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# Bio-antioxidants Activity: Their Mechanisms and Measurement Methods

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**Abstract**

Bioactive food components (such as phenolic compounds, carotenoids, anthocyanins, tochopherols, ascorbic acid, flavonoids, minerals, enzymes and pectin) are active ingredients in food or dietary supplements, which proven to have an important role in health, and are safe for human consumption. These compounds exert their antioxidant effects by different mechanisms such as single electron transfer or hydrogen atom transfer, and their efficiencies can be evaluated by several methods such as ferric reducing ability of plasma, oxygen radical absorbance capacity assay, total radical trapping antioxidant parameter, total oxidant scavenging capacity, conjugated dienes, lipoxygenase activity inhibition assay, chemiluminescence, deoxyribose, tocopheroxyl radical attenuating ability, and nitric oxide radicals trapping. In this review, these mechanisms and methods will be discussed in details.

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## 1. Introduction

Bioactive food components are active ingredients in food or dietary supplements, which are derived from vegetable, animal and microbial sources, including materials necessary for human major nutritional needs, and materials proven to play a role in the health and safety of human beings. All active components in foods such as micro- and macro-nutrients should be considered as bioactive food compounds. Bioactive compounds are classified into different groups according to their distinctive chemical structures and functions (e.g. phenolic compounds and their subclasses such as flavonoids). The biological activity of a chemical group is affected not only by the differences in chemical composition and structure, but also by such factors as bioavailability, distribution and metabolism. In these experiments, all positive and negative constructive effects of bioactive food components should be investigated in scientific models of risk-benefit; however, toxic components should not be considered as bioactive compounds [1]. Microbial products including modified proteins and amino acids can be mentioned as natural antioxidants [2, 3].

Functional foods (that are beneficial for health, and help reduce the risk of chronic diseases) include conventional foods containing bioactive natural materials such as fiber, foods enriched with bioactive substances like probiotics and antioxidants, and synthetic commercial nutrients like prebiotics [1, 4].

This study aimed to review the antioxidant properties of bioactive compounds, involved mechanisms and effective factors in bio-antioxidant effects, and at last several practical methods for assessing their antioxidant activity.

## 2. A Brief about Oxidation Reactions

Oxidizing characteristics of oxygen have a vital role in different biological actions including electron transport in ATP production. While, oxygen is essential for life, it can have destructive effects by means of cellular material oxidation [5]. Auto-oxidation and thermal oxidation of lipids,

cellular oxidation pathways, and numerous physiological and biochemical processes in human body under normal circumstances produce two groups of radicals; reactive oxygen species and reactive nitrogen species [6]. Thus, they attempt to obtain or lose electrons in the body, and consequently, cause damages to DNA, proteins, lipids and carbohydrates [4, 7, 8]. Also, free radicals participate in the mechanism of cytochromes [9]. The lack of anti-oxidative capacity causes imbalance and therefore, high concentrations of reactive oxygen species cause damages to cellular lipids, proteins, and nucleic acids as well as their routine performances [10, 11, 12]. Human body always requires antioxidant sources continuously [13]. Vitamins C and E, carotenoids, xanthophylls, tannins, several amino acids, and total phenolic are involved in the antioxidant index of a sample [14, 15, 16].

Antioxidants' functions can be divided into two categories: Chain breaking and path inhibitors. Antioxidants sources in biological systems are categorized as follows: enzymes, large molecules, small molecules, some hormones [17]. Under different conditions and concentrations, antioxidants such as vitamin E and vitamin C can act as antioxidant, peroxidant or pro-oxidant [18, 19].

## 3. Main Chemical Reactions of Antioxidants

Antioxidants are capable of inactivating radicals with two major mechanisms, hydrogen atom transfer (HAT) and single electron transfer (SET). Methods using HAT mechanism include: oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP), total oxidant scavenging capacity (TOSC), chemical luminescence, α-carotene bleaching, low density lipoprotein (LDL) oxidation, and methods using SET mechanism are: ferric reducing antioxidant power (FRAP), TRAP and copper reduction. Total phenolic method (Folin-Ciocaltue method= FC) is among the methods using both HAT and SET mechanisms [17].

## 4. Factors Affecting Antioxidant Mechanisms and Capacity

Ascorbic acid, scurvy preventive agent, is the most abundant water-soluble antioxidant and a heat sensitive vitamin [20]. The antioxidant effect of ascorbate is attributed to its capacity to remove reactive oxygen species by reaction with superoxide radicals, hydroxyl radicals, hydrogen peroxide, and single oxygen through the HAT mechanism. Also, this compound removes RNS and prevents nitration reactions. Biological studies of vitamin C have shown its protection role against DNA mutations [21].

