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Vitamins as an Antioxidants

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الاهداء

دائما في كل امورك ثق بالله وتوكل واعلم أن الله سيكون معك بفضل الله سبحانه وتعالى وبفضل اهلي امي وابي لم يتبقى سوى أيام قليلة على إختتام مسيرتي الدراسية بتخرجي وفي هذه المناسبة اتقدم بالشكر لكل من ساندني في مسيرتي الدراسية شكرا لكل من قدم لي النصيحة شكرا لكل من أعطى لي الامل في مواصلة مشواري وابارك لكل اصدقائي الغوالي هذا التخرج واتمنى لهم كل التوفيق وفي النهاية اهدي تخرجي لاهلي جميعا أبي الغالي وامي واخوتي.

شكر وتقدير

لابد لنا ونحن نخطو خطواتنا الأخيرة في الحياة الجامعية من وقفة نعود إلى أعوام قضيناها في رحاب الجامعة مع أساتذتنا الكرام الذين قدموا لنا الكثير باذلين بذلك جهودا كبيرة في بناء جيل الغد لتبعث الأمة من جديد ...

وقبل أن نمضي تقدم أسمى آيات الشكر والامتنان والتقدير والمحبة إلى الذين حملوا أقدس رسالة في الحياة ...

إلى الذين مهدوا لنا طريق العلم والمعرفة ...

إلى جميع أساتذتنا الأفاضل.......

"كن عالما .. فإن لم تستطع فكن متعلما ، فإن لم تستطع فأحب العلماء ،فإن لم تستطع فلا تبغضهم"

وأخص بالتقدير والشكر:

الدكتورة : زينب نجم العبادي

Contents:

Summary

Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and well-being. Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. They are found in many foods, including fruits and vegetables. Although oxidation reactions are crucial for life, they can also be damaging; plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxides. Traditional herbal medicines, dietary foods were the main source of antioxidant for ancient peoples that protected them from the damage caused by free radicals. Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials of antioxidant supplements including beta-carotene, vitamin A, and vitamin E singly or in different combinations suggest that supplementation has no effect on mortality or possibly increases it.

1.1 Introduction

Antioxidants are believed to play a very important role in the body defense system against ROS (1,2). In another term antioxidant is "any substance that, when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate (3,4). It has been reported that an antioxidant is "any substance that delays, prevents or removes oxidative damage to a target molecule(4). Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. 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 study presents some information about the vitamins(E and C) as an antioxidant/antiradicals and their role in our body (4).

Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Antioxidants are found in many foods, including fruits and vegetables. Although oxidation reactions are crucial for life, they can also be damaging; plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase (4).

1.2 Free Radicals and Antioxidants:

Free radicals are atoms or molecules containing one or more unpaired electrons, making them very “reactive” (5). Biologically relevant free radicals are activated atoms or groups of atoms (usually containing oxygen or nitrogen) with an odd (unpaired) number of electrons. In a non-radical compound, all orbits are occupied by two electrons. When a chemical reaction breaks the bonds that hold paired electrons together, free radicals are produced. Therefore in a ‘free radical’ compound, there is a single unpaired electron in the outer orbit . A single excited electron is searching to become part of a paired set and will steal an electron from another, nearby atom to accomplish this pairing. During this theft, the original free radical becomes stable while the neighboring atom, by losing an electron, becomes a free radical itself. This new free radical will then seek out another atom to steal from, creating a chain reaction The extreme reactivity driven by a desire to acquire another electron underlies their ability to interact with and ultimately damage tissue. Biologically relevant molecules such as DNA, proteins, lipids and carbohydrates are damaged. Free radical collective terms are reactive oxygen species (ROS) and reactive nitrogen species (RNS) and include not only the oxygen or nitrogen radicals, but some non-radical reactive derivatives of oxygen and nitrogen (5).

1.3 Antioxidant defenses in the organism:

As a small part the oxygen consumed for aerobic processes will be converted into superoxide anion (19), which will have to be scavenged or converted into less reactive (and harmful) molecules. The main enzymes that regulate this process are Superoxide dismutase (SOD) Glutathione Peroxidase (GSH-Px) and Catalase (Figure 1). When ROS overproduction or chronic hyperglycemia occurs, the activity of these enzymes is insufficient, leading to more ROS and RNS formation and activation oxidative stress pathways (19).

