Advances on antioxidants in research and applications

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Abstract. Antioxidants play a significant role in the prevention and treatment of numerous chronic diseases as they prevent oxidative stress and maintain reduction-oxidation (redox) equilibrium in the human body by eliminating reactive free radicals effectively. This study focused on the types and applications of antioxidants and discussed the existing problems with regard to the practical applications of antioxidants. Also, it presented a review of the latest research on antioxidants in China and abroad and performed a comprehensive, objective analysis of relevant research on antioxidants.

1 Introduction

As the standard of living improves, the global average life expectancy has increased and yet, there are still a huge number of deaths in relation to noninfectious diseases such as cardiovascular disease, stroke, diabetes, and cancer [1]. A previous study shows that these chronic diseases are associated with oxidative stress caused by free radicals, and antioxidants allow for the elimination of free radicals [2]. Therefore, in order to improve human health, it is of great importance to obtain a profound understanding of natural antioxidants.

Reactive oxygen species (ROS) are constantly formed in cells and serve as a regulator of cell growth and proliferation, intracellular chemical reactions, and other metabolic processes. However, high concentrations of ROS may induce oxidative stress and invoke damage to cell structures. Dr. Harman proposed the well-known free-radical theory of aging in 1956 [5], which has been extended and perfected by a massive number of studies. The theory, at its core, postulates that long-term oxidative stress is attributed to an imbalance between free radicals and antioxidants in the body and specifically, an excess of free radicals in the body's cells, which brings cumulative oxidative damage to cellular macromolecules (e.g., proteins, lipids, carbohydrates, DNA) and promotes cell aging and the cytopathic effect [3-4]. Oxidative stress in the body is considered a major mechanism that induces the development and progression of diverse chronic diseases (inflammation, cardiovascular disease, cancer, etc.): when extracellular lipids in the body (especially in the blood) are oxidized by free radicals, cytotoxic oxidative products are formed and trigger inflammation by interfering normal cell functions, thereby leading to a range of chronic diseases. Taking heart disease for example, high content of low-density lipoprotein (LDL) entails a great risk of cholesterol oxidation, which forms numerous cholesterol oxidation products that alter cholesterol metabolism-

related cell functions and bring cardiovascular plaques to the heart. Fruit and vegetables are good sources of dietary fiber, minerals, and trace elements and contain a variety of active antioxidants. An adequate intake of high-antioxidants fruit and vegetables is a major approach to maintain redox balance in the body [6]. The WHO recommends a minimum of 400 g of fruit and vegetables per day for the prevention of chronic diseases and supplement of trace elements [7]. Also, epidemiological studies demonstrate that a regular intake of food loaded with antioxidants (e.g., fruit and vegetables, cereals, etc.) can effectively reduce the risk of chronic diseases [8-11].

2 Types of active antioxidants

Antioxidants are found in plants, animals, alga, fungi, and bacteria. Antioxidant mainly includes carotenoids, vitamins, polyphenols, polysaccharides, alkaloids, and polypeptides. Specifically, high-antioxidant content plants include spices, Chinese medicinal herbs, tea, and fruits and vegetables; propolis is the major animal source of antioxidants; marine microalgae and seaweed are rich in antioxidants; such fungi as *Russulaceae*, *Strophariaceae*, *Polypore*, and *Morchella* are loaded with antioxidants; as to bacteria, antioxidants mainly exist in *Rhodobacter sphaeroides*, *Rhodopseudomonas palustris*, and *Rhodospirillum rubrum*. The current studies largely focus on antioxidant carotenoids, vitamins, and polyphenols.

2.1 Carotenoids

Carotenoids, a class of natural pigments, have a conjugated polyene structure with the carbon skeleton of conjugated carbon-carbon double (C=C) bonds that determine the pigment properties of carotenoids and allow for reactions with free radicals and singlet oxygen.

