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ASTAXANTHIN: A POTENTIAL CAROTENOID

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ABSTRACT

Astaxanthin, a member of the carotenoid family, is a dark-red pigment which is the main carotenoid found in the marine world of algae and aquatic animals. Astaxanthin, is present in many types of seafood, including salmon, trout, red sea bream, shrimp and lobster, as well as in birds such as flamingo and quail. Synthetic Astaxanthin dominates the world market but recent interest in natural sources of the pigment has increased substantially. Common sources of natural Astaxanthin, are the green algae *haematococcus pluvialis*, the red yeast, *Phaffia rhodozyma*, as well as crustacean byproducts. Astaxanthin possesses unusual antioxidant property which has caused a surge in the nutraceutical market of the encapsulated products. Numerous studies have shown that astaxanthin has potential health-promoting effects in the prevention and treatment of various diseases, such as cancers, chronic inflammatory diseases, metabolic syndrome, diabetes, diabetic nephropathy, cardiovascular diseases, gastrointestinal diseases, liver diseases, neurodegenerative diseases, eye diseases, skin diseases, exercise-induced fatigue, male infertility, and renal failure. In this article, the currently available scientific literature regarding the most significant activities of astaxanthin is reviewed.

INTRODUCTION: The ketocarotenoid astaxanthin, 3, 3′-dihydroxy-b,b-carotene- 4, 4′-dione, belongs to the family of xanthophylls, the oxygenated derivatives of carotenoid. The synthesis of xanthophylls in plants is derived from lycopene.

Astaxanthin is ubiquitous in nature, especially in the marine environment [1] and is a red pigment common to many marine animals, such as salmonids, shrimp, lobsters, and crayfish, contributing to the pinkish-red color of their flesh [2]. Its main role is to provide the desirable reddish-orange color in these organisms as they do not have access to natural sources of carotenoids.

In addition to its effect on color, one of the most important properties of AX is its antioxidant property which has been reported to surpass those of β-carotene or even α-tocopherol [3]. Due to its antioxidant activity, AX can serve as a potent free-radical scavenger. Moreover, Astaxanthin has been found to provide many essential biological functions, including protection against lipid-membrane peroxidation of essential polyunsaturated fatty acids and proteins, DNA damage and UV light effects; it also plays an important role in immunological defense [4, 5, 6]. This has stirred great interest in Ax and promoted numerous research studies concerning its potential benefits to humans and animals.

Astaxanthin has been found and identified in several microorganisms including the microalgae *Haematococcus pluvialis*, *Chlorella zofingiensis*, and *Chlorococcum* sp., the red yeast *Phaffia rhodozyma*, and the marine bacterium *Agrobacterium aurantiacum* [7]. There has been growing interest in the use of astaxanthin as a food-coloring agent, natural feed additive for the poultry industry and for aquaculture, especially as a feed supplement in the culture of salmon, trout, and shrimp. The use of AX in the aquaculture industry is important from the stand point of pigmentation and consumer appeal but also as an essential nutritional component for adequate growth and reproduction.

Much work has also been focused on the identification, production and utilization of natural sources of AX (algae, yeast, and crustacean byproducts) as an alternative to the synthetic pigment which currently covers most of the world markets. This review paper aims to provide an updated overview of the most important chemical, biological and application aspects to this unusual carotenoid underlying its relevance to the growing industry of nutraceutical products.

Chemical structure of Carotenoids: Carotenoids comprise a family encompassing more than 600 pigments which are synthesized de novo in higher plants, mosses, algae, bacteria, and fungi [8]. Although carotenoids are largely diffused in the plant and animal kingdoms, they are synthesized *de novo* only in higher plants and protists [8]. Animal carotenoids mainly result from metabolic transformation (oxidation and/or reduction) of those present in the food chain. Aquatic animals constitute a considerable rich source of carotenoids, which are in many cases responsible of organoleptic characteristics considered by the consumer (bright colours in lobster, shrimp, salmon, fish eggs) [9, 10].