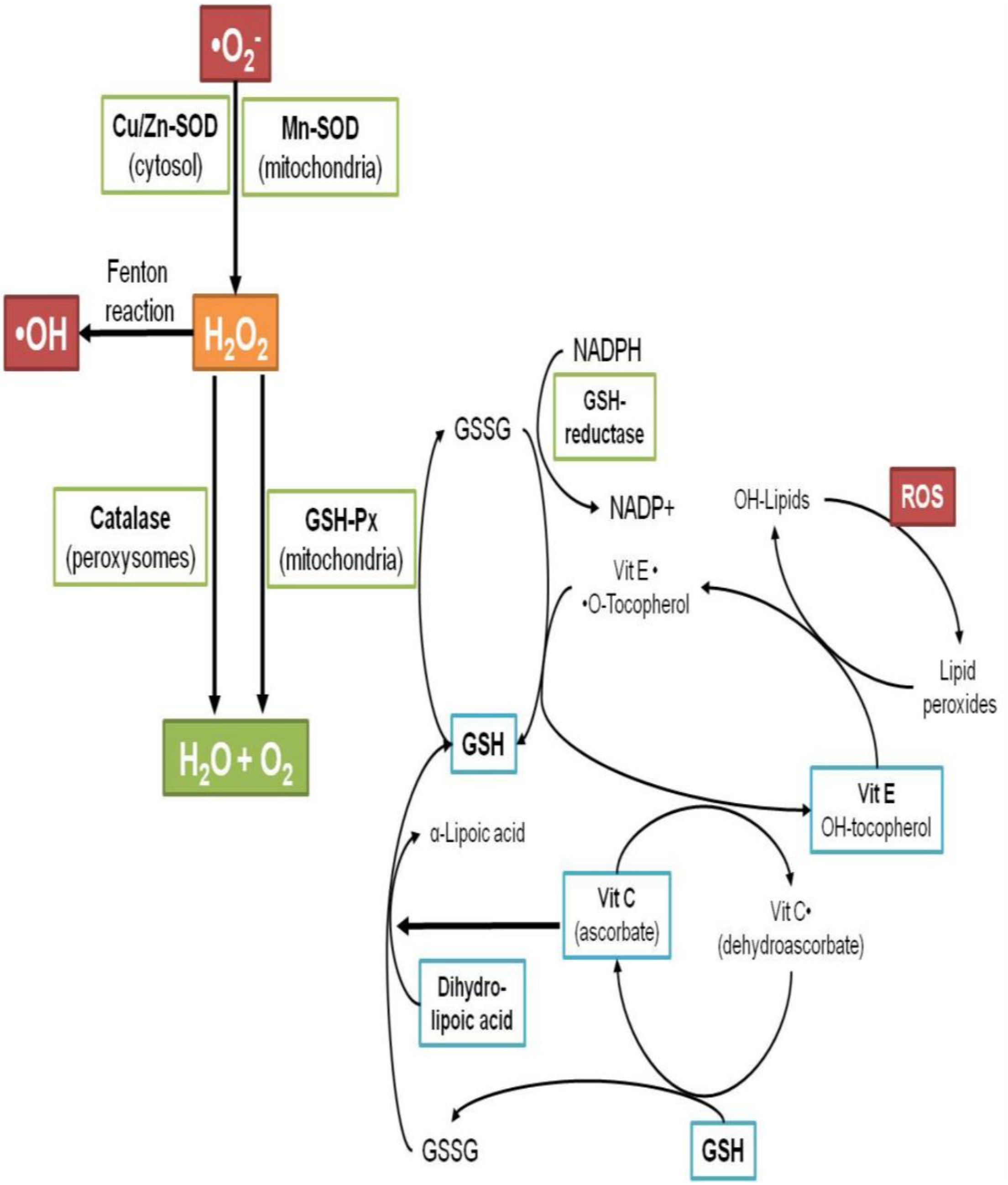

Figure 1. Antioxidant defenses in the organism.

