UTILIZATION OF NATURAL ANTIOXIDANTS AND THEIR EFFECTS ON HUMAN HEALTH

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Abstract

Natural antioxidants as a nessecary biocompounds to protect human body and it can be used in the food industry. Antioxidants are considered as of synthetic antioxidants such as BHT, BHA and TBHQ and natural antioxidants such as flavonoids, isoflovonoids, saponins, tannins, minerals, vitamins. tocopherols, some enzymes, sterols, volatile oils and poly-unsaturated fatty acids.

Vegetables, fruits, cereals, legumes and herbs are the main sources of natural antioxidants and it can use genetic engenering and tissue culture to increase the natural antioxidants.

These phytochemical (natural abntioxidants) can have completely an overlapping mechanisms of action including modulation of detoxification enzyme, stimulation of immune system, modulation of cholesterol synthesis and hormone metabolism, reduction of blood pressure, prevented the onset of chronic diseases, antioxidant control to cystic fibrosis, antihyperilipidemic, antihyperglycemic, anticancer, antibacterial and antiviral effects.

Introduction:

In the recent year, there has been a global trend toward the use of natural phytochemicals present in natural sources, such as vegetables, fruits, herbs, cereals, grains and oilseeds, as antioxidants and functional food (Wang *et al.,* 1997 and Lee *et al.,* 2002). Natural antioxidants can be used in the food industry, and there is evidence that substances may exert their antioxidant effects within the human body. It effectively prevents premature lipid oxidation. Once isolated from animal or plant materials and used in processed food, lipids autoxidise readily. As a result, organoleptic and nutritional quality is reduced, and even toxic products may be formed. The retardation of autoxidation is therefore a key to high product quality. Because most consumers prefer natural food additives over synthetic ones, natural antioxidants are of increasing importance (Krings and Berger, 2001).

Major sections consisting of antioxidants vitamins (including not only vitamin E and C, but also carotenoids and retinoids), flavonoids, chlorophyll derivatives, polyphenols, hormones (such as, melatonin), phytochemicals, metabolities (such as, coenzyme-Q, α-lipoic acid and uric acid), enzyme (catalase, peroxidase, superoxide dimutase), glutathione, protein hydrolysates (protect lipids from oxidation), amino acids (arginine, histidine, cysteine, tryptophan, lysine, methionine and threonine), essential fatty acids (linoleic, linolenic, omega fatty acids), sterols, terpenes, alkaloids, tannins and saponins (Larson, 1988). These phytochemicals can have complementary and overlapping mechanisms of action, including modulation of detoxification enzymes, stimulation of immune system, reduction of platelet aggregation, modulation of cholesterol synthesis and hormone metabolism, reduction of blood pressure, prevented the onset of chronic diseases antioxidant, control to cystic fibrosis, antihyperlipidemic, antihyperglycemic, anticancer, antibacterial and antiviral effects (Byers and Perry, 1992; Cadenas and Packer, 1996; Rayner, 1998; Lampe, 1999; Leuschner and Ielsch, 2003 and El-Hadidy, 2004).

Various medicinal plants, herbs, spices, tea, grains, fruits and vegetables are the main sources of natural antioxidants (Vinson *et al.,* 1995 and 1998). Sies and Stahl (1995), Simon (1997), and Haslam (1998) indicated to become of current interest because of their potential amelioration of diseases simply by improving the dietary intake of nutrients with antioxidant properties, such as vitamin E and vitamin C, β-carotene and carotenoids, and plant phenolics, such as tannins and flavonoids. As such the food industry faces a challenge to include antioxidative phytochemicals in foods.

The most important dietary phenolics are phenolic acids (including hydroxybenzoic and hydroxycinamic acids), polyphenols (hydrolysable and condensed tannins) and flavonoids. Phenols protect plants from oxidative damage. King and Young (1999) also, studied extensively as antioxidant protectants for humans.

The distinctive properties of each antioxidant group (tocopherol, carotenoids, flavonoids, and vitamin C) highlight the importance of a varied complement of antioxidants in the diet. Among the groups of antioxidant molecules, the flavonoids are somewhat distinctive because of their different roles in plants vs. humans. Carotenoids, tocopherols and ascorbate are all recognized as playing a role of antioxidants in both plants and humans. However, flavonoids do not appear function as antioxidants in plants, and instead serve various ecological roles, such as protectants, attractants and repellents. It seems simply fortuitous that the flavonoids,

which can make a substantial contribution to the human diet, may be beneficial to health because of their antioxidant characteristic (Kushad, 2000).

Medical interest in antioxidants emerges from their capeability to prevent human diseases (cancer, diabetes, heart diseases, etc.) and the presence of such antioxidants in fresh fruits and vegetables has rekindled interest in lipid peroxidation in plant tissue. But the linkage among lipid peroxidation, membrane deterioration, and plant disorders have not yet been convincingly demonstrated. The most plausible explanation for the role of antioxidants in the prevention of human or plant disease is the prevention of propagation of peroxidation in membrane lipids (Shewfelt and del Rosario, 2000).

2- Types of Antioxidants

2.1- Polyphenols

Polyphenols are effective antioxidants in a wide range of chemical oxidation systems, being capable, for example of scavenging peroxyl radical, alkyl radicals, superoxide, hydroxyl radicals, nitric oxide and peroxynitrite in aqueous and organic environments (Duthie and Crozier, 2000). In a similar manner to vitamin E, this activity is essentially due to the ease with which an hydrogen atom from an aromatic hydroxyl (OH) group can be donated to a free radical and the ability of an aromatic compound to support an unpaired electron due to delocalization around the π-electron system.

2.1.1- *Phenolic compounds*

Phenolic compounds are commonly found in both edible and non-edible plants and they have been reported to have multiple biological effects including antioxidant activity. Crude extracts of vegetables, herbs, fruits, cereals and other plant materials rich in phenolics are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufactures, and

consumers as the trend of the future is moving toward functional food with specific health effects (Kahkonen *et al.*, 1999).

Phenolics are able to act as antioxidants in different ways. Hydroxyl phenols are good metal ion chelators. The implication of this is that metal-catalyzed non-enzymaztic free radical generation is thus suppressed in the presence of suitable phenolics. Also, phenolic structures often have the potential to interact strongly with proteins, mediated both by their hydrophobic benzenoid rings and the hydrogen-bonding potential of the phenolic hydroxyl groups. As mentioned, this gives phenolics the potential to act as enzyme inhibitors. Sometimes, as in the case of various tannins and other polyhydroxy-substituted phenolics, this interaction can be largely non-specific and there is a relatively general inhibitory effect. In other cases, discrete interaction occurs between individual phenolics and the active site of enzymes, and a more specific type of inhibition results. This ability of certain phenolics to modify selected enzyme activities is presumed to have a role in the physiological action of these phenolics, though the picture is yet incomplete, since the multiple interrelated actions of phenolics, some structure-specific, some non-specific, make it very difficult to obtain a detailed understanding of their mode of action (*Aruoma et al.*, 1996 and Arouma, 1997). Phenolic may also inhibit oxidation by chelating divalent metal ions and thus reducing the formation of free radicals induced by Fenton reactions (Robards *et al.*, 1999).

Studies on phenolic composition and antioxidant action of vegetable extracts have been reported. Beans, followed by beet, corn, and broccoli, showed highest total phenol content per fresh weight among 22 vegetable analyzed. By using a LDL oxidation method and a combined measure of the quality and quantity of phenol antioxidants present in the vegetables (per fresh weight), kidney and pinto beans were also ranked at the top in respect to the antioxidant ability. Garlic, yellow and red onion, asparagus, snaps bean, beet, potato, and broccoli were also evaluated among 10 most potent vegetable (Vinson *et al.*, 1998).

2.1.2- Flavonoids

In 1930 a new substance was isolated from oranges, which had been believed to be a number of a new class of vitamins, and was designated as vitamin P. When it became clear that this substance was a flavonoid (rutin), a flurry of research began in an attempt to isolate the various individual flavonoids and to study the mechanism by which flavonoids act. Moreover, flavonoids can be divided into various classes on the basis of their molecular structure. The four main groups of flavonoids are flavones, flavonones, catechins, and anthocyanins. The flavones and catechins seem to be the most powerful flavonoids for protecting the body against reactive oxygen species (Justesen *et al.*, 1998; and Justesen and Knuthsen, 2000).

Flavonoids belong to a group of natural substances with variable phenolic structures and are found in fruits, vegetables, grains, roots, stems, leaves, flower, tea, and wine. These natural products have been known for their beneficial effects on health long before flavonoids have been isolated as the effective compounds (Hertog *et al.*, 1992 a, b and 1993; Miura *et al.*, 1994; Mc-Donald *et al.*, 1998; and Chu *et al.*, 2000).

Flavonoids are widely distributed in the plant kingdom. They are mainly produced as a pigment of many shades, and play an important role in normal growth, development, and defense of plants. At the biochemical level, flavonoids act as enzyme inhibitors, provide defense against ultraviolet radiation, are chelating agents for metals, and act as reducing agents (Harris, 1992; Rice-Evan *et al.*, 1995). Furthermore, Flavonoids are synthesized only in plants. Dietary intake comes only through the food chain, and is obtained from fruits, vegetables and green leaves, and beverages such as bear, tea, coca and coffee. Flavonoids are also found in several medicinal plants and herbal remedies. They have been reported to exhibit a wide range of biological effects, such as antibacterial, antiviral, antiinflammatory, antiallergenic, anticancer and vasodilatory actions. They are known to inhibit lipid peroxidation and platelet aggregation and to affect capillary permeability and fragility. Metabolically, flavonoids inhibit the activity of various enzyme systems; including cyclooxygenase and lipoxygenase, act as antioxidants, free radical scavengers and chelators of actions. Intake of flavonoids is correlated with low incidence of coronary

disease and heart disease (Chu *et al.*, 2000; and Knekt *et al.*, 1996 and 2002).

A wide range of different biological activities, including antibacterial, antiviral, antithrombotic, vasodilatory, antiinflammatory, antiallergic and anticarcinogenic effects mediated by different mechanisms, are associated with flavonoid compounds. Several studies in human that have predicted the effect of flavonoids on cardiovascular risk, some showed on inverse association. In addition, flavonoids inhibit lipid peroxidation (LPO), platelet aggregation and the activity of enzyme systems including cyclooxygenase and lipoxygenase (Middleton and Kandaswami, 1993; Hertog *et al.*, 1993; Hanasaki *et al.*, 1994; Frankel, 1995; Halliwell *et al.*, 1995; and Cook and Samman, 1996).

The average daily flavonoid intake in the Netherlands is estimated to be 23 mg/d. Intakes of flavonoids exceed those of vitamin E and β-carotene, whereas the average intake of vitamin C is three items higher than the intake of flavonoids. Flavonoids intakes seem to vary greatly between countries; the lowest intake (≈ 2.6 mg/d) are in Finland and the highest intake (68.2 mg/d) is in Japan (Nijveldt *et al.*, 2001).

Over 4000 different flavonoids have been identified within the major flavonoids classes, which include flavonols, flavones, flavanones, catechin, anthocyanidins and isoflavonoids. Flavonoids are absorbed from the gastrointestinal tract of human and animals, and are excreted either unchanged or as flavonoid metabolites in the urine and feces. Flavonoids are potent antioxidants, free radical scavengers, and metal chelators and inhibit lipid peroxidation. The structure requirement for the antioxidant and free radical scavenging functions of flavonoids include a hydroxyl group in carbon position three, a double bond between carbonyl group in carbon position four, and poly-hydroxylation of the A and B aromatic rings. Hanasaki *et al.* (1994), and Cook and Samman (1996) have been reported to exhibit a wide range of biological effects, including antibacterial, antiviral, antiinflammatory, antiallergic and vasodilatory action.

In total, the flavonoid database includs values of 9 flavones (kaempferol, quercetin, myricetin, isorhmnten, apigenin, chrysin, luteolin, sibelin, and rutin), 6

flavanones (fisetin, hesperetin, naringin, naringenin and taxifolin), 3 catechins (catechin, epicatechin, and epigallocatechin gallate), and 6 anthocyanins (cyaniding, delphinidin, malvidin, pelargonidin, peonidin, and petunidin) (Knekt *et al.*, 2002).

The nutritional importance of vegetables and fruits, Kanner *et al.* (1994), and Vinson and Hontz (1995) investigated the flavonoids and vitamin C content of these products and their changes with post harvest treatments (modified atmospheres, minimal processing, domestic cooking, etc.). There is considerable evidence for the role of antioxidant constituents of vegetables and fruits in the maintenance of health and disease prevention.

Recent work is beginning to shed light on the relation of flavonoids and other dietary phenolic constituents to these protective effects. They act as antioxidants by virtue of the free-radical scavenging properties of their constituent hydroxyl groups. The extended conjugation across the flavonoid structure and an increasing number of the hydroxyl groups enhance the antioxidant properties, allowing them to act as reducing agents, hydrogen or electron-donating agents, or single-oxygen scavengers (Gil *et al.*, 1999).

The antioxidant effects of several other substances in plants such as flavonoids, which are even more potent antioxidants than vitamin C and E. One study tested the effect of supplements of vegetables and fruits extracts, including sources of flavonoids, on lipid peroxidation. The supplement included dried vegetable juice extract from parsley, spinach, cabbage, broccoli, kale, beets, carrots and tomatoes; and fruit juice extracts from apples, oranges and peaches. Plasma lipid peroxide concentration in the 15 subjects decreased from 16.85 to 3.13 μmol/l within 1-week and remained in this range through the additional 3-weeks of treatment (Lampe, 1999).

Quercetin, the most common flavonoid in higher plants, seems to contribute to the mutagenicity of kaempferol in the presence of microsomal metabolizing systems. Quercetin inhibits a number of enzymes, inhibits smooth muscle contraction and proliferation of rat lymphocytes. Although it is antiinflammatory, antibacterial, antiviral and antihepatotoxic, it exhibits mutagenic activity and allergenic properties

(Middleton and Kandaswami, 1993; Manach *et al.*, 1994 a,b; and Halliwell *et al.*, 1995).

Quercetin is one of the most abundant flavonoids in the human diet. There are many beneficial properties of quercetin, namely its antibacterial, antiviral, antioxidant, antiproliferative, antiinflammatory and anticarcinogenic effects. In food quercetin is present as glycosylated forms, mainly as β-glycosides. The nature of glycosylation is known to markedly influence the efficiency of quercetin absorption (Plumb *et al.*, 1998; and Crespy *et al.*, 2002). The same authors showed that in rats, as in humans, the absorption of rutin is delayed compared to that of quercetin, because rutin must be hydrolyzed by the microflora in the distal intestine prior to absorption of the aglycone. As the microflora also degrade the aglycone to phenolic acids, the absorption of rutin is less efficient than that of quercetin.

Many natural phenolics function in plants to discourage attack by fungal parasites, herbivorous grazers and pathogens. Many are also toxic and mutagenic in cell culture system and their consumption to excess by mammals could cause adverse metabolic reactions. For example, the unexpected increase in antioxidants enzymes detected by Nielsen *et al.* (1999) may reflect an adaptive response by the endogenous antioxidant defense system in the face of an impose stress incurred by the consumption of apeginin-rich parsley.

Apigenin suppressed 12-O-tetradecanoylphorbol-1,3-acetate (TPA)-mediated tumor promotion of mouse skin, as did curcumin, a dietary pigmented polyphenol, possibly through suppression of protein kinase C activity and unclear oncogene expression (Line *et al.*, 1997). Apigenin is antibacterial, antiinflammatory, diuretic, hypotensive, and also promotes smooth muscle relaxation (Duthie, 1999).

