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Ankita Chib

Division of Food Science and Technology, SKUAST-J, Chatha, Jammu and Kashmir, India

Neeraj Gupta

Division of Food Science and Technology, SKUAST-J, Chatha, Jammu and Kashmir, India

Anju Bhat

Division of Food Science and Technology, SKUAST-J, Chatha, Jammu and Kashmir, India

Nadira Anjum

Division of Food Science and Technology, SKUAST-J, Chatha, Jammu and Kashmir, India

Garima Yadav

Division of Food Science and Technology, SKUAST-J, Chatha, Jammu and Kashmir, India

Corresponding Author:

Ankita Chib

Division of Food Science and Technology, SKUAST-J, Chatha, Jammu and Kashmir, India

Role of antioxidants in food

Ankita Chib, Neeraj Gupta, Anju Bhat, Nadira Anjum and Garima Yadav

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Abstract

Antioxidant is any substance that delays, prevents or removes oxidative damage to a target molecule. It can be found in many foods, including fruits and vegetables. The role of antioxidants in foods is to retard or control oxidation. The process of autoxidation and development of rancidity in foods involves a free radical chain mechanism via initiation, propagation and termination steps. While radicals are produced in the 'initiation' step, they react with unsaturated fatty acids by abstracting a hydrogen atom from a site which requires the least energy that is the allylic or diallylic position in the 'propagation' steps. The reactions in the propagation step make up a chain reaction until a 'termination' reaction occurs. Due to high stability and low volatility, it helps to maintain the level of nutrients, the texture, colour, taste, freshness, functionality, aroma, and appeal to consumers such as the older person. Various antioxidants found in food viz. natural antioxidants, synthetic antioxidants, dietary antioxidant, endogenous antioxidant play an important role in preservation of food. Vitamin C, Vitamin E, α -carotene, Lycopene, Polyphenol etc. is main sources of antioxidants. They may be present in foods as endogenous factors or may be added to preserve their lipid components from quality deterioration. The most widely used antioxidants in foods include butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), propyl gallate (PG) and tertiary butyl hydroquinone (TBHQ). These antioxidants may be used at 200 ppm in bulk oils and at 200 ppm, based on the lipid content of other foods. The benefits of antioxidants include whole foods and beverages (e.g., acai berry, gogi berry, green tea) as well as isolated substances sold primarily as dietary supplements (e.g., vitamin C, lycopene, selenium) or added to foods (e.g., vitamin E). It reduces the risk of developing certain diseases such as; cancer, heart disease, stroke, and arthritis etc.

Keywords: Antioxidant, free radicals, autoxidation, termination, nutritional value

Introduction

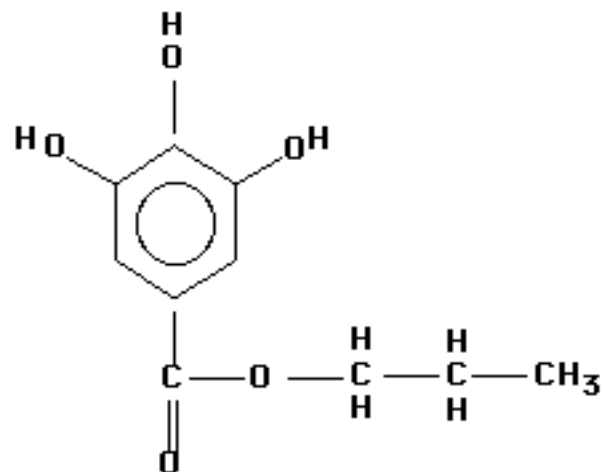
Antioxidants in foods are important for our health and eating five to seven serves each day of fresh fruit and vegetables has been show to help protect against heart disease, cancers, and other disease. Consumed large amount of fresh fruits and vegetables is the best source of antioxidants. Antioxidant is "any substance that delays, prevents or removes oxidative damage to a target molecule" (Halliwell *et al.*, 2007) [16]. Antioxidants are compound or substances that prevent deterioration of lipids during processed or in the processed food by scavenging the free radicals. It increases the oxidative stability of food, control free radicals, control the pro-oxidants & other oxidative intermediates. Antioxidants are phytochemicals, vitamins and nutrients that protect our cells from damage caused by free radicals. It is capable of stabilizing or deactivating, free radicals before they attack cells.

