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ANTIOXIDANTS OBTAINED FROM NATURAL SOURCE

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Abstract:

Natural antioxidants are widely distributed in food and medicinal plants. These natural antioxidants, especially polyphenols and carotenoids, exhibit a wide range of biological effects, including anti-inflammatory, anti-aging, anti-atherosclerosis and anticancer. The effective extraction and proper assessment of antioxidants from food and medicinal plants are crucial to explore the potential antioxidant sources and promote the application in functional foods, pharmaceuticals and food additives. The present paper provides comprehensive information on the green extraction technologies of natural antioxidants, assessment of antioxidant activity at chemical and cellular based levels and their main resources from food and medicinal plants.

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INTRODUCTION:

Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. An antioxidant achieves this by slowing or preventing the oxidation process that can damage cells in the body. This it does by getting oxidized itself in place of the cells. Thus an antioxidant can also be termed as a reducing agent.

Although, antioxidants are sold in various forms as dietary supplements there is no clinical evidence in favor of antioxidants as beneficial in maintaining health and preventing disease. However, there is a lot of anecdotal evidence that those who partake of antioxidant-rich food are better protected against problems such as heart disease, macular degeneration, diabetes, and cancer.

Antioxidants are either hydrophilic or hydrophobic. Water soluble or hydrophilic antioxidants are active in the blood plasma while the water insoluble antioxidants protect the cell membranes.Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive species. A variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables and tea, etc. This review presents some information about the antioxidant/antiradicals and their role in our body and also their presence in spices and herbs.

Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and well being. Regular consumption of anti-oxidative vegetables and fruits has been recognized as reducing the risk of chronic disease. It is a well-known fact that citrus fruits (oranges, lemons, etc.) contain a high amount of natural antioxidants, such as vitamin C. Blueberries, strawberries, grapes, plums, prunes, red beans, spinach, kale, broccoli flowers, alfalfa sprouts, and more have been proven to contain a high amount of antioxidants and have been incorporated into many dietarys.

EFFECTS OFANTIOXIDANTS:

Some of the antioxidants when taken in excess in diet may cause more harm than good. For example, when a person takes in excessive amounts of strong reducing agents as antioxidants, he or she may develop deficiency of several minerals like iron and zinc. The absorption of these minerals is prevented from the gastrointestinal tract.

Relatively strong reducing acids can have antinutrient effects by binding to dietary minerals and preventing them from being absorbed. Notable examples are oxalic acid, tannins and phytic acid, which are high in plant-based diets. Calcium and iron deficiencies are not uncommon in diets in developing countries where less meat is eaten and there is high consumption of phytic acid from beans and unleavened whole grain bread.

Similarly oxalic acid is present in cocoa, chocolate, spinach, turnip and rhubarb and tannins are present in cabbage, tea and beans. Excess of these in diet may prevent mineral absorption.

Eugenol, an antioxidant present in oil of cloves, also possesses toxic effects in high levels. Toxicity associated with high doses of water-soluble antioxidants such as ascorbic acid are less of a concern, as these compounds can be excreted rapidly in urine.[34] More seriously, very high doses of some antioxidants may have harmful long-term effects. The beta-carotene and Retinol Efficacy Trial (CARET) study of lung cancer patients found that smokers given supplements containing beta-carotene and vitamin A had increased rates of lung cancer

SOME CONDITIONS CAUSED BY ANTIOXIDANTS INCLUDE:

- Deterioration of the eye lens, which contributes to blindness
- Inflammation of the joints (arthritis)
- Damage to nerve cells in the brain, which contributes to condition

such as Parkinson's or Alzheimer's disease

- Acceleration of the ageing process
- Increased risk of coronary heart disease, since free radicals encourage

Low-density lipoprotein (LDL) cholesterol to stick to artery walls

- Certain cancers, Triggered by damaged cell DNA.