Nielsen *et al.* (1999) studied the effect of intake of parsley *(Petroselinum crispum)*, containing high levels of the flavone apigenin, on the urinary excretion of flavones and on biomarkers for oxidative stress. They found that the fraction of apigenin intake excreted in the urine was 0.58% during parsley intervention. Erythrocyte glutathione reductase and superoxide dismutase activities increased during intervention with parsley as compared with the levels on the basic diet, whereas erythrocyte catalase and glutathione peroxidase activities did not change.

Oxidative modification of LDL may play an important role in development of atherosclerosis. α-tocopherol functions as major antioxidant in human LDL. Zhu *et al.* (2000) tested whether four natural flavonoids (kaempferol, morin, myricetin, and quercetin) would protect or regenerate α-tocopherol in human LDL. All flavonoids showed a varying protective activity against depletion of α-tocopherol in LDL, with kaempferol and morin being less effective than myricetin and quercetin. The addition of flavonoids to the incubation mixture after 5min demonstrated a significant regeneration of α-tocopherol in human LDL. The protective activity of four flavonoids to LDL is related to the number of location of hydroxyl groups in the B ring as well as the stability in sodium phosphate buffer.

Flavonoids have long been recognized to possess antiallergic, anti-inflammatory, antiviral, antiproliferative, and anticarcinogenic activities. This is consistent with the inhibitory effects of these flavones (Kaempferol< quercetin< myricetin) on platelet aggregation induced by a combination of adenosine diphosphate (ADP), collagen, and platelet activating factor. The average intake of all flavonoids combined was ≈ 1g/d (Prior and Cao, 2000).

Flavonoids and other plant phenols are extensively metabolized by colonic bacteria: the ring structure is cleaved, giving a rage of phenolic acids which are then absorbed. Human studies showed that only about 1% of a well-absorbed flavonoid was excreted with an intact flavonoid backbone into urine (Hollman, 2001).

Absorption of flavonoids from the diet was long considered to be negligible, because most flavonoids, except for catechins, are present in plants bound to sugar as β-glycosides. Glycosides were considered too hydrophilic for absorption by passive diffusion in the small intestine, thus only aglycones were considered absorbable (Hollman *et al.*, 1995; Cook and Samman, 1996; and Plumb *et al.*, 1998).

Metabolism generally lowers the antioxidant activity of the dietary phenols, because mostly the phenolic hydroxyl groups are affected by metabolism. Conjugation of these hydroxyl groups with glucuronic acid, sulphate or glycine decrease their antioxidant activity. Ring fission also lower the antioxidant activity. The antioxidant activity of hydroxylated phenylacetic acids, major metabolites of quercetin, is much

lower than that of the parent quercetin, but similar to that of vitamin E *(Manach et al.*, 1994 a,b; Cook and Samman, 1996; and Nijveldt *et al.*, 2001)

Some enzymes, such as various cytochrome P450 isoforms, lipoxygenases, cyclooxygenases and xanthine oxidase, are potentially pro-antioxidant and can generate radicals. Certain flavonoids and phenylpropanoids are effective inhibitors of these enzymes. For example, quercetin inhibits 5-lipoxygenase. Certain flavonoids and purpurogallin derivatives have also been found to inhibit xanthine oxidase (Parr and Bolwell, 2000).

2.1.3-Catechins

Tea is one of the cheapest and most popular non-alcoholic beverages worldwide. Tea catechins have a variety of pharmacologic effects, i.e. antioxidative, antimutagenic,anticarcinogenic, anticancer promoting, antiinflammatory, antimicrobial and hypolipidemic. Catechins are the major components of tea; they constitute ≈30% of the dry weight of green tea and 9% of the dry weight of black tea (Harbowy and Balentine, 1997). Tea extracts are powerful antioxidants owing mainly to the presence of flavonols epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate (Salah *et al.*, 1995). These compounds believed to have physiological effects by acting as free radical scavengers (Quartley *et al.*, 1994). Tea catechins are more effective scavengers of free radicals, with more effective catechins having a galloyl moiety at C3 (Rice-Evans and Miller, 1996) and trihydroxy structure in the B ring (Nanjo *et al.*, 1996).

More recently, it was reported that ethanolic extract of green tea strongly inhibit oxidation of canola oil (Chen *et al.*, 1995; and Zandi and Gordon, 1999), and tea catechins retard oxidation of marine oils with a similar effectiveness to synthetic antioxidants (Wanasundara and Shahidi, 1996).

Tea is considered a major dietary contributor in reducing mortality from coronary heart disease in elderly men (Hertog *et al.*, 1993). Catechins, whether from tea or other sources, may reduce the risk of the ischemic heart disease mortality but not of stroke (Arts *et al.*, 2001).

Elevated plasma cholesterol is a major risk factor in the development of heart disease. Epidemiological studies have identified an inverse association between the consumption of green tea and plasma cholesterol, a major risk factor in the development of heart disease. The purified tea catechins, epicatechin gallate and epigallocatechin gallate, have also been observed to lower plasma cholesterol in mice and rats models of dietary-induced hypercholesterolemia (Imai and Nakachi, 1995; Tijburg *et al.*, 1997; and Chan *et al.*, 1999).

Oxidative modification of LDL may play an important role in the development of atherosclerosis. α-tocopherol functions as a major antioxidant in human LDL. Zhu *et al.* (2000) tested whether green tea catechins would protect or regenerate α-tocopherol in human LDL. They suggested that green tea catechin as a mixture or the individual epicatechin derivatives can be absorbed and circulated into the blood, where they may function as antioxidants protecting LDL from oxidation either directly, by protecting LDL from the attack of free radicals, or *via* the mechanism of maintaining and regenerating α-tocopherol. All of growing evidence so far suggests the recycling of α-tocopherol by water-soluble antioxidants including ascorbate, thiols and green tea epicatechin derivatives may be important mechanism for the enhanced antioxidant protection of LDL and the reduced risk of cardiovascular diseases.

Green tea contains various antioxidative flavan-3ols (tea catechins), which exert potent inhibitory effects on LDL oxidation *in vitro* and *in vivo* in humans. Miura *et al.* (2001) suggested that chronic ingestion of tea extract prevents the development of atherosclerosis without changing the plasma lipid level in apo-E deficient mice, probably through the potent antioxidative activity of the tea.

Brusill *et al.* (2001) determined the effects of green tea on the expression of the hepatic low density lipoprotein (LDL) receptor, a cell surface protein involved in the control of plasma cholesterol. Incubating human HepG2 liver cells in culture with green tea increased both LDL receptor binding activity and protein. An ethyl acetate extract of green tea, containing 70% (w/w) catechins, also increased the LDL receptor binding activity, protein and mRNA, indicating that the effect was at the level of gene transcription and that the catechins were the active constituents. Green tea

decreased the cell cholesterol concentration (-30%) and increased the conversion of sterol regulated elements binding protein (SREBP-1) from the inactive precursor from the active transcription-factor form. Consistent with this, the mRNA of 3-hydroxy 3-methylglutryl COA reductase, the rate-limiting enzyme in cholesterol synthesis, was also increased by green tea.

Physiological intakes of specific dietary polyphenols, such as catechin may play an important role in cancer chemoprevention. Ebeler *et al.* (2002) showed that dietary catechin significantly delayed tumor onset; a positive, linear relation was observed of dietary catechin or plasma catechin and metabolite concentrations. They showed that. Catechin 0.5 - 4 mmol/kg diet, was absorbed by mice, had no adverse health effects, and delayed tumor onset in a linear dose-dependent manner in a transgenic animal model of neurofibromatosis. Plasma concentrations of total catechin and metabolite increased with increasing catechin concentrations in the diet and were positively correlated with the delay in time of tumor onset.

2.1.4- Tannins

Tannins have been defined as, any phenolic compound of sufficiently high molecular weight containing sufficient hydroxyl and other suitable groups (i.e., carboxyl's) to form effectively strong complexes with protein other macro-molecules (Horvarth, 1981).

It well known that plant polyphenols act as free radical scavengers *in vitro;* tannins occur naturally in plant foods such as tea leaves, grape seeds, vegetables, and fruits juices, and exhibit antioxidants effects (Uchida *et al.*, 1991 and Constantino *et al.*, 1992).

Recently, there has been considerable interest in the ability of certain tannins to act as scavengers of free radicals (Yokozawa *et al.*, 1998) and antioxidants (Hagerman *et al.*, 1998), raising the possibility of their medicinal use.

Vegetable tannins are water-soluble phenolic compounds which can cause precipitation of alkaloids gelatin, and other protein in addition to phenolic reactions.

They can be classified into hydrolyzable and non-hydrolyzable, or condensed tannins. Hydrolyzable tannins contain either gallotannins or ellagitannins. Upon hydrolysis by acids, bases, or certain gallic enzymes, gallotannins yield glucose and gallic acid. Ellagitannins contain gallic acid and a molecule of hexahydroxy diphenic acid linked to glucose ester. Upon hydrolysis, the hexahydroxy diphenic acid undergoes lactonization to produce ellagic acid. Condensed tannins are structure more complex. They are mainly the polymerized products of flavan-3-ols and/or flavan-3, 4-diols (Chung and Wei, 1997).

Most of the condensed tannin-protein complexes remained insoluble under conditions similar to those in the stomach and the small intestine, suggesting that histatins may act as a defense against dietary tannin in humans (Naurato *et al.*, 1999).

Remesy *et al.* (1996) studied the nutritional interest of tannins and their classification, polymerization, food sources, intestinal absorption, transport in plasma and metabolism, protection against cardiovascular diseases, association with fiber and cholesterol elimination, oxidation of low density lipoproteins, benefits to blood circulation and protection against cancer. The authors suggested that research is required into polyphenols bioavailability and the mechanisms underlying their biological effects.

Tebib *et al.* (1994 a,b) showed that the protective effect of grape seed tannins against plasma cholesterol and LDL cholesterol. The hypothesized would exert a beneficial effect against oxidant stress.

The lipid peroxidation in plasma and tissues was significantly reduced in the presence of supplemented polymeric tannins as much as presence of vitamin E. It is therefore likely that polymeric grape seed tannins function as antioxidants *in vivo* (Tebib *et al.*, 1997).

Plumb *et al.* (1998) reported that proanthocyanidins were shown to scavenge $O^{\cdot}_2$, $OH^{\cdot}$ in aqueous solutions, often as efficiently as quercetin or butylated hydroxyl toluene (BHT).

Frei (1995) reported that the protective role of proanthocyanidins of wine and tea polyphenols against cardiovascular disease by inhibiting of LDL oxidation and platelet aggregation. Santos-Buelga and Scalbert (2000) added that LDL is oxidized by free radicals generated from endothelial cells, monocycle-derived macrophages and smooth muscle cells, resulting in several chemical and physical changes of LDL and rapidly accumulated by resident macrophages, thus oxidized LDL initiates and promotes atherogenesis in several ways. An association between proanthocyanidins consumption and cardiovascular disease has never been examined in humans, but several epidemiologists have studied the effects of the consumption of black tea rich in tannin-like phenolic polymers on cardiovascular disease. The same authors cited that proanthocyanidins inhibited the radical peroxidation of lipids by several mechanisms, either by scavenging the radical used to indicate lipid peroxidation or the lipid peroxyl radicals themselves, or by inhibiting the formation of the initiating radical through chelating of the formation of the transition metal Fe^{+++} or Cu^{++} added together with a reducing agent to generate $OH^{\cdot}$ radicals. However, in some experiments addition of Cu^{++} together with polyphenols accelerate the peroxidation of methyl linoleate.

El-Shemy *et al.* (2000) studied the effect of tannins presented in soy bean and faba bean on some body constituents and enzymes activity of male albino rats. They found that addition of soybean and faba bean as well as tannins to the basal diet caused a decrease in the average gain in body weight, alteration of food intake and food efficiency and slight differences between treatment and weight of organs. While, they found no change in the level of nucleic acid (RNA and DNA) in the different organs (liver, kidney, and brain), and normally changes in alkaline phosphatase and transaminases enzymes.

2.1.5- Saponins

Saponins are complex groups of compounds that occur naturally in plants, and are used in plants as raw material for food production. Saponins are glycosides with steroid or triterpene aglycon. Three mains types of steroid aglycones are known:

the spirostan, furostan and nautigenin derivatives. The most well known triterpene aglycones are derivatives of oleanan (Mahato *et al.*, 1988; and Takechi *et al.*, 1992).

Saponins can not penetrate the intestinal wall, but work within the small and large intestine. They bind the cholesterol making it unavailable for reabsorption, then it passed on into the colon and eventually excreted and this forces the liver to produce more bile acid. To produce bile acid (which could be from endogenous cholesterol), the liver must remove cholesterol from the blood stream, leaving less to build-up in the arteries. In this way saponins cause a depletion of body cholesterol by preventing its reabsorption, thus increasing its excretion (Oakenfull *et al.*, 1984), in much the way as other cholesterol-lowering drugs, such as cholestyramine (Lacaille-Dubois and Wanger, 1996).

Sidhu and Oakenfull (1986) reported that saponins may also interact with bile acids; the two compounds form micelles due to the interaction of hydrophobic segments of the molecules. These complex formation are not absorbable (hinders the cholesterol absorption in the small intestine).

Stewart *et al.* (1987) reported that the toxicity of saponins vary widely depending on the animal and consumption conditions. They added that saponins are very toxic to fish. Some saponins are lethal even in a dilution 1: $2X10^5$ however; the toxicity of saponin administrated orally is much lower for the warm-blooded animal. The toxic level is generally over 50-100 mg kg^{-1}, this low level of toxicity is attributed to the absorption rate of saponins. In contrast, to per oral administration, the parenteral use of saponins has a strong toxic effect. The toxicity of individual saponins ranging from 0.67 to 50 mg kg^{-1} (vary widely).

Saponins bind bile acid, it has been reported that saponins diet lower cholesterol concentrations. Potter *et al.* (1993) explained the formation of insoluble saponin-cholesterol complexes and its effects on lipid metabolism of animals and human have been known for a relatively long times. Saponins (triterpene saponins) confirmed the complex formation with cholesterol, ergosterol, and 7-dehydrocholesterol. Saponins may also interact with bile acids. As an additional effect, the binding of bile acids by saponins is mentioned. This is confirmed by increased

bile acid excretion in feces if diet contains higher amounts of saponins. The increased bile acid excretion needs a higher production of bile acid, which could be formed from endogenous cholesterol and so reduces the remaining cholesterol quantity.

Oakenfull and Sidhu (1990) reported that saponins could lower plasma cholesterol values by directly inhibiting absorption of cholesterol from the small intestine or indirectly inhibiting the reabsorption of bile acids, therapy increasing fecal excretion of bile acids. Reduced entry of cholesterol or bile acids into the enterohepatic circulation results in the stimulation of cholesterol synthesis mainly by liver. In certain foodstuffs naturally occurring compounds tocotrienols may suppress hepatic cholesterologensis, such foodstuffs could be combined with saponin-containing foods to control hypercholesterolemia more effectively.

Harwood *et al.* (1993) reported that natural and synthetic saponins inhibit cholesterol absorption and reduce plasma cholesterol levels in experimenta; animals and are therefore of potential pharmacologic utility in the treatment of hypercholesterolemic. They evaluated the effects of the synthetic saponin, beta-tigogenin cellobioside (Tiqueside, CP-88818), on male golden Syrin hamsters. It was found that tiqueside inhibits cholesterol absorption without interfereing with enterohepatic bile acid recirculation.

Kinitia (1996) indicated that spirostanol saponins are more active than furostanol saponins in screening for the ability to remove excess cholesterol from blood.