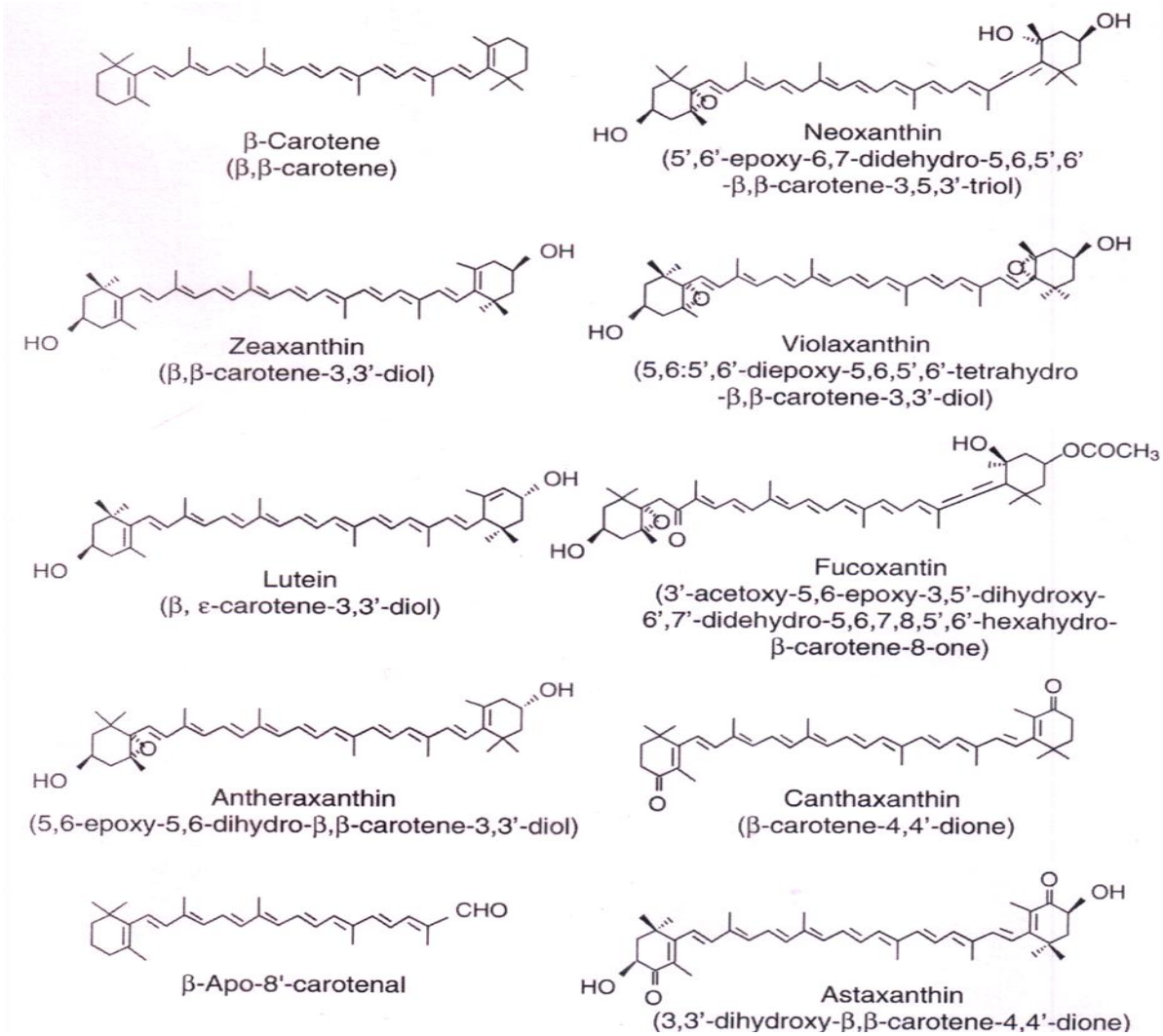

FIG. 1: CHEMICAL STRUCTURE OF SOME CAROTENOIDS (Source : Urich 1994)

Moreover, among the large number of pigments present in living organisms (i.e. chlorophylls, anthocyanins and porphyrins), carotenoids play an important role in protecting cells against photosensitised oxidation [11]. The structure of carotenoids is derived from lycopene. The majority are hydrocarbons of 40 carbon atoms which contain two terminal ring systems joined by a chain of conjugated double bonds or polyene system [12].