These are termed reactive oxygen species (ROS) and they exhibit two unpaired electrons in different orbitals at their highest energy level, which makes them susceptible to the formation of radicals (Pham-Huy *et al.*, 2008) [30]. ROS is a collective term that describes free radicals derived from oxygen, such as superoxide anion (O_2^{\bullet}), hydroxyl radical (HO^{\bullet}), peroxy radical (RO_2^{\bullet}) and alkoxy radical (RO^{\bullet}), as well as hydrogen peroxide, a non-radical species resulting from oxygen metabolism ($H_2 O_2$) (Circu *et al.*, 2010) [8].

Antioxidants prevent oxidation of stable compounds by neutralizing free radicals. Antioxidants significantly delay or inhibit oxidation of oxidizable substrates at low concentration, compared to the higher contents of lipids and proteins in foods (Halliwell and Gutteridge 2001) [17]. The antioxidants are naturally present in foods during processing. Antioxidants may be present in foods as endogenous factors or may be added to preserve their lipid components from

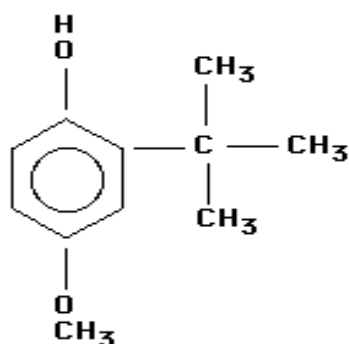
quality deterioration. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary-butylhydroquinone (TBHQ) are commonly used in food formulations. Food manufacturers have used food-grade antioxidants to prevent quality deterioration of products and to maintain their nutritional value (Shahidi, *et al.*, 1997) [33]. Antioxidants are not only in food additives but are also to be found in food supplements and levels should be measured, as such, in body tissues and fluids (Franco *et al.*, 2017) [13].

In the 20th century, antioxidants entered in the widely emerging food industry as an important means to limit the degradation of stored foods as a result of oxidation process. The most widely used antioxidants in foods include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary-butylhydroquinone (TBHQ). In 1940's, the use of synthetic antioxidants in foods and BHA was found to retard oxidation and the effectiveness of several alkyl esters of gallic acid was unravelled. Later, in 1954, BHT was also approved for food use in the USA and TBHQ was commercialized in 1972 (Barlow *et al.*, 1992) [2].