Steroidal glycosides have growing interest owing to the wide range of their biological action on living organism, including antidiabitic, antitumor, antitussive and platelet aggregation inhibitor activities. Sang *et al.* (2001) added the *Allium* genus ~500 species is a rich source of steroidal saponins as well as sulfur containing compounds. The leaves of Chives have been used for treatment of abdominal pain, diarrhea, hematemesis, snakebite and asthma.

Okoli and Akah (2000) tested the acute toxicity of the crude methanol extract (CE) of *Culcasia scandens* and its fractions. Phytochemical analysis of the extract and

its fractions revealed the prescience of saponins. They indicated that the median lethal dose of the crude extract in mice was greater than 5g/kg.

Matsuura (2001) found that the saponin fractions from garlic lowered plasma total and LDL cholesterol concentrations without changing HDL cholesterol levels in a hypercholesterolemic animal model. The author suggested that special consideration should be given to steroid saponins, as well as organosulfur compounds, in biological and pharmacological studies of garlic and its preparations.

2.2- Carotenoids

Carotenoids are class of lipophilic pigments that are found in nature in a wide variety vegetables and fruits. There is general interest in their metabolism and potential biologic and health-related function (s) because of well established correlation between increased of vegetables and fruits consumption and decreased risk of chronic diseases such as cancer and atherosclerosis. Epidemiologic studies that have examined disease risk and intake of specific carotenoids have suggested an inverse correlation between plasma levels of carotenoids and the incidence of coronary heart disease (Gey *et al.*, 1991; and Kardinal *et al.*, 1995).

American consume, on average, ≈ 6mg carotenoids daily (Chug-Ahuja *et al.*, 1993), with the amounts of the major carotenoids lutein, β-carotene, and lycopene ≈ 1.3, 1.8, and 2.6 mg/d, respectively.

Erdman Jr (1999) showed that healthy young men and women (mean age: 22y) in Netherlands consumed carefully controlled diets high (490 g/d) or low (130 g/d) in vegetables, the latter being provided with and without a supplement of highly bioavailable β-carotene (6mg/d) and lutein (9 mg/d). The diets provided equivalent amounts of protein, fat, fiber, and vitamin E, but differed in vitamin C and carotenoids contents. Measured outcomes included changes in plasma carotenoids and vitamin C, *ex vivo* estimation of the oxidizability of LDL. The author added that the bioavailabilities of β-carotene (14%) and lutein (67%) when comparing the high-vegetable diet with the low-vegetable diet plus the supplemental carotenoids.

De-Pee *et al.* (1998) reported that only 7% relative bioavailability of β-carotene from green leafy vegetable. They added a relative bioavailability of 23% for β-carotene from leafy vegetables plus carrots.

A new carotenoid-rich product was formed by entrapment of *Dunaliella salina* (halo-tolerant microalgae). The highest concentrations of β-carotene are found in the *Dunaliella salina,* reaching level of up to 100g/kg on a dry weight basis (Leach *et al.*, 1998).

Romanchik *et al.* (1995) showed that carotenoids are transported in plasma with lipoprotein, predominantly LDL. Because carotenoids have the ability to function as antioxidant, it is possible they could function in part to protect LDL from oxidation. Such protection might be important in the development of atherosclerosis. Plasma (and hence LDL) levels of β-carotene have been successfully increased using β-carotene supplements.

Carotenoids are dietary antioxidants transported with plasma lipoproteins, primarily LDL. Lutein, β-cryptoxanthin, lycopene and β-carotene, the four major plasma carotenoids, were destroyed before the formation of lipid peroxidation products. The rates of destruction of the individual carotenoids were differed; lycopene was destroyed most rapidly and lutein most slowly. Upon oxidation of β-carotene-enriched LDL, the rates of destruction of β-carotene, lycopene and lutein were slowed and lag times before the initiation of lipid peroxidation increased from 19 to 65 min. Neither effect was observed in LDL enriched with lutein or lycopene. Thus, β-carotene was unique among the carotenoids studied in having a small, but significant effect on LDL oxidation *in* vitro (Romanchik *et al.*, 1997).

The constituent of vegetables and fruits, β-carotene consistently has been associated with a reduced risk of cancer at several sites, and its intakes are highly correlated with vegetables and fruit consumption. β-carotene can inhibit carcinogenesis, although not at all cancer stages and not at all cancer sites (van Poppel, 1993).

A number of both human and animal studies with dietary supplementation of β-carotene can protect tissue against oxidative damage. β-carotene can protect against lipid peroxidation in cell cultures. Parenteral β-carotene has been shown to protect guinea pigs against oxidative damage occurring in ascorbate deficiency and dietary supplementation with β-carotene has been shown to protect mice from lipid peroxidation induce by methyl mercuric chloride (Allard *et al.*, 1994).

Long-term dietary consumption of vegetables and fruits high in β-carotene is associated with a lowered risk for cancer and heart disease. In addition, in rats, mice, and hamsters, supplementation with carotenoids prior cancer to initiation by ultraviolet (UV) or chemical carcinogens prevent tumorigenesis (Bertram and Bortkieicz, 1995).

Antioxidants as β-carotene may be beneficial for reducing or delaying age-associated decreases in T cell- mediate immune function and subsequent onset of disease (Garewal *et al.*, 1992).

Carotenoids are pigmented constituents present in most vegetables and fruits. Nutritional interest was initially on the provitamin-A carotenoids, particularly in vegetables, as they provide the major source of dietary vitamin A in most countries. Expanded interest in plant carotenoids was stimulated in the 1980's by epidemiological and laboratory studies indicating they may have anti-carcinogenic, anti-ulcer or anti-aging properties. While, the initial focus was with β-carotene, many of the vegetables and fruits suggested as being protective were poor sources of β-carotene but good sources of oxygenated xanthophylls which have little vitamin A activity (Wills and Rangga, 1996).

Carotenoids might have beneficial effects on health are in part related to their ability to quench singlet oxygen. Also, carotenoids act as chain-breaking antioxidants under physiological conditions of oxygen tension (Omaye *et al.*, 1996). Since carotenoids can act as antioxidants, there has been interest in carotenoids for possible anticarcinogenic activity; however, they may have anticarcinogenic activity due to some other property.

Carotenoids appear to be involved in protection against both singlet oxygen and triplet oxygen (as radical chain-breaking antioxidants). Carotenoids are most effective biological quenchers of $^1O_2$. Singlet oxygen is known to be capable of damaging DNA (Beutner *et al.*, 2001).

2.3- Vitamins

2.3.1- *Tocopherols*

Antioxidant vitamins, especially vitamin E, metabolize free radicals and reduce the risk of disease out comes. Free radical damage has been implicated in the casual pathway of lipid peroxidation, a major factor in atherogenesis and coronary heart disease. Vitamin E, the major lipid-soluble, chain-breaking antioxidant has been associated with a reduction in lipid (Gaziano, 1994 and Mitchinson, 1994).

Although the process of absorption of all the eight vitamin E homologues in diet is similar, the α-form predominates in blood and tissue despite some of the other forms being more potent antioxidants in chemical systems (Dutta-Ray *et al.*, 1994).

Vegetable fats and oil are major sources of dietary vitamin E. consequently the current trend to reduce fat consumption is accompanied by a reduction of the intake of vitamin E. in addition, the absorption of vitamin E is thought to be dependent on the hydrolysis of dietary lipids in the small intestine. It is therefore conceivable that a lower dietary fat intake also diminshes the intestinal absorption of vitamin E (Brink *et al.*, 1996). The author's added the apparent absorption of vitamin E from a very low fat meal varied, depending on the vitamin E concentration, from 73% to 83%. The magnitude on the vitamin E absorption was not significantly different from that from meals containing a high amount of fat. Liver vitamin E status was equal in rats fed on the very low fat meals compared with those fed on the high fat meals. They concluded that, when very low fat or low fat products are used as a replacement for full-fat products, addition of vitamin E to these products, as DL-α-tocoheryl acetate, might be useful in meeting the vitamin E requirements.

Vitamin E prevents oxidative modification of LDL and oxidation of LDL neither could nor be detected until all endogenous vitamin E was consumed (Jessup *et al.*, 1990); thus antioxidant intake would be expected to retard the development of atherosclerosis.

High serum concentrations of vitamin E as well as a higher dietary intake of vitamin E have been associated with a reduced risk of atherogenesis, coronary heart disease and stork. Serum concentrations of vitamin E were significantly and inversely related to angina in a case control study of men aged 35-54 (Donnan *et al.*, 1993). Antioxidants, including vitamin E have also been associated with a reduced risk of certain cancer (Kirkpatrick *et al.*, 1994).

Dietary vitamin E was reported to be significantly and inversely related to coronary mortality in men and women aged 30-69y over 14% (Knekt *et al.*, 1994). Bolton-Smith *et al.* (1992) found that the risk of undiagnosed coronary heart disease was significantly lower in the highest quintile of dietary vitamin E intake in men and women aged 40-59y.

Cellular antioxidant enzymatic defenses exist, such as superoxide dimutase (SOD), which dissimulates the superoxide anion to hydrogen peroxide and oxygen, and catalase (CAT), and glutathione peroxidase (GSH-Px), which detoxify hydrogen peroxide. Elsewhere, the protective role of glutathione (GSH) as antioxidant and detoxifying agent has been demonstrated; it is a ubiquitous compound which is rapidly synthesized in many tissues (Larson, 1988).

Clinical trails have shown that dietary supplementation with vitamin E led to increase the resistance of LDL to *ex* vivo (Abbey *et al.*, 1993 and Wander *et al.*, 1996). Another dietary means of reducing the susceptibility of LDL to lipid peroxidation is the partial substitution of dietary poly-unsaturated fatty acid (PUFA) by mono-unsaturated fatty acid (MUFA) (Berry *et al.*, 1991). Indeed, MUFA are more resistant to oxidative modification that PUFA and unlike saturated fatty acids, are neutral or have hypolipidemic effect. Hence, it can be expected the requirements of dietary antioxidants for protecting LDL are related to the amounts and type of dietary fats.

Low-density lipoprotein cholesterol oxidation decreased significantly in blood taken from subject receiving ≥400IU/ day but not ≤200IU/ day α-tocopherol (Fuller *et al.*, 1996).

The elevated level of free radicals during a hypercholesterolemic pathological state with vitamin E deficiency could be due either to their overproduction and/ or the decrease in the activity of their metabolizing enzyme (SOD, CAT and GSH-Px) (Tebib *et al.*, 1994 a,b and 1997).

Vitamin E acts as a lipophilic chain-breaking antioxidant, while water-soluble chain-breaking antioxidants, such as vitamin C or uric acids suppress the oxidation of LDL initiated by aqueous radical. Hirano *et al.* (1997) suggested that vitamin radicals (tocopheryl radical) act as peroxidants during the autoxidation of LDL. It was also shown that the shortened lag time induced by higher doses of vitamin E was restored when lipid- and water-soluble antioxidant were added simultaneously, which suggests that vitamin E radicals derived from vitamin E are subsequently reduced by vitamin C to regenerate vitamin E. thus, the interaction between lipid- and water-soluble antioxidants provides an important function in maintaining LDL resistant to oxidation.

Nutrients and other constituents of vegetables and fruits have potential to affect almost all aspects of immune system (Kubena and Mc-Murray, 1996). Vitamin E modulates synthesis of prostaglandins and other host defenses, which are immune response (Romach *et al.*, 1993 and Bendich, 1993).

Vitamin E supplements, known to enhance immune activity, might have activated latent autoimmune diseases in his patients (e.g. asthma, food allergy, diabetes, rheumatoid, arthritis, multiplesclerosis and lupus) (Daily and Zemel, 1995).

In animal models diabetes has been induced by substances that produce free radical, such as alloxan, streptozocin, and other whereas free radical scavengers been effective in preventing diabetes in animal models (Davie *et al.*, 1992). Specifically, alloxan has produced hydrogen peroxide *in vitro* and may interact with membrane glucose receptor or the site of glucose-stimulated insulin release. In mice, several hydroxyl radical scavenges have blocked the diabetogenic action of alloxan.

Insulin sensitivity increased when either normal subjects or patients with NIDDM consumed pharmacologic doses of vitamin E (Paolisso *et al.*, 1993 a,b).

Paolisso *et al.* (1994) explained that, elevated fasting insulin concentrations and insulin resistance have been associated with NIDDM, obesity, atherosclerosis, and hypertension. Insulin resistance has been associated with subclinical atherosclerosis as measured by B-mode ultrasound of the carotid artery. It has been postulated that oxidative stress and an excess of free radical activity may play a role in the pathogenesis of diabetes and its complications.

Vitamin E supplementation in persons with and without NIDDM has been associated with better insulin sensitivity and improved metabolic control (Paolisso *et al.*, 1994 and Sharma et al., 2000).

Ceriello *et al.* (1996) showed that vitamin E supplementation (1.000 IU/d for 10 days) in healthy adults significantly increased glutathione (GSH) levels as compared to controls. They explained that vitamin E is the most important natural antioxidant. It prevents oxidation of GSH and the protein-SH group during the redox reaction. Vitamin E has been inhibiting protein kinase stimulation due to diabetes. Vitamin E is particularly effective in protecting LDL from oxidation. Low levels of GSH and vitamin E in diabetes can be due to their exhaustion during detoxification of free radicals produced by cell membrane lipid peroxidation. Control of diabetes is likely to improve GSH levels by truncating the effect of hyperglycemia in causing oxidative stress. GSH and vitamin E, both isolated or in combination from formidable defense against free radical. Since the use of vitamin E supplementation significantly decreased oxidative stress, a possible role of vitamin E supplementation is suggested in reducing free radical induced oxidant injury in diabetes mellitus.

In diabetic patients, the risk of atherosclerosis is 3- to 4- fold higher than in nondiabetic persons and cardiovascular disease is the major cause of premature death. The process of peroxidation of PUFA in lipoproteins can be inhibited by vitamin E when given either *in vitro* or *in vivo*. Indeed, evidence from clinical trials and epidemiologic studies suggests that vitamin E supplementation is associated with a reduced risk of cardiovascular disease. Engelen *et al.* (2000) added, in the patients with type 2 diabetes, vitamin E supplementation led to a decrease in the susceptibility of LDL to *in vitro* peroxidation and to a decrease of *in vivo* peroxidation products in young (mean age: 12y) patients with type1 diabetes. The recommended dietary

allowance of 10 mg α-tocopherol (equivalents 15 IU). Vitamin E supplementation decreased lipoprotein susceptibility to *in vitro* oxidation by copper but had no effect on other cardiovascular risk factors, such as lipid profiles, glycation, and glycemic control.

Carr *et al.* (2000) showed that, vitamin E, mainly α-tocopherol, is the major fat-soluble antioxidant present in the LDL particle. On average, 5-9 vitamin E molecules are carried by each LDL particle are believed to protect LDL from oxidative damage. Vitamin E in LDL particles acts as a chain-breaking antioxidant and prevents lipid peroxidation of PUFA and modification of proteins in LDL by reactive oxygen species (ROS).

Vitamin E is the principal lipid-soluble antioxidant in human plasma and lipoproteins. Epidemiologic studies have shown that dietary vitamin E consumption is inversely associated with the development of coronary artery disease in both men and women. One proposed mechanism for the putative cardioprotictive effect of vitamin E has been the inhibition of LDL oxidation. Moreover, vitamin E inhibited platelet aggregation appears to be protein kinase C (PKC) dependent. In fact, vitamin E improved endothelial function in part due to the inhibition PKC stimulation. This activity of vitamin E was examined in platelets, and vitamin E inhibited platelet aggregation in part through a mechanism that involves PKC. While, the platelet inhibitory activity of vitamin E was independent of its antioxidant action because platelet inhibition was still observed with isoforms of vitamin E that were devoid of antioxidant activity (Freedman and Keaney Jr, 2001).