Two groups have been singled out as the most important: the carotene which are composed of only carbon and hydrogen; and the xanthophylls which are oxygenated derivatives, in the later, oxygen can be present as OH groups (as in zeaxanthin), or a oxy-groups (as in canthaxnthin): or in combination of both (as in AX). The polyene system gives these carotenoids their distinctive molecular structure, chemical properties, and light-absorption characteristics [13].

Different Chemical Forms of Astaxanthin: Astaxanthin has unique chemical properties based on its molecular structure. Astaxanthin has two carbonyl groups, two hydroxyl groups, and eleven conjugated ethylenic double bonds. The presence of the hydroxyl and keto moieties on each ionone ring explains some of its unique features such as the ability to be esterified and a higher antioxidant activity and a more polar nature than other carotenoids [14]. Astaxanthin may act as a strong antioxidant by donating the electrons and reacting with free radicals to convert them to more stable product and terminate free radical chain reaction in a wide variety of living organisms [14,15]. In its natural state, astaxanthin is usually associated with other molecules. Free astaxanthin is particularly susceptible to oxidation [14].

Therefore, astaxanthin in nature is either conjugated with proteins or esterified with one or two fatty acids to form monoester and diester forms, producing an array of colors in different organisms[14,16]. It is the chromophore in the blue, green, and yellow pigments of lobsters. In other cases, astaxanthin may simply be dissolved in the lipid fraction of complex molecules such as lipoproteins, or it may actually be bound chemically to molecules such as fatty acids to form esters. Reddening of some snow algae and Haematococcus is the result of such esters accumulating in cytoplasmic lipid droplets .

Whether free or complexed, the atoms comprising an astaxanthin molecule can be oriented in different ways, producing different isomers .The different isomeric forms which can be classified accordingiy to stereoisomers, geometic isomers, and free or esterified forms . In the astaxanthin molecule,each double bond from the polyene chain may exist in two configurations as geometric isomers cis or trans [13, 15,16]. Most carotenoids found in nature are predominantly all trans-isomers [12, 13]. The trans-Astaxanthin is readily isomerized to cis-trans mixtures, especially the 9-cis and 13-cis unhindered isomers for steric reasons [13].

Although astaxanthin exists mainly as trans-astaxanthin esters of various fatty acids, cis-astaxanthin esters are also detected in the algal pigment extracts. In addition to forming geometric isomers, and considering that each molecule has two chiral centers in C-3 and C-3′, AX may present three configurational isomers: two enantiomers (3R, 3′ R and 3S, 3′ S) and a meso form (3R, 3′ S) [17]. From all these isomers, the 3S, 3′ S is the most abundant in nature [18]. Synthetic AX consists of a racemic mixture of the two enantiomers and the meso form [17]. Three types of optical isomers can be found in crustacean.

Synthetic AX is an identical molecule to that produced in living organisms and it consists of a mixture 1:2:1 of isomers (3S, 3S′), (3R, 3S′), and (3R, 3R) respectively. It is the main carotenoid used worldwide in the aquaculture industry. However, the growing demand for natural foods and the high cost of synthetic pigments has stimulated the search for natural sources of AX with potential for industrialization. Only a few sources of microbial origin can compete economically with synthetic AX: the green microalgae *Haematococcus pluvialis* and the red yeast *Phaffia rhodozyma*. Their manufacturing methods have been reviewed by [18, 19, 20].