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The conjugated double-bond structure makes carotenoids an important part of the biological system for their antioxidant and pigment properties. There are two general classes of carotenoids: carotenes and xanthophylls. β -carotene and lycopene are the most prominent compounds of carotenes, which contain only carbon and hydrogen atoms in their structures. Hence, in the strict sense, these compounds are actually hydrocarbons. Xanthophylls, at the molecular level, contain oxygen presented either as hydroxyl groups and/or as hydrogen atoms substituted by oxygen atoms. The group of xanthophylls includes lutein and zeaxanthin that contain $-\text{OH}$ groups in their structures, canthaxanthin and echinenone with $=\text{O}$ groups, and astaxanthin having both $-\text{OH}$ and $=\text{O}$ groups in their structures. Further, some carotenoids (e.g., violaxanthin, diadinoxanthin) contain epoxy groups while others (e.g., dinoxanthin, fucoxanthin) have acetyl groups in their structures. Both of the above carotenoids also contain the $\text{C}=\text{C}=\text{C}$ (allene) group that is unique to natural products [12]. It has been proved that carotenoids can reduce the risk of senile macular degeneration, cataract, coronary heart disease, and certain types of cancers [13]. Hence, β -carotene, lutein, and lycopene are often found in dietary supplements designed to benefit human health [14]. Presently, there is ample evidence showing that plants, microalgae, and fungi contain carotenoids; in the meantime, numerous methods have been developed and perfected to extract different carotenoids [15]. Marine microalgae and seaweed (microalgae), including *Haematococcus pluvialis*, *Chlorella vulgaris*, *Dunaliella salina*, *Spirulina maxima*, *Mallomonas*, and *Nitzschia*, are natural sources of such carotenoids as β -carotene, lutein, astaxanthin, zeaxanthin, violaxanthin, and fucoxanthin [16]. These carotenoids mainly are distributed in chloroplasts, vesicles, intercellular substances or bound to macromolecules to fulfill diverse biological functions [17]. As biotechnology advances forward, carotenoid biosynthesis has been heatedly discussed, with photosynthetic and nonphotosynthetic systems such as microalgae, blue-green algae, bacteria, fungi, and yeasts being used as the hosts in carotenoid biosynthesis [18].

2.2 Vitamins

Vitamins are a group of organic compounds that are found in plants and animals, especially different-colored fruit and vegetables, animal livers, and whole milk. Although vitamins are neither the composition of cells or the organic tissue nor an energy source, they play an essential role in maintaining cell metabolic activity and health by regulating the body's metabolism. Abundant evidence has proved that Vitamin A, C, and E are antioxidant compounds [19] that mitigate the damage caused by oxidative stress. Vitamin A is a class of lipid-soluble compounds that include retinol, retinal, retinoic acid, and provitamin A (β -carotene). Cod-liver oil, animal livers, and green vegetables are primary sources of vitamin A. Extracellular retinoic acid inhibits the formation of NO residues by downregulating interleukin-

2 (IL-2) gene expression [20]. Vitamin A deficiency will cause night blindness (nyctalopia). Vitamin C, also known as L-ascorbic acid and ascorbic acid, is a water-soluble vitamin produced in plants and most animals through metabolism. Citrus fruit, apples, apricots, carrots, cherries, pomegranates, and peaches are great sources of vitamin C [21]. Anahita et al. [22] found that vitamin C in pomegranate juice was up to 57.8 ± 0.59 mg/100 g. As a natural quencher of free radicals, vitamin C allows for effective elimination of ROS, superoxide radicals, and sulfur radicals. However, vitamin C cannot be made by the human body and so 90% of our daily vitamin C intake comes from fruit and vegetables. An adequate intake of vitamin C is very important to prevent and delay degenerative changes caused by oxidative reactions [23]. Vitamin E is a fat-soluble nutrient found in a variety of food, particularly in fruit, vegetables, and beans. Wheat germ oil is the best source of vitamin E, including tocopherols and tocotrienols. With isoprenoid side-chain containing three double bonds, tocotrienols are different from tocopherols in terms of antioxidant properties. Musalmah et al. [24] found that α -tocopherol reduced free radicals and increased peroxidase to accelerate the rate of wound closure in normal and diabetic rats.