2.3.2- *Ascorbic acid*

Ascorbic acid (AA) is water-soluble antioxidant formed from a six-carbon compound derived from glucose. It is easily oxidized to form a free radical, semidehydroascorbic acid, which is relatively stable. Further oxidation generates diketogulonic acid, which has no biological function. The antioxidant activity of ascorbic acid is due to the case of its loss of electrons, making it very effective in biological systems. Because it is an electron donor, it serves as a reducing agent for many reactive oxidant species. It protects compounds in the water-soluble portion of

cells and tissues, and reduces tocopherol radicals back to their active form at the cellular membrane. It is also a cofactor for reduction of Fe^{3+} to Fe^{2+}. The interactions of ascorbic acid with minerals and with tocopherol are reminders of synergism in biological systems (Klein and Kurilich, 2000).

In both plant and animal systems L-AA interacts enzymatically and non-enzymatically with damaging oxygen radicals and their derivatives, so-called reactive oxygen species (ROS). The biological importance of the antioxidant behaviour of L-AA is that unlike other low-molecular-weight antioxidants (α-tocopherol, uric acid, carotenoids, flavonoids, etc.), L-AA is able to terminate radical chain reactions by disproportionation to non-toxic, non-radical products, i.e., dehydroascorbic acid (DHA) and 2,3-diketogulonic acid. Indeed, one of the most important features of the non-enzymatic antioxidant activity of L-AA, is its involvement in the regeneration of the lipophilic, membrane-associated with α-tocopherol (vitamin E, α-chromanoxy), radical. L-AA, also referred to as ascorbate or vitamin C, is required nutrient for human. Ascorbate is synthesized by plants and most mammals but not by human and nonhuman primates, guinea pigs, the Indian fruit bat, several birds, and some fish. Primates lack the enzyme gulonolactone oxidase, which catalyzes the last enzymatic step in ascorbate synthesis.

The current recommended dietary allowance (RDA) for vitamin C for adult nonsmoking men and women is 60 mg/d, which is based on a mean requirement of 46 mg/d to prevent the deficiency disease scurvy. However, recent scientific evidence indicates that an increased intake of vitamin C is associated with a reduced risk of chronic disease such as cancer, cardiovascular disease, and cataract, probably through antioxidant mechanisms. Therefore, the biochemical, clinical and epidemiologic evidence to data for a role of vitamin C in chronic disease prevention. The totality of the reviewed data suggests that an intake of 90-100 mg vitamin C/d is required for optimum reduction of chronic disease risk in nonsmoking men and women. This amount is about twice the amount on which the current RDA for vitamin C is based, suggesting a new RDA of 120 mg vitamin C/d (Carr and Frei, 1999).

Vitamin C is a cofactor for several enzymes involved in the biosynthesis of collagen, carnitine and neurotransmitters. Procollagen-proline 5-dioxygenase (praline

hydroxylase) and procollagen-lysine 5-deoxygenase (lysine hydroxylase), 2 enzymes involved in procollagen biosynthesis; require vitamin C for maximal activity. Posttranslational hydroxylation of praline and lysine residues by these enzymes is essential for the formation and secretion of stable collagen helixes. Two dioxygenases involved in the biosynthesis of carnitine also require vitamin C as a cofactor for maximal activity. Carnitine is essential for transport of activated long chain fatty acids into the mitochondria: as a result, vitamin C deficiency results in fatigue and lethargy, early symptoms of scurvy. In addition, vitamin C is used as a cofactor for catecholamine biosynthesis, in particular the conversion of dopamine to norepinephrine catalyzed by dopamine β-monooxygenase (Phillips and Yeowell 1997; and Carr and Frei, 1999).

Vitamin C is an important water soluble antioxidant in biological fluids. An antioxidants has been defined as "any substrate that, when present at low concentrations compared to those of an oxidizable substance substrate (e.g., proteins, lipids, carbohydrates and nucleic acids), significantly delays or prevents oxidation of that substrate" (Halliwell, 1996). The definition proposed by the Panel on Dietary Antioxidants and Related Compounds of the Food and Nutrition Board (2000) is that "dietary antioxidants is a substance in foods that significantly decreases the adverse effects of reactive oxygen species, reactive nitrogen species, or both on normal physiological function in humans". Vitamin C readily scavenges reactive oxygen and nitrogen species, such as superoxide and hydroperoxyl radicals, aqueous peroxyl radicals, singlet oxygen, peroxynitrite, nitrogen dioxide, nitroxide radicals, and hypochlorous acids, thereby effectively protecting other substrates for oxidative damage. Vitamin C has been recognized and accepted by the US Food and Drug Administration (FDA) as one of 4 dietary antioxidants, the other 3 being vitamin E, the vitamin A precursor β-carotene, and selenium, an essential component of the antioxidant enzyme glutathione peroxidase and thioredoxin reductase.

Vitamin C can also act as co-antioxidant by regenerating α-tocopherol from α-tocopheroxyl radical, produced via scavenging of lipid-soluble radicals. This is a potentially important function because *in vitro* experiments have shown that α-tocopherol can act as pro-oxidant in the absence of coantioxidants such as vitamin C. Vitamin C has also been shown to regenerate urate, glutathione, and β-carotene *in*

vitro from their respective one-electron oxidation products, i.e. urate radicals, glutathiyl radicals, and ß-carotene radical cations (Bowry *et al.*, 1995; and Edge and Truscott, 1997).

Ascorbic acid is the most abundant and effective water-soluble antioxidant in human plasma and is believed to be of major importance for protection against diseases and degenerative processes caused by oxidative stress (Meister, 1992).

Vitamin C inhibits chemical synthesis of nitrosamines (most of which are animal carcinogenesis) in the gastric contents, but inhibition is not competing until dietary intakes reach ≈100 mg (Tannenbaum *et al.,* 1991). Considerable evidence from both epidemiologic studies and clinical trails suggests that recommended daily allowance (RAD) may reduce the risk or risk factors for chronic diseases such as, heart disease and cancer (Losonczy *et al.*, 1996).

Recommended dietary allowances in USA (men and women 60 mg/d, pregnancy 70 mg/d and lactation 95 mg/d) and RDA for vitamin C are raised to 200mg/d (Levine *et al.*, 1996). Blanchard *et al.* (1997) suggested that doses of vitamin C above 200 mg do not increase blood levels of the vitamin significantly and may be excreted. They measured blood levels of vitamin C when the dose given was 200 mg and then again when 2500 mg was administered and they found negligible absorption increases between the lower and higher doses.

Ascorbate is reversibly oxidized with the loss of one electron to form the free radical, semidehydroascorbic acid, which is further oxidized to dehydroascorbic acid. Dehydroascorbic acid can be reduced to ascorbate *via* the same intermediate radical, or the ring structure of dehydroascorbic acid can irreversibly hydrolyze to yields diketogulonic acid. The latter can be metabolized further to form oxalate, threonate, xylose, xylonic, and lynxonic acid. Dehydroascorbic acid is unstable in aqueous solution, with a half-life at 37°C of approximately 6-20min as a function of concentration (Washko *et al.*, 1993).

Ascorbate is often called an outstanding antioxidant. In chemical terms this is simply a reflection of its redox properties as according agent. In physiologic terms

this means that ascorbate provides electrons for enzymes, for chemical compounds that are oxidants, or for other electron acceptors. In addition to its redox potential, other properties of ascorbate make it an excellent electron donor in biological system. First, its intermediate free radical is relatively non-reactive, especially with oxygen. Second, the ascorbate oxidation product dehydroascorbic acid is reduced by cells to ascorbate, which then becomes available re-use. (Park and Levine, 1995).

Ascorbate is very effective at preventing lipid peroxidation from aqueous peroxyl radicals and from oxidants released by polymorphonuclear cells. Ascorbate prevents both initiation and propagation of metal ion-induced oxidation of human LDL. LDL oxidation is thought to be a key process in the initiation of atherosclerosis. In addition, ascorbate may decrease LDL-induced leukocyte adhesion, and endothelial dysfunction may also be improved by ascorbate in both smokers and patients with coronary artery disease (Heirtzer *et al.*, 1996 and Levine *et al.*, 1996).

Diabetes often has lower levels of antioxidants, which can increase the risk of diabetic complications such as cardiovascular disease. The cellular uptake of vitamin C is promoted by insulin and inhibited by high blood sugar; and as diabetics have low insulin levels, they are at greater risk of vitamin C deficiency. Most studies have found people with diabetes to have at least 30% lower vitamin C concentration than people without the disease. Levels seem to lower in diabetic people as results of the disease than as a result of poor dietary intake (Sinclair *et al.*, 1994).

Elevated fasting insulin concentrations and insulin resistance have been associated with (NIDDM), obesity, atherosclerosis, and hypertension, and some research suggested that the antioxidant vitamin may help to reduce insulin resistance (Meyer *et al.*, 1997).

High intake of vitamin C from food raises beneficial HDL cholesterol and lower serum triglycerides. Low vitamin C levels have higher total and harmful LDL cholesterol levels and lower beneficial HDL cholesterol levels. Vitamin C also helps to protect blood fats and artery walls against oxidative damage by free radical, and seems to have beneficial effects on clotting. Vitamin C supplements may protect against the development of cardiovascular disease (Losonczy *et al.*, 1996).

High blood sugar levels in diabetes cause a compound known as sorbitol to be manufactured from glucose. This contributes to the progression of diabetic complications. Vitamin C has been shown to reduce levels of sorbitol in diabetics. Cunningham *et al.* (1994) investigated the effect of two different doses of vitamin C supplements (100 or 600 mg) on young adults with type I normalized sorbitol levels in those diabetes in 30 days.

Lysy and Zimmerman (1992) showed that blood vitamin C concentrations in person with diabetes were not significantly lower than concentrations in person without diabetes. In the other study by (Schorah *et al.*, 1996) called, persons with diabetes actually had higher blood concentrations than the comparison group. In several studies, it seems likely that results were influenced by the provision of extensive dietary instruction to persons with diabetes that promoted the benefits of consuming fruit and vegetables.

Several explanations for reduced serum vitamin C concentrations in persons with diabetes might be considered: (1) renal re-absorption of vitamin C may be reduced by hyperglycemia, (2) blood glucose may compete with vitamin C for uptake into certain cell and tissue, (3) cellular regulation of vitamin C may be impaired and (4) increased oxidative stress may deplete antioxidant reserves (Will and Byers, 1996; and Will *et al.*, 1999).

Armdtrong *et al.* (1996) showed an inverse correlation between vitamin C and glucose tolerance, suggesting a role for vitamin C in glucose disposal. In NIDDM (type 2) patients, vitamin C supplementation has been shown to improve lipid and glucose metabolism. Suppiementation-induced increases in ascorbic acid paralleled the decline in LDL-cholesterol and insulin concentrations in fasting plasma .Improved insulin action may lead to lower v-LDL and LDL concentration, and to a more favorable LDL subclass distribution, potentially increasing resistance of LDL to oxidation as small, dense LDL subclasses have been shown to be more susceptible to oxidation (Jari *et al.*, 1999 and Hamilton et al., 2000).

2.4- Sterols

The total plant sterol composed of large number of individual sterols. These can divide into subgroups based on the structure of the ring system (i.e., the number and location of double bonds and methylation at the C-4 position) and side chain (alkylation, double bonds). The most common sterols in vegetables and fruits are 4-desmethylsterols, such as sitosterol, capmesterol, stigmasterol, and avenasterol. Monomethyl and dimethyl sterols serve mainly as precursors of the end-products (Nes, 1987). Plant sterol occur both as free sterols and as bound conjugates, i.e. fatty esters (mainly C-16 and C-18 fatty acids), esters of phenolic acids, glycosides (most commonly with β-D-glucose) and acetylated glycosides (esterified at 6-hydroxy group of the sugar moiety) (Wojciechowski *et al.*, 1991). Generally, the plant sterol content in a given plant may vary depending on many factors, such as genetic background, growing conditions, tissue maturity and post-harvest changes (Piironen *et al.*, 2000a).

The major dietary sources of plant sterols are seeds and oil, but the sterol content of plant varies with their geographic location and climate. The most common plant sterols are β-sitosterol, campesterol and stigmasterol (Pollak, 1985). The intake of plant sterol in a Western diet is 200-400 mg/d (Jones *et al.*, 1997), and vegetarian diet may provide twice that amount (Ling and Jones, 1995). Plant sterols are obtained from the diet by intestinal absorption. The absorption rate for sistosterol is about 5%, but higher absorption rates of 10% have been reported for campesterol (Heinemann *et al.*, 1993).

In plants, sterols have both a structural function and a metabolic role. They are integral membrane components which serve to regulate the fluidity and permeability of membranes, affecting various membrane functions, such as simple diffusion, carrier mediated diffusion and active transport across membrane, as well as modulating the activities of membrane-associated proteins including enzymes, receptors, and signal transduction components. In addition, they are precursors of other bioactive steroids. They are biogenic precursors of so-called brassinosteroids, a special class of growth substances, and substrates for the synthesis of numerous secondary plant metabolites (Days and Goad, 1993; and Clouse, 2000).

Phytostrerols are thought to promote human health through cholesterol or as quenchers of singlet oxygen providing protection of skin from lipid peroxidation on exposure to UV and ionizing radiation (Hetherington and Steck, 1999).

Cereal products are recognized as significant plant sterol sources, whereas plant sterol content of vegetables, given on fresh weigh basis, is considerably lower (Piironen *et al.*, 2000b and Normen *et al.*, 2001). The most abundant sterols in these natural sources are 4-desmethylsterols, such as sitosterol, campesterol, stigmasterol, Δ5-avenasterol, and Δ7-avenasterol; sitosterol was the predominant sterol. Other sterols like saturated stanols and sterols synthesized earlier in the biosynthesis pathway, such as the 4-monomethyl and 4,4-dimethyl sterols, usually occur in lower amounts. In cereals, plant sterol occur as free sterol, steryl esters with fatty acids, or phenolic acids, steryl glycosides, and acetylated steryl glycosides. The level of these components vary in different cereals and in different parts of the kernel (Toivo *et al.*, 2000).

Only the common vegetable oils corn, canola, and sesame have phytosterol levels comparable to those in ginseng (Abidi *et al.*, 1999). Phytosterol contents in ginseng were as follows: squalene, oxidosqalene, campesterol, stigmasterol, clerosterol, β-sitosterol, β-amyrin, Δ5-avenasterol, Δ5,24,25-stigmasterol, lupeol, Δ7-sitosterol, Δ7-avenasterol, 24-methylene-cycloaratanol, and citrostandienol (Beveridge *et al.*, 2002).

Normen *et al.* (2001) reported, high consumption of fruits and vegetables, which seldom have a sterol concentration above 200-300mg/kg fresh weight, also contributes substantially to the total sterol intake. In their study the contribution of fruits and vegetables to the total plant sterol intake was calculated to be on average 21% total sterols.

Plant sterols are one of the food components currently being especially actively studied. They have decreased serum cholesterol levels in several studies (Hallikainen and Uusitupa, 1999) and they may also be beneficial in preventing colon cancer (Awad and Fink, 2000). Their potency in decreasing serum cholesterol levels

and thus protecting against cardiovascular disease has led to development of functional foods enriched in plant sterol. In the products currently on the market, plant sterols are derived either from tall oil or soy. The beneficial health effects of plant sterols led to a rise in interest in plant sterols of various natural materials and on the means to optimize these levels (Piironen *et al.*, 2000a). The most important natural sources of plant sterols in human diets are oils and margarines.

