative reactions.
- Numerousotherantioxidant phytonutrients present in a wide variety of plant foods. For instance, oligomericproanthocyanidins present in grape seed extracts evidenced by clinical trials revealed fifty times more potential than vitamin C in regards with bioavailability and tissue repair mechanism [13].

VII. EXTRACTION AND ISOLATION

The increasing preference for natural foodshas obliged the food industry to includenatural antioxidants in various products to delay oxidative degradation of lipids, improve quality and nutritional value of foods, and replace synthetic antioxidants. To be consideredasanantioxidant,acompound must be free of any toxicological or physiological effect, no impairment of any strong odor or flavor to base food, and must haveconsiderableantioxidantpotentialevenat low concentration. To fulfill these pertaining aims, one has to extract and isolate these antioxidants reasonably from the natural sources. The aim of extraction and isolation is to concentrate the antioxidant components of the raw material, apart from inert substances, so that the product of the extraction could be added to the food in smaller quantities. Currently, extraction is being carried out using traditional methods including Soxhlet extraction, solid-liquid and liquid-liquid extraction. These methods have been associated with high solvent consumption, longer extraction times and an increased riskof thermal degradation of labile components. Soxhlet extraction has been the most regarded amongst all the conventional extraction methods even though it requires lengthy extraction times (up to 24 h or more) and high energy consumption. Additionally, the extended extraction times severely decreases efficiency, which is a tremendous liability in terms of commercial applicability. Alternative novel extraction procedures are now being practiced and studied to reduce extraction time and solvent consumption, increase extraction efficiency and improve antioxidant recovery. Some of these novel methods include supercritical fluid extraction (SFE),acceleratedsolventextraction(ASE) (ASEisa new extraction technique that is similar in principle to Soxhlet extraction, but the use of elevated temperature and pressure with ASE allows the extraction to be completed within a short time and

with a small quantity of solvent), ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE). Optimization and standardization of the extraction process is desperately needed to combat the increasing trend of antioxidant demand and usage.

VIII. DETERMINATION OF ANTIOXIDENT POTENTIAL

To satisfythe intended use in fetching benefits in health or food preservation, it is essential to assess antioxidant potential of the pertaining compound. Antioxidant capacity of the compound or extract could be determined in- vitro as well as in-vivo.

8.1. In-Vitro Estimation of Antioxidant Activity

Dependingonthemechanism,thein-vitroestimationsofantioxidantsaredesignedon following principles:

- Estimationbyfreeradicalscavengingcapacity
- Estimationbyreductionofmetalions
- Estimationby inhibitionoflipid peroxidation in plasma
- Estimationusingculturedcellagainst oxidative stress

8.2. Estimation by Free Radical Scavenging Capacity

The primary mode of action of an antioxidant isscavengingfreeradicalseitherbyhydrogen atom or single electron transfer. On the basisof this mechanism, antioxidant assays have been designed and utilized to assess potentiality of compound as an antioxidant. In these assays free radicals are added/generated and the potency is calculated on the basis of decrease in concentration of free radicals. The scavenging activity is either conducted versus stable radical like 2,2-diphenyl-1- picrylhydrazyl (DPPH) ordoneoncomparison basis with other standard antioxidants like trolox, BHT, tocopheroal, gallic acid etc.Table 2 explains few of the assays with the stated approach to evaluate antioxidant potential.

Table2: MethodsandApproachestoMeasureAntioxidantActivityBasedonDifferentFreeRadicals.