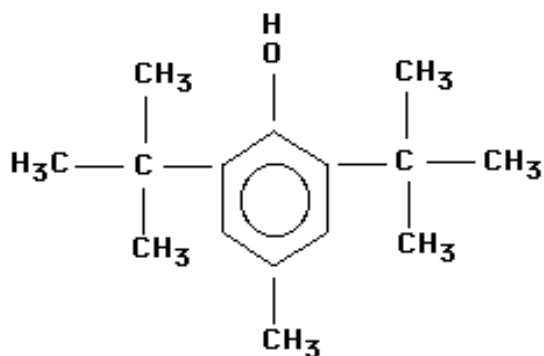


PG

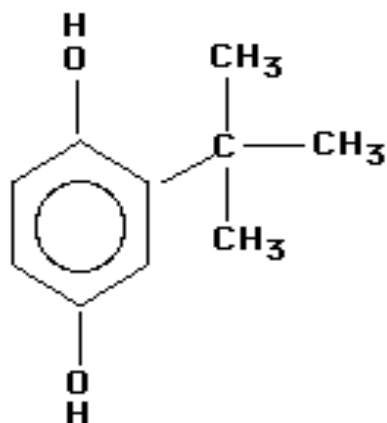
The role of antioxidants in foods is to retard or control oxidation. The process of autoxidation and development of rancidity in foods involves a free radical chain mechanism via initiation, propagation and termination steps. While radicals are produced in the 'initiation' step, they react with unsaturated fatty acids by abstracting a hydrogen atom from a site which requires the least energy, that is the allylic or diallylic position in the 'propagation' step (Wagner *et al.*, 1990) [39]. The reactions in the propagation step make up a chain reaction until a 'termination' reaction occurs. In addition to autoxidation, lipid quality deterioration in foods may occur under photooxidative conditions, oxidation via a lipoxygenase-assisted process, or oxidation under thermal conditions as experienced under high temperatures such as those endured during frying of foods. Many of the products formed as a result of oxidation of foods, regardless of the conditions experienced, are similar in nature. Thus, all oxidation processes should be controlled in order to protect food lipids from deterioration and off-flavour formation (Shahidi, 1997) [33].



BHT



BHA



TBHQ

Mechanism of Antioxidants on action

The possible mechanisms of action of antioxidants were first explored when it was recognized that substance with anti-oxidative activity is likely to be the one that itself readily oxidized. An antioxidant can be defined as: "any substance that, when present in low concentrations compared to that of an oxidizable substrate, delays or inhibits the oxidation of that substrate" (Rosenblat M, *et al.*, 2006) [32]. A free radical can be defined as, "any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital and capture electrons from other substances in order to neutralize themselves" (Halliwell *et al.*, 1999). The existence of an unpaired electron results in certain common properties shared by most of the radicals (Anbudhasan *et al.*, 2014) [1].

There are two principal mechanisms of action have been proposed for antioxidants. The first is a chain-breaking mechanism by which the primary antioxidants donate electrons to the free radicals present in the system, example lipid radicals (Mahantesh SP *et al.*, 2012) [23]. Chainbreaking antioxidants act by scavenging free radicals and donating hydrogen atoms (Beer D, *et al.*, 2002) [4]. The second mechanism involves removal of ROS and RNS initiator by quenching chain initiator catalyst (Terefe 2016) [38]. Preventative antioxidants are generally metal chelators and reductants capable of sparing other antioxidants in vivo (Beer *et al.*, 2002) [4]. These reactive species are capable of causing

damage to the vital biological molecules such as deoxyribonucleic acid (DNA), proteins, carbohydrates, and lipids (Young IS and Woodside JV, 2001) [41].

Chain Reactions of Free Radicals

The mechanism of chain reactions can be divided into the three stages: initiation, propagation and termination (Polumbryk *et al.*, 2013) [26].

On the first stage of oxidation reaction from biological systems RH are formed radicals R· as a result of abstraction of a hydrogen atom H· :

Initiation Stage:

- 1) $RH \rightarrow R\cdot + H\cdot$
- 2) $R\cdot \rightarrow R\cdot + O_2 \rightarrow ROO\cdot$
- 3) $2ROOH \rightarrow ROO\cdot + RO\cdot + H_2O$

After initiation, propagation of the free radical chain occurs, in which molecule of oxygen from environment react with reactive radical species, resulting in formation of peroxides and peroxy radical ROO·. These intermediates may further propagate free radical reactions.

Propagation Stage

- 1) $R\cdot + O_2 \rightarrow ROO\cdot$
- 2) $ROO\cdot + RH \rightarrow ROOH + R\cdot$
- 3) $RO\cdot + RH \rightarrow ROH + R\cdot$

In the last stages, interaction of two radicals may lead to formation of non-radical adduct and termination of free radical chain.

Termination Stage

- (1) $R\cdot + R\cdot \rightarrow R-R$
- (2) $R\cdot + ROO\cdot \rightarrow ROOR$
- (3) $ROO\cdot + ROO\cdot \rightarrow ROOR + O_2$
- (4) Antioxidants + $O_2 \rightarrow$ Oxidized antioxidants (Ingold KU, 1996) [20]

Antioxidants can slow lipid oxidation by inactivating or scavenging free radicals, thus inhibiting initiation and propagation reactions (Rosenblat *et al.*, 2006) [32]. The antioxidants function by the very simple and effective method of donating hydrogen atom to free radicals and thus terminating their life (Hamid *et al.*, 2010) [18].