Phytosterols taken as dietary supplements, or a supplemental ingredients in foods, reduced serum cholesterol and LDL-cholesterol levels. The mechanism involved may relate to inhibition of dietary and biliary cholesterol absorption from the intestinal lumen (Jones *et al.*, 1997 and 1999). In addition to a cholesterol-lowering effect, phytosterols have been suggested to possess anti-inflammatory, antibacterial, antifungal, antiulcerative, and antitumor activities (Ling and Jones, 1995 and Akihisa *et al.*, 2000). They also effective in the treatment of begin prostatic hyperplasia, hyperglycemia, and colon cancer (Pegel, 1997).

Agren *et al.* (2001) showed that both serum total and LDL cholesterol level of patients with rheumatoid arthritis were significantly lowered by a vegan diet containing on average 732mg of sitosterol and 164mg of campesterol per day with an omnivorous diet.

2.5- Volatile oils

Natural antioxidants of plant origin are generally classified as vitamins, phenolic compounds including phenolic acids and flavonoids, and volatile compounds in herbs and spices. These natural antioxidants are becoming increasingly important, not only in food but also in preventive medicine. In medicine, natural antioxidants are one of the important sources in curing diseases associated with oxidative damage. Only a few volatile extracts of herbs and spices obtained by distillation and liquid-liquid continuous extraction were assessed for their antioxidant activities. Typical assays for antioxidants activities of plant extracts and their components are performed in hydrophilic system such as water or alcohols. However, volatile components in plant extracts are dissolved in less polar or non-polar solvents such as

dichloromethane and hexan (Farag *et al.*, 1989; Aruoma *et al.*, 1996; and Lee *et al.*, 2002).

El-Abbasy (2001) showed that ether extract of rosemary, black cumin, cardamom and anise gave a good antioxidant activities on the samn during accelerated incubation at 63±21°C. However, fennel oil may be had a prooxidant effect when added to samn at a concentration of 200ppm.

Lee and Shibamoto (2002) evaluated antioxidant activities of volatile extracts isolated from thyme, basil, rosemary, chamomile, lavender and cinnamon by two independent assays: the aldehyde/carboxylic acid assay and the conjugated diene assay. They reported that the antioxidant activities of the extracts decreased in the following order in both of lipophilic assay system : thyme > basi l> rosemary > chamomile > lavender and cinnamon. They added, thyme and basil extracts inhibited the oxidation of hexanal for 40 days at the levels of 10μg/ml and 50μg/ml, respectively. The extract of thyme and basil were effective in retarding methyl linoleate deterioration at 40°C, with activity increasing with concentration in the range 10-200μg/ml. At a concentration of 50μg/ml, thyme extract was similar in antioxidant activity to BHT and α-tocopherol in the conjugated assay.

2.6- Polyunsaturated fatty acids

The first evidence for modern or near-modern humans at various fossil localities appears from before 30,000 to after 100,000 years ago; however, the earliest occurrences are restricted to Africa. It is certainly possible that genetic modifications in lipid trafficking coupled with ready access to the essential nutrients, long-chain polyunsaturated fatty acid (LC-PUFA) included, helped various African Homo populations make the precocious leap to modernity (Conroy *et al.*, 1998). There must have been enough LC-PUFA available in the diet to (1) provide many generations of hominids with fuel for fetal/infant development as well as childhood and adults needs, (2) allow for the fact that substantial amounts of PUFA would almost certainly have been oxidized for energy requirements, (3) both explain and allow for our inefficient conversion of linoleic acid (LA) to arachidonic acid (AA) and α-linolenic acid (LNA) to decosahexaenoic acid (DHA) (Chen *et al.*, 1995; Cunnane and Anderson, 1997 and Broadhurst *et al.*, 1998).

The importance of reducing omega-6 polyunsaturated fatty acids (PUFAs) even as the omega-3 PUFAs are increase in the diet of adults and newborns for optimal brain and cardiovascular health and function. This is necessary to reduce adverse effects of excesses of AA. Such excesses can occur when too much LA and AA are present in the diet and an adequate supply of dietary omega-3 fatty acids is not available. The adverse effects of too much AA and its eicosanoids can be avoided by two interdependent dietary changes. First, the amount of plant oils rich in LA, the parent compound of the omega-6 class, which is converted to AA, needs to be reduced. Second, simultaneously the omega-3 PUFAs need to be increased in the diet. LA can be converted to arachidonic acid and the enzyme, δ-6 desmutase, necessary to desaturate it, is the same one necessary to desaturate LNA, the parent compound of the omega-3 class; each competes with the other for this desaturase. The presence of LNA in the diet can inhibit the conversion of the large amounts of LA in the diets of Western industrialized countries which contains too much dietary plants oils rich in omega-6 PUFAs (e.g. corn safflower, and soybean oils). The increase of LNA, together with eicosapentaenoic acid (EPA, 20:5, n-3) and docosahexaenoic acid (DHA, 22:6, n-3), and reduction of vegetable oils with high LA content, are necessary to achieve a healthier diet in these countries (Simopoulos *et al.*, 1999 and 2000).

Habitual consumption of modest amounts of fish may be associated with reduced mortality from coronary heart disease. Although in these epidemiological surveys the intake of LC-PUFAs of the n-3 variety has been calculated to be relatively small, n-3 fatty acids may accumulate over time to achieve the biological effects that were reported with larger intakes of fish oils seen in populations that consume much fish (Kromhout *et al.*, 1985). Such effects include lower plasma lipids, diminished thrombogenicity, and possibly reduced blood pressure (Singer *et al.*, 1986; Nestel, 1987 and Von Schacky 1987). That this may indeed occur is suggested by the inverse correlation between prevalence of coronary heart disease and content of EPA in platelets-an index of long-term dietary habits. EPA is the major n-3 fatty acid of fatty fish in Northern Oceans but can also be derived from elongation and desaturation of the n-3 fatty acid of plant origin, LNA (Adam *et al.*, 1986).

Fish oil rich in n-3 fatty acids (DHA and EPA) whereas, Gerbi *et al.* (1999) suggested that fish oil therapy may be effective in the prevention of diabetic neuropathy.

2.7- Amino acids, peptides and enzymes

Many amino acids have been tested for their antioxidant activity, especially in food-based systems. Among the amino acids for which antioxidant activity has been claimed are arginine, histidine, cysteine, tryptophan, lysine, methionine, and threonine (Riisom *et al.*, 1980; and Larson 1988). The literature reports are often very confusing, with data suggesting that some amino acids may exhibit antioxidant potential under some conditions of temperature, pH, or oxygen concentration but have no effect or actually promote oxidation of linoleic acid at pH 9.5 and to promote it at pH 7.5 in others. For example, alanine and histidine were reported to inhibit the oxidation of linoleic acid at pH 9.5 and to promote it at pH 7.5.

In 1888, de Rey-Pailhade observed that yeast cells contain a substance that is responsible for the formation of hydrogen sulfide when the cells are crushed with elemental sulfur. He obtained further evidence that the same substance is present in a number of tissues, e.g., fish and beef muscle, beef liver, fresh sheep blood, sheep brain, egg white, lamb small intestine, and tips of fresh asparagus, the substance was named *philothion*. Hopkens suspected that *philothion* was dipeptides containing glutamate and systeine. In 1921 Hopkens, named *philothion* as glutathione (GSH). Hopkins and Kendall *et al.* (1929) discovered that glutathione was actually a tripeptide and that the peptide contained glycine (Glu-Cys-Gly).

Glutathione peroxidase is specific for its hydrogen donor, reduced glutathione, but may use a wide range of substrates extending from H_2O_2 to organic hydroperoxides. The cytosolic and membrane-bound monomer GSH phospholipid hydroperoxide-glutathione peroxidase and the distinct tetramer plasma glutathione peroxidase are able to reduce phospholipid hydroperoxides without the necessity of previous hydrolysis by phospholipase A_2. The protective action of phospholipid hydroperoxide-glutathione peroxidase against membrane damaging lipid peroxidation has been directly demonstrated (Thomas *et al.*, 1990 and Meister, 1992).

Hayes and Pulford (1995) showed reduced GSH is a major cellular electrophile conjugator as well. Glutathione S-transferases catalyze the reaction between the –SH group of GSH and potential alkaylating agents, thereby neutralizing their electrophilic sites and rendering them more water soluble. Glutathione S-transferases represent a major group of phase II detoxification enzymes.

Glutathione (GSH) has emerged to be one of the most fascinating endogenous molecules virtually present in all animal cells often in quite high concentrations. In addition to the detoxicant, antioxidant, and cysteine-reservoir functions of cellular glutathione, the potential of this ubiquitous thiol to modulate cellular signal transduction processes has been recently evident. Lowered tissue GSH levels have been observed in several disease conditions. Sen (1997) showed that restoration of cell GSH levels in a number of these conditions have proven to be beneficial. Thus, strategies to boost cell glutathione level are of marked therapeutic significance. He added that availability of cysteine, a precursor for glutathione synthesis, inside the cell is a critical determinant of cellular glutathione level. N-acetylcysteine and α-lipoic acid are two pro-glutathione agent that have remarkable clinical potential. The ability of these two clinical drugs to enhance cellular glutathione level, coupled with their favorable effect on the molecular biology of HIV infection may make them useful tools for AIDS treatment.

The human hepatoma cell line, hepG2, retains many of the xenobiotic metabolizing enzymes found in normal hepatocytes, including an inducible glutathione S-transferase (GST). The isoforms of GST that is induced by xenobiotics in this cell line is GSTA1-1. Williamson *et al.* (1997) examined the ability of extracts from wide variety of fruits and vegetables to induce GST activity in hepG2 cells. They found that extracts from cruciferous vegetables (broccoli, Brussels sprouts, cabbage) were the most potent inducers, but this was dependent on the variety. Most of the extracts from fruits, with the exception of grapefruit, were poor inducers. The results showed that extracts from cruciferous vegetables are effective inducers of human GST, in agreement with previous studies on GST in animals and cell lines derived from animals.

Many one-electron processes have been described that convert O_2 to its radical anion reduction product, O_2^-, superoxide. Superoxide dismutase (SOD) catalyze the conversion of O_2^- to H_2O_2 and oxygen. This reaction is quite rapid even without enzymic catalysis at ordinary physiological pH's, although O_2^- is quite stable above pH 11 or so; nevertheless, virtually all aerobic organisms that have been examined contain SOD. Dhindsa *et al.* (1981) reported that SOD is a powerful enough catalyst to increase rate of the reaction by several orders of magnitude at physiological pH's. They added, superoxide, like H_2O_2, is not directly toward most organic compounds (at least not as an oxidant), but it probably gives rise to more reactive oxygen species of higher potential toxicity.

2.8- Minerals

The trace elements, copper (Cu), manganese (Mn), zinc (Zn), and selenium (Se) act as cofactors of antioxidant enzymes to protect the body from oxygen free radical (OFR) that are produced during oxidative stress. It is necessary to maintain a balance between the harmful pro-oxidant components produced and the antioxidant compounds that counter these effects. A delicate balance also exists for the redox trace elements such as Cu, which can initiate free radical reactions but is also a cofactor of Cu/Zn-superoxide dimutase (SOD), a free radical scavenging enzyme. Selenium is found to be most severely deficient in traumatized patients who need adequate supplementation during parenteral micro-nutrition to assist the free radical scavenging activity of glutathione peroxidase and the immune system (Leung, 1998).

A number of enzymes with antioxidant functions require trace elements such as Se, Cu, Zn, and Fe from the diet as cofactors. In general these micronutrients are recognized as being essential because: (1) a deficiency causes a defined disease to develop, e.g. serve vitamin C deficiency causes scurvy combined vitamin E and Se deficiencies cause myopathies and neuropathies, moreover such conditions can be reversed by repletion with the appropriate micronutrients, (2) they are readily absorbed and likely to be near the biomolecules in the cell where oxidative damage is to occur, (3) in fulfilling their role as antioxidants, they do not cause marked damage to cellular processes *in vivo,* (4) in nutritionally relevant amounts, they moderate

markers of oxidative stress and/or disease risk (Duthie, 1999).

The antioxidant enzymes are metalloenzymes, which contain trace minerals for which vegetables and fruits are significant sources. Mitochondrial superoxide dimutase (SOD) is a manganese-containing enzyme. Glutathione peroxidases (GSH-Px) are selenium-dependent enzymes. Vegetables and fruits are rich source of manganese, but not typically significant sources of selenium. However, selenium is found in plant tissues in amounts proportional to the mineral concentration of the soil in which plant grows (Steinmetz and Potter, 1996; and Lampe, 1999).

Copper (Cu), the transition metal, plays a key role as a cofactor regular in the transcription and post translation of Cu/Zn SOD, as illustrated in yeast. Lack of Cu^{2+} reduces the synthesis of Cu/ Zn SOD mRNA as well as the insertion of this ion into the apoenzyme to form the active enzyme (Harris, 1992). They added, diets deficient in Cu or the use of metal ion chelators have reduced the expression of Cu/Zn SOD activity in human tissues. Proteins such as metallothionein and ceruloplamin bind Cu ions to prevent this transition metal from catalyzing hydroperoxide decomposition to free radicals.

Manganese (Mn) is required for activity of the mangano-enzyme (Mn SOD) to protect the mitochondrial membrane from free radical damage. Most of Mn SOD is located in the mitochondrial matrix, but can occur in the liver cytosol as "extra" Mn-SOD in case of copper deficiency in which more of Mn SOD is formed to compensate for less Cu/ Zn SOD. Mammalian Mn SOD exists in human liver as a tetramer subunit with 4 moles Mn per mole enzymes (Kurobe *et al.*, 1990).

The recommended daily allowance (RDA) according to WHO (1990) for selenium is a mere 55 to 70 µg/d for female and male adults respectively, while 10 µg/d for infants (0-6 months) and the RDA for children between 4 to 10 years, is 20 to 30 µg/d for good health. Exceeding doses of Se more than 200 µg/d can cause skin rashes, hair and nail breakage, fatigue, and stomach upset. Excessively large dose (more than 1000 µg/d) can lead to serious side effects, including liver damage and potentially fatal heart problems.

Selenium (Se) and vitamin E may have important roles in biological process of importance for the cardiovascular system that not necessarily relate to their antioxidant function (Arthur et al., 1993; and Dutta-Ray *et al.*, 1994).

The addition of Se and vitamin E to the diet of cholesterol-fed rabbits has been shown to a beneficial effect on lipid concentrations and atherosclerotic lesions (Stone *et al.*, 1994).

Hypercholesterolemia in human subjects is frequently related to an increased risk of atherosclerosis. Even if human studies on relationships between Se and plasma lipids and lipoproteins are inclusive, animal studies that Se deficiency results in a significant increase in plasma cholesterol (Kuo *et al.*, 1995).

The epidemiologic association of higher Se intakes with reduced cancer risk and the antioxidant role of Se in glutathione peroxidase (as well as several other possible mechanisms) have provided a basis for research on possible anticarcinogenic effect of Se. Several selenium compounds have anticarcinogenic activities in a variety of animal models when administered at amounts greater than those associated with nutritional need. 200 μg selenium significantly decreased total and lung cancer mortality and total colorectal and prostate cancer incidence (Clark *et al.*, 1996).

Selenium deficiency results in increased concentrations of plasma cholesterol and apo E. Both could be explained by an increase in the HDL, fraction. Vitamin E deficiency alone had no significant effect on plasma lipid, lipoprotein and apo E concentration. Se deficiency combination with vitamin E deficiency leads to an increase in plasma LDL and Apo E concentrations (Nassir *et al.*, 1996).

