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**Antioxidants**

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**1- Classiﬁcation of Antioxidan**

 Antioxidants can be classiﬁed into two major types based on their source, i.e., natural and synthetic antioxidants (schematic representation of the classiﬁcation of antioxidants is shown in Fig. 6.1

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**1-1 Natural Antioxidants**

 Natural antioxidants either are synthesized in human body through metabolic process or are supplemented from other natural sources, and their activity very much depends upon their physical and chemical properties and mechanism of action. This can be further divided into two categories, i.e., enzymatic antioxidants and nonenzymatic antioxidants

 **2.1.1 Enzymatic Antioxidants :**

 Enzymatic antioxidants are uniquely produced in the human body and can be sub-divided into primary and secondary antioxidant

**a- Primary Antioxidants** Primary antioxidants mainly include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) as described below.Superoxide Dismutase Superoxide dismutase (SOD) enzyme is found in both the dermis and the epidermis. It removes the superoxide radical (O2.−) and repairs the body cells damaged by free radical. SOD catalyzes the reduction of superoxide anions to hydrogen peroxide (6.1). SOD is also known to compete with nitric oxide (NO) for superoxide anion, which inactivates NO to form peroxynitrite.

**b- Secondary Antioxidant:-**

 Secondary antioxidant includes glutathione reductase (GR) and glucose-6- phosphate dehydrogenase (G6PDH). G6PDH generates NADPH. GR is required to recycle the reduced glutathione (GSH) using secondary enzyme GR and NADPH

Glutathione is a cysteine containing peptide-type antioxidant and is synthesized in the body cells. The thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. A high level of glutathione is found in the cells (~3,100 μg/g of tissue)), maintained in the reduced form (GSH) by the enzyme GR, and in turn reduces other metabolites and enzyme sys-tems, such as ascorbate. Due to its high concentration and its role in maintaining redox state in the cells, it is considered one of the most important cellular antioxi-dants..

 **2.1.2 –Nonenzymatic:-**

 AntioxidantsThey are a class of the antioxidants which are not found in the body naturally but are required to be supplemented for the proper metabolism (Raygani et al. 2007). Some of the known nonenzymatic antioxidants are minerals, vitamins, carotenoids, polyphenols, and other antioxidants as listed below.

**a-Minerals**

 Minerals are required in the body cells for the proper functioning of the enzymes. Their absence is known to affect the metabolism of many macromolecules. They include selenium, copper, iron, zinc, and manganese. They act as cofactors for the enzymatic antioxidants

 Selenium (Se) Selenium is also a very important component of enzymatic antioxidant. In the presence of selenium (Se), glutathione peroxidase (GPx) plays a protective role against oxidation of lipid and protects the cell membrane and takes part in H2O2 and lipids’ hydroxyperoxide metabolism. Hence, Se behaves like vitamin E and can be substituted in place of vitamin E and is used to prevent the risk of cancer and cardiovascular diseases (Sikora et al. 2008).Copper (Cu), Zinc (Zn), and Manganese (Mn) SOD is a class of enzyme that consists of different types of SODs, depending upon their metal cofactor such as Cu–Zn and Mn. Cu–Zn SOD is found in the cytosol having Cu and Zn at their active sites which helps in proton conduction, whereas Mn-SOD is found in mitochondria and has Mn at its active site. These metals are responsible for SOD’s antioxidant activities.

**b-Vitamins**

 Vitamins form the class of micronutrients required for the proper functioning of the body’s antioxidant enzyme system, such as vitamin A, vitamin C, vitamin E, and vitamin B. They cannot be synthesized in our body and hence need to be supple-mented in the diet.Vitamin A Vitamin A is helpful in night vision and in maintenance of epithelial cells in mucus membranes and skin. Because of its antioxidant properties, it assists immune system also and is found in three main forms: retinol, 3,4-didehydroretinol, and 3-hydroxyretinol. The main sources of this include sweet potatoes, carrots, milk, egg yolks, and mozzarella cheese.Vitamin C Vitamin C is water soluble and is also called as ascorbic acid. It is found in fruits (mainly citrus), vegetables, cereals, beef, poultry, ﬁsh, etc. It is helpful in preventing some of the DNA damage caused by free radicals, which may contribute to the aging process and the development of diseases, such as cancer, heart disease, and arthritis.Vitamin E Vitamin E is a lipid-soluble vitamin. This consists of eight different forms such as α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol.

**Carotenoid**

 Carotenoid consists of β-carotene, lycopene, lutein, and zeaxanthin. They are fat- soluble colored compounds found in fruits and vegetables. β-Carotene is found mostly in radish-orange-green color food items including carrots, sweet potatoes, apricots, pumpkin, mangoes, and cantaloupe along with some green and leafy veg-etables, including collard greens, spinach, and kale. Lutein is abundant in green leafy vegetables such as collard greens, spinach, and kale (Hamid et al. 2010). Lutein is best known for its role in protection of retina against harmful action of free radicals and also prevents atherosclerosis (Sikora et al. 2008).Although lycopene, lutein, canthaxanthin, and zeaxanthin do not possess provi-tamin A activity, β-carotene is known as a precursor for vitamin A (Fang et al. 2002). Tomato is a good source of lycopene and spinach is a good source of zeaxan-thin. It has been shown that lycopene is a potent antioxidant and is the most effective compound in removing singlet oxygen found in tomatoes, watermelon, guava, papaya, apricots, pink grapefruit, and other foods