Polyphenols are the main plant components with antioxidant activity [13], and the hydrogen donating property of polyphenol compounds is responsible for preventing the production of free radical causing lipid peroxidation [22]. Generally, phenolic compounds in low concentrations show antioxidant behavior. In higher concentrations, they display pro-oxidant behavior, and again by further increasing of the concentration, they show antioxidant behavior [23, 24, 25]. Flavonoids also chelate metal ions, and therefore, prevent them from participating in the production of free radicals [26, 27]. Polyphenolic compounds are implicated in large number of biological effects including anti-bacterial, anti-inflammatory, anti-histamine, anti-hepatitis, anti-viral, anti-cancer, anti-coagulation, and vessel protection [6, 28].

Carotenoids, especially α-carotene, quench single oxygen [12]. They are efficient quenchers against reactive oxygen species, particularly peroxyl radical. Gene regulation is one of the most apparent mechanisms in some pathogenic processes, which is influenced by carotenoids. Carotenoids are relatively resistant to thermal decomposition [29].

In human nutrition, selenium, copper, zinc, iron and manganese are essential minerals for optimizing the response of antioxidant enzymes. Prosthetic groups that are present in heme proteins are similar to the antioxidant enzymes of catalase, peroxidase and superoxide dismutase. Research findings have shown that the amount of anthocyanins increases with the increase of zinc level; these compounds prevent membrane lipid peroxidation as a plant protective system against oxidative stress [30].

Vitamin E (α-tocopherol) is the best single oxygen quencher that acts as a chain breaking antioxidant [12].

Antioxidant activities of glutathione and uric acid are exerted by scavenging free radicals and antioxidant abilities of isothiocyanates (including anti-nutritive compounds) inactivate single oxygen [31].

## 5. Practical Methods for Assessing Antioxidants

Total phenolic content reducing capacity is measured by Folin-Ciocalteu method, which is

well related to the reducing and antioxidant abilities of phenolic compounds. Phenolic compounds react with FCR only in alkaline conditions (sodium carbonate solution at pH=10) [15, 20, 32].

Flavonoids in food systems act as free radical scavengers and terminate chain radical reactions during the triglyceride oxidation; they also exert various biological effects (such as antioxidant, anti-inflammatory), and protect vascular actions [28]. Flavonoid content was measured using colorimetric method provided by Lenucci *et al*. (2006) with some modifications [21, 23].

Analysis of ascorbic acid in vegetables and fruits was carried out according to the works the Ngee Wen *et al.* (2010) and Sahari *et al.* (2004) [24, 33-34].

Spectrophotometric methods with UV-VIS detectors at 400-600nm wavelengths are usually used for determining peroxide values. These procedures usually use oxidation of Fe (II) to Fe (III) to react with different reagents, and produce colored complexes [35, 36, 37-38].

The FRAP method is related to the reduction of [Fe (III)-(2, 4 and 6-three (2-Pyrydyl)-s-Tryazyn=$TPTZ)_2$] complex to a colored product [Fe (II)-$(TPTZ)_2$] by an antioxidant and usually in non-physiological conditions at low pH of about 3.6 [24, 39- 40]. Generally, FRAP mechanism, as compared to the mixed SET and HAT, is based more on electron transfer; so, its combination with other methods for the diagnosis of the dominant mechanisms of various antioxidants is very useful.

The ORAC mechanism is a HAT system, which is monitored by means of spectrophotometer. ORAC is applied to assess the antioxidant capacity of hydrophilic compounds, and with some modifications, it is used to determine the antioxidant capacity of hydrophobic compounds [16, 41, 42]. The ORAC method measures the inhibition of peroxyl radical oxidation generated by antioxidant activity, and thus reflects the antioxidants' radical chain breaking activity through H atom transmission. In this method, the peroxyl radical reacts with a fluorescent probe to form a non-fluorescent product that can be determined easily by fluorescence assessment.

TRAP method shows the ability of antioxidant compounds to interfere with the reaction between the peroxyl radicals derived from AAPH or ABAP (2, 2′-azobis (2-amidinopropane) dihydrochloride) and the target probe. Antioxidants expand the retardation time to advent oxidized probe, and their ability is measured by percentage of reaction reduction. TRAP values are usually expressed as retardation time or sample reaction time in comparison to the corresponding times for trolox.
Copper reduction method is based on the reduction of Cu (II) to Cu (I) by the combined antioxidant action of all samples (reducing agents). CUPRAC (copper reduction antioxidant capacity) values are comparable with total equivalent antioxidant capacity = TEAC values for polyphenols, while FRAP values are usually significantly lower.