Bioavailability and safety of Astaxanthin: Carotenoid absorption strongly depends on a number of factors that are not entirely understood. Bioavailability of carotenoids also depends on their structures; in general, polar carotenoids (e.g. free astaxanthin) tend to be of higher bioavailability than apolar species (e.g. b-carotene and lycopene) [21]. It has been reported that astaxanthin from *H. pluvialis* shows better bioavailability than b-carotene from *Spirulina platensis*

and lutein from *Botryococcus braunii* [15]. In addition, cis-astaxanthins accumulate preferentially in blood plasma compared with the trans-form due to apparent shorter chain lengths [21]. Xanthophyll esters seem to be of low bioavailability, but there is a scientific controversy [21]. Studies suggested that xanthophyll esters were hydrolyzed in the small intestine for absorption in humans [22].

Recently, Sugawara *et al.,* [22] found the enzymatic esterification of xanthophylls such as astaxanthin in intestinal cells at a lower rate, and suggested that the esterification of xanthophylls was mediated by enzymatic activity after intestinal absorption. The esterified xanthophylls were likely to be incorporated into the lipid core in chylomicron and carried into a variety of tissues including the skin. In addition, by esterifying xanthophylls into highly nonpolar products, intestinal cells might be protected from the cytotoxic effects of xanthophylls. The presence of astaxanthin esters in *H. pluvialis* might be an added advantage to influence the higher bioavailability of astaxanthin [15].

Health Benefits of Astaxanthin:

Antioxidant properties and Oxidative Stress: The presence of the hydroxyl (OH) and keto (C=O) moieties on each ionone ring explains some of its unique features, namely, a higher antioxidant activity. In recent years, a number of studies on astaxanthin have in vitro and in vivo demonstrated its antioxidant effect, for example, the quenching effect on singlet oxygen, a strong scavenging effect on superoxide, hydrogen peroxide and hydroxyl radicals and an inhibitory effect on lipid peroxidation [3,11].

In addition to these, several other biological activities of astaxanthin, including anticancer, anti-inflammatory, anti-diabetic, immuno-modulatory activities and a neuroprotective effect, also have been reported [23]. This capacity of astaxanthin to quench ROS forms the basis of the ‘‘antioxidant role’’ hypothesis. ROS are highly reactive and can damage biologically relevant molecules, such as DNA, proteins and lipids. ROS covers both free radicals (e.g., superoxide, hydroxyl, nitric oxide) and non-radical oxidants (e.g., hydrogen peroxide, hypochlorous acid, singlet oxygen).

Most of endogenously produced ROS (about 90%) are normal by-products of mitochondrial activity during aerobic metabolism [24]. Apart from cell metabolism, another important source of ROS is the immune response. During an infection, the immune system cells are activated, which usually implies a certain degree ROS production [24, 25]. The lymphocytes constantly generate ROS as a way to combat invading pathogens, whereas macrophages and neutrophils phagocytose and destroy foreign particles through an oxidative mechanism termed respiratory burst that also involves the production of ROS.

Even though ROS are produced as part of the killing mechanism, this may be potentially harmful for the host organism, increasing the cost of immune response. The imbalance between ROS and antioxidant defenses is defined as oxidative stress, and is involved in relevant processes such as ageing and several degenerative diseases [26]. Free radicals can damage DNA, proteins and lipid membranes. Oxidative damage has been linked to number of ailments.