**Polyphenols**

 Polyphenols is a class of the phytochemicals that possess marked antioxidant activi-ties. Their antioxidant activities depend on their chemical and physical properties which in turn regulates the metabolism depending on their molecular structures (Ajila et al. 2011). These consist of phenolic acids, ﬂavonoids, gingerol, curcumin, etc. (Amit and Priyadarsini 2011).Flavonoid is a major class of polyphenolic compound and is mostly found in vegetables, fruits, grains, seeds, leaves, ﬂower, bark, etc. Some of the spices, such as ginger and turmeric, are also good sources of polyphenolic compound, e.g., gin-gerol is obtained from the rhizomes of ginger, whereas curcumin (diferuloylmeth-ane) is the main bioactive component of turmeric and is known to possess good antioxidant activity. Curcumin is an excellent scavenger of ROS, such as O2.− radi-cals, lipid peroxyl radicals (LO2.), OH radicals, and nitrogen dioxide (NO2.) radi-cals, which induced oxidative stress. Curcumin has been shown to inhibit lipid peroxidation and has been shown to increase GSH levels also in epithelial cells which lead to lower ROS production (Biswas et al. 2005

**1-2 Synthetic Antioxidants**

 Synthetic antioxidants are artiﬁcially produced or synthesized using various tech-niques. Basically they are polyphenolic compounds mainly that capture the free radicals and stop the chain reactions. Polyphenolic derivatives usually contain more than one hydroxyl or methoxy group. Ethoxy quinine is the only heterocyclic, N-containing compound reported to be used as antioxidant in the food, especially animal feed. Mostly reported synthetic phenolic antioxidants are p-substituted, whereas the natural phenolic compounds are mostly o-substituted. The p- substituted substances are preferred because of their lower toxicity. Synthetic phenolic antioxi-dants are always substituted with alkyl groups to improve their solubility in fats and oils and to reduce their toxicity (Wanasundara and Shahidi 2005). These synthetic compounds possessing antioxidant activity are commonly used in pharmaceuticals, as preservatives for cosmetics and to stabilize the fat, oil, and lipid in food (Gupta and Sharma 2006).These new ﬁndings about the synthetic antioxidants have led the researches to develop new synthetic antioxidants in terms of their water solubility, stability, and non-toxicity. Characteristics of some of the known synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethylenediami-netetraacetic acid (EDTA), 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (ethoxy-quin), propyl gallate (PG), and tertiary butylhydroquinone (TBHQ), are given below (Hamid et al. 2010))

**1.2.1. BHA**

 It is a monophenolic, lipid-soluble antioxidant, better used for the lipid oxidation in animal fat compared to vegetable oil (Wanasundara and Shahidi 2005)

**1.2.2. BHT**

 It is also a monophenolic fat-soluble antioxidant but is more stable than BHA at high temperature, and both act synergistically. Many commercially available anti-oxidant formulations contain both of these antioxidants. BHA interacts with peroxy radicals to produce a BHA phenoxy radical which in turn may remove a hydrogen atom from the hydroxyl group of BHT. BHA is regenerated by the hydrogen radical provided by BHT. The BHT radicals so formed can react with a peroxy radical and act as a chain terminator (Wanasundara and Shahidi 2005)

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**1.2.3. EDTA**

 EDTA is a common sequestrant, water-soluble antioxidant added to foods, body care, and household products. It binds with trace minerals, such as copper, iron, and nickel, that may be present in the food product. If not inactivated, these minerals may lead to discoloration,

rancidity, and textural breakdown. When added as an antioxidant, EDTA prevents oxygen from causing color changes and rancidity

1.2.4 Ethoxyquin

 It is as an antioxidant primarily used to protect carotenoid oxidation in animal feeds, vegetables (potato etc.), and fruits (apples and pears) during storage.

**1.2.5 PG**

 It is an ester formed by the condensation of gallic acid and propanol. It acts as an antioxidant which is used as a food additive to protect mainly oils and fat in the food products.6.2.2.6 TBHQTBHQ is a highly effective diphenolic antioxidant. In foods, it is used as a preserva-tive for unsaturated vegetable oils and many edible animal fats. It does not cause discoloration even in the presence of iron and does not even change ﬂavor or odor of the material to which it is added. It is used industrially as a stabilizer to inhibit auto-polymerization of organic peroxides. It is also used as a corrosion inhibitor in biodiesel. In perfumery, it is used as a ﬁxative to lower the evaporation rate and improve stability. It is also added to varnishes, lacquers, resins, and oil ﬁeld additives. It can be used alone or in combination with BHA or BHT (Said et al. 2002).

**1.3 Sources of Antioxidants**

 Antioxidants can be derived from two main sources: natural source such as fruits, vegetables, cereals, legumes, beverages, spices, and animals (Table 6.1) and from agro-industry, e.g., waste and processing industry (Table 6.2)

 **1.3.1.Natural Sources**

**1.3.2 Agro-industry**

 In recent past, agro industry is also found to be one of the major sources for the production of antioxidants. They can be derived either from the waste produced from the agro industries or as a by-product during the processing of the food material.Food processing processes generally produce large amount of waste as well as by-products along with reasonable quantity of efﬂuent. These food processing by- products, agro-industrial waste, and efﬂuents typically consist of high amounts of proteins, sugars, and lipids along with speciﬁc organic compounds as well. Therefore, this could be used as a cheap and abundant source of ﬁne chemicals and secondary metabolites. Valuable natural antioxidants, antimicrobial agents,.

 **1.4.-Mechanism of Antioxidant Activity**

There are mainly three types of mechanism known for the antioxidant activity, viz., chain breaking, preventive, and synergetic. Schematic representation of these mech-anisms is given in Fig. 6.4a–c.6.5