Various types of antioxidants:

In present time various types of antioxidant found in food viz. natural antioxidants, synthetic antioxidants, dietary antioxidant, endogenous antioxidant which plays important role in preservation of food.

Natural Antioxidants: Natural antioxidants are those oxidants that are found in natural sources, such as fruits, vegetables and meats (Grozea, 2012) [15]. There are several common natural antioxidants which are found in everyday foods, the most common of which being vitamin C (ascorbic acid), vitamin E (tocopherols), vitamin A (carotenoids), various polyphenols including flavonoids, anthocyanins, lycopene (a type of carotenoid), and coenzyme Q10, also known as Ubiquitin, which is a type of protein (Yadav, *et al.*, 2016) [40]. Natural antioxidants are found in most fresh foods.

Table 1: Sources of some natural antioxidants

Compounds	Natural Sources
Ascorbic acid	Citrus fruits, green peppers, leafy green vegetables, etc.
Tocopherol	Vegetable oils, walnuts, peanuts, almonds, seeds, etc.
β- carotene	Mangoes, papaya, pumpkin, peppers, etc.
Anthocyanin	Berries, cherries, red onion, red beans, etc.
Lycopene	Carrots, pumpkin, grapes, watermelon, etc.
Extracts	Extract from green tea, rosemary, sage, clove, oregano, thyme, oat, rice bran, etc.

Synthetic Antioxidants: Synthetic antioxidants are chemically synthesized since they do not occur in nature and are added to food as preservatives to help prevent lipid oxidation (Shahidi *et al.*, 1992) [34]. Some of the synthetic antioxidants currently permitted for use in foods include BHT, BHA, propyl gallate (PG) and tertiary butylhydroquinone (TBHQ).

- **BHA:** Butylated hydroxy anisole is a mixture of two isomers. Referred to as a 'hindered phenol' because of the proximity of the tertiary butyl group to the hydroxyl group. This may hinder the effectiveness in vegetable

oils, but increase the 'carry through' potency for which BHA is known.

- **Propyl Gallate:** Three hydroxyl groups make it very reactive. Lower solubility. Tend to chelate trace minerals such as iron and form colored complexes. They are heat labile, especially under alkaline conditions.
- **BHT:** Butylated hydroxy toluene is also a 'sterically hindered' phenol Susceptible to loss through volatilization in high temperature applications.
- **TBHQ:** Tertiary-butylated hydroquinone is an extremely potent antioxidant. It had been used extensively in non food applications prior to gaining approval in food.

Table 2: Sources of some Synthetic Antioxidants

Compounds	Sources
BHA	Bakery products, Meat products, Spices, Cereals, Yeast, Vegetable oils, Beverage mixes, Dessert mixes, Nuts, etc
PG	Lard, shortening, vegetable oils, cereals, package liners, animal feeds, etc.
BHT	Breakfast cereals, baked goods, Potato chips, Vegetable Oils, Snack foods, Butter, Margarine, Frozen seafoods, Chewing gum base, etc
TBHQ	Dry cereals, Edible fats, Pizza toppings Potato chips, Poultry, meat, etc.

Dietary Antioxidants: The dietary antioxidants such as ascorbates, tocopherols and carotenoids are well known and there is a surplus of publications related to their role in health (Boskou *et al.*, 2005) [5]. The health benefits of fruits and vegetables are largely due to the antioxidant vitamins supported by the large number of phytochemicals, some with greater antioxidant properties (Dimitrios, 2006) [10]. Vitamin C, vitamin E, β -carotene and other carotenoids and oxycarotenoids, e.g., lycopene and lutein are among the most widely studied dietary antioxidants.