2.3. Polyphenols

Polyphenols are a structural class of compounds characterized by the presence of large multiples of phenolic hydroxyl groups. In plants, polyphenols are secondary metabolites that originate from the shikimic acid pathway. There is a variety of polyphenols that have different structures. Broadly, these compounds are divided into four classes, including flavonoids, phenolic acids, stilbenes, and lignans. Generally, leaves, roots, peel, and pulp are rich sources of polyphenols. In Zhang et al. [25], polyphenols extracted from *Ilex latifolia* Thunb were 187.86 mg gallic acid equivalents (GAE) per g dry material plant (mg GAE/g plant material), through liquid chromatography-mass spectrometry (LC-MS) analysis revealed that, which contained quinic acid, 3-caffeoylquinic acid, 5-caffeoylquinic acid, shikimic acid, 4-caffeoylquinic acid, rutin, hyperoside, 3,4-di-caffeoylquinic acid, 3,5-di-caffeoylquinic acid, and 4,5-di-caffeoylquinic acid; these compounds not only acted as inhibitors against pro-inflammatory cytokines (TNF- α , IL-1, IL-6) and MAPKs (ERK and JNK) and NF- κ B activation but reduced the production of NO and COX-2 in RAW 264.7 cells. Gao et al. [26] extracted 14.90 ± 0.7 mg GAE/g polyphenols from rice husk and found that the bound fraction of rice husk exhibited higher antioxidant than common fruit and grain while the free fraction displayed low cytotoxicity. Considering that resveratrol and flavonoids in wine are proved to be effective in the prevention and control of coronary artery disease, it is believed that polyphenols have favorable anti-inflammatory and antioxidant effect.

3 Antioxidant applications

Since antioxidants allow for removal of free radicals accumulated in vivo and in vitro, they delay the aging process by reducing the damage brought by oxidative stress to the body and achieve shelf life extension of food and drugs by preventing oxidation. Hence, antioxidants have gained extensive applications in the pharmaceutical, cosmetic, health food, and food industries.

3.1. Pharmaceutical industry

In developed countries, infectious diseases were no longer the major threat during the past decade; instead noninfectious diseases namely chronic diseases such as cardiovascular disease and cancer, contributed to most illness and death cases. Numerous studies show that excessive accumulation of free radicals in the body is a major cause of relevant chronic diseases. On this basis, antioxidants are considered to have favorable antibacterial activity and anticancer effect as they can effectively remove free radicals from the body. Deepti et al. [27] extracted polyphenols from a colored avocado seeds using methanol and found the extract having a relatively high oxygen radical absorbance capacity, which made it an effective inhibitor against lipid peroxidation; in the in vitro experiment, the extract reduced the viability of human breast (MCF7), lung (H1299), colon (HT29), and prostate (LNCaP) cancer cells, downregulated the expression of cyclin D1 and E2 in LNCaP cells and induced apoptosis in LNCaP cells. Flavonoid glycoside, as a member of the flavonoids, is a type of polyphenols found in wild chrysanthemum, ginkgo leaves, orange peel, and tea leaves. Fahima et al. [28] extracted flavonoid glycosides from prunus armeniaca and found the extract with high antibacterial activity against Gram-positive and Gram-negative bacteria. Li et al. [29] created supermolecules by mixing the aqueous solution of flavonoid glycosides and berberine through self-assembly, which displayed enhanced inhibition against *Staphylococcus aureus* and increased efficiency in biofilm eradication, thus providing a new idea for the application of antioxidants in the pharmaceutical industry. Also, there are studies on the use of antioxidants in developing medications for cardiovascular disease [30], diabetes [31], and coronary artery disease [32]. So far, despite the extensive clinical use of synthetic antioxidants like Probucol, very little has been done on clinical application and treatment based on natural antioxidants.

3.2 Cosmetic industry

The following antioxidants are found in human skin cells: glutathione peroxidase, superoxide dismutase, catalase, and non-enzyme antioxidants of low molecular weight such as vitamin E subtypes, vitamin C, glutathione, uric acid, and ubiquitin. Aging of skin is the result of in vivo and in vitro mechanisms of oxidation, including the in vitro factors such as ultraviolet radiation and smoking,

and the in vivo factors like accumulation of free radicals in the aging process. As the human skin cells become less efficient in restoring the skin and fighting oxidation, loose skin, wrinkles, and senile plaques may appear. Antioxidants in cosmetics may help regulating the cell antioxidant regulatory system and improve the anti-oxidation capacity, facilitate the removal of free radicals and delay aging of skin. Presently, such antioxidants as ginkgo biloba extracts, resveratrol, α -arbutin, vitamin E, and polysaccharides are commonly found in cosmetics. Ginkgo biloba extracts are added in a wide range of skincare products because there are multiple antioxidant activity substances such as flavonoids, terpenoids, polysaccharides, and alkaloids [33]. A previous study showed that ginkgo biloba extracts could protect hairless mice from the damage to their skin protection barriers and erythema induced by ultraviolet radiation [34]. Besides, resveratrol is a proven antioxidant that enables elimination of free radicals and inhibits NADPH and adenosine 5'-phosphate(ADP)-Fe³⁺-dependent lipid peroxidation and ultraviolet light-induced lipid peroxidation [35]. Considering its great value in cosmetic applications, resveratrol has been included in the *International Nomenclature of Cosmetic Ingredients* (INCI) and the *International Cosmetic Ingredient Dictionary and Handbook* (2010). The global cosmetic industry is undergoing rapid development recently. Against this backdrop, a great diversity of antioxidants have been applied in the field, including enzymes (e.g., catalase, superoxide dismutase, glutathione peroxidase, aldosterone reductase) and non-enzyme antioxidants (e.g., vitamin A, vitamin C, vitamin E, carotenoids, glutathione). In the vitamin industry, the global demand for vitamins reaches tens of thousands of tons a year. Yet, the vitamins are largely consumed by the feed industry, only a limited proportion is contributed by the cosmetic industry, which indicates the vast potential for future development in the applications of antioxidants.