Methods	Principle of measurement	Methodology	References
DPPH scavenging assay	Ability to scavenge DPPH radicals generated in a model system	The reaction is set up between DPPH radicals and test ompound, incubated for 30 min in dark followed by measuring absorbance at 517 nm.	[14]
Hydroxyl radical scavenging assay	Ability to scavenge hydroxyl radicals generated in a model system. H_2O_2 is the source for hydroxyl radicals	The test compound is mixed with reaction buffer and incubated for 1 h at 37°C. To the mixture, tri- chloroacetic acid and thio- barbituric acid are mixed and kept in boiling water bath for 10 min, followed by cooling to room temperature and measure the absorbance at 532 nm. The reaction mixture prepared by $FeCl_3$, ascorbic acid, ethylene diamine tetra acetate (EDTA) and deoxyribose in phosphate buffer (pH 7.4), and H_2O_2 in phosphate buffer. Each of these solutions were mixed well and used as	[15]

Methods	Principle of measurement	Methodology	References
		reaction buffer for analysis.	
Superoxide radical scavenging assay (SOSA)	Ability to scavenge superoxide radicals generated in a model system. Phenazine methosulphate is the source of superoxide radicals generated in a model system.	The reaction was started by adding Phenazine methosulphate (PMS) to the mixture of nitro blue tetrazolium (NBT), reduced Nicotinamide Adenine Dinucleotide (NADH) and sample. Then the reaction mixture is incubated at 25°C for 5 min and absorbance of reaction was measured at 560 nm against blank. The decrease in bsorbance indicates increase in SOSA.	[16]
Oxygen radical absorption capacity (ORAC)	Ability to scavenge oxygen radical (peroxy radicals). Oxygen radicals are generated using 2,2'- azobis(2-amidino-propane) dihydrochloride.	In ORAC, fluorescent probe (like Fluorescein) and test sample are incubated in fluorescent tubes for 30 min at 37°C. Followed by addition of 2,2'-azobis(2-midino-propane) dihydrochloride and eading fluorescence at excitation485nm and Emission520nm.	[14]
Total radical trapping antioxidant parameter (TRAP)	Calculate the amount of time that oxygen intake is required for oxidation caused by 2,2'-Azobis(2-aminopropane) hydrochloride thermal breakdown (AAPH).	The organic substrate used for TRAP measurement can be plasma or lipid. This involves incubating AAPH at 37°C for the experiment, standard samples, and control (which does not include the test sample). The amount of time that the oxidizable substrate takes to absorb oxygen is determined after introducing AAPH. Oxygen electrodes are used to control the time. Trolox is used to express the activity. There is an alternative method for TRAP, though, in which the protein R phycoerythrin's (R-PE) fluorescence is used to track the rate of peroxidation caused by AAPH. The lag-phase in the TRAP experiment caused by plasma in the same plasma sample is contrasted with that produced by Trolox.	[18,19]
Trolox equivalent	Based on the neutralization of the radical cation of 2,2'-Azinobis(3-	ABTS radicals that have been produced and a test substance are used to set up	[14]

Methods	Principle of measurement	Methodology	References
antioxidant capacity (TEAC)	ethylbenzthiazoline)-6-sulfonic acid (ABTS) and the mM concentration of a Trolox solution with an antioxidant capacity equal to 1.0-mM solution of the test material.	the reaction. It is then left in the dark for 30 minutes, and the absorbance at 734 nm is measured.	
Total oxyradical scavenging capacity (TOSC)	Measures the amount of ethylene produced when hydroxy radicals produced from the thermal homolysis of 2,2′-azobis-amidinopropane (ABAP) oxidize α-keto-γ-methiolbutyric acid (KMBA).	Gas-tight vials are filled with the test substance, ABAP, and KMBA. Ethylene production is monitored using gas chromatography and a flame ionization detector after a predetermined amount of time. The outcome is represented as follows: TOSC = 100 - (production of ethylene in test/production of ethylene in control X 100).	[20]