The effects of Se supplementation include its ability to decrease the incidence of cancer of the lung, colon/rectum, and prostate, although it has no effect on basal cell or sequamous cell carcinoma of the skin. Se deficiency with other diseases, such as human immuno-deficiency virus and acquired immuno-deficiency disease and cancer (Taylor *et al.*, 1997).

Bengoumi *et al.* (1998) and El-Nadi *et al.* (1999) reported the toxic level of

selenium to plants, animals, and humans which more than 0.4-5 ppm on dry matter. They added the normal requirements in non-lactating cows between 0.1-0.2 ppm DM and toxicity level is 0.5 ppm DM.

The trace mineral selenium is an essential nutrient of fundamental importance to human biology. It is a key component of number of functional selenoproteins required for human health. There is evidence that Se has an antioxidant role through the selenoenzyme glutathione peroxidase. Se is also involved in thyroid metabolism through the enzyme, type I and II iodothyronineiodinase, although the latter is not itself a selenoenzyme. Selenoproteins have also been associated with maintenance of fertility and possibly some anticancer effect (van Bakel *et al.*, 2000).

Se deficiency has been suggested to have a role in the a etiology of pathologies, such as oxidative stress or inflammatory conditions, diabetes mellitus, hepatopathies and HIV infection (van Bakel *et al.*, 2000; and Murphy and Cashman 2001).

Iron (Fe) deficiency anemia is one of the most prevalent nutritional deficiencies affecting the world's population today, especially among women and children in developing countries. Most nutrition programs aimed at decreasing the incidence of Fe deficiency utilize fortification of the diet with various Fe compounds.

Iron in its ferrous complexes state is an essential component of hemoglobin, but in its ionized free state it can cause oxidative work with hydrogen to produce peroxide toxic hydroxyl radicals. Fe, the transition metal acts as an important mediator in cell injury accompanying oxidative stress. The formation of extremely reactive hydroxyl radicals ($^{\cdot}OH$) occurs by means of the Fenton reactions: Fe (II) + $H_2O_2 \rightarrow$ Fe (III) + $^{\cdot}OH$ + OH^- in which ferrous Fe reduces peroxide. The hydroxyl radical can rapidly interact with DNA, protein and lipids to induce oxidative injury. *In vivo,* the transport protein transferrin bids with Fe to keep it form participating in trace element-dependent reactions, and this chelation acts as an important antioxidant mechanism. Transferrin receptor (TR), a plasma protein receptor, allows control of the intracellular Fe homeostasis to maintain little or no "free" Fe in plasma. For patients who require Fe supplementation to their total parenteral nutrition (TPN)

solutions, free-Fe admixtures with bound-Fe such as Fe dextran to protect against spontaneous generation of hydroxyl radical (Meneghini, 1997; and Leung, 1998).

Magnesium (Mg) deficiency affects the activities of various enzymes which require the ion as cofactor and use high-energy phosphate bonds in glucose metabolism. It has been shown that diabetes mellitus is the chronic disease most frequently associated with hypomagnesaemia. Reis *et al.* (2001) showed that Mg is involved in glucose homeostasis in rats, so that Mg depletion leads to increased insulin secretion, which is prevented by Mg supplementation. In diabetes mellitus hypomagnesaemia may act by improving β-cell glucose sensitivity and thus participating in the exacerbated pancreatic response to hyperglycemia.

3- Interaction of antioxidants and their effects on human diseases

Antioxidants are important in prevention of pollution damage to plants, and disease, prevention in both plants and animals. Reactive oxygen molecules may cause damage when consumed by denaturing proteins, damaging nucleic acids and membranes. Oxidative and free radical processes have been related to cancer initiation and promotion. Damage can be prevented by α-tocopherol (vitamin E), ascorbic acid (vitamin C), β-carotene and glutathione (GSH). Diets high in antioxidant vitamins may help to reduce risk of such diseases (Byers and Perry, 1992; and Al-Saikhan *et al.*, 1995).

In the recent years great interest has been focused on the antioxidant vitamins (vitamin C and E, and β-carotene), particularly because of their likely role in the prevention of CHD and cancer. Antioxidant vitamins can counteract the oxidizing effect of lipids by scavenging oxygen free radicals which have been found as major promoters of such diseases. Vegetables and fruits are the main sources of antioxidant vitamin, making these foods essential to human heath (Abushita *et al.*, 1997).

Antioxidants are compounds that protect cellular systems from the potentially harmful effects of processes that can cause excessive oxidants. By implication they may inhibit the pathogenesis of the many diseases which involve oxidative reactions (Diplock *et al.*, 1998). Antioxidants can be of endogenous and exogenous origin and

contribute to the complex and integrated biological antioxidant defense system, which normally protects cells from the injurious effects of oxidation. This is achieved by directly scavenging reactive O and N free radical species, by metabolizing peroxides to non-radical products and by chelating metal ions to prevent the generation of oxidizing species.

The main sources of oxidants and oxygen-free radicals in cells are mitochondria, phagocytes, peroxisomes, and cytochrome P-450 enzymes. Mitochondria produce superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) as consequence of normal respiration. The "respiratory burst" of phagocytes is associated with the production of $O_2^{\cdot-}$, H_2O_2, nitrite oxide ($NO^{\cdot}$) and hypochlorite ($CLO^{\cdot}$). Peroxisomes degrade fatty acids and other substances to give H_2O_2, while cytochrome P-450 enzymes catalyse a range of oxidation reactions involving endogenous and exogenous substrates (e.g. steroid hormone synthesis, detoxification reactions) (Bramley *et al.*, 2000). In addition, low-wave length electromagnetic reaction such as gamma rays can split water in the body to generate hydroxyl radical ($OH^{\cdot}$), while ultraviolet light can cleave to O-O covalent bond in H_2O_2 to give two $OH^{\cdot}$ radicals.

The production of free radicals in the body may be beneficial, as in the case of the respiratory burst or the relaxation of smooth muscle, or it may be harmful, leading to inflammation and tissue damage. Membrane phospholipids are especially vulnerable to damage by free radical. On being attacked by a free radical, a membrane polyunsaturated fatty acids (PUFA) (R^1H) gives up a loosely bound hydrogen atom becomes a fatty acids radical ($R^{1\cdot}$). This re-arranges spontaneously to conjugated diene and takes up molecular oxygen, becoming a highly reactive peroxyl radical ($R^1OO^{\cdot}$). This can attack a second PUFA (R^2H), resulting in the formation of hydroperoxide (R^1OOH) and other fatty acid radical ($R^{2\cdot}$). The new fatty acid radical can then take up oxygen and repeat the cycle over again, and as a consequence, a chain reaction becomes established. The hydroperoxides produces during the so-called "propagation" phase may split in the presence of iron or copper to yield peroxyl ($R^1OO^{\cdot}$) or alkoxyl ($R^1O^{\cdot}$) radicals, which accelerate the chain reaction, or they may break down to aldehydes, ketones, alkanes and other products, which may bind to,

and disrupt, cellular macromolecules such as DNA and proteins, or induce inflammatory reactions (Halliwell, 1996 and Bramley *et al.*, 2000).

Eriksson and Kohvakka (1995) studied the effect of magnesium and vitamin C supplements on metabolic control were assessed in 56 diabetics. The study involved a 90 day run-in period followed by two 90 d treatment periods, during which patients received 600 mg of magnesium and 2g vitamin C/d. The results showed that vitamin C supplementation improved glycemic control, fasting blood glucose, cholesterol and triglyceride levels.

Oxidative modification of LDL-cholesterol is thought to play key role during atherosclerosis. Plants contain a variety of antioxidants (including vitamin C and E and carotenoids) that can inhibit oxidation of LDL-cholesterol (Fuhrman *et al.*, 1997).

Steinberg and Chait (1998) showed that vitamin E was highly correlated with β-carotene. Plasma total peroxyl radical trapping potential values did not change in response to supplementation. This study indicate that an antioxidant-supplemented drink [tomato-based juice supplemented with vitamin C (600 mg), vitamin E (400 mg) and β-carotene (30 mg)] can reduce lipid peroxidation and susceptibility of LDL to oxidation in smokers and may Amelia rate the oxidative stress of cigarette smoke. Oxidative processes, such as the oxidative modification of LDL appear to play important role in the development of atherosclerosis. Oxidized LDL also affects the expression of several proteins involved in the initiation and progression of atherosclerosis.

The intake of polyunsaturated fatty acid (PUFA) at larger amounts than those required to prevent edificial on risk factors associated with CHD such as, thrombosis and hyperlipidemia. In particular, lipophilic substance as vitamin E, β-carotene, lycopene and ubiquinol that are present within particles are major determinants of their resistance to oxidative degradation (Esterbauer *et al.*, 1992).

α-tocopherol and carotenoids are important, plant-derived functionally related antioxidants in mammals. It has been reported that a low circulating concentration of these compounds is an additional risk factor for the development of lung cancer in

smokers (Krinsky, 1993). Concentration of both α-tocopherol and β-carotene were significantly lower in lung cancer patients than in control subjects.

Diets rich in vegetables and fruits inhibit the development of major diseases such as coronary heart diseases (CHD) and certain cancer (Ness and Powles, 1997). This beneficial effect is ascribed, in part, to the antioxidants in such foods which protect biomolecules in our cells, such as lipids, proteins and DNA, from oxidative damage by reactive free radicals; such damage is implicated in the pathogenesis of many clinical conditions. Until recently, most attention has focused on the role of the well recognized nutritional antioxidants, vitamin E, vitamin C and carotenoids, in the prevention of these potentially deleterious reactions.

Rose *et al.*, (1986) added the raw and fresh vegetables showed the most protection against cancer risk, with 87% of studies finding a protective association and one study each showing a null or positive association. Study findings are highly consistent for lettuce, leafy green and Cruciferous vegetables, carrot and citrus fruit with >70% reporting a protective role. For all other vegetable and fruit >60% of studies found a protective effect against total cancer risk.

Anticarcinogenic agent found in vegetable and fruit include carotenoids, vitamin C and E, dietary fiber, selenium, glucosinolates and indols, isothiocynates, flavonoids, phenol, and plant sterols. These and other agents display both complementary and overlapping mechanisms of action, include induction of detoxifying enzymes, antioxidants effects, inhibition of the formation of nitrosamine, binding and dilution of carcinogens in the digestive tract alteration of hormone metabolism, and others (Steimetz and Potter, 1991).

Block *et al.* (1992) reviewed more than 200 epidemiological investigations (cohort and case-control) and found a statistically significant protective effect of vegetable and fruit consumption in most dietary studies. More than 90% of studies correlating vegetable and fruit intake with cancers of the upper respiratory and digestive tracts (oral cavity, pharynx, larynx and esophagus) and lungs observed protective effects; as did >80% of studies of colorectal and ovarian cancers. The results were less consistent for bladder and breast cancers (60% and 57%

agreement, respectively), whereas for prostate cancer only 29% studies reported a protective association.

Diets rich in fresh vegetables and fruits protect against several common epithelial neoplasms. The relative risks (RRs) for most common neoplasms ranged from 0.2 to 0.5 for the highest compared with the lowest tertile of vegetable intake. Protective effects of vegetable are also observed against hormone-related neoplasms. Higher intake of fruits was related to a reduced RR for cancers of the oral cavity and pharynx, esophagus, stomach, or larynx, as well as of the urinary tract, although protection was less evident for other digestive tract sites, as well as for other epithelial cancers. For upper respiratory and digestive tract cancers, population attributable risks for fresh vegetable and fruit intake ranged from 18% to 40% in men from 15 to 30% women; attributable risks for vegetable and fruit intake, combined with tobacco and alcohol, exceeded 85% for men and 55% women (Tavani and La-Vecchina, 1995).

Diets rich in vegetables and fruits, and the antioxidant nutrients they contain, are protective against a wide range of chronic diseases including CHD and cancer. The role of vegetables and fruits and the antioxidant vitamin they contain, in the relation to the prevention CHD. Vegetables and fruits are protective against CHD, official reports setting out dietary recommendations began to quantify the desirable amount of vegetables and fruits that people should consume (Rayner, 1998).

The present studies were to measure the plasma concentrations of various antioxidants (vitamin A, C and E; β-carotene and other carotenoids; and selenium and zinc) and some lipid peroxidation indexes in an HIV-positive population, either a symptom or with AIDS, and to compare these results with those age matched zero negative control subjects (Constans *et al.*, 1995; Delmas-Beauvieux *et al.*, 1996; and Allard *et al.*, 1998).

Losonczy *et al.* (1996) examined vitamin E and C supplement use in relation to mortality risk. They found that use of vitamin E reduced the risk of all-cause mortality and risk of coronary disease mortality. Also, use of vitamin E at two points in time was also associated with reduced risk of total mortality compared with that in

persons who did not use any vitamin supplement. Effects were strongest for coronary heart disease mortality. Simultaneous use of vitamin E and C was associated with a lower risk of total mortality and coronary mortality. Adjustment for alcohol use, smoking history, aspirin use and medical conditions did not substantially alter these findings. These finding are consistent with those for younger person and suggest protective effects of vitamin E supplements in the elderly.

The antioxidant capacities of various flavonoids and vitamin C against the oxidative DNA damage produced *ex vivo* in human lymphocytes by hydrogen peroxide. Flavonoids and polyphenolic compounds whose consumption has been linked to protection against heart disease and cancers (Hertog *et al.*, 1992 a,b).

Quercetin, myricetin, kaempferol, rutin and vitamin C are powerful antioxidants in the oxidants in the oxidation of LDL and provide a possible mechanism for the beneficial epidemiologic effect of dietary vegetables and fruits on heart disease (Vinson and Hontz, 1995).

The protective effect of vitamin C against DNA damage at this concentration was significantly less than that of all the flavonoids except apeginin, quercetin-3-glucoside and rutin. The free flavonoids are more protective than the conjugated flavonoids (i.e. quercetin compared with its conjugated quercetin-3-glucoside) (Noroozi *et al.*, 1998).

In different herbs (*Allium sp.* and *Labiatae, Umbelliferae* and *Zingiberaceae* families), a wide variety of active phytochemicals, including the flavonoids, terpenoids, lignans, sulfides, polyphenols, carotenoids, saponins, sterols. Several of these phytochemicals either inhibit nitrostation or formation of DNA adducts or stimulates the activity of protective enzymes such as the phase II enzyme glutathione transferase. Many of these herbs contain potent antioxidant compounds that provide significant protection against chronic diseases. These compounds many protect LDL cholesterol from oxidation, inhibit cyclooxygenase and lipoxygenase enzymes, inhibit lipid peroxidation or have antiviral or antitumor activities (Craig, 1999).

4- Utilization of natural antioxidants

4.1- *Antimicrobial agents*

Natural antioxidants and enzymes effectively prevent premature lipid oxidation. Once isolated from animal or plant materials and used in processed foods, lipids autoxidise readily. As a result, organoleptic and nutritional quality is reduced, and even toxic products may be formed. The retardation of autoxidation is therefore a key to product quality. Because most consumers prefer natural food additives over synthetic ones, natural antioxidants are of increasing importance (Krings and Berger, 2001; and Negi and Jayaprakasha, 2003).

Although significant advances have been made in food preservation, spoilage and pathogenic bacterial growth during food preparation, storage and distribution remains a serious problem. Most approaches to food preservation employ physical and/or chemical methods that reduce pre- and post-treatment bacterial contamination of foods, and include additives, packaging or storage conditions that retard or inhibit the growth of food borne bacterial pathogens, and/or reduce processing requirements for their elimination in foods are desired (Bowles and Jay, 1993; and Bowles and Miller, 1993a; and Leuschner and Ielsch 2003).