SOD is considered a first-line defense against ROS. This enzyme is present in nearly all cells, and converts •O2 - into H2O2. Mitochondrial and bacterial SOD contain Mn, while cytosolic SOD is a dimer containing Cu and Zn. As the H2O2 may still react with other ROS, it needs to be degraded by either one of the other two antioxidant enzymes, GSH-Px or catalase (20, 21). GSH peroxidase is located in the mitochondria. It catalyzes degradation of H2O2 by reduction, where two gluthathione (GSH) molecules are oxidized to glutathione disulfide (GSSG) (21).

1.4 Function of antioxidants:

The Food and Drug Administration (FDA) defines antioxidants only as dietary supplements to be taken in addition to normal food consumption in an effort to prevent these diseases (6). Antioxidants are known to play a key role in the protective influence exerted by plant foods (7,8,10). Regular consumption of vegetables and fruits has been recognized as reducing the risk of chronic diseases (9). Studies demonstrate that an antioxidant-rich diet has a very positive health impact in the long run Sin et al. (11). Recently, antioxidants have attracted considerable attention in relation to radicals and oxidative stress, cancer prophylaxis and therapy, and longevity(12). All antioxidants are working in concert as a team, the (antioxidant system), responsible for prevention of the damaging effects of free radicals and toxic products of their metabolism. However, the antioxidant (team) acts to control levels of free radical formation as a coordinated system where deficiencies in one component impact the efficiency of others (13). Four possible mechanisms have been suggested (14) by which antioxidants function to reduce the rate of oxidation of fats and oils. These are hydrogen donation by antioxidants, electron donation by antioxidants, addition of lipid to the antioxidants and formation of a complex between lipid and antioxidants. Among food components fighting against chronic diseases, great attention has been paid to phyto-chemicals, plant derived molecules endowed with steady antioxidant power. The cumulative and synergistic activities of the bioactive molecules present in plant food are responsible for their enhanced antioxidant properties (14).

1.5 Various types of Antioxidants:

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

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 (23). Vitamin C, vitamin E, and beta carotene, Beta carotene and other carotenoids and oxycarotenoids, e.g., lycopene and lutein are among the most widely studied dietary antioxidants. In extracellular fluids vitamin C is considered the most important water-soluble antioxidant. It is capable of neutralizing ROS in the aqueous phase before lipid peroxidation

is initiated. Vitamin E, a major lipid-soluble antioxidant, is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation. Vitamin C has been cited as being capable of regenerating vitamin E (24). Beta carotene and other carotenoids are also believed to provide antioxidant protection to lipid-rich tissues. Research suggests beta carotene may work synergistically with vitamin E (25). 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 (26-29).

Synthetic antioxidant: Synthetic antioxidants are chemically synthesized since they do not occur in nature and are added to food as preservatives to help prevent lipid oxidation (30). These antioxidants fall into two major categories depending on their mode of action primary antioxidants and secondary antioxidants. The primary antioxidants, which prevent the formation of free radicals during oxidation, can further include three major categories.

1.6 Vitamins as Antioxidants :

An antioxidant is any substance that significantly reduces or impairs the oxidation of a substrate once present in small amounts. The term oxidizable substrate includes every type of molecule found in *vivo* (31). Antioxidant vitamins are readily available in food items; however, most of them are not available in sufficient quantities to meet the daily recommended amount for humans or animals. Thus, in order to avert problems associated with chronic deficiencies, supplemental doses have to be taken to meet up the shortages. Most of the classical deficiency syndromes associated with these vitamins are not seen in the western countries. However, chronic deficiencies are common and associated with a lot of complex health problems especially in the elderly (32). Antioxidant vitamins generally enhance different aspects of cellular and non-cellular immunity. The antioxidant function of these vitamins could, at least in part, enhance immunity by maintaining the functional and structural integrity of important immune cells. A compromised immune system will result in reduced animal production efficiency through increased susceptibility to diseases, thereby leading to increased animal morbidity and mortality.

