



# ANTIOXIDANTS: AN OVERVIEW ON THE NATURAL AND SYNTHETIC TYPES

Emad M. Atta<sup>[a,b]\*</sup>, Nawal H. Mohamed<sup>[c,d]</sup> and Ahmed A. M. Abdelgawad<sup>[d]</sup>

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Antioxidants were used to prevent oxidation process in foods which lead to rancidity and browning, DNA oxidation and have many positive physiological effects in human. The concentration and the absorption mechanism of natural antioxidants are important in obtaining the maximum beneficial effect. The sources of antioxidants must be carefully considered to maximize absorption and avoid the toxicity of higher concentration of synthetic groups. A general lack of information about antioxidants indicates by a survey of the general public. An organized effort to educate individuals about foods rich in natural antioxidants and the ability to recognize the major synthetic antioxidants on food labels would be highly beneficial, though more research needs to be done to fully understand their physiological effects.

\* Corresponding Authors

E-Mail: emadata@yahoo.com

[a] Chemistry Department, Faculty of Science, Jazan University, Jazan, KSA

[b] Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Egypt

[c] Chemistry Department, Faculty of Science, Girls' Section, Jazan University, Jazan, KSA

[d] Aromatic and Medicinal Plants Department, Desert Research Center, Cairo, Egypt

## Introduction

Antioxidants go through several processes before they can have consumed by the people. Researchers in the middle of the twenty-first century after doing many researches found that life span of people increases by the normal consumption of anti-oxidants and also it prevents several fatal diseases. At the end of 19<sup>th</sup> century, antioxidants are used for several industrial processes like prevention of metal corrosion, rubber vulcanization. The scientists found that these substances or anti-oxidants protects the metal from corrosion and limited the oxidation of the metals.<sup>1-2</sup>

Antioxidants are widely used as an ingredient in dietary supplement for promoting good health and preventing diseases like cancer, cardiovascular disease. In addition, they are also used as preservatives for foods. This indeed happened in the mid. 20<sup>th</sup> century. It all started with the attempts by scientists to extend the life of foods. By combining antioxidants with foods which have high unsaturated fat, the tests were able to prevent the onset of rancidity - a nasty process by which the unsaturated fats break down and produce a rancid-like smell and taste.<sup>3</sup> As this process continued; new information was brought to light, it was quickly discovered that a few of the key and vital vitamins - essential in the human diet - were actually able to be classified as antioxidants too. This meant that over the past 1000 years, people had been consuming antioxidants on a daily basis.<sup>4</sup>

The aim of this review is to explore the different types of natural and synthetic antioxidants, the suggested mechanism and physiological effects of each type as well, in addition to

the potential health risks associated with consuming the synthetic antioxidants. On the other hand, an overview on the definitions, uses, popular methods to assay antioxidant activity was stated.

## Oxidation of foods

### Oxidation process

Most foods are made up of several organic compounds that can easily undergo oxidation. Lipids (such as fats, oils, and waxes) in general have the greatest tendency to lose electrons. Auto-oxidation of lipids in food Triggered by exposure to light, heat, ionizing radiation, metal ions or metallo-protein catalysts can have a deteriorating effect on the food colour, flavour, texture, quality, and safety. Fats contained in food are chemically composed of triglycerides and oxidation leading to the rancidity of foods occurs at the unsaturated sites of the triglycerides.<sup>5</sup>

### Oxidants

The most common oxidants in biological systems are free radicals. Free radicals are atoms, molecules or ions with unpaired electrons that are highly unstable and active towards chemical reactions with other molecules. An unpaired electron in these free radicals, causes them to seek out and capture electrons from other substances in order to stabilize themselves. Although the initial attack causes the odd electron to be paired, another free radical is formed in the process, causing a chain reaction to occur.<sup>6</sup>

In the biological systems, the free radicals are often derived from oxygen, nitrogen and sulphur molecules. These free radicals are parts of groups of molecules called reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulphur species (RSS). For example, ROS includes free radicals such as superoxide anion ( $O_2^{\cdot-}$ ), perhydroxyl radical ( $HO_2^{\cdot}$ ), hydroxyl radical ( $OH^{\cdot}$ ), nitric oxide and other species such as hydrogen peroxide ( $H_2O_2$ ), singlet oxygen ( $O_2$ ), hypochlorous acid (HOCl) and peroxy nitrite

(ONOO<sup>-</sup>).<sup>7</sup> RNS are derived from nitric oxide through the reaction with O<sub>2</sub><sup>•</sup> to form ONOO<sup>-</sup>. RSS are easily formed from thiols by reaction with ROS.<sup>8</sup> ROS are produced during cellular metabolism and functional activities.

### Formation of free radicals in cells

Free radicals can be formed in 3 ways, (i) by homolytic cleavage of covalent bond of a normal molecule, with each fragment retaining one of the paired electrons, (ii) by loss of single electron from normal molecule and (iii) by addition of a single electron to a normal molecule. They are constantly being generated in vivo.<sup>9</sup>

Four endogenous sources appear to account for most of the oxidants produced by cells.