Many naturally occurring compounds found in edible and medicinal plants, herbs, and spices have been shown to posse's antimicrobial functions, and could serve as a source of antimicrobial agents against food pathogens (Deans and Ritchie, 1987).

Phenolic compounds and their subclasses, such as cumarins, flavonoids, tannins, saponins, and essential oil, have antimicrobial function (Kubo *et al.*, 1993).

A variety of naturally occurring plant components have been demonstrated to antimicrobial. For example, several hydrocynnamic acid derivatives, aldehyde and

ketones have been shown antibacterial, antifungal and in some instances to potent anticarcinogenic agents. Activity of these compounds is dependent upon aromaticity, carbon chain length, number and location of carbonyl groups in the hydrocarbon structure, and other reactive groups (Bowles and Miller, 1993b).

The presence and growth of fungi in foods may cause food spoilage and result in a reduction in its quality and quantity. *Aspargillus* species are xerophilic fungi and are responsible for many cases of food and feed contamination cases. *A. niger* is commonly involved in fruit spoilage, while *A. flavus* produces aflatoxins. Natural plant extracts may provide an alternative way to protect food or feed from fungal contamination, such as garlic bulbs, green garlic, ginger, hot peppers, Chinese parsley, and basil (Yin and Cheng, 1998).

Plants possessing antimicrobial principals in human diet include the Allium species: *A. satium* (garlic), *A. cepa* (onion), and *A. porrum* (leek) (Beuchat and Golden, 1989 and Leuschner and Ielsch, 2003).

The nature of SH groups of proteins is probably important in the antimicrobial activity of thiosulfinates. Garlic and cabbage extracts contains S-Methyl-L-Cysteine (Block *et al.*, 1992; and Kyung and Fleming, 1994). Garlic extract 1-2% inhibit microbial growth and higher concentrations were germicidal. Gram-negative rod-shaped bacteria were more resistant to the antimicrobial activity of garlic (Shim and Kyung, 1999).

Autoclaved cabbage juice was an inhibitory material to growth of *Staphylococcus aureus.* S-Methyl-L-Cysteine sulfoxide (SMCSO) inhibited the growth of *S. aureus.* Methyl methanosulfonate ($MMTSO_2$), a thermal breakdown product of SMCSO, completely inhibited growth of *S. aureus* at 10 ppm (Kyung *et al.*, 1994).

Allyl isothiocynate (AITC) isolated from mustard, horseradish, cabbage, bussels, turnip and kale had a minimum inhibitory concentration (MIC) of 50 to 1,000 ppm for bacteria and 1-4 ppm for non-xerotolerant yeast, and, against xerotolerant yeasts at 50 ppm, and it retarded but did not prevent growth (Shofran *et al.*, 1998).

Extracts from each of pepper, lettuce, cress, and carrot were prepared and their effects on 4 types of pathogenic and toxic bacteria were examined. The results were as follow: (1) pepper extract has the highest effect on *Listeria monocytogenes, Staphylococcus aureus,* and *Salmonella* spp. compared with *E. coli.* (2) *Salmonella* spp. is sensitive to all studies concentrations of the lettuce extract in comparison with the others. (3) increasing the amount of cress extract to 2000ppm gives an excellent destructive effect of the investigated pathogenic bacteria, and slight enhancement with respect to *E.coli.* (4) carrot extract has the best destructive effect on pathogenic bacteria, especially, *E. coli,* in compared with other extract at 1000, 2000, and 3000ppm. (5) all extracts were suitable substances as safe additive and nutrient foods and dairy products for consumer health and natural antimicrobial growth as a safety food additives and flavors agent (Gomaa and El-Shawaf, 1998).

Zeitoun (2000) studied the antibacterial effect of the carrot juice and the distillate of carrot juice, against two strains of *Listeria monocytogenes.* He observed the carrot juice was bacteriostatic at concentration of 0.4% (v/v) and was bactericidal above this concentration. There was significant difference ($P < 0.05$) between the concentration of 0.5% and 0.7% (v/v) for both strains used in this study.

Tannins inhibit many fungi, bacteria, and viruses and serve as a natural defense against microbial infections in vegetables and fruits. However, the antimicrobial properties of tannic acid are lost upon ripening. Some tannin components can inhibit activity of human immunodeficiency viruses (HIV) (Chung and Wei, 1997).

Raticilffe and Mc-Millan (1999) discussed the benefits of tannins to manipulate gut microflora by increasing growth of beneficial bacteria and suppress harmful bacteria.

Chattopadhyay *et al.* (2001) indicated that crude methanol-extract, methanol-aqueous extract and n-butanol part of the crude extract of *Alstonia macrophylla* leaves which contains tannins showed anti-microbial activity against *Staphylococcus aureus, Staphylococcus saprophticus, Streptococcus faecalis. Escherichia coli, Proteus mirabilis, Trichophyton rubrum, Trichophyton mentagrophytes var. mentagrophytes,*

and *Microsporum gypseum*. The author found that the minimum inhibitory concentration values ranged from 64-1000 µg/ml.

Ogunwande *et al.* (2001) analyzed the methanol extract of *Butyrospermum paradoxum* and it was revealed the presence of tannin in leaves and root bark. The methanol extract of the leaves and root bark not exhibited activity against *Klebsiella sp.* and *Shigella sp.* but it present activity against different bacteria namely; *Pseudomonas aeruginosa, Esherichia coli, Salmonella typhi, Staphylococcus aureus, Pseudomonas salnecerium, and Bacillus cereus.* Also, the methanol extract exhibited activity against fungi; *Fusarium oxysporum and Candida albicans.* The authors observed that spectrum of antimicrobial activity provide some support for the traditional uses of this plant.

A crude saponin fraction from white asparagus bottom cut strongly inhibited the growth of some kinds of fungi. Interestingly, *Candida albicans,* which is responsible for contamination in food processing and *Candidiasis*, and some species of *Trichophyton, Microsporum,* and *Epidermophyton.* Shimoyamada *et al.* (1996) added a new antifungal which saponin was isolated from white asparagus, suppressed the growth of *Epidermophyton floccosum, Trichophyton rubrum, Trichophyton mentagrophytes* and *Microsporum gypsum* at levels of 0.5-5 µg/ml. However, its activity against *Candida albicans* was relatively lower than crude saponin fraction (minimum inhibitory concentration (MIC) 30 µg/ml) and did not inhibit the growth of *Saccharomyces cervisiae.*

Lacaille-Dubois and Wanger (1996) found that steroidal saponins have some interesting biological and pharmacological activities including antifungal and antibacterial influences.

Hemicellulosic acid hydrolysates of lignocellulosic materials were extracted with organic solvents (ethyl acetate and diethyl ether) to remove part of phenolic derived from lignin. The phenoilc compounds extracted by solvent showed antioxidant activity contain inhibitor of microbial growth and fermentation that can hinder or even prevent fermentation (Cruz *et al.*, 1999).

Lopez-Malo *et al.* (1997) evaluated the effect of incubation temperature (10-30°C), pH (3-4) and vanillin (4-hydroxy-3-methylbenzaldehyde) concentration (350-1200 ppm) on the growth of *Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus* and *Aspergillus parasiticus* using potato-dextrose agar adjusted to water activity (a_w) 0.98. The generation time and the radial growth rates were significantly affected by the three studied variables ($P<0.001$). The inhibitory conditions (no growth after 30 days) depend on the type of mold. *A. niger*, the most resistant species, was inhibited at 15°C, pH 3.0 and 1000 ppm vanillin. For *A. ochraceus*, the most sensitive, the inhibitory conditions in presence of 500 ppm vanillin were pH 3.0 and temperature ≤25°C or pH 4.0 with temperature≤15°C. They concluded natural vanillin can be used as an antimicrobial agent to prevent mold spoilage in processes.

Ebeid *et al.* (1997) tested BHA, BHT and TBHQ at concentrations 0.0, 100, 150, 200 and 400 ppm for antimicrobial activity against *Staph. aureus, Salmonela typhimurium, E. coli* and *Bacillus cereus* (as food pathogenic bacteria) in nutrient broth at 37°C for 48 h. The antimicrobial activity varied with the type of antioxidant and strain type. BHA and BHT at >200 ppm were bacterial on all strains. TBHQ was less effective and was bactericidal at > 400 ppm.

4.2- In food packaging

Lipid peroxidation in fats and fatty foods not only causes chemical spoilage in foods but also produces free radicals active oxygens such as peroxyl and hydroxyl radicals (Tagi, 1987). Free radical attack the unsaturated fatty acids in the biomembrane; resulting in membrane lipid peroxidation, decrease in membrane fluidity, loss of enzyme and receptor activity and damage to membrane protein leading to cell inactivation. Also, free radical attack DNA and cause mutation leading to cancer (Diplock *et al.*, 1994).

Lipid oxidation causes a decrease in nutritional value of lipids, in their safety and appearance. Nowadays, various synthetic and natural antioxidants are used in prevention or retardation of lipid oxidation. Recently, some negative side effects of the commonly used synthetic antioxidants have been established that has shifted the consumer interest to the natural products as a less harmful alternative to the

synthetic ones.

So identifying antioxidants that stop or reduce the generation of free radical chain reactions is very important. Synthetic antioxidants, i.e. butylated hydroxyl anisol (BHA), butylated hydroxyl toluene (BHT), Butylated hydroquinone (BHQ) and propyl gallate (PG) are used in food industries at low concentrations. It was reported that synthetic antioxidants possess carcinogenic effect by stimulation of DNA synthesis and induction of enzymes; producing neoplasms of the non-glandular sequamous cell protein of the stomach and hepatocellular tumors. Thus, the importance of natural antioxidants has been greatly increased, as safer and less adverse reaction (Williams *et al.*, 1990; Duve and White, 1991).

Antioxidants are major ingredients which play an important role in manufacturing, packaging and storage of lipid containing foods. Synthetic antioxidants are usually used in food industry to reduce deterioration and rancidity of oils and fats. There has been growing concern regarding the possible activity of such synthetic antioxidants to cause liver damage. Therefore, development of safer, inexpensive natural antioxidants is essentially (Yen and Due, 1993).

Oxidation of lipids has detrimental effects on color, flavor, texture and nutritional values of foods. Addition of phenolic antioxidants such as BHA, BHT or TBHQ control lipid oxidation in foods. These compounds possess antimicrobial activity against a variety of bacteria, molds, viruses and protozoa (Hettiarachchy *et al.*, 1996). However, use of such compounds has been related to health risks resulting in strict regulations over their use in food product.

Synthetic antioxidants as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary-butyl hydroquinone (TBHQ) and propyl gallate have been shown to cause heath hazard, such as enlarge of liver size, increase liver microsomal enzyme activity and convert some ingested materials into toxic or carcinogenic substances, especially when they are added in excessive amounts (Williams *et al.*, 1990).

Generally, synthetic antioxidants, such as BHA and BHT have restricted use in foods as these synthetic antioxidants are suspected to be carcinogenic. Therefore, the search for natural antioxidants, especially of plant origin, has greatly increased in recent years.

There are many naturally occurring compounds that have antioxidant properties. Among these are tocopherols, lecithins, ascorbic acid and citric acid. Tocopherols are perhaps the best known of these compounds. Tocopherols are effective antioxidants, improving the stability of many fats and oils. They are present in the most plants and, therefore, important oxidative stability to many vegetable oils. Adding tocopherols to vegetable oils usually does not improve oxidative stability significantly. Tocopherols work well in food products that are deficient in antioxidants, such as animal fats and butterfat. Generally, antioxidants can protect a fat or oil from oxidation but cannot rejuvenate an already oxidized fat or oil. Addition of antioxidants can effectively inhibit oxidation and extend the shelf life of food products. Proper selection of antioxidant(s) and thorough dispersion into the fat or oil portion of the product can ensure adequate protection against oxidation (Byrd, 2001).

4.3- In fermented dairy products

Nutrients, their kinds and proportions, are all-important in determining what organism is most likely to grow. Consideration must be given to: foods for energy, foods for growth and accessory food substance, or vitamins, minerals, which may be necessary for energy or growth factors. Jay (1986) showed that the members of the family *Lactobacillaceae* are around thirty five species. Some are employed in the production of fermented milk, such as *Acidophilus* and the *Bulgaricus* milks. Some are important in cheese making. He added that *Micrococcus*, belongs to the family *Micrococcaceae* and contains at least nine species. Several species are associated with dairy products, through which they enter processed meats such as frankfurters. Also lactic acid bacteria, such as *Streptococcus lactics* are homofermenters, all members share the property of producing lactic acid from hexoses. The Streptococci generally make up around 90% of mixed dairy starter population, and good starter culture can convert of the lactose to lactic acid. The titratable acidity may increase to 0.8-1.0%, calculated as lactic acid, and the pH usually drops to 4.3-4.5 (Collins,

1972).

Many strains of antioxidant-producing moulds have been isolated from various foods. Some of the strains belonging to species of *Aspergillus, Pencillium* and *Rhizopus* are likely to produce antioxidants in their culture filtrates (Ishikawa, 1992). Yen and Lee (1996 and 1997), investigated the antioxidants activity of extracts from 10 strains of moulds that used in fermented foods. According to those results, the ethyl acetate extracts from *Aspergillus candidus,* showed strong antioxidant activity.

Gomaa and El-Shawaf (1998 and 1999) studied the effect of crude extracts (1000, 3000, 5000 ppm) from each carrot, cress, pepper and tomato on the growth and activity of four species of bacteria are used of dairy products, i.e. *Streptococcus lactis, Lactobacillus bulgaricus, Micrococcus* sp. and *Pseudomonas fluorescens* were examined. When crude cress extract was added, it led to increase growth and activity of *P. fluorescens* and *L. bulagricus* as compared with *S. lactis* and the least effect was found with *Micrococcus* sp. On the other hand, crude cress extract resulted more considerable growth of *L. bulagricus* and *S. lactis* than each crude extracts of pepper or carrot. Whereas, tomato had a lower effect as growth factor.

The consumption of fermented dairy products could lead to reduction in serum cholesterol levels. El-shewey and El-abbassy (1998) showed that feeding on yoghurt or Biograde (new yoghurt-like product) reduced cholesterol, total lipids, triglycerides, LDL, GOT, GPT, acid and alkaline phosphatase, creatinine and uric acid while increased HDL in blood serum of hypercholesterolemia albino rats.

Volpe *et al.* (2001) observed the yoghurt enriched with plant sterols (1g plant sterol extracted from soybean/day) significantly reduced, in a dose-dependent manner, serum cholesterol and LDL levels and LDL: HDL ratio ($P<0.001$), whereas no changes in HDL and triacylglycerol levels. They concluded a low fat yoghurt-based drink moderately enriched with plant sterols may lower total cholesterol and LDL effectively in patients with primary moderate hypercholesterolemia.

The antioxidative activity and antimicrobial agents of methanolic, chloroformic, acetonic and hexanoic extracts of orange peel was tested in buffalo's

ghee and buffalo's butter. The crude extract was added to ghee at three levels (0.05, 0.01 and 0.15%) as antioxidant compared with Antrancine as synthetic antioxidant, and also it was added to butter at three levels (0.1, 0.2 and 0.3%) as antimicrobial agent to pathogenic and non-pathogenic microorganisms. Results showed that chloroformic extract was the most active antioxidant in ghee than other extracts. The inhibition activity extract was high on *Staphylococcus aureus*, *Pesudomonas fluorescence*, *Listeria monocytogenes* and *Salmonella* sp., while inhibition activity was lower on *Aspergillus flavus*, *E. coli* and non-pathogenic microorganisms. Generally, extract was best at 0.3% in butter as antimicrobial agent against bacterial food poisoning (El-Shawaf and Gomaa, 2000).