One of the protective effects of vitamin C may partly be mediated through its ability to reduce circulating glucocorticoids. The suppressive effect of corticoids on neutrophil function in cattle has been alleviated with vitamin C supplementation. Vitamin C and E supplementation resulted in a 78% decrease in the susceptibility of lipoproteins to mononuclear cell-mediated oxidation (33). Ascorbic acid is reported to have a stimulating effect on phagocytic activity of leukocytes, on function of the reticulo endothelial system, and on formation of antibodies. Ascorbic acid levels are very high in phagocytic cells with these cells using free radicals and other highly reactive oxygen containing molecules to help kill pathogens that invade the body. In the process, however, cells and tissues may be damaged by these reactive species. Ascorbic acid helps to protect these cells from oxidative damage(33).

Mechanism of antioxidant vitamin action: Aerobic microorganisms are shielded from ROS and NOS produced from oxidative stress by a diverse mechanism involving multiple antioxidants which have different functions and roles (34). While all antioxidants are either micro or macro molecules such as proteins and enzymes, they all proffer several defensive strategies against damage caused by oxidant damage (35). The first defensive action involves the prevention of ROS/NOS production by catalyzing the breakdown of hydroperoxidase and hydrogen peroxides to hydroperoxides and water. In the second defensive action, the antioxidants neutralize ROS/NOS before they induce cellular injury to the cells. Thirdly, damage caused to membranes and tissues are repaired by these antioxidant compounds or enzymes. Hence, antioxidants act cooperatively and synergistically in a dynamic defensive network to cope with oxidative stress (34).

1.7 Types of Antioxidants Vitamins:

1.7.1 Vitamin E

Vitamin E is a group of eight antioxidant lipophilic molecules, four of which are tocopherols and four of which are tocotrienols. It is mostly found in green vegetables, grains, nuts and various vegetable oils, as well as in eggs and milk. Although it is commonly known today for its antioxidant properties, the first biological role attributed to vitamin E was its necessity for fetal survival (15).

Today vitamin E is known to possess many biological properties, including antioxidant activity and the ability to modulate protein function and gene expression. The evidence linking vitamin E and cancer risk is less extensive than that for vitamin C and carotenoids. Until recently, a lack of reliable information on the vitamin E content in foods has impeded epidemiologic studies of dietary vitamin E intake. Instead, most researchers have used blood vitamin E levels as a biomarker of vitamin E nutriture. The findings of these blood studies have been inconsistent; some have shown an inverse association between vitamin E levels and cancer risk, whereas others have found no association (15).

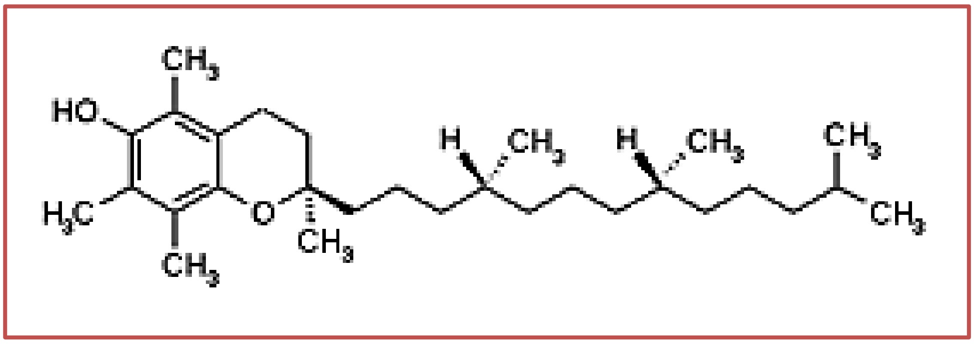

Figure 2: The structure of alpha-tocopherol form of vitamin E

1.7.2 Vitamin C

As opposed to vitamin E, vitamin C (L-ascorbate) is a hydrophilic molecule, and, therefore, it is found mostly in bodily fluids. Vitamin C is abundant in fruits and vegetables and they serve as the main source for dietary vitamin C intake. However, modern food processing methods lead to the loss vitamin C, as well as many other vitamins and nutrients (16). Isolated in1928, vitamin C was recognized as the bioactive molecule that was missing in the diet of sailors, causing scurvy (17). Vitamin C is known to take part in many physiological processes, and has been proposed to have a beneficial or therapeutic role in immune responses, cardiovascular disease and cancer (16). As noted above, the U.S. studies of female nurses and male health professionals did not find any association between vitamin C supplementation and coronary risk However, several other studies have shown effects of vitamin C. For example, the Basel prospective study showed that Swiss men with low blood vitamin C levels had an increased risk of dying from a heart attack during 12 years of follow-up compared with men with normal blood vitamin C levels. Studies comparing different European populations indicate that coronary heart disease mortality is higher in those with blood vitamin C levels that are almost in the deficient range (16).