8.3. Estimation by Reduction of Metal Ions

In an autoxidation or free radical chain reaction, transition metals function as a catalyst. Metal ions can be chelated by antioxidants, especially phenolic compounds, and reduced by non-phenolic chemicals using a single electron transfer pathway. Two well-known assays called Ferric Reducing/Antioxidant Power (FRAP) and Cupric Ion Reducing Antioxidant Capacity (CUPRAC) have been developed and are used in the evaluation of antioxidant activity. They are based on the action mechanism against metal ions. This indicates the compound's ability to lower the valency states of cupric [Cu(II)] and ferric [Fe(III)]. A combination of 2,4,6-TPTZ [2,4,6-tri (2-pyridyl)-1,3,5-triazine] solution in hydrochloric acid + ferric chloride + acetate buffer (pH 3.6) is used in FRAP to assess potency. Spectrophotometer measurements of the blue color generated are made at 593 nm [21]. It is ironic that while Fe(II) and Cu(I) are less reactive ions than Fe(III) and Cu(II) in the breakdown of hydrogen peroxide and hydroperoxides, antioxidants will nevertheless have a prooxidant effect by lowering the levels of ferric and cupric ions.

8.4. Estimation by Inhibition of Liquid Peroxidation of Plasma

The biological significance of this approach lies in its utilization of plasma as the reaction medium for assessing the antioxidant effectiveness of lipophilic and hydrophilic compounds as well as their interaction with biological fluids. The test animal is given an antioxidant first, and then their blood plasma is isolated for additional analysis. Alternatively, the test animal is given an antioxidant first, and then their plasma is separated for analysis. A measure of lipid peroxidation in human plasma, cholesteryl linoleate hydroperoxide is one of the testing parameters. The ability of an antioxidant to scavenge free radicals is not always correlated with its ability to suppress lipid peroxidation, as Niki [22] demonstrated.

8.5. Estimation using Cultured Cell Against Oxidative Stress

In order to assess the protective effects of antioxidants against different oxidative stressors and to clarify the underlying mechanisms of oxidative stress, cultured cells are frequently used as a substrate. According to the estimation, the antioxidants in the human body suppress the production of reactive oxygen species (ROS), oxidation of lipids, proteins, and DNA, and cell

death. This approach involves either pre-incubating the antioxidants to incorporate them into the cells or adding them to the culture medium concurrently with the stressor [22I].

The benefit of employing cultured cells is that the antioxidant effects can be assessed using a range of distinct stressors and cell types for a given condition. It should be mentioned that the challenge of obtaining experimental animals in the future might be solved by cultured cells.

8.6. In-Vivo Estimation of Antioxidant Activity

The function and potential of antioxidants in-vivo must be demonstrated in order to address the health benefits of antioxidants. The purpose of these investigations is to clarify, comprehend, and assess the action, efficacy, and capability of antioxidants in the human system. Analysis of human and experimental animal bodily fluids and tissues following antioxidant ingestion is used in these investigations. It is useful to evaluate the bioavailability of different antioxidants and the impact of dosage and duration to demonstrate the positive effects on health. Numerous diseases have been linked to oxidative stress, and controlling the amount of stress in the biological system is a major function of antioxidants. The most reliable way to determine the effectiveness and capacity of antioxidants in vivo is to look at how antioxidant chemicals affect the degree of oxidation in biological fluids and tissues. The following are typically employed as biomarkers to estimate antioxidant potential:

- Saliva, tears, cerebrospinal fluids, erythrocytes, and plasma [22]
- Oxidative products of lipids including ratio of cis,trans/ trans,transfatty acids (as free radicals induces isomerisation of unsaturated lipids from cis to trans form) [23]
- Sugars and proteins that have undergone oxidative modification[24]
- Strand breaks of DNA and oxidative products of DNA bases [25]

Levels of oxidation products of antioxidants like tocopheryl quinine, 5- nitro-γ-tocopherol,allantoin,andbiopyrrin, antioxidant enzymes including superoxide dismutase, catalase, glutathione reductase, etc., and ratio ofits oxidized/reduced forms, i.e., glutathione (GSSG/GSH), tocopheryl quinine/tocopheryl hydroquinone and ubiquinone/ubiquinol [22].

- The estimation of antioxidants typically has the following disadvantages:
- Because assays are sensitive to experimental details, the same substance or biomarker may yield different results when measured in various labs.
- The association between various assays of the same drug is not very strong. For example, Cao and Prior [26] found no link between ORAC and TEAC, nor between FRAP and TEAC, but a weak but substantial correlation between serum ORAC and FRAP.
- External interference factors, such as temperature, duration, and light intensity throughout the reaction period, as well as extract composition, affect every antioxidant assay.
- Wide fluctuations in antioxidant activity unit expression.