- (i) Normal aerobic respiration in which mitochondria consume O<sub>2</sub>, reduces it by sequential steps to produce O<sub>2</sub>, H<sub>2</sub>O<sub>2</sub>, and <sup>-</sup>OH as byproducts.
- (ii) Bacteria or virus infected cells get destroyed by phagocytosis with an oxidative burst of nitric oxide (NO), O<sub>2</sub><sup>-</sup>, H<sub>2</sub>O<sub>2</sub> and OCl.
- (iii) Peroxisomes produce H<sub>2</sub>O<sub>2</sub> as a byproduct of fatty acid and other lipid molecular degradation, which is further degraded by catalase. Evidence suggests that, certain conditions favor escape of some of the peroxide from degradation, consequently releasing it into other compartments of the cell and increasing oxidative stress leading to DNA damage.
- (iv) Animal Cytochrome P<sub>450</sub> enzymes are one of the primary defense systems that provides protection against natural toxic chemicals from plants, the major source of dietary toxins. Even these enzymes are protective against acute toxic effects from foreign chemicals, yet they may generate some oxidative byproducts that damage DNA.<sup>10-13</sup>

### Effect of oxidants on body tissue

Excessive amounts of free radicals can have deleterious effects on many molecules including protein, lipid, RNA and DNA since they are very small and highly reactive. ROS can attack bases in nucleic acids, amino acid side chains in proteins and double bonds in unsaturated fatty acids, in which <sup>-</sup>OH is the strongest oxidant. ROS attacking macromolecules is often termed oxidative stress. Cells are normally able to defend themselves against ROS damage through the use of intracellular enzymes to keep the homeostasis of ROS at a low level.

However, during times of environmental stress and cell dysfunction, ROS levels can increase dramatically, and cause significant cellular damage in the body. Thus, oxidative stress significantly contributes to the pathogenesis of inflammatory disease, cardiovascular disease, cancer, diabetes, Alzheimer's disease, cataracts, autism and aging.<sup>14-17</sup> In order to prevent or reduce the ROS induced oxidative damage, the human body and other organisms have developed an antioxidant defense system that includes

enzymatic, metal chelating and free radical scavenging activities to neutralize these radicals after they have formed. In addition, intake of dietary antioxidants may help to maintain an adequate antioxidants status in the body.

### Antioxidants

In foods, antioxidants have been defined as 'substances that in small quantities are able to prevent or greatly retard the oxidation of easily oxidisable materials such as fats,<sup>18</sup> therefore, in food science antioxidants are usually equated with chain-breaking inhibitors of lipid peroxidation, but not exclusively so. Many antioxidants have been studied and are used in a wide range of foods including beverages. Therefore, for foods and beverages, antioxidants are molecules that can be equated with the protection of macromolecules from oxidation.<sup>19</sup> In biological systems the accepted definition is that antioxidant is any substance that, when present at low concentrations compared to those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate.<sup>20-21</sup> This is a broader definition encompassing many vulnerable macromolecules (e.g. DNA, lipids and proteins) that can be affected by oxidation. In biological terms, it is accepted that any molecule that can retard or prevent the action of oxidants could be considered to be an antioxidant.<sup>22</sup> Such a broad definition means that compounds that inhibit specific oxidizing enzymes, react with oxidants before they damage molecules, sequester dangerous metal ions or even repair systems such as iron transport proteins, can fit the definition. Antioxidants can also be defined as substances that trap harmful forms of oxygen and prevent them from damaging cells.<sup>23</sup> Mechanistic definitions of antioxidants are usually focused on the ability to be a hydrogen donor or an electron donor. Many of the frequently cited assays of antioxidant capacity can be broadly categorized as either hydrogen transfer assays or single electron transfer reaction based assays. These assays measure the radical scavenging capacity or the reducing ability, respectively, not the preventative antioxidant capacity of the sample.<sup>24</sup>

### Antioxidants process

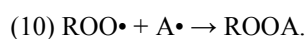
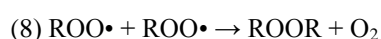
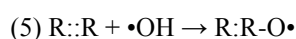
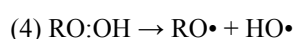
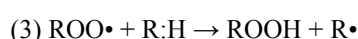
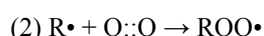
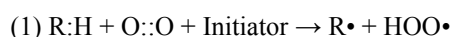
Antioxidants block the process of oxidation by neutralizing free radicals. In doing so, the antioxidants themselves become oxidized. How do they work? The two possible pathways are chain-breaking and preventive.<sup>25</sup>

**Chain-breaking:** When a free radical release or abstracts an electron, a second radical is formed. This molecule then turns around and does the same thing to a third molecule, continuing to generate more unstable products. The process continues until termination occurs, either the radical is stabilized by a chain-breaking antioxidant such as β-carotene and vitamins C and E, or it simply decays into a harmless product.

**Preventive:** Antioxidant prevents oxidation by reducing the rate of chain initiation. That is, by scavenging initiating radicals, such antioxidants can thwart an oxidation chain from ever setting in motion. They can also prevent oxidation by stabilizing transition metal radicals such as copper and iron.

## Mechanisms

If a compound inhibits the formation of free alkyl radicals in the initiation step, or if the chemical compound interrupts the propagation of the free radical chain, the compound can delay the start or slow the chemical reaction rate of lipid oxidation. The initiation of free radical formation can be delayed by the use of metal chelating agents, singlet oxygen inhibitors and peroxide stabilizers. The propagation of free radical chain reaction can be minimized by the donation of hydrogen from the antioxidants and the metal chelating agents.<sup>26</sup>



**Scheme 1.** Mechanism of antioxidants' action.

## Antioxidant Defenses

Antioxidant means "against oxidation." Antioxidants work to protect lipids from peroxidation by radicals. Antioxidants are effective because they are willing to give up their own electrons to free radicals. When a free radical gains the electron from an antioxidant it no longer needs to attack the cell and the chain reaction of oxidation is broken.<sup>27</sup> After donating an electron an antioxidant becomes a free radical by definition. Antioxidants in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive. The human body has an elaborate antioxidant defense system. Antioxidants are manufactured within the body and can also be extracted from the food which humans eat such as fruits, vegetables, seeds, nuts, meats, and oil.