The following pathological conditions have all been linked to free radicals [27]:

- Cancer
- Aging (including immune deficiency with aging and premature aging disorders)
- Radiation injury
- Alcohol damage
- Ischemia-reperfusion injuries
- Inflammatory-immune injuries (including vasculitis from drugs and hepatitis B virus, idiopathic and membranous glomerulonephritis, and autoimmune diseases)
- Reactions induced by drugs and toxins
- Amyloid diseases
- Affecting the brain senile dementia, neurotoxin reactions, hyperbaric oxygen effects, Parkinson's disease, cerebral trauma, hypertensive cerebrovascular' injury, allergic encephalomyelitis and other demyelinating diseases, neuronal ceroid lipofuscinoses, ataxia-

telangiectasia syndrome, potentiation of traumatic injury, aluminum overload

- Affecting the heart and cardiovascular system-atherosclerosis, stroke, peripheral circulation problems, alcohol cardiomyopathy
- Affecting the kidney-renal graft rejection, nephritic antiglomerular basement membrane tlisease, heavy metal nephrotoxicity, aminoglycoside nephrotoxicity
- Affecting joints--rheumatoid arthritis
- Affecting the gastrointestinal tract and liver endotoxin liver injury, carbon tetrachloride liver injury, diabetogenic action of alloxan,free fatty acid-induced pancreatitis, abetalipoprotein-emia, nonsteroidal antiinflammatory drug-induced lesions
- Affecting the skin-sunburn and solar radiation injury, thermal injury, porphyria, contact dermatitis, effects of photosensitive dyes
- Affecting the eyes-age-related macular degeneration, ocular hemorrhage, degenerative retinal damage, cataractogeuesis, retinopathy of rematurity, photic retinopathy.

Astaxanthin as Powerful Antioxidant: To fight ROS and protect themselves from oxidative damage, organisms rely on a relatively complex antioxidant system composed of endogenously produced compounds, including low molecular weight antioxidants, enzymes and some other proteins without enzymatic functions, plus some food-derived antioxidants. Within an ecological and evolutionary context, and oxidative stress may play a key role in life-history evolution because increased oxidative damage is likely to be a significant constraint in many biological processes.

Astaxanthin's antioxidant activity has been demonstrated in several studies. In some cases, astaxanthin has up to several-fold stronger free radical antioxidant activity than vitamin E and b-carotene [24, 28]. It has been generalized that astaxanthin has an antioxidant activity, as high as ten times more than other carotenoids such as zeaxanthin, lutein, canthaxantin, and b-carotene, and 100 times more than a-tocopherol, and thus has been dubbed a ''super vitamin E'' [13]. Astaxanthin has unique chemical properties based on its molecular structure. The presence of the hydroxyl and keto moieties on each ionone ring is responsible for its higher antioxidant activity [29]. The oxo function is capable to resonance-stabilize carbon-centered radicals, which may explain the powerful antioxidative properties of astaxanthin without pro-oxidative contributions [30].

Astaxanthin catches radicals not only at the conjugated polyene chain but also in the terminal ring moiety. Goto *et al*. [31] suggested that the hydrogen atom at the C3 methine in the terminal ring was a radical trapping site. Although the unsaturated polyene chain of astaxanthin trapped radicals only in the membrane, the terminal ring of astaxanthin could scavenge radicals both at the surface and in the interior of the phospholipid membrane. The unique properties of astaxanthin have been associated with its potent antiperoxidation activity [31].

Recently, it was reported that astaxanthin could inhibit lipid peroxide formation and enhance the antioxidant enzyme status in glycated protein/iron chelate-exposed endothelial cells by suppressing reactive oxygen species generation [32]. The antioxidant properties of astaxanthin are believed to have a key role in several other properties such as protection against UV-light photooxidation, inflammation, cancer, ulcer's *Helicobacter pylorii* infection, aging and age-related diseases, or the promotion of the immune response, liver function and heart, eye, joint and prostate health.

Astaxanthin as a Photoprotectant: Exposure of lipids and tissues to light, especially UV-light, can lead to production of singlet oxygen species and subsequently free radicals and photo-oxidative damage of these lipids and tissues [33]. Carotenoids have an important role in nature in protecting tissues against UV-light mediated photo-oxidation and are often found in tissues directly exposed to sunlight. Astaxanthin can be significantly more effective than b-carotene and lutein at preventing UV-light photooxidation of lipids [25]. Oxidative damage to the eye and skin by UV light has been widely documented [34, 35] and thus, the unique UV protection properties of astaxanthin could be very important for eye and skin health.