3.3 Food industry

Lipid oxidation is the main factor that affects the quality of food and imposes a real challenge to food manufacturers and scientists. Lipids are prone to oxidation in the presence of heat, light, enzymes, metal, metalloprotein, and microorganisms, which leads to flavor reversion and loss of bioactive molecules such as essential amino acids and lipid-soluble vitamins [36]. Antioxidants are widely used in the food industry as preservatives to prevent food deterioration due to oxidation during processing, storage, and transportation. Because of their high stability and low volatility, antioxidants can effectively maintain the nutrient substances in food, preserve the textures, colors, odors, freshness, functions, and fragrance of food and extend the shelf life [37].

3.4 Health food industry

It is demonstrated that antioxidants can prevent such chronic diseases as atherosclerosis, neurodegeneration,

and aging. Hence, antioxidants are extensively used for developing healthy food products. Zhao et al. [38] investigated the correlations between the total dietary intake of carotene, vitamin C, and vitamin E and the risk of mortality from all causes, including cancer, cardiovascular disease, and other relevant diseases and discovered that the total carotene and vitamin C intake from diet was inversely associated with deaths from all causes and cardiovascular disease in China's middle-aged or elderly population. It is believed that broccoli, carrots, and tomatoes are the most functional health food as they are rich in sulforaphen, β -carotene, and lycopene, respectively. These natural antioxidant compounds can delay aging and reduce the risk of cancer and yet, only a very limited amount of these antioxidants is available through food intake, especially for the elderly population [39]. For this reason, many health food products containing a variety of antioxidants have emerged in the market. Additionally, since China is endowed with rich sources of Chinese medicinal herbs loaded with antioxidants, both the health food industry and consumers are attracted to health food products made of Chinese medicinal herbs, which represents a promising future of applying Chinese medicinal herbs to health food production.

4 Summary and prospects

Antioxidants are extensively used in the pharmaceutical, health food, cosmetic, and food industries as they are widely available and allow for the elimination of free radicals from the body, inhibition against ROS formation, enhancement of the body's endogenous antioxidant defense system, and prevention and control of chronic diseases. Previous studies on antioxidants as components of drugs largely focused on in vitro experiments, making it difficult to evaluate the consistency between in vitro and in vivo experimental results. Further, the application and development of antioxidants are hindered by the facts that in vivo molecular mechanisms of antioxidants remain unclear and that there is a severe lack of clinical studies that explore the pharmacological effect and drug action of antioxidants.

At present, the production of antioxidants still relies heavily on chemical synthesis, which pollutes the environment to a certain extent and entails a risk of performance and safety problems in relation to chemical residues in food and drugs. Antioxidants will never be comprehensively applied to the pharmaceutical, cosmetic, health food, and food industries until these drawbacks have been overcome. The sustainable development policy and rocketing development of biotechnology urge that environmentally-friendly antioxidant production techniques must be devised. Natural antioxidants are worth research and development and have a wide market prospect as they are easily available, safe, and beneficial to human health. Meanwhile, guidelines and a management system must be set up to ensure the safe use of antioxidants, especially in the pharmaceutical and food industries. In addition, there are many detection standards for antioxidant content in China, but the

evaluation standards for antioxidant biological activity and efficacy are seriously inadequate. There are many methods for evaluating antioxidant activity, while the results of different methods are significant differences. It is impossible to uniformly evaluate the activity of antioxidant products on the market. Therefore, the establishment of relevant standards should be strengthened to promoting the development of antioxidant industry.

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