β -carotene and other carotenoids are also believed to provide antioxidant protection to lipid-rich tissues. Research suggests that β -carotene may work synergistically with other vitamins (Percival 1996) [27]. In plants, flavonoids serve as protectors against a wide variety of environmental stresses while, in humans, flavonoids appear to function as “biological response modifiers”. Flavonoids have been demonstrated to have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity (Yadav *et al.*, 2016) [40].

Endogenous Antioxidants: In addition to dietary antioxidants, the body relies on several endogenous defense mechanisms to help protect against free radical-induced cell damage. The antioxidant enzymes-glutathione peroxidase, catalase, and superoxide dismutase (SOD)-metabolize oxidative toxic intermediates and require micronutrient cofactors such as selenium, iron, copper, zinc, and manganese for optimum catalytic activity. It has been suggested that an inadequate dietary intake of these trace minerals may compromise the effectiveness of these antioxidant defence mechanisms. (Percival, 1996) [27]. Glutathione, an important water-soluble antioxidant, is synthesized from the aminoacids glycine, glutamate, and cysteine. Glutathione directly quenches ROS such as lipid peroxides, and also plays a major role in xenobiotic metabolism (Jacob, 1995) [21].

Lipoic acid, yet another important endogenous antioxidant, categorized as a “thiol” or “biothiol,” is a sulfur-containing molecule that is known for its involvement in the reaction that catalyzes the oxidative decarboxylation of alpha-keto acids, such as pyruvate and alpha ketoglutarate, in the Krebs cycle (Yadav *et al.*, 2016) [40].

Functions of antioxidants

- Antioxidants such as vit- C & vit- E boost our immune system.
- Certain phytochemicals have beneficial effect on heart diseases.
- Antioxidants lower the level of LDL cholesterol, thus preventing plaque deposition in the blood vessels.
- It is beneficial in cancer prevention.
- Antioxidants neutralize substances that can damage the genetic material by oxidation.
- It reduces the free radicals.
- Protects cells against premature and abnormal ageing.
- It helps to fight age related molecular degeneration.

Antioxidants components in food

There are many food components with antioxidant properties, such as α -tocopherol, γ -tocopherol, tocotrienol, ascorbic acid, β -carotene, and other substances like ubiquinol, and phenolic compounds (Mataix-Verdú *et al.*, 2009) [24]. Therefore, nutrition plays a very important role in the maintenance of antioxidant enzymes because some micronutrients such as selenium, copper, iron, manganese and zinc function as cofactors or are included as part of their prosthetic groups. If micronutrient is insufficient, the enzyme defense system can be impaired (Fang *et al.*, 2002) [14]. Phenolic compounds and ascorbic acid are the most important natural antioxidants. Carotenoids, protein-related compounds, Maillard reaction products, phospholipids, and sterols also show natural antioxidant activities in foods.

Antioxidants obtained through diet can act in different ways: first, preventing the excess of free radicals, thus avoiding oxidative damage to the cell. Secondly, after damage has occurred, antioxidants can control free radical levels preventing further damage thereby alleviating some symptoms caused by oxidative stress (Delgado *et al.*, 2010) [9].

Polyphenols are a heterogeneous group of molecules consisting of aromatic rings substituted with a hydroxyl group. Phenolic compounds are classified in several types depending on their structure. The most important are: phenolic acids and flavonoids (mainly found in vegetables), are found in the surface layers of vegetables, fruits, cereals and other seeds. Their function is to protect the lower layers from oxidation (Zavaleta *et al.*, 2005) [42].

These compounds are secondary metabolites produced by plants and they are part of a defense mechanism against ultraviolet radiation and aggression by pathogens. The longterm consumption of plants rich in polyphenols provides protection against cancer, cardiovascular disease, diabetes, osteoporosis and neurodegenerative diseases according to epidemiology studies and meta-analysis. In food, polyphenols contribute to acidity, astringency, color, flavor, odor and oxidative stability. There are also substances with estrogenic activity (phytoestrogens) like isoflavones, lignans and the stilbene resveratrol, whereas others, like tanins, are able to bind to metals and proteins, affecting their bioavailability and leading to some unspecific effects such as antimicrobials or the prevention of neurodegenerative diseases (Scalbert *et al.*, 2005) [35].