Astaxanthin and Eye Health: Two of the leading causes of visual impairment and blindness are age-related macular degeneration (AMD) and age-related cataracts. Both diseases appear to be related to light-induced oxidative processes within the eye [34, 36]. It is therefore not surprising that factors related to oxidation have been shown in epidemiological studies to be related to an elevated risk for AMD, drusen formation, blurred vision, loss of visual acuity, loss of dark vision.A high dietary intake of carotenoids, specifically lutein and zeaxanthin from spinach, kale, and other leafy green vegetables) is associated with a reduced risk for both nuclear cataracts and AMD [37, 38].

Lutein and zeaxanthin, two carotenoid pigments closely related to astaxanthin, are concentrated in the macula of the eye [39]. The structure of astaxanthin is very close to that of lutein and zeaxanthin but has a stronger antioxidant activity and UV-light protection effect [25]. Astaxanthin is concentrated in the cone cells of the fovea, the center of macula. Astaxanthin protects against oxidative and free radical damage in eye.It protects rods, cones and DHA containing membranes. Astaxanthin is a potent inhibitor of DHA oxidation and DHA content of the eye is 30% of dry mass.Animal studies [40] demonstrated that astaxanthin is capable of crossing the blood–brain barrier and, similar to lutein, will deposit in the retina of mammals.

Nagaki *et al*. [41] found that 6mg of astaxanthin from *H. pluvialis* per day could improve eye fatigue in visual display terminal workers. It was shown that astaxanthin might increase retinal capillary blood flow in both eyes in normal volunteers and intraocular pressures remained unchanged during the supplementation period [42]. In addition, Izumi-Nagai *et al*. [43] concluded that astaxanthin treatment, together with inflammatory processes including NF-kB activation,subsequent upregulation of inflammatory molecules, and macrophage infiltration, significantly suppressed the development of choroidal neovascularization capable of leading to severe vision loss and blindness. Astaxanthin was found to be capable of providing appreciable protection for vulnerable tryptophan residues and beta high-crystallin against oxidative stress, and thus capable of protecting porcine lens crystallins against oxidative damage and degradation by calcium-induced calpain [44].

Liao *et al*., [45] reported that astaxanthin could interact with selenite, whose accumulation in the lens might cause cataract formation directly, and thus could delay selenite induced lens crystalline precipitation and attenuate selenite induced cataractogenesis in rats. Nakajima *et al*., [46] found that astaxanthin had neuroprotective effects against retinal ganglion cell damage. Recently, Cort *et al*., [47] showed that astaxanthin significantly decreased the percent of apoptotic cells on the retina in rats with elevated intraocular pressure .This study confirmed the role of oxidative injury in elevated intraocular pressure and highlighted the protective effect of astaxanthin in ocular hypertension [47].

Astaxanthin and Antidiabetic Activity: Diabetes mellitus is strongly associated with oxidative stress, which can be a consequence of increased free radical production, reduced antioxidant defenses or both [48]. Oxidative stress induced by hyperglycemia possibly causes the dysfunction of pancreatic b-cells and various forms of tissue damage in patients with diabetes mellitus [49]. It was found that astaxanthin could diminish the oxidative stress caused by hyperglycemia in the pancreatic b cells, significantly improve glucose tolerance, increase serum insulin levels, and decrease blood glucose levels, indicating that astaxanthin might exert beneficial effects on pancreatic b-cell function and could protect pancreatic b-cells against glucose toxicity by preventing the progressive destruction of these cells [49].