## Types of Antioxidants

Antioxidant system includes, antioxidant enzymes (e.g., SOD, GPx and reductase, CAT, etc.), nutrient-derived antioxidants (e.g., ascorbic acid, tocopherols and tocotrienols, carotenoids, glutathione and lipoic acid), metal binding proteins (e.g., ferritin, lactoferrin, albumin, and ceruloplasmin) and numerous other antioxidant

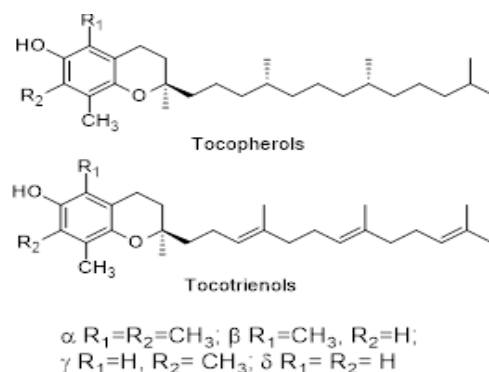
phytonutrients present in a wide variety of plant foods. Dietary antioxidants, such as water-soluble vitamin C and phenolic compounds, as well as lipid-soluble vitamin E and carotenoids, present in vegetables contribute both to the first and second defense lines against oxidative stress.<sup>28</sup>

## Natural antioxidants

Natural antioxidant system is sorted in two major groups, enzymatic and non-enzymatic.

**Non-enzymatic antioxidants:** Non-enzymatic antioxidants include direct acting antioxidants, which are extremely important in defense against oxidation stress. Most of them, including ascorbic and lipoic acid, polyphenols and carotenoids, are derived from dietary sources. The cell itself synthesizes a minority of these molecules. Indirectly acting antioxidants mostly include chelating agents and bind to redoxmetals to prevent free radical generation.<sup>29</sup>

Vitamin E is a generic description for all tocopherol (Toc) and tocotrienol (Toc-3) derivatives. Tocopherols have a phytyl chain, while tocotrienols have a similar chain but with three double bonds at positions 3',7' and 11'. Both tocopherols and tocotrienols have four isomers, designated as  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -, which differ by the number and position of methyl groups on the chromanol ring.<sup>30</sup> All of these molecules possess antioxidant activity, although  $\alpha$ -tocopherol ( $\alpha$ -Toc) is chemically and biologically the most active.<sup>31-32</sup>  $\alpha$ -Tocopherol is the major vitamin E *in vivo* and exerts the highest biological activity. Tocopherols are present in polyunsaturated vegetable oils and in the germ of cereal seeds, whereas tocotrienols are found in the aleurone and subaleurone layers of cereal seeds and in palm oils.



**Figure 1.** Structure of vitamin E.

It has been reported that  $\alpha$ -Toc-3 possessed 40- to 60-fold higher antioxidant activity than  $\alpha$ -Toc against ferrous iron/ascorbate- and ferrous iron/NADPH-induced lipid peroxidation in rat liver microsomes<sup>33</sup> and that  $\alpha$ -Toc-3 exhibited greater peroxy radical scavenging potency than  $\alpha$ -Toc in liposomal membranes.<sup>34</sup>

The antioxidant reaction of  $\alpha$ -tocopherol is not a reaction with oxygen. Many molecules react with oxygen, but they do so without being antioxidants.  $\beta$ -Carotene, for example,

readily reacts with oxygen, but it is by no means an efficient antioxidant.<sup>35</sup> The basis of an antioxidant reaction is not the removal of oxygen but the interception of the autoxidation radical chain process which is not perpetuated by oxygen but by the fatty acid.

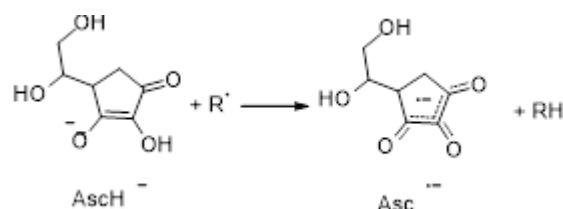
$\alpha$ -Tocopherol reacts with fatty acid peroxy radicals, the primary products of lipid peroxidation, and intercepts the chain reaction.<sup>36</sup> What makes  $\alpha$ -tocopherol such a highly efficient antioxidant is (i) that it reacts with the peroxy radical extremely fast, much faster than to allow for the peroxy radical to do any other reactions; (ii) it takes away the radical character from the oxidizing fatty acid and prevents it from further radical reactions; (iii) in the antioxidant reaction,  $\alpha$ -tocopherol is turned into a fairly stable radical. Under normal circumstances, it will only react with another radical (either a tocopheroxyl radical or a fatty acid peroxy radical) to form stable, non-radical products. In this setting,  $\alpha$ -tocopherol is the most powerful lipid soluble antioxidant known, and only recently novel synthetic antioxidants have been developed that surpass  $\alpha$ -tocopherol's antioxidant capacity.<sup>37</sup> Chemically, abstraction of the 6-OH hydrogen yields a tocopheroxyl radical. Tocopherol can be restored by reduction of the tocopheroxyl radical with redox-active reagents like vitamin C (ascorbate) or ubiquinol.<sup>38-42</sup> In homogeneous solution phase autoxidation, the tocopheroxyl radical will react with a second peroxy radical to give non radical products. This second reaction leads to the destruction of a tocopherol as an antioxidant. Thus, one molecule of  $\alpha$ -tocopherol can terminate two autoxidation chains.