It is crucial to employ a range of techniques for evaluating antioxidant capacity due to the many mechanisms behind lipid and antioxidant interactions, the complexity of varied biological and food-related systems, and the inconsistent nature of the analytical data. The assessment of antioxidant capacity ought to be standardized using the following four steps, after the suggestion made by Becker et al. [27]: The first four objectives are the following: (1) quantifying and potentially identifying phenolic compounds; (2) quantifying radical scavenging capacity and reduction potential; (3) assessing the ability to prevent or inhibit lipid oxidation in model biological and food systems; and (4) conducting storage studies using real

antioxidants included in the food product, or human intervention studies using pertinent biomarkers.
Moreover, when targeted for food processing, thermal stability of antioxidant as well as synergistic action, in presence of more than one, should also be evaluated.

IX. TOXICOLOGICAL ASPECTS

Antioxidants are well-known for reducing oxidative stress and the diseases it causes, but misuse and neglect can have toxicological consequences. Researchers have noted that natural antioxidants have a wide range of adverse effects and a hazard of toxicity [28]. In theory, all antioxidants function as pro-oxidants at a particular concentration. Studying the effects of antioxidants with regard to dosage, pro-oxidant action, adverse effects, bioavailability, and interactions with other nutrients is essential to ensuring their effectiveness.
The benefits and drawbacks of antioxidants have been shown in several research. Vitamin C intake before exercise was observed by Jakeman and Maxwell [29] to speed up the recovery of muscle strength. On the other hand, antioxidant supplements may hinder the recovery process after activity that damages muscles, according to Urso and Clarkson [30]. Reports have also been made on the pro-oxidant properties of vitamins C and E [28]. Because these vitamins are pro-oxidants, they produce transition metal ions.
It has been documented that vitamin E supplements' pro-oxidant properties can result in deadly myocardial infarctions [18I,31] and block glutathione S-transferase P 1-1 (GST P 1-1) [32]. Furthermore, it has been noted that elevated vitamin E levels worsen weakened blood coagulation [33]. Some hazardous metabolites of vitamin E, such as quinines, which are extremely cytotoxic, were described by Bast and Haenen[28]. Nonetheless, it has been documented that 2,7,8-trimethyl-2-(β-carboxyethyl)-6-hydroxychroman is another metabolite with potent nutraceutical effects [28,34]. As the cyclooxygenase enzyme (COX-1) is linked to serious gastrointestinal tract damage, γ-tocopherol is an efficient inhibitor of this enzyme [28].
It has been observed that β-carotene functions as an antioxidant in low-oxidative stress situations, but in high-stress scenarios, it promotes lipid peroxidation [35]. Furthermore, carcinogenesis is aided by the unstable oxidized metabolites of β-carotene. Dihydrolipoic acid, a lipoic acid metabolic product, has pro-oxidant properties similar to those of vitamins C and E [28]. Commonly used antioxidant caffeineic acid has the potential to function as a pro-oxidant when heated. Indeed, during the initial stages of the breakdown of caffeic acid, highly reactive cations were produced, which had an impact on the system's oxidative status as well as its reaction pathway [36].
The facts list on the consequences of using antioxidants excessively emphasizes the need for thorough toxicological studies and particular criteria to rule out toxicological effects. Public education about the balanced benefits and toxicological aspects of antioxidants is imperative.

X. CONCLUSION

It is crucial to employ a range of techniques for evaluating antioxidant capacity due to the variety of mechanisms underlying lipid and antioxidant interactions, the complexity of heterogeneous biological and food-related systems, and the inconsistent nature of analytical results on antioxidant potency. Furthermore, it has been demonstrated that antioxidants have a pro-oxidant and harmful effect, which necessitates further study to improve comprehension and establish regulatory guidelines.

XI. REFERENCES

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