Application of non-conventional techniques in the extraction of antioxidants from some foods

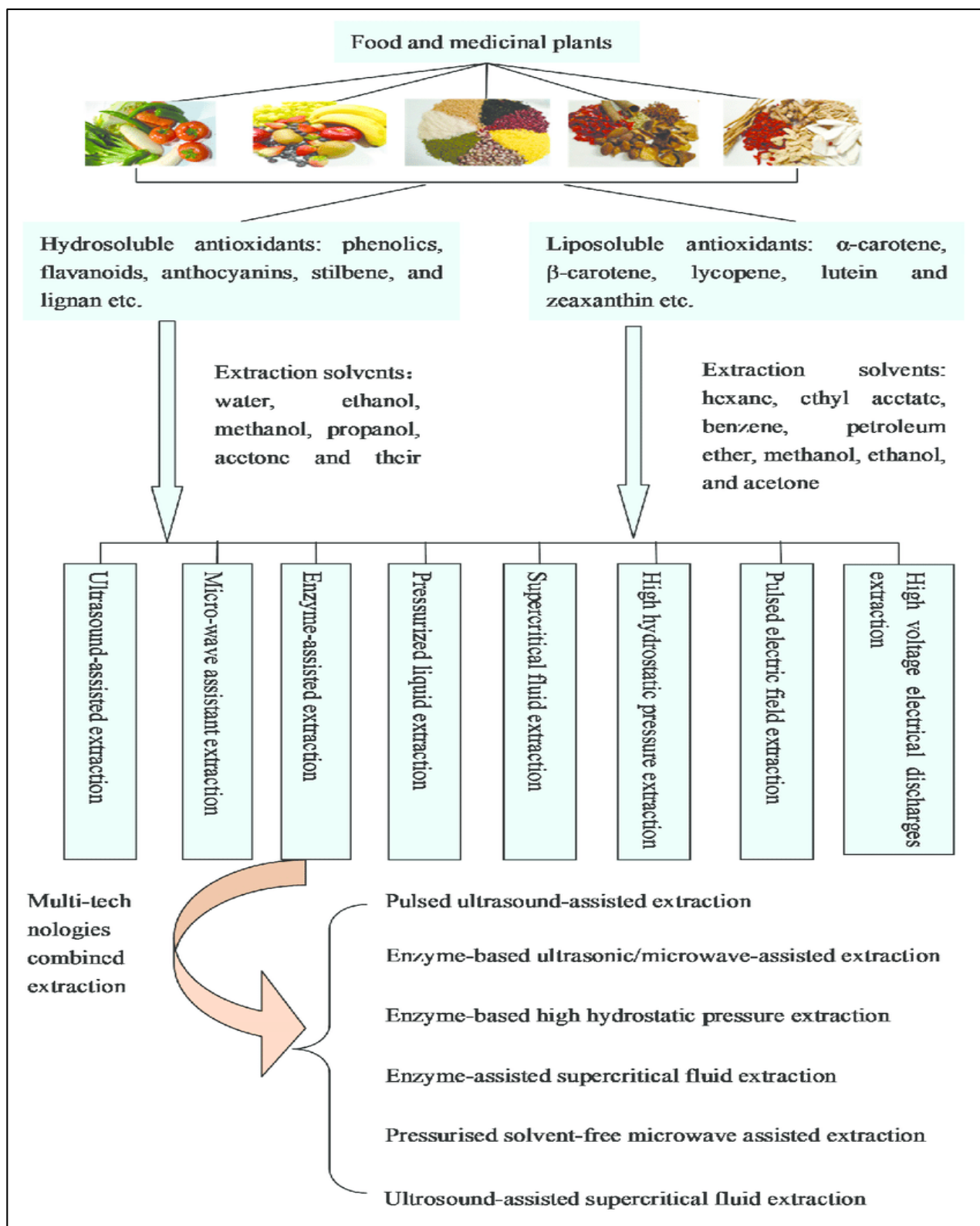


Fig. 1: The outline of extraction of antioxidants from foods and medicinal plants.