The main source for dietary uptake of vitamin E is plant food (vegetables, fruits, seeds, and seed oils). Sunflower seeds, olive oil, and almonds are rich sources of  $\alpha$ -tocopherol. While other seeds and seed oils generally contain more  $\gamma$ -tocopherol than  $\alpha$ -tocopherol, the opposite is true for green leaves.  $\beta$ -Tocopherol and  $\delta$ -tocopherol are the least abundant, and so, in general, are the different tocotrienols.<sup>43</sup>

Vitamin C (ascorbic acid and ascorbate) is a six-carbon lactone which is synthesized from glucose by many animals. Vitamin C is a water-soluble vitamin. As such, it scavenges free radicals that are in an aqueous (water). When there is insufficient vitamin C in the diet, humans suffer from the potentially lethal deficiency disease scurvy.<sup>44</sup>

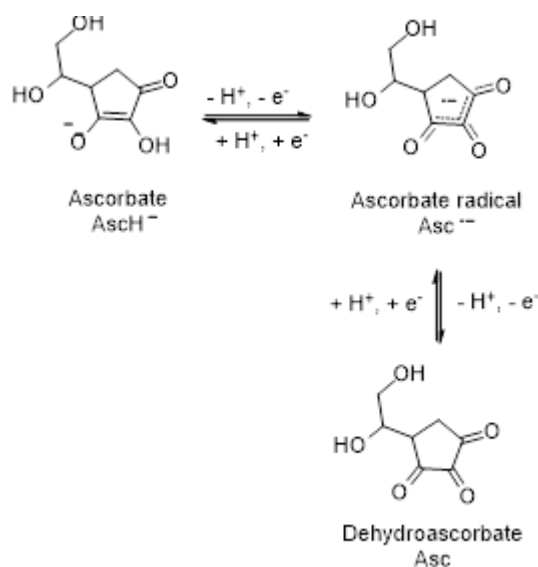
Vitamin C is an electron donor (reducing agent or antioxidant), and probably all of its biochemical and molecular functions can be accounted for by this function. Vitamin C acts as an electron donor for 11 enzymes.<sup>45-46</sup> Gastric juice vitamin C may prevent the formation of *N*-nitroso compounds, which are potentially mutagenic.<sup>47</sup> High intakes of vitamin C correlate with reduced gastric cancer risk,<sup>48</sup> but a cause and effect relationship has not been established. Vitamin C protects low-density lipoproteins *ex vivo* against oxidation and may function similarly in the blood.<sup>49</sup>

Vitamin C plays an important role in the production of collagen. Collagen gives your skin elastic properties. As people get older, their skin contains lower levels of collagen. Most anti-aging creams, therefore, include plenty of Vitamin C. This keeps the skin young and healthy by improving elasticity.



**Scheme 2.** Formation of ascorbate radical.

A common feature of vitamin C deficiency is anaemia. The antioxidant properties of vitamin C may stabilize folate in food and in plasma, and increased excretion of oxidized folate derivatives in human scurvy was reported.<sup>50</sup> Vitamin C promotes absorption of soluble non-haem iron possibly by chelation or simply by maintaining the iron in the reduced (ferrous,  $\text{Fe}^{2+}$ ) form.<sup>51-52</sup> The effect can be achieved with the amounts of vitamin C obtained in foods. However, the amount of dietary vitamin C required to increase iron absorption ranges from 25 mg upwards and depends largely on the amount of inhibitors, such as phytates and polyphenols, present in the meal.<sup>53</sup> Vitamin C (ascorbate,  $\text{AscH}^-$ ), for example, can donate a hydrogen atom to a free radical molecule thereby neutralizing the free radical (ascorbic acid, generally acts as an antioxidant by donating hydrogen atoms from its own hydroxyl groups in order to quench reactive radical species) and generating double bonds in place of the lost hydrogen to make up for the lost electron density. However, once this occurs, the Vitamin C molecule itself is oxidized, and so it is reduced back into a useable form of the Vitamin C molecule by a variety of enzymes, including glutathione.



**Scheme 3.** Oxidation of Vitamin C.

But the ascorbic acid free radical is very stable because of its resonance structure.<sup>54</sup> In general, recent literature on the interaction between vitamin C and vitamin E has provided strong support for the non-enzymatic regeneration of  $\alpha$ -tocopherol from the  $\alpha$ -tocopheroxyl radical, formed when  $\alpha$ -tocopherol scavenges a peroxy radical ( $\text{ROO}^\bullet$ ), by ascorbic acid.

Vitamin C is found in many fruits and vegetables.<sup>55</sup> Citrus fruits and juices are particular important sources of vitamin C but other fruits including cantaloupe, honeydew melon,



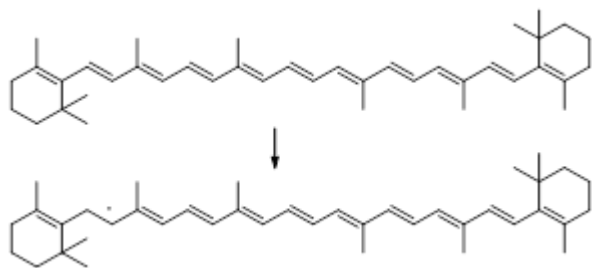
cherries, kiwi fruits, mangoes, papaya, strawberries, tangelo, watermelon, and tomatoes also contain variable amounts of vitamin C. Vegetables such as cabbage, broccoli, Brussels sprouts, beansprouts, cauliflower, kale, mustard greens, red and green peppers, peas, tomatoes, and potatoes may be more important sources of vitamin C than fruits. This is particularly true because the vegetable supply often extends for longer periods during the year than does the fruit supply.