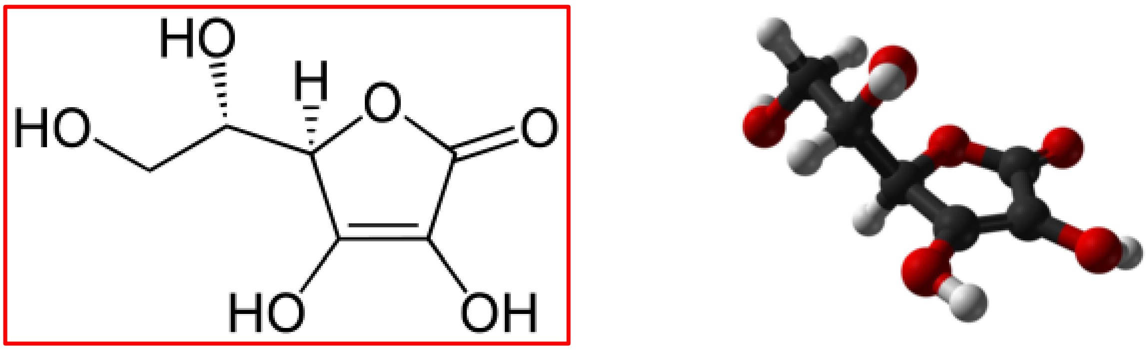

Figure 3: Structure of L-Ascorbic acid

Tocopherols and tocotrienols secured as lipophilic radical scavengers stability of membranes and longevity of seeds. Protection of aqueous compartments seems to have been linked to diversification in nitrogen excretion with uric acid as an important antioxidant in mammals together with ascorbic acid related to herbivore/omnivorous differentiation(18).

The principal antioxidant vitamins for tissue defense against free-radical damage includes vitamins E, C and β-carotene. Selenium would be the mineral most specifically related to antioxidant function. There are at least 35 antioxidant Se proteins and include selenoprotein P, five glutathione peroxidases and three thioredoxin reductases (TrXR1). Most recent research indicates TrXR1 reduces harmful ROS and facilitates gene expression of other cytoprotective antioxidants (22).

Chemistry of L-Ascorbate and Antioxidant Activity

L-Ascorbate's unique structure that includes two adjacent hydroxyl groups and a carbonyl makes this molecule an excellent hydrogen or electron donor. Therefore, it takes part as a co-factor in many enzymatic reactions, and also acts as a plasma localized anti-oxidant. Once oxidized, ascorbate is turned into ascorbate free radical (AFR), a molecule that is relatively stable due to electron delocalization. Although AFR can donate another electron, it does not undergo further oxidation. Rather, it is reduced back to ascorbate via NADH-dependent and independent mechanisms. AFR accumulation, resulting from increased oxidative conditions, leads to a reaction between two AFR molecules that form one molecule of ascorbate and one molecule of dehydroascorbate (DHA). DHA itself can either be reduced back to ascorbate, or hydrolyzed to gulonic acid (36). L-Ascorbate fulfills the requirements of an antioxidant, since it can react with radicals and terminate their reaction. Indeed, in the cellular environment where its concentrations are high and recycling mechanisms are abundant, L-ascorbate protects the cell from oxidative stress (37). However, L-ascorbate radical can also serve as an electron donor, and actually accelerate redox reactions in the presence of transition metals such as iron or copper. Thus, in the atherosclerotic plaque where ferric iron is present, vitamin C might serve as a pro-oxidant rather than as an anti-oxidant (38).