GOOD SOURCE OF ANTIOXIDANT & THEIR ROLES:

Vitamin C

Sweet red peppers are one of the top sources of vitamin C, with one cup of raw peppers supplying 190 milligrams, or at least 200 percent of your recommended daily intake. You'll get at least 100 percent of your recommended daily intake from one cup of orange juice, green peppers, broccoli and Brussels sprouts. A one-cup serving of tomatoes, cauliflower, asparagus, cantaloupe, raspberries,

blackberries and grapefruit, as well as one sweet potato and orange, provides at least 25 percent of men's and 32 percent of women's recommended daily intake.

Vitamin E

The best sources of vitamin E are sunflower seeds, almonds, hazelnuts, peanuts, pinto beans, white beans and vegetable oils, such as safflower, canola and corn oil. You will get 2 to 7 milligrams of vitamin E from 1 cup of cooked beans, a 1-ounce serving of nuts and seeds and 1 tablespoon of vegetable oil. Those values represent 13 to 47 percent of your recommended daily intake. Some foods that are rich in vitamin C also supply at least 10 percent of your daily intake of vitamin E, including raspberries, blackberries, sweet peppers, broccoli and tomatoes.

Phytiochemcals

Antioxidant phytochemicals, such as flavonoids, carotenoids and resveratrol, are easily identified because of their pigments that give fruits and vegetables their red, orange, yellow, blue and purple colors. Dark blue and purple fruits, including grapes, blackberries and blueberries, are good sources of resveratrol. Pumpkin, carrots, tomatoes, red peppers and oranges contain carotenoids, which impart shades of yellow, orange and red. Dark green vegetables, especially leafy greens, are also good sources of antioxidant carotenoids, but their green chlorophyll hides other colors.

Beta carotene:

The most studied of more than 600 different carotenoids that have been discovered, beta-carotene (lycopene, carotenes) protects dark green, yellow and orange vegetables and fruits fromsolar radiation damage. It is thought that it plays asimilar role in the body. Carrots, squash, broccoli,sweet potatoes, tomatoes, kale, collards, cantaloupe, peaches, and apri- cots are particularly rich sources of beta-carotene25.

Selenium:

Selenium probably interacts with every nutrient that affects the antioxidant balance of the cell. This mineral is thought to help fight cell damage by oxygen-derived compounds and thus may help protect against cancer. It is best to get selenium through foods,26 as large doses of the supplement form can be toxic. Good food sources include fish, shellfish, red meat, grains, eggs, chicken, and garlic. Vegetables can also be a good source if grown in selenium-rich soils.

Antioxidant activity of aromatic plants:

Consumer interest in natural food additives, havereinforced the interest in natural antioxidants. Herbsand spices are harmless sources for obtaining naturalantioxidants. A very important compound in herbs ofLamiaceae family is rosmarinic acid, showing highscavenging DPPH potential, this being related to thepresence of four hydroxyl groups in its molecule36.Oregano is particularly rich, 55000 ppm, in thiscompound and peppermint and lemon balm alsocontain high amounts, about 30000 and 37000 ppm,respectively17. Curcuma zedoaria (Berg.) Rosc.(Zingiberaceae) has long been used as a chinese folkmedicine. The essential oil of its dried rhizome wasmoderate to good in antioxidant activities by threedifferent methods, good in reducing power andexcellent in scavenging effect on 1,1-diphenyl-2-Picrylhydrazyl radical but low in chelating effect onferrous ion. Although epicurzerenone and curzerenewere found with moderate to good antioxidantactivity, the compounds 5-isopropylidene-3,8-dimethyl-1(rH)- azulenone was responsible for betterantioxidant properties37. However, naturalcurcuminoids were also isolated from Curcumalonga and showed reducing antioxidant activities.