Ultrasound-Assisted Extraction (UAE)

Ultrasound assisted extraction (UAE) has been applied widely in the last three decades as an efficient extraction method in the food and pharmaceutical industries (Esclapez *et al.*, 2011)^[11]. During the ultrasound assisted extraction of bioactive components from plant materials, the high temperature and pressure would be destroy the cell walls, to facilitate the release of bioactive compounds from plant cell walls and enhance the mass transport. The heat transfer of UAE is from

outside of the plant cell to the inside, which is in the opposite direction of microwave assisted extraction. Ultrasound frequency, intensity, temperature, and time can directly affect both extraction efficiency and yields. In addition, types of solvent, solvent volume, as well as sample characteristics such as moisture content of the sample and particle size are also the important factors for effective extraction (Talmaciu *et al.*, 2015)^[37].

Microwave-Assisted Extraction (MAE)

Microwave is an electromagnetic radiation. During MAE, microwave can deliver energy to solvent and plant matrix and the energy can be absorbed by molecules inside plants, particularly the polar molecules. The severe thermal, localized pressures and mechanical stress caused by microwave significantly change the physical properties of the cell walls and finally result in rupture of cell walls and release of target components (Zhang *et al.*, 2011) [43].

Enzyme-Assisted Extraction (EAE)

Enzymes have the properties of high specificity and high efficiency. Enzyme-assisted extraction (EAE) is a potential green extraction method because of the mild extraction conditions and barely any effect on the environment (Cheng *et al.*, 2015) [6]. The enzymes could degrade the compositions and destroy the structural integrity of plant cell wall, which enhance the release of bioactive compounds. Cellulase, pectinase, hemicellulase and β -glucosidase are extensively used in the EAE. These enzymes can be obtained from various materials such as bacteria, fungi, vegetable and fruit extracts, or animal organs (Liu *et al.*, 2014) [22].

Pressurized Liquid Extraction (PLE)

PLE is based on the use of solvents at elevated temperature and pressure to extract target components from various matrices (Mustafa *et al.*, 2011) [25]. By elevating the pressure, the temperature of solvent under liquid state can be above its boiling point at normal temperature, which can enhance mass-transfer rate and promote the solubility of the analytes. The wide ranges of temperature from room temperature to 200 °C and pressure from 35 to 200 bar can be applied in PLE. When the extraction solvent is water, PLE is also called sub-critical water extraction (SWE). When the water is heated to 200–250 °C in SWE, it can be maintained in liquid state, while the dielectric constant (ϵ) of water is decreased from 80 to 30–25, which is close to the dielectric constant of some organic solvents such as ethanol or methanol. The closed dielectric constants mean the similar polarity of the organic solvent. Although not viable for every application, the use of SWE can be regarded as an effective alternative to organic solvents in some applications. Due to free of organic solvents, SWE is perceived as the “greenest” of the PLEs (Herrero *et al.*, 2015) [19].

Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction (SFE) as a sustainable green technology has been extensively applied since the past decades. Over the critical pressure and temperature, the solvent can be transformed into the supercritical state, which shows liquid-like (solvent power, negligible surface tension) and gas-like (elevated diffusivity and low viscosity) properties (Radzali *et al.*, 2014) [31]. Even though PLE and SFE have in common that they conduct under medium-to-high pressures, SFE operate using solvents at temperatures and pressures above their critical points, whereas PLE is based on the use of liquids at temperatures above their normal boiling points (Talmaciu *et al.*, 2015) [37]. Compared with normal liquids, supercritical fluids could enhance transport properties, which can diffuse easily through solid materials and therefore obtain faster extraction rates (Silva *et al.*, 2016) [36]. SFE utilizes the outstanding physicochemical properties of supercritical fluids (SF) to extract targets components from various matrices. SFE basically contains two major steps: firstly, the soluble compounds from the plant material are

extracted by the supercritical solvent, then these compounds are separated from the supercritical solvent by rapidly reducing the pressure, increasing the temperature, or both (Pereira *et al.*, 2016) [28].