1.8 Antioxidant Vitamins and Disease:

As mentioned above previously, vitamins C, E, and A constitute the non-enzymatic defense against oxidative stress, by regenerating endogenous antioxidants (Figure 1). Vitamin C has a role in scavenging ROS and RNS by becoming oxidated itself. The oxidized products of vitamin C, ascorbic radical and dehydroascorbic radical are regenerated by glutathione, NADH or NADPH. In addition, vitamin C can reduce the oxidized forms of vitamin E and gluthatione (39). Vitamin E is a fat-soluble vitamin which may interact with lipid hydroperoxides and scavenge them. It also participates, together with vitamin C, in gluthatione regeneration by interaction with lipoic acid (40). Table 1 lists the best food sources of each of the major dietary antioxidants. As Table 1 indicates, two categories of foods — fruits and vegetables — are particularly important. These foods are the principal sources of both vitamin C and carotenoids. They also contribute substantial amounts of vitamin E to the diet as well as non-nutrient antioxidants. Authorities in several countries have recommended that everyone should consume at least five servings of fruits and vegetables daily, since population studies have revealed a lower incidence of certain degenerative diseases, such as cardiovascular disease and some forms of cancer, in subjects consuming larger amounts of fruits and vegetables. Unfortunately, the diets typically consumed in many parts of the world, including most of Western Europe and North America, fall far short of this goal (40).

1.9 Mechanisms of Antioxidants:

Antioxidants are effective via various mechanisms including:

1) preventive antioxidants,

2) free radical scavengers,

3) sequestration of elements by chelation and

4) quenching active oxygen species .

- **Preventive antioxidants** – These antioxidants suppress formation of free radicals; for example catalase (Fe containing) and glutathione peroxidase (Se containing), two antioxidant enzymes, decompose hydrogen peroxide, preventing formation of oxygen radicals.
- **Free radical scavengers:** These antioxidants confer stability to the 'reactive' species by donation of an electron and become oxidized themselves to form a more stable radical. For example α-tocopherol (vitamin E) scavenges peroxyl radicals and is converted to a tocopherol radical. Illustrating antioxidant interactions, the vitamin E becomes "re-activated" by ascorbic acid donating an electron which in turn forms an ascorbate radical in the process.
- **Sequestration of metal by chelation** - Although trace minerals are important dietary constituents, they can act as pro-oxidants (promote free radical formation). Since trace minerals such as Fe and Cu can propagate the formation of more reactive radicals they are kept bound to transport proteins such as transferrin or ceruloplasmin, as this renders them less available to contribute to radical or pro-oxidant formation.
- **Quenching of active oxygen species** - Antioxidants can convert active oxygen species to more stable forms, for example, carotenoids and vitamin E stabilize singlet oxygen radicals, forming less reactive hydrogen peroxide.

1.10 Sources of Antioxidants (Vitamin C, Vitamin E, and β-carotene) :

It is well known that fruit juices, beverages and hot drinks contain high amounts of antioxidants, like polyphenols, vitamin C, vitamin E, β-carotene, and lycopene (41). The consumption of fruit juices, beverages and hot drinks was found to reduce the morbidity and mortality caused by degenerative diseases (42-45). The recommendations based on epidemiological studies are such, that fruits, vegetables and less processed staple foods ensure the best protection against the development of diseases caused by oxidative stress, such as cancer, coronary heart disease, obesity, type 2 diabetes, hypertension and cataract (46). The explanation consists in the beneficial health effect, due to antioxidants present in fruit and vegetables (47).

Table 1: Food source of antioxidant vitamins

Vitamin E

Best sources:	vegetable oils, cold-pressed seed oils, wheat germ
Other significant sources:	vegetables, fruits, meat/poultry/fish

Vitamin C

Fruits:	especially citrus fruits, strawberries, cantaloupe melon
Vegetables:	especially tomatoes, leafy greens, cabbage-family vegetables such as broccoli and cauliflower

Carotenoids

ß-Carotene:	yellow-orange vegetables and fruits, dark green vegetables
α-Carotene:	Carrots
Lycopene:	Tomatoes
Lutein and zeaxanthin:	dark green leafy vegetables, broccoli
ß-Cryptoxanthin:	citrus fruits

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