Other Antioxidant Sources

Dark chocolate contains antioxidant flavonoids, but you need to buy chocolate that contains cacao solids. You won't get flavonoids from white chocolate and most types of milk chocolate. Coffee, wine and tea also contain flavonoids. Selenium is found in nuts, beans and animal products, including fish, chicken, pork, beef and milk. Top sources of manganese include nuts, beans, pineapple, brown rice and sweet potatoes.

allium sulphur compounds – leeks, onions and garlic

anthocyanins – eggplant, grapes and berries

beta-carotene – pumpkin, mangoes, apricots, carrots, spinach and parsley

catechins – red wine and tea

copper – seafood, lean meat, milk and nuts

cryptoxanthins – red capsicum, pumpkin and mangoes

flavonoids – tea, green tea, citrus fruits, red wine, onion and apples

indoles – cruciferous vegetables such as broccoli, cabbage and cauliflower

isoflavonoids – soybeans, tofu, lentils, peas and milk

lignans – sesame seeds, bran, whole grains and vegetables

lutein – green, leafy vegetables like spinach, and corn

lycopene – tomatoes, pink grapefruit and watermelon

manganese – seafood, lean meat, milk and nuts

polyphenols – thyme and oregano

selenium – seafood, offal, lean meat and whole grains

vitamin A – liver, sweet potatoes, carrots, milk, and egg yolks

vitamin C – oranges, blackcurrants, kiwifruit, mangoes, broccoli, spinach, capsicum and strawberries

vitamin E – vegetable oils (such as wheatgerm oil), avocados, nuts, seeds and whole grains

zinc – seafood, lean meat, milk and nuts

zoochemicals – red meat, offal and fish. Also derived from the plants that animals eat.

ANTIOXIDANT ACT ON DISEASES:

A diet high in antioxidants may reduce the risk of many diseases, including heart disease and certain cancers. Antioxidants scavenge free radicals from the body cells, and prevent or reduce the damage caused by oxidation.

The protective effect of antioxidants continues to be studied around the world. For instance, men who eat plenty of the antioxidant lycopene (found in tomatoes) may be less likely than other men to develop prostate cancer. Lutein, found in spinach and corn, has been linked to a lower incidence of eye lens degeneration and associated blindness in the elderly. Flavonoids, such as the tea catechins found in green tea, are believed to contribute to the low rates of heart disease in Japan.

Plant foods are rich sources of antioxidants. They are most abundant in fruits and vegetables, as well as other foods including nuts, wholegrains and some meats, poultry and fish.

Antioxidants action on atherosclerosis:

Lipoprotein oxidation is a key early stage in the development of atherosclerosis. Oxidized LDL is known to promote atherogsenesi through foam cell formation and inflammatory responses. Free radicals have been implicated in the oxidative modification of LDL and several basic research studies strongly suggest that progression of the atherosclerotic lesions can be delayed by intervention with anti-oxidants. It has been advised that general population consume a balanced diet with more emphasize on antioxidants. However there are discrepancies in epidemiological evidence and clinical trails, and few longterm trials have evaluated the effect of antioxidants in men at initially low risk of cardiovascular disease.

Antioxidants action on ocular disease:

Oxidative processes are thought to be an important contributing factor in the development of both cataracts and the age-related disorder of the retina, maculopathy. Oxidation, induced mainly by exposure to UV light, is believed to be a major cause of damage to the proteins of the lens. The oxidized protein precipitates and causes cloudiness of the lens. Antioxidants and antioxidant enzymes inactivate harmful free radicals and proteases degradation and remove the damaged portion from the lens, but the oxidative damage occurs at a faster rate. The oxidized protein may therefore accumulate, and with time, the damage becomes irreversible.

Antioxidants in prevention of skin aging:

The reactions which add hydroxyl groups to the amino acids proline and lysine in the collagen molecule, via prolylhydroxylase and lysyl hydroxylase, both require vitamin C as a cofactor. Hydroxylation allows the collagen molecule to assume its triple helix structure, making vitamin C essential to the development and maintenance of scar tissue, blood vessels, and cartilage. In addition, topically applied vitamin C seems to enhance the mRNA level of collagens I and III, their processing enzymes, and the tissue inhibitor of matrix metalloproteinase 1 in the human dermis.