### $\beta$ -Carotene

Carotenoids are a widespread group of naturally occurring fat-soluble colorants. In developed countries, 80-90% of the carotenoid intake comes from fruit and vegetable consumption. Of the more than 700 naturally occurring carotenoids identified, only six of them ( $\beta$ -carotene,  $\beta$ -cryptoxanthin,  $\alpha$ -carotene, lycopene, lutein and zeaxanthin) account for more than 95% of total blood carotenoids.  $\beta$ -Carotene (BC) is a naturally occurring orange-colored carbon-hydrogen carotenoid, abundant in yellow-orange fruits and vegetables and in dark green, leafy vegetables.<sup>56</sup> It is also the most widely distributed carotenoid in foods.<sup>57</sup> BC undergoes trans (E) to cis (Z) isomerization,<sup>58</sup> whereas the (all-E)-form is the predominant isomer found in unprocessed carotene rich plant foods.<sup>59-60</sup>

Nutrition has a significant role in the prevention of many chronic diseases such as cardiovascular diseases (CVD), cancers, and degenerative brain diseases.<sup>61</sup> The consumption of food-based antioxidants like BC seems to be useful for the prevention of macular degeneration and cataracts.<sup>62</sup> It is also available in synthetic forms and is commercially processed from substances such as palm oil and algae. BC has potential antioxidant biological properties due to its chemical structure and interaction with biological membranes.<sup>63</sup> It is well-known, that BC quenches singlet oxygen with a multiple higher efficiency than  $\alpha$ -tocopherol.<sup>64</sup> In addition, it was shown that (Z)-isomers of BC possess antioxidant activity *in vitro*.<sup>65-67</sup>

The other strategy with which antioxidants prevent oxidation is to use double bonds to donate electron density. As the electrons in double bonds are less tightly held to the molecule, they are more easily available for donation. Generally, antioxidants that use this strategy are non-polar, and contain hydrocarbon chains of moderate length. Three prominent natural antioxidants that utilize this method are carotene, lycopene, and vitamin A. Carotene and lycopene have very similar mechanisms of antioxidant activity, as both have similar chemical structures and fall into the carotenoid family of molecules.



**Scheme 4.** Oxidation of carotene via the donation of an electron from a double bond.

### Phenolic antioxidants

Phenolic compounds are a large group of the secondary metabolites widespread in plant kingdom. They are categorized into classes depending on their structure and subcategorized within each class according to the number and position of hydroxyl group and the presence of other substituents. The most widespread and diverse group of the polyphenols are the flavonoids which are built upon C6-C3-C6 flavone skeleton. In addition, other phenolic compounds such as benzoic acid or cinnamic acid derivatives have been identified in fruits and vegetables.<sup>68-69</sup>

Phenolic compounds, especially flavonoids, possess different biological activities, but the most important are antioxidant activity, capillary protective effect, and inhibitory effect elicited in various stages of tumor.<sup>70-73</sup> Phenolics are able to scavenge reactive oxygen species due to their electron donating properties. Their antioxidant effectiveness depends on the stability in different systems, as well as number and location of hydroxyl groups. In many *in vitro* studies, phenolic compounds demonstrated higher antioxidant activity than vitamins and carotenoids.<sup>74-75</sup>

The major antioxidative phenolics in plants can be divided into four general groups viz., phenolic acids (gallic, protocatechuic, caffeic, and rosmarinic acids), diterpenes (carnosol and carnosic acid), flavonoids (quercetin and catechin), and volatile oils (eugenol, carvacrol, thymol, and menthol).<sup>76</sup> Phenolic acids generally act as antioxidants by trapping free radicals whereas flavonoids can scavenge free radicals and metal chelates as well.<sup>77</sup>

Many mechanisms have been proposed for polyphenol prevention of oxidative stress and ROS/RNS generation both *in vitro* and *in vivo*. Radical scavenging by polyphenols is the most widely published mechanism for their antioxidant activity. In this radical scavenging mechanism, polyphenols sacrificially reduce ROS/RNS, such as  $\cdot\text{OH}$ ,  $\text{O}_2^{\cdot-}$ ,  $\text{NO}^{\cdot}$ , or  $\text{OONO}^{\cdot}$  after generation, preventing damage to biomolecules or formation of more reactive ROS.<sup>78-80</sup> The spatial arrangement of substituents is perhaps a greater determinant of antioxidant activity than the flavone backbone alone. Consistent with most polyphenolic antioxidants, both the configuration and total number of hydroxyl groups substantially influence several mechanisms of antioxidant activity.<sup>81-83</sup>

### Selenium

Selenium (Se) is an essential trace element and its deficiency in humans has been linked to increased risk of various diseases, such as cancer and heart diseases. Good food sources of selenium include fish, shellfish, red meat, grains, eggs and chicken. Vegetables can also be a good source if grown in selenium-rich soils. 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, as large doses of the supplement form can be toxic. The level of Se generally depends on its level in soil.<sup>84</sup> Selenium is a mineral, not an antioxidant nutrient. However, it is a component of antioxidant enzymes. Since its discovery as an important component of antioxidant enzymes, such as glutathione peroxidase (GPx), thioredoxinreductase (TrxR) and