Antioxidant Activity Determination**Radical scavenging activity (RSA %) assay:**

Free radical scavenging activity (RSA) of the sample was measured using the method described by El-Hamzy and Yaseen (2014). An aliquot of the sample solution (40 μ l) was mixed with 2.9 ml of 0.1 mM DPPH in methanol solution, incubated for 30 min at 25°C in dark; the decrease in the absorbance at 517 nm was measured. Methanol was used as blank. Antioxidant activity was expressed as percentage inhibition of the DPPH radical and was determined by the following equation:

$$\text{Scavenging activity (\%)} = 1 - (A_s/A_0) \times 100.$$

Where: is the Absorbance of sample and is the Absorbance of control.

Control: 40 μ l of methanol mixed with 2.9 ml of DPPH methanol solution.

The ferric reducing antioxidant power (FRAP) measurement:

Antioxidant activity was measured using the ferric reducing antioxidant power reported by Benzie and Strain, (1996) [3]. The FRAP assay reduces ferric ion (Fe+3) to ferrous ion (Fe+2). (Fe+2/TPTZ) forms a blue complex color, which increases the absorption at 593 nm. The FRAP reagent contained 2.5 ml of a 10 mmol/l TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mmol/L HCL plus 2.5 ml of 20 mmol/L FeCl₃.6H₂O and 25 ml of 0.3 mol/L acetate buffer (pH 3.6) and was freshly prepared and warmed at 37°C prior to use. Aliquots of 0.1 ml sample solution were mixed with 3 ml FRAP reagent and the absorbance of reaction mixture at 593 nm was measured spectrophotometrically at 593 nm after incubation at 37 °C for 10 min. A standard calibration curve was prepared using freshly prepared ammonium ferrous sulphate (25–1600 μ M Fe+3).

Uses in technology**Food preservatives:**

Antioxidants used as food additives to help guard against food deterioration. Exposure to oxygen & sunlight are the 2 main factors in the oxidation of food. Vitamin C and Vitamin E are natural preservatives.

Synthetic antioxidants such as Propyl gallate, Tertiary butyl hydro quinone (TBHQ), Butylated hydroxy anisole (BHA), Butylated hydroxy toluene (BHT). Unsaturated fats are used by drying, smoking, salting or fermenting.

Industrial uses:

Antioxidants are frequently added to industrial products. The common uses in industries are stabilizers in fuels & lubricants to prevent oxidation.

Risk of Antioxidants

Even though there are many benefits to using antioxidants, too much can also cause harm.

- Increase the risk of mortality
- Increase the risk of cardiovascular disease
- increased risk of hemorrhagic stroke

Because the body produces its own antioxidants so adding too many external antioxidants can disrupt the homeostatic balance your body tries to maintain which leads to complications (Coila *et al.*, 2011) [7].

Benefits of Antioxidants

1. Reduce the amount of free radicals in our body.
2. Reduce the risk of developing certain diseases such as; cancer, heart disease, stroke, cataracts, Parkinson's, Alzheimer's and arthritis (Phillip, 2012) [29].
3. Prevent direct cell damage caused by the chain reaction that free radicals initiate.
4. Reduce the signs of aging by preventing the oxidation of skin cells.
5. It improves our digestion.

Conclusion

Antioxidant plays an important role to reduce the amount of free radicals and to prevent cancer, and other disease. They also have role in slowing ageing process and preventing heart disease. It derived from food and medicinal plants have been increasingly investigated for their various nutritional function and health benefits. It increasing intake of dietary antioxidants may help to maintain an adequate antioxidant status. So antioxidants are very much necessary for our body. But our body can't manufacture these chemicals, so they must be supplied through diet.

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