MECHANISMS OF ACTION:

Dietary antioxidants reduce oxidative stress by scavenging free radicals by three main mechanisms (variations are possible depending on the reactive species involved). Tocopherols and most polyphenols donate a hydrogen ion, carotenoids quench oxygen singlets and ascorbate transfers electrons (reviewed in Vertuani et al. 2004; Halliwell and Gutteridge 2006). The end result of these actions is that the free radical is neutralized. The efficiency with which an antioxidant destroys free radicals is called anti-oxidant potency and is measured in Trolox equivalent antioxidant capacity; Trolox is a synthetic analogue of topheroocl. After reducing the free radicals, antioxidants are oxidized. At this stage, most antioxidants are fairly stable and relatively innocuous molecules that are subsequently catabolized and

excreted. In contrast, carotenoid radicals are rather noxious pro- oxidants that may oxidize other biologically important molecules. How- ever, if other antioxidants are present (usually vitamin C or E), they usually reduce the oxidized carotenoids, which are thereby recycled Therefore, the function of carotenoids as antioxidants isdebatable and always contingent on the presence of other antioxidants.[32,33,18]
low molecular weight antioxidant are small molecules that frequently infiltrate cells, accumulate at high concentrations in specific compartments associated with oxidative damage, and then are regenerated by the cell . In human tissues, cellular low molecular weight antioxidant are obtained from various sources. Glutathione, nicotinamide adenine dinucleotide reduced form, and carnosine are synthesized by the cells; uric acid and bilirubin are waste products of cellular metabolism; ascorbic acid , tocopherols and polyphenols are antioxidants obtained from the diet.

Among these low molecular weight antioxidant, a considerable attention was focused on ascorbic acid (AA), known for its reductive properties and for its use on a wide scale as an antioxidant agent in foods and drinks ; it is also important for therapeutic purposes and biological metabolism.

Vitamin C can be easily oxidized, its degradation being accelerated by heat, light and the presence of heavy metal cations [57-59]. Thus, due to its content variation, vitamin C represents an important quality indicator of foodstuffs [59] and contributes to the antioxidant properties of food [60-64].

Special attention has been dedicated to the study of antioxidant action mechanism.

The excess free radicals circulating in the body oxidize the low density lipoproteins (LDL), making them potentially lethal; the excess free radicals can also accelerate aging processes and have been linked to other very serious pathologies, such as brain stroke, diabetes mellitus, rheumatoid arthritis, Parkinson's disease, Alzheimer's disease and cancer. Physiologically, the oxygenated free radicals are among the most important radical species. Reactive oxygen species (ROS) comprise species with a strong oxidizing tendency, both of a radical nature (the superoxide radical, the hydroxyl radical) and a non-radical nature (ozone, hydrogen peroxide) [65].

A number of chemical and physical phenomena can initiate oxidation, which proceeds continuously in the presence of (a) suitable substrate(s), until a blocking defence mechanism occurs [66]. Target substances include oxygen, polyunsaturated fatty acids, phospholipids, cholesterol and DNA [67].

The essential features of oxidation via a free radical-mediated chain reaction are initiation, propagation, branching and termination steps [66]. The process may be initiated by the action of external agents such as heat, light or ionizing radiation or by chemical initiation involving metal ions or metalloproteins [68].

Secondary or preventative antioxidants are compounds that retard the rate of oxidation. This may be achieved in a number of ways, including removal of substrate

METHODS OF TESTING ANTIOXIDANTS:

Spectrometric Techniques:
Spectrometric techniques rely on the reaction of a radical, radical cation or complex with an antioxidant molecule capable to donate a hydrogen atom.

The DPPH method:
DPPH (2,2-diphenyl-1- picrylhydrazyl) is a stable free radical, due to the delocalization of the spare electron on the whole molecule. Thus, DPPH does not dimerize, as happens with most free radicals. The delocalisation on the DPPH molecule determines the occurence of a purple colour, with an absorbtion band with a maximum around 520nm.
When DPPHreacts with a hydrogen donor, the reduced (molecular) form (DPPH) is generated, accompanied by the disappearance of the violet colour. Therefore, the absorbance diminution depends linearly on the antioxidant concentration. Trolox is used as standard antioxidant.

The spectrophotometric method with DPPH was applied to antioxidant capacity determination in fruit juices and fruit (guava) extracts . The standard curve was linear between 25 and 800mM Trolox. Results are expressed in µM Trolox Equivalents/g fresh mass. Antioxidant activity of guava fruit methanol extracts, as determined by the DPPH method are comprised between 16.2 ± 1.0 and 32.0 ± 5.1µM TE/ fresh mass.