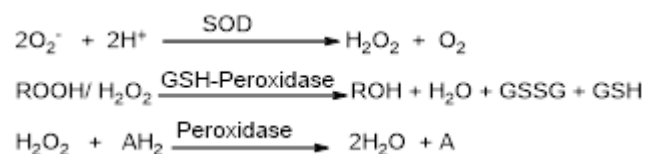
iodothyroninedeiodinases (IDD), there has been an increased interest in the study of other Se-containing proteins (selenoproteins) or enzymes (selenoenzymes).<sup>85</sup>

The selenoenzymes that are found to have strong antioxidant activity include six groups of the GPx-GPx1, GPx3, GPx4, GPx5 and GPx6. These GPx play a significant role in protecting cells against oxidative damage from ROS and RNS, which include superoxide, hydrogen peroxide, hydroxyl radicals, nitric oxide and peroxynitrite.<sup>86-87</sup> The other essential antioxidant selenoenzymes are the TrxR where they use thioredoxin (Trx) as a substrate to maintain a Trx/TrxR system in a reduced state for removal of harmful hydrogen peroxide.<sup>88-89</sup> There are three types of TrxR that have been identified, and these include cytosolic TrxR1, mitochondrial TrxR2 and spermatozoa-specific TrxR, (SpTrxR).<sup>90-91</sup> Increasing evidence suggests that selenoprotein may also play a significant role in antioxidant defense system in preventing attack from harmful ROS and RNS.<sup>92-93</sup>

### Enzymatic antioxidants

Antioxidant enzymes are capable of stabilizing, or deactivating free radicals before they attack cellular components. They act by reducing the energy of the free radicals or by giving up some of their electrons for its use, thereby causing it to become stable. In addition, they may also interrupt with the oxidizing chain reaction to minimize the damage caused by free radicals. By reducing exposure to free radicals and increasing the intake of antioxidant enzyme rich foods or antioxidant enzyme supplements, our body's potential to reducing the risk of free radical related health problems is made more palpable.<sup>94</sup> Antioxidant enzymes are, therefore, absolutely critical for maintaining optimal cellular and systemic health.

The repair enzymes that can recreate some antioxidants are SOD, GPx, glutathione reductase (GR), CAT and the other metalloenzymes. SOD, CAT, and GPx constitute a mutually supportive team of defense against ROS. While SOD lowers the steady-state level of O<sup>2-</sup>, catalase and peroxidases do the same for H<sub>2</sub>O<sub>2</sub>.



**Scheme 5.** Mechanism of action of enzymatic antioxidant.

The antioxidant enzymes—GPx, heme peroxidase, CAT, and SOD—metabolize oxidative toxic intermediates and require micronutrient cofactors such as selenium, iron, copper, zinc, and manganese for optimum catalytic activity.<sup>95</sup>

The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) serve as the primary line of defence in destroying free radicals. Glutathione plays several roles in the body e.g., it improves the effectiveness of Vitamin C. SOD first reduces (adds an electron to) the radical superoxide (O<sub>2</sub><sup>-</sup>) to form hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and oxygen (O<sub>2</sub>). Catalase and

GPx then work simultaneously with the protein glutathione to reduce hydrogen peroxide and ultimately produce water (H<sub>2</sub>O). The oxidized glutathione is then reduced by another antioxidant enzyme glutathione reductase. Together, they repair oxidized DNA, degrade oxidized protein, and destroy oxidized lipids (fat-like substances that are a constituent of cell membranes). Various other enzymes act as a secondary antioxidant defense mechanism to protect from further damage.<sup>95</sup>

SOD is the antioxidant enzyme that catalyzed the dismutation of the highly reactive superoxide anion to O<sub>2</sub> and to the less reactive species H<sub>2</sub>O<sub>2</sub>. Peroxide can be destroyed by CAT or GPx reactions.<sup>96-98</sup> In humans, there are three forms of SOD, cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (EC-SOD).<sup>99-100</sup> SOD destroys O<sup>2-</sup> by successive oxidation and reduction of the transition metal ion at the active site in a Ping Pong type mechanism with remarkably high reaction rates.<sup>101</sup> All types of SOD bind single charged anions such as azide and fluoride, but distinct differences have been noted in the susceptibilities of Fe, Mn or Cu/Zn-SODs. Cu/Zn-SOD is competitively inhibited by N<sub>3</sub><sup>-</sup>, CN<sup>-</sup>,<sup>102</sup> and by F<sup>-</sup>.<sup>103</sup>

SOD is found in our skin and it is essential in order for our body to generate adequate amounts of skin-building cells called fibroblasts. Among the common natural sources of SOD are cabbage, Brussels sprouts, wheat grass, barley grass and broccoli.

Catalase (CAT) is an enzyme responsible for the degradation of hydrogen peroxide. It is a protective enzyme present in nearly all animal cells. The functions of Human erythrocyte catalase include catalyzing the decomposition of H<sub>2</sub>O<sub>2</sub> to water and oxygen. It is a tetramer of 4 polypeptide chains. As with the chemical antioxidants, cells are protected against oxidative stress by an interacting network of antioxidant enzymes.<sup>104</sup>

Glutathione peroxidase (GPx) is an enzyme that is responsible for protecting cells from damage due to free radicals like hydrogen and lipid peroxides. The GPx contains a single selenocysteine (Sec) residue in each of the four identical subunits, which is essential for enzyme activity.<sup>105</sup> There are five GPx isoenzymes found in mammals. The function of glutathione peroxidase, therefore, is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free H<sub>2</sub>O<sub>2</sub> to water. Levels of GPx in the body are closely linked with that of glutathione, the master antioxidant. Glutathione (GSH for short) is a tripeptide that not only protects the cells against ill effects of pollution; it is also acts as the body's immune system boosters. It is present in high concentrations in the cells and plays a pivotal role in maintaining them in reduced state lest they suffer damage by oxidation by free radicals.