TOSC method determines the antioxidants absorbance capacity especially against three major oxidants, including hydroxyl radicals, peroxile radicals and peroxy nitrite. In this method, α-keto-γ-methiol butyric acid is oxidized to form ethylene. Time analysis of ethylene formation is followed by headspace GC analysis, and antioxidant capacity is determined through the antioxidants' ability to prevent ethylene formation towards the control reaction [43].

Fresh LDL is isolated from blood samples, and oxidized by Cu (II) or other substances such as AAPH, forming conjugated dienes that are monitored spectrophotometrically at 234nm wavelength. The effects of antioxidant compounds with HAT mechanism are evaluated by reducing the absorption amounts at 234nm. This method is appropriate for assessing a simple fatty acid, and the primary stage of lipid peroxidation [44, 45].

Antioxidants react non-specifically with lipoxygenase through scavenging the radical intermediates and reduce their iron section [13]. The phenolic antioxidant's activity is probably related to their reducing properties, which allow them to act as reducing agents or hydrogen atoms donors. In addition to their ability to chelate metals, they inhibit lipoxygenase and scavenge free radicals [28]. Enzymatic lipid peroxidation is measured by means of spectrophotometer with an increase in the lipid hydroperoxide absorption at 234nm [13].

In a simple and cheap method, deoxyribose sugar decomposes with exposure to the $OH^{\bullet}$ generated by radiation or by Fenton systems. If the complex mixture of products is heated under acidic conditions, malone aldehyde is formed, which is identified by its ability to react with thiobarbitoric acid and the formation of pink material. In addition, deoxyribose is used to determine the $OH^{\bullet}$ formed in biological systems. It is likely that the decomposition rate of deoxyribose increases in the

presence of reducing agents such as ascorbic acid in the reaction mixture [4, 5, 46].

Tocopheroxyl radical attenuating ability is a quick and easy method for screening the inhibitor's ability to prevent LDL peroxidation. The method is based on the antioxidants' capacity to reduce α-tocopheroxyl radicals' contents. α-tocopherols are produced by UV light, and measured by electron spin resonance spectroscopy. The tocopheroxyl radical attenuating ability method using $CCl_3O_2^{\bullet}$ is expensive because it needs electron spin resonance machine [24].

Nitric oxide radicals trapping method is based on this assumption that sodium nitroprusside, which reacts with environmental oxygen to produce nitrite ions in an aqueous solution at physiological pH, slowly produces nitric oxide. Nitric oxide trapping and competing with oxygen decrease the production of nitrite ions. The absorption at 546nm is read versus the solvent. Quercetin is used as the control for comparison [47]. Proxy nitrite can cause lipid peroxidation, oxidation of methionine and sulfhydryl residues in proteins decrease in antioxidants and damage to DNA.

The antioxidant activity is monitored through the main reaction of HAT by a decrease in the chemical luminescence intensity caused by luminal and superoxide anion radical. Luminol is the most important indicator component for trapping oxidants, and intensifying long lasting and stable emissions. A continuous light output depends on the constant production of free radicals derived from p-idophenol, luminol and oxygen. Also, the light emission is sensitive to radical scavenger antioxidants interferences. The antioxidant capacity is assessed as the emission light declines [48].

## 6. Conclusion

Peroxidation of lipids in the cell membrane, caused by free radicals, is critically associated with heart diseases, cancers, aging, etc. This harmful procedure can be inhibited by bio-antioxidants' activities in biosystems. It is well accepted that ascorbic acid, polyphenols, carotenoids, α-tocopherol, minerals as selenium, copper, zinc, iron, manganese and other compounds like glutathione, uric acid and isothiocyanates can exert notable antioxidant properties against free radicals. These antioxidants are capable of inactivating radicals with the two major mechanisms of HAT and SET. HAT-based methods measure the ability of antioxidants to disable free radicals by hydrogen donating. SET-based methods detect the ability of antioxidants to transfer single electron to reduce any compound, including metals, carbonyls and radicals. Principal mechanisms applied by antioxidant compounds include the reduction of metals such as iron and copper; scavenging free radicals; creating complexes with metal pro-oxidants (chelating); quenching single oxygen; and stimulating anti-oxidative defense enzymatic activities. Some methods are usable to evaluate the activity of bio-antioxidants, which were discussed in this review in details. In brief, methods using HAT mechanism include ORAC, TRAP, TOSC, chemical luminescence, α-carotene bleaching, and LDL oxidation. Methods using SET mechanism include FRAP, TRAP and copper reduction. Total phenolic method (FC) uses both HAT and SET mechanisms.

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