The ABTS method:
The s ABTS cation radical which absorbs at 743 nm (giving a bluish-green colour) is formed by the loss of an electron by the nitrogen atom of ABTS (2,2'-azino-bis(3- ethylbenzthiazoline-6-sulphonic acid). In the presence of Trolox (or of another hydrogen donating antioxidant), the nitrogen atom quenchethe hydrogen atom, yielding the solution decolorization.
ABTS can be oxidized by potassium persulphate or manganese dioxide , giving rise to the ABTS cation

radical whose absorbance diminution at 743 nm was monitored in the presence of Trolox chosen as standard antioxidant.

The spectrophotometric method based on the absorbance diminution of ABTS cation radical was applied to antioxidant content determination in guava fruit extracts, fruit and vegetable extracts, soft beverages, alcoholic beverages, tea and coffee. The standard curve was linear between 25 and 600µM Trolox. The values of the total antioxidant capacity of guava extracts ranged between 22.3 ± 0.9 and 37.9 ± 3.4µM TE/ fresh mass.

The antioxidant activity of soft beverages, as determined by the ABTS method is comprised between 0.09 mM Trolox/liter for Cola and 3.30mM Trolox/liter for grapefruit juice.

The FRAP (ferric reducing antioxidant power) method:

The FRAP (ferric reducing antioxidant power) method relies on the reduction by the antioxidants, of the complex ferric ion-TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-triazine). The binding of Fe2+ to the ligand creates a very intense navy blue color. The absorbance can be measured to test the amount of iron reduced and can be correlated with the amount of antioxidants. Trolox or ascorbic acid were used as references.

The total antioxidant activity of white- and yellow-flesh nectarines was evaluated by FRAP method, the results being expressed as Ascorbic Acid Equivalent Antioxidant Capacity, AEAC). Thea values ranged between 14.4 and 104.5mg/100 of fruit.

The ORAC (oxygen radical absorption capacity) assay:

the method measures the antioxidant scavenging activity against the peroxyl radical, induced by 2,2'-azobis-(2-amidino-propane) dihydrochloride (AAPH), at 37°C. Fluorescein was used as the fluorescent probe. The loss of fluorescence was an indicator of the extent of the decomposition, from its reaction with the peroxyl radical. Antioxidant activity of guava fruit methanol extracts were determined by the ORAC method. The standard curve was linear between 0 and 50mM Trolox.

FUTURE PROSPECTIVE OF ANTIOXIDANT:

By modifying visual and taste cues between food types, we can establish the proximate mechanisms that animals use to discern antioxidants in food. Environmental variation in the availability of antioxidants and individual variation in food selection may result ininterindividual differences in antioxidant intake that may explain, at least partially, intra and interspecific differences in resource allocation to self-maintenance or reproduction.

We should develop a more complete structure for therelevance of dietary antioxidants for the expression of life history traits and trade-offs, we suggest that future studies should consider the full range of available antioxidants, their possible interactions, their environmental availability, and the potential for interindividual differences in anti-oxidant intake and uptake. This is because more dietary antioxidants than traditionally perceived may affect crucial life history traits, such as fertility, growth, immunity, senescence and the expression of sexually selected traits. Surprisingly, the most common and potent antioxidants, flavonoids and phenolic acids, are those that have received the least attention. Given their ubiquity in food items, we urge researchers to include these potentially important antioxidants in evolutionary ecology as well as ecological immunology studies.

CONCLUSION:

The most important free radical in biological systems is radical derivatives of oxygen with the increasing acceptance of free radical as commonplace and important biochemical intermediate. Antioxidants are believed to play a very important role in the body defense system against reactive oxygen species (ROS), which are the harmful byproducts generated during normal cell aerobic respiration . Increasing intake of dietary antioxidants may help to maintain an adequate antioxidant status and, therefore, the normal physiological function of a living system. To protect the cells and organ systems of the body against and complex antioxidant reactive oxygen species, humans have evolved a highly sophisticated protection system. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals.

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