Combination of certain antioxidants like glutathione, vitamin C and E, selenium and glutathione peroxidase are very powerful in helping the body fight against the free radicals. GSH ensures that the red blood cells remain intact and protect the white blood cells (which are responsible for immunity). Glutathione is found in vegetables and fruit, but cooking will significantly reduce its potency. Taking it as a supplement is a good idea.

Glutathione-S-transferase (GST) is a family of enzymes comprising a list of cytosolic, mitochondrial and microsomal proteins which are capable of multiple reactions with multitude of substrates, both endogenous or xenobiotic. GSTs contribute to the phase II biotransformation of xenobiotics by conjugating these compounds with reduced glutathione to facilitate dissolution in aqueous cellular and extra cellular media and from there, out of the body. GSTs catalyse conjugation of reduced glutathione via the sulfhydryl group, to electrophilic centers on a wide variety of substrates. This activity is useful in the detoxification of endogenous compounds such as peroxidised lipids as well as metabolism of xenobiotics.

Glutathione reductase (GR) plays an indirect but essential role in the prevention of oxidative damage within the cell by helping to maintain appropriate levels of intracellular glutathione (GSH). GSH, in conjunction with the enzyme glutathione peroxidase (GP), is the acting reductant responsible for minimizing harmful hydrogen peroxide cellular levels. The regeneration of GSH is catalyzed by GR. GR catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione, using  $\beta$ -nicotinamide dinucleotide phosphate (NADPH) as the hydrogen donor. Molecules such as NADPH act as hydride donors in a variety of enzymatic processes. NADPH has been suggested to also act as an indirectly operating antioxidant, given its role in the re-reduction of GSSG to GSH and thus maintaining the antioxidative power of glutathione.

### Chemical pathways of natural antioxidants

After absorption, all antioxidants undergo certain chemical reactions in order to protect other compounds from oxidation. Most natural antioxidants have areas of high electron density within themselves in order to prevent other molecules from remaining as radicals for extended periods of time.

Natural antioxidants donate electrons from two major electron-rich sources: hydroxyl groups and double bonds. After donating electrons, natural antioxidants undergo additional chemical reactions in order to facilitate their breakdown. The first major method that several antioxidants use in order to prevent oxidation in other compounds is to donate electrons from their hydroxyl (-OH) groups.<sup>106</sup>

### Sources of natural antioxidants

Various antioxidants are supplied to human body through diet, both vegetarian as well as non-vegetarian. Vitamins C and E,  $\beta$ -carotene and coenzyme Q are the most common antioxidants of diet, out of which, Vitamin E is present in vegetable oils and found abundantly in wheat germ. It is fat soluble vitamin, absorbed in the gut and carried in the plasma by lipoproteins. Out of 8 natural isomeric forms of vitamin E,  $\alpha$ -tocopherol is the most common and potent isomeric form. Being lipid soluble, vitamin E can effectively prevent lipid peroxidation of plasma membrane.<sup>107-108</sup>

Plants (fruits, vegetables, medicinal herbs) may contain a wide variety of free radical scavenging molecules such as phenolic compounds (phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins etc.), nitrogen compounds (alkaloids, amines, betalains etc.), vitamins, terpenoids (including carotenoids) and some other endogenous metabolites which are rich in antioxidant activity.<sup>109-112</sup>

### Synthetic antioxidants

Synthetic antioxidants are chemically synthesized compounds since they do not occur in nature and are added to food as preservatives to help prevent lipid oxidation. Due to the inherent instability of natural antioxidants, several synthetic antioxidants have been used to stabilize fats and oils. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were originally developed to protect petroleum from oxidative gumming.<sup>113</sup> However, these compounds have been used as antioxidants in human foods since 1954 and are perhaps the most common antioxidants used in those foods today.<sup>114</sup> BHT and BHA not only have similar names, but similar structures and antioxidant activity and are often used together in fats and oils. Despite the fact that both BHT and BHA are included in the list of substances that are "generally accepted as safe". Certain chronic toxicity studies have implicated BHT as potential tumor promoter when fed at high levels.<sup>115-116</sup> In contrast, BHA and BHT, may both be important inhibitors of carcinogenesis, probably by way of their antioxidant function.<sup>117</sup> Thus, there have been some attempts to remove these antioxidants, TBHQ (tert-butylhydroxyquinone) is another synthetic antioxidant which is widely used in the feed industry. Like BHT and BHA, TBHQ has a benzene ring or phenol structure. Other examples of synthetic antioxidants are propyl gallate (PG), dodecyl gallate (DG), octylgallate (OG) and ethylene diaminetetraacetic acid (EDTA).

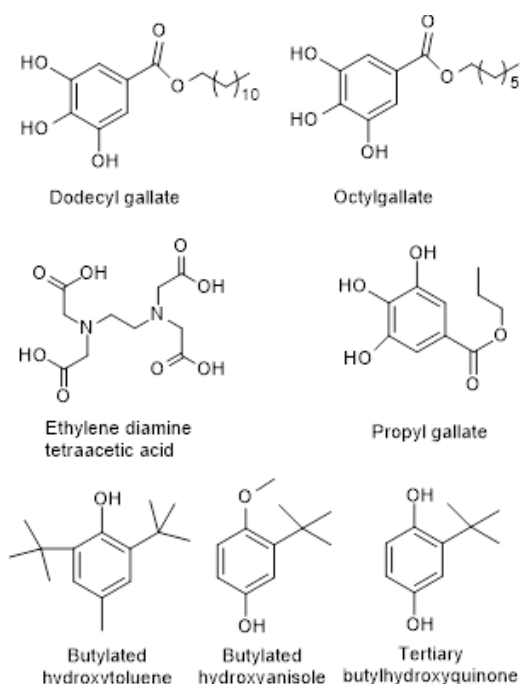


Figure 2. Structures of some synthetic antioxidant



### Methods for assessing in vitro antioxidant activity

Due to the increasing interest in these biological molecules for consumers, food scientists and the medical fraternity, a quick and easy method for determining antioxidant capacity would be most useful. The most promising methods used to evaluate antioxidant properties were summarized by Krishnaiah et al.<sup>118</sup>