4.4- In bakery products

Rogers *et al.* (1993) studied the stability of added β-carotene in selected bakery products. β-carotene was added to each product at a level of ~1mg of β-carotene per serving. β-carotene losses during baking ranged from ~20% in bagels and cakes, to ~30% in cookies. No farther loss of β-carotene occurred during storage at room temperature.

Park *et al.* (1994) fortified bread with three forms of ascorbic acid including fat-coated ascorbic acid, L-ascorbate 2-polyphosphate and crystalline ascorbic acid, again at the level of 64 mg of ascorbic acid/100g flour. At this level, one slice of bread (28g) would provide 20% of the US adult RDA (60 mg/d) of vitamin C, if no vitamin C was lost in bread making. Among the three forms of ascorbic acid tasted, fat-coated ascorbic acid gave the highest retention of ascorbic acid during bread making and storage of bread at 25°C. When all-*rac*-α-tocopheryl acetate was incorporated into bread formulation as the source of vitamin E, it showed good stability during processing and storage of bread.

The same authors (1997 a and b) fortified white bread with coated-fat L-ascorbic acid, cold-water-dispersible β-carotene and cold-water-dispersible all-*rac*-α-tocopheryl at levels of 64, 5 and 100mg, respectively, of active ingredient/100g of flour. The freshly baked pup-loaves retained 76, 67 and 96% of added antioxidant, respectively. One serving size (one slice 28g) of three-day-old bread fortified with

one of the three antioxidants was concluded to provide 7, 120-150 and 13-16%, respectively, of the adult RDA for vitamin C, E and A.

The total plant sterol content (free sterol and covalently bound structures) of the main cereals cultivated in Finland were determined by (Piironen *et al.*, 2001). Furthermore, sterol contents were determined for different flour and bran fractions in the milling process of wheat and rye, as well as plant sterol contents in various milling and retail bakery products. They found that when two cultivars rye, wheat, barley, and oats grown in the same year were compared, the highest plant sterols was observed in rye (95.5 mg/100g), whereas the total sterols contents of wheat, barley, and oats were 69.0, 76.1, and 44.7 mg/100g, respectively. In addition, the 10 rye cultivars and breeding lines compared had total sterol contents of 70.7-85.6 mg/100g. Rye bread with whole meal rye flour as the main or only ingredient was a good source of sterols. Sterol content was higher than that of wheat bread, whereas plant sterol content of other bakery products was affected by type and amount of fat used in baking.

4.5- In meat products

Oxidation is a major cause of deterioration of food because of its negative effects on organoleptic qualities (flavor, color, etc.). Oxidation of lipids can also have a marked negative effect on nutritional value, and may be responsible for the production of toxic compounds capable of triggering metabolic disorders such as mutagenesis, carcenogensis, circulatory disorders and ageing (Kanner, 1994; Gray *et al.*, 1996; and Ruiz *et al.*, 1999).

In recent years, interest in the applications of naturally occurring antioxidants in muscle foods has increased, particularly since the use of synthetic antioxidants has become less acceptable. A range of substances have been investigated as potential antioxidants in meat products. These include nutritive antioxidants (such as α-tocopherol, β-carotene and vitamin C), spice extracts and muscle dipeptides (Chan *et al.*, 1994).

The production of off-flavors and odours, loss of polyunsaturated fatty acids (PUFA), fat-soluble vitamins and pigments and lower consumer acceptability are direct result of lipid oxidation of muscle foods (Morrissey *et al.*, 1994).

Buffalo meat nuggets (BMN) were incorporated with 500 ppm sodium ascorbate, 10 ppm α-tocopherol acetate and 0.5% sodium tripolyphosphate while processing. Sahoo and Anjaneyulu (1997) reported that use of natural antioxidants and vacuum packaging extended the shelf life of BMN from 10 to 30 days under refrigerated storage.

The color and color stability of meat is important for consumers and retailers. Meat color is affected by the amount and chemical state of the pigment myoglobin, which after oxidation results in the unattractive colored metmyoglobin. Lipid oxidation is an important factor for deterioration of meat and meat products, in particular in frozen meat. Arnold *et al.* (1993) showed that in beef, vitamin E retards the oxidation of myoglobin, and thus the loss of attractive color. Asghar *et al.* (1991) found that pork chops that had previously been frozen and thawed had better color stability during storage when vitamin E was added to the feed (100 or 200 ppm).

Color stability is one of the most important quality attributes contributing to meat shelf-life and studies have been carried out in laboratory to explain the deterioration of the bright red color beef. In France, the consumption of turkey meat is rapidly increasing and the maintenance of color and lipid stability of over-wrapped meat is important. Lipid oxidation causes the development of rancidity and "warmed-over flavors" and membrane phospholipids are the sites where oxidative changes are initiated in meat. It is well known that to decrease these oxidative processes, vitamin E is a highly-efficient antioxidant in cell membranes, acting as a chain-breaker. Experiments have shown that vitamin E reduced lipid oxidation in chicken meat (Sheehy *et al.*, 1993) and pork during storage. In frozen turkey meat have shown that dietary vitamin E lowered thiobarbutric acid (TBARS) values.

Muscles naturally contain antioxidant system which remains active for a short time after death (Chan and Deker, 1994). Meat processing has taken advantage of such antioxidant capacity but has also introduced antioxidant additives and techniques (vacuum processing, vacuum packaging, etc.) to prevent oxidative damages.

Skeletal muscle is particularly susceptible to oxidative reactions since it contains high contaminations of prooxidants (transition metals, haem-containing proteins, i.e. myoglobulin, haemoglobin) and lipid membrane which contain higher percentages of polyunsaturated fatty acid (PUFA) (Kanner, 1994).

Lipid oxidation in meat products can be controlled or minimized, by the addition of commercial synthetic antioxidants or natural antioxidants. (Gray *et al.*, 1996). Consumers and processors are concerned about the safety of synthetic food additives. This has led to renewed interest in natural products and increased research using natural antioxidants. The use of natural antioxidants has the advantage that they are readily accepted by the consumer, considered to be safe and not "chemical", and co safety tests are required by the legislation if the food component that is "Generally Recognized As Safe (GRAS)".

Lipid oxidation is one of the major problems encountered in meat processing following cooking and subsequent refrigerated or frozen storage. It affects the quality of the product due the loss of desirable color, odor and flavor and a reduced shelf life. The rate of lipid oxidation can be effectively retarded by the use of antioxidants (Ruiz *et al.*, 1999). Natural antioxidants are of main interest nowadays. Synthetic antioxidants were widely used in the meat industry but consumers concern over their safety and toxicity pressed the food industry to find sources of antioxidant.

The only antioxidant additives specifically allowed in meat product in Italy are ascorbic acid and some ascorbate at a maximum level of 0.2%. Tocopherols are allowed for some types of fats and oils (maximum 0.03 %) and for fresh sausages for frying (maximum 0.01). Ascorbate are widely used in meat products as they have positive effects on color stability of exposed surfaces, as demonstrated by dipping steaks in solutions of ascorbic acid. Tocopherols, though, are less useful because of their cost and because their lipo-solubility prevents uniform distribution in meat/fat mince. Nitrites act also antioxidants and, more importantly, antimicrobial agents which inhibit the growth and toxin production of *Clostridium botulinum* (Freybler *et al.*, 1993). Their use in meat products has been questioned for many years but a real substitute has still to be found, especially for its antimicrobial properties.

Vitamin E is a potent biological antioxidant obtained only through the diet. The major function of vitamin E, a lipid soluble compound in phospholipid membranes, is to act as a chain breaking antioxidant by inactivating free radicals in cell membranes (Monahan *et al.*, 1994). Much research on the relationship between vitamin E content and meat quality and the effects of increasing the vitamin E content of muscle by dietary mean has been carried out.

Antioxidants have been used for over 50 years to avoid or at least delay, the autoxidation process (Cuvelier *et al.*, 1994). However, due to concerns about toxicological safety of synthetic antioxidants with natural antioxidative substances. Mansour and Khalil (2000) found that adding freeze-dried extracts from ginger rhizomes, fenugreek seeds and potato peel to beef patties control lipid oxidation and color changes during cold storage. Ginger rhizome extract had an antioxidant activity comparable to the commercial antioxidants.

Wu *et al.* (1982) isolated a natural antioxidant extract from rosemary leaves which had greater antioxidant activity than BHA and equal to BHT. Further fractionation identified carnosol to be one of the active antioxidant components in rosemary. The antioxidant properties of spices are related mainly to their phenolic structure, thus their antioxidant action is similar to synthetic phenolic antioxidants (Cuvelier *et al.*, 1994). In added benefit of rosemary extract is the antibacterial effects they are reported to exhibit in meat systems.

Some of the strongest natural antioxidants can be found in leaves of rosemary. Murphy *et al.* (1998) and Gey (1998) investigated the antioxidant properties of rosemary oleoresin extract in pre-cooked roast beef slices during refrigerated (3°C) and frozen (-20°C) storage. They reported that improved both the sensory characteristics and oxidative stability of aseptically processed beef gels.

The present research was designed to evaluate the antioxidant properties of some compounds from vegetable sources and compare their effect with sodium ascorbate. Mincing, cooking and maturing for long times are known to expose muscle foods to oxidative stress, and, therefore, a test with two pork products, salame Milano and mortadella, both cured, minced and rich in fat (about 25-30%) were carried out;

the former is raw fermented and the latter cooked (Novelli *et al.*, 1998).

Natural antioxidants often have multiple modes of action that have not been fully clarified but the main functions have been identified. Catechin and sesamol are phenolic antioxidants and act as free radical acceptors and chain breakers. Catechin is extracted from green tea leaves and sesame seeds. Phytic acid is a natural compound present in seeds and cereal grains. It is a powerful inhibitor of iron-catalysed hydroxyl radical formation and lipid peroxidation since it acts by chelating free iron. Phytic acid has also been shown to function as a hypocholesterolemic agent (Zhou and Erdman, 1995). Phytic acid is water soluble, catechin is only partially water soluble and sesamol is fat soluble.

Tang *et al.* (2002) studied the effect of dietary tea catechins supplementation at levels of 50, 100, 200, 300 mg/kg feed on oxidative stability and on the protection of α-tocopherol in long-term stored (-20°C X 12 months) chicken breast and thigh meat. Added tea catechins showed significantly high antioxidant activity in oxidized docosahexanaenoic acid liposomes than the control. In addition to chelating effects on Fe^{+2}, tea catechin showed strong scavenging capacity for 1,1-diphenyl-2-picrylhydrazyl free radical. The strong free radical-scavenging ability plus the iron-chelating effects of tea catechins provide a plausible mechanism for the antioxidant effects of added tea catechins in the *in vitro* meat system.

In vivo antioxidant effects of several organosulfur compounds derived from garlic, onion, Chinese leek against lipid-associated oxidation have been studied by Borek (2001). These authors reported that these antioxidant effects were due to the activation and modification of several enzymes such as 3-hydroxy-3-methylglutaryl-COA reductase, glutathione-transferase and catalase.

The antioxidant and antimicrobial protection of diallyl sulfide (DAS), diallyl disulfide (DADS), s-ethyl cysteine (SEC) and n-acetyl cysteine (NAC) in ground beef against discoloration, lipid oxidation and microbial contamination were studied by Yin and Cheng (2003). The exogenous addition of these garlic-derived organosulfur compounds significantly delayed both oxymyoglobin and lipid oxidations. The antioxidant protection from these compounds was dose-dependant, and showed significantly greater antioxidant activity than α-tocopherol. The presence of DAS ad

DADS in ground beef significantly reduced total aerobes and inhibited the growth of five inoculated pathogenic bacteria, *Salmonella typhi., E. coli, Listeria monocytogenes, Staph. aureus* and *Campylobacter jejuni.*

Fruits and vegetables may contain components that exert antimicrobial effect. Bower *et al.* (2003) studied beef jerky formulated with 15% raisins produced conditions inhibitory to pathogenic bacteria by decreasing pH to 5.4 and a_w to 0.64. Storage of vacuum-packaged raisin-beef-jerky (10 weeks; 30°C). Raisins have a high concentration of phenolic compounds, with accompanying high levels of antioxidant activity. The product received favorable sensory ratings for appearance, texture and flavor, comparable to the non-raisin control.

Steimetz and Potter (1991) and Tannenbaum *et al.* (1991) reported that the effect of antioxidants from fruits and vegetables such as carotenoids, flavonoids, sterols, selenium and vitamin E and were inhibited the formation of nitrosamine. They added, the protective effect of vitamin C on the formation of nitrosamines in the gastrointestinal tract from nitrite added to meats suggest that vitamin C is a very effective protective agent against harmful nitrogen oxide.

5-Recommendations

Natural antioxdiants can have complementary and overlapping mechanisms of action, including modulation of detoxification enzymes, stimulation of immune system, reduction of platelet aggregation, modulation of cholesterol synthesis and hormone metabolism, reduction of blood pressure, prevented the onset of chronic diseases antioxidant, control to cystic fibrosis, antihyperlipidemic, antihyperglycemic, anticancer, antibacterial and antiviral effects. Natural antioxidants can be used in the food industry, and there is evidence that substances may exert their antioxidant effects within the human body. Its effectively prevent premature lipid oxidation. Once isolated from animal or plant materials and used in processed food, lipids autoxidise readily. As a result, organoleptic and nutritional quality are reduced, and even toxic products may be formed. The retardation of autoxidation is therefore a key to high product quality. Because most consumers prefer natural food additives over synthetic ones, natural antioxidants are of increasing importance.

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الإستفادة من مضادات الأكسده الطبيعيه و أثرها علي صحة الإنسان

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تعتبر مضادات الأكسده من المركبات الحيويه الضروريه للمحافظه علي صحة الإنسان كما أنها تلعب دورا هاما في العديد من مجالات التصنيع الدوائي و الغذائي. قد تكون مضادات الأكسده إصطناعيه مثل (BHT,BHA, TBHQ) و قد تكون طبيعيه مثل (الكاروتينات- البولي فينولات – الفلافونيدات و الأيزوفلافونيدات- الصابونينات – التانينات – بعض المعادن مثل السلينيوم – بعض الفيتامينات مثل الأسكوربيك و التوكوفيرولات- بعض الأنزيمات – الإستيرولات – الزيوت الطياره- الأحماض الدهنيه غير المشبعه). ولكن الأبحاث الحديثه أوضحت مخاطر مضادات الأكسده الإصطناعيه لما لها من أثر مسرطن يظهر أثرها علي المدي الطويل.

تتواجد مضادات الأكسده الطبيعيه بصورها الفعالة في الخضراوات و الفاكهة و الحبوب و البقوليات و الأعشاب الطبيه و العطريه و الأسماك البحريه و الطحالب. و قد أمكن الآن زيادة إنتاجها من خلال التقنيات الحديثه كزراعة الأنسجه و الهندسه الوراثيه.

و تعمل مضادات الأكسده علي الإرتباط بما يعرف بالشقوق (الشوارد) الحره Free Radicals سواء كانت ناتجه عن التمثيل الغذائي داخل جسم الإنسان أو عن التلوث البيئي الناتج عن عوادم السيارات والمصانع أو عن فساد بعض الأغذيه مثل تزنخ الزيوت. و تعتبر الشقوق الحره أحد العوامل القويه في ظهور أمراض العصر كالسرطانات و الأورام و إرتفاع مستوي السكر و الكولستيرول في الدم و أمراض القلب وتصلب الشرايين ، كما تعمل علي تلف الكبد و الكلي و المخ. كما أنها تلعب دورا هاما كمضادات لنمو و نشاط بعض الميكروبات التي تسبب الفساد والتسمم الغذائي