### Uses of antioxidants in technology

Epidemiological studies have been reported that many of antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial and antiviral activities to greater or lesser extent.<sup>119</sup>

In many cases, increased oxidative stress is a widely associated in the development and progression of diabetes and its complications which are usually accompanied by increased production of free radicals or failure of antioxidant defense.<sup>120</sup> Though the intake of natural antioxidants has been reported to reduce risk of cancer, cardiovascular diseases, diabetes and other diseases associated with aging, there is considerable controversy in this area.<sup>121</sup>

Leukocytes and other phagocyte destroy bacteria, parasites and virus-infected cells with NO, O<sub>2</sub>, H<sub>2</sub>O<sub>2</sub>, and OCl<sup>-</sup>, which are powerful oxidants and protect humans from infection. However, they also cause oxidative damage and mutation to DNA and participate in the carcinogenic process if remain unchecked.

It has been reported that antioxidants modulate the pathophysiology of chronic inflammation up to some extent.<sup>122</sup> Moreover, experiments and studies infer that antioxidants are needed to scavenge and prevent the formation of ROS and RNS, out of them, some are free radicals while some are not.<sup>123</sup> There is growing evidence that oxidative damage to sperm DNA is increased when there is vitamin C insufficiency in diet.<sup>10</sup> This strongly suggests the protective role of antioxidant in our daily diet.<sup>124</sup>

The antioxidants control gene behaviour and prevent diseases. The antioxidant network is body's built-in intelligence. It constantly monitors the health of each of the trillions of cells in your body. Whenever a problem is detected, antioxidants will turn on the appropriate gene, which, in turn, activates the cells that it needs to solve the problem. For example, antioxidants direct genes to alert the immune system when there are invading viruses are detected. The immune system then creates more white blood cells to kill the viruses. But the process begins with the antioxidant network. Because antioxidants can help regulate dangerous genes, it opens up the possibility to treat diseases at their root cause, by suppressing bad genes before they can do harm, using antioxidants-the ultimate preventive medicine.

### Food Preservatives

Antioxidants are used as food additives to help guard against food deterioration.<sup>125</sup> Consequently, packaging of fresh fruits and vegetables contains an ~8% oxygen atmosphere milk and milk products like cheese; meat, fish and their products; spices and other dry foods like sugar, honey, beverages, and chewing gum.<sup>126</sup> Besides the direct addition to food items, the antioxidants can be used to preserve food by preventing the degradation of food packaging during processing and storage. Thus, antioxidants can be added to packaging materials like paper, polyethylene, plastic and paperboard preventing the oxidation of the material itself, or allowing the added antioxidants to migrate into the packaged food inside and prevent oxidation there.<sup>127-128</sup>

Antioxidants are an especially important class of preservatives.<sup>129</sup> These preservatives include natural antioxidants such as ascorbic acid and tocopherols as well as synthetic antioxidants such as, t-butylhydroquinone, BHA and BHT.<sup>130-132</sup> Antioxidant preservatives are also added to fat-based cosmetics such as lipstick and moisturizers to prevent rancidity.

### Industrial uses

Antioxidants are frequently added to industrial products. They are widely used to prevent the oxidative degradation of polymers such as rubbers, plastics and adhesives that causes a loss of strength and flexibility in these materials. Polymers containing double bonds in their main chains, such as natural rubber are especially susceptible to oxidation and ozonolysis. Oxidation and UV degradation are also frequently linked, mainly because UV radiation creates free radicals by bond breakage. The free radicals then react with oxygen to produce peroxy radicals which cause yet further damage, often in a chain reaction.

### Health benefits and risks

Due to the power of natural antioxidants to prevent the generation of free radicals, it has been found that they are particularly useful in preventing certain diseases. However, though it is apparent that natural antioxidants have many positive effects on health, it should also be taken into consideration that they could also have harmful effects if taken in excess.

### Conclusions

In foods that may undergo oxidation, antioxidants, function as an inhibitor of oxidation reactions through various mechanisms. Nevertheless, some foods are deficient in natural antioxidants and can easily deteriorate during processing or in storage, necessitating the use of synthetic antioxidants. However, most synthetic antioxidants are effective at low concentrations, and the addition of higher levels may lead to a pro-oxidant effect. Additionally, large doses of synthetic antioxidants have been reported to impart safety problems.



Therefore, caution must be taken when selecting and adding antioxidants in food systems. Meanwhile, the safety of natural antioxidants should not be taken for granted as antioxidants from natural sources are attracting more and more attention.

The best way to get a variety of antioxidants in the diet is to eat foods that represent all the colours of the rainbow. Each color provides its own unique antioxidant effects. Bright orange, deep yellow fruits and vegetables like carrots, sweet potatoes, and apricots provide one type of antioxidant. Red foods like tomatoes provide another. Green vegetables, such as broccoli and cabbage, and blue or purple foods, like blueberries, each have their own antioxidant packages.

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