Based on the strong correlation between oxidative stress and immune dysfunction in diabetic patients,Otton *et al.* [50] recently studied the antioxidant effects of astaxanthin in the reactive oxygen/nitrogen species metabolism of lymphocytes isolated from alloxan-induced diabetic rats. The results showed that astaxanthin could be a good adjuvant in prophylaxis or recovery of lymphocyte dysfunctions associated with diabetic patients, especially when focusing on the re-establishment of the redox balance and a hypothetical antiapoptotic effect in lymphocytes [50]**.** Nakano *et al*.[51] compared the effect of astaxanthin in combination with other antioxidants such as ascorbic acid and a-tocopherol against oxidative damage in streptozotocin induced diabetic rats, and indicated that astaxanthin in combination with a-tocopherol could ameliorate oxidative injury through the suppression of oxidative

stress induced by diabetes. On the contrary, a high dose of ascorbic acid intake was found to increase lipid peroxidation in diabetic rats [51]. Nishigaki *et al*. [52] recently found that astaxanthin could inhibit the nonenzymatic glycation and glycated protein/iron chelate-induced cytotoxicity in human umbilical-vein endothelial cells by preventing lipid and protein oxidation and increasing the activity of antioxidant enzymes *in vitro*.

In addition, Hussein *et al*. [53] investigated the effects of astaxanthin in a metabolic syndrome animal model of spontaneously hypertensive corpulent rat, and found that astaxanthin significantly lowered the levels of blood glucose, nonesterified fatty acids and triglycerides, and significantly increased the levels of high-density lipoprotein cholesterol and adiponectin, indicating that astaxanthin ameliorates insulin resistance and improve insulin sensitivity by mechanisms involving the increase of glucose uptake, and by modulating the levels of circulating adiponectin and blood lipids.

Recently, Bhuvanesswari *et al.,* [54] showed that significant elevation in both glucose and insulin levels induced by a high fat plus high fructose diet in mice was abolished by astaxanthin supplementation, also indicating that astaxanthin could substantially improve insulin sensitivity.

Astaxanthin and Skin Health: Excessive exposure of unprotected skin to sunlight results in sunburn and can also lead to photo-induced oxidation,inflammation, immunosuppression, aging and even carcinogenesis of skin cells. Pre-clinical studies show that typical dietary antioxidants, such as a-tocopherol, ascorbicacid or b-carotene, could reduce such damage [55, 56, 57]. Astaxanthin is believed to protect the skin and eggs of salmon against UV-light photo-oxidation [58, 59]. These findings suggest that astaxanthin has an excellent potential as an oral sun-protectant.

It was reported that preincubation with synthetic astaxanthin or an algal extract containing 14% of astaxanthin could prevent ultraviolet A-induced alterations in cellular dismutase activity and decrease in cellular glutathione content [60]. Camera *et al*., [61] compared the modulation of ultraviolet A-related injury by astaxanthin,canthaxanthin, and b-carotene for systemic photoprotection in human dermal fibroblasts, and found that astaxanthin exhibited a pronounced photoprotective effect and counteracted ultraviolet A-induced alterations to a significant extent,and uptake of astaxanthin by fibroblasts was higher than that of canthaxanthin and b-carotene, indicating that astaxanthin had a superior preventive effect toward photooxidative changes.

Recently, Suganuma *et al*., [62] examined the effects of astaxanthin on the induction of matrix metallo proteinase-1 and skin fibroblast elastase by ultraviolet A treatment of cultured human dermal fibroblasts, and showed that astaxanthin could interfere with ultraviolet A-induced matrix-metalloproteinase-1 and skin fibroblast elastase/neutral endopeptidase expression. These studies suggest that topical or oral administration of astaxanthin might prevent or minimize the effects of ultraviolet A radiation such as skin sagging or wrinkling [60, 62].

Astaxanthin and Inflammation: In inflammation-related clinical conditions such as Crohn's disease, toxic reactive oxygen species (ROS) are released by phagocytic leucocytes at the site of inflammation (intestinal mucosa and lumen). These, plus increased concentrations of neutrophils at the site of inflammation, create a pro-oxidative balance that leads to lower levels of antioxidant vitamins and increased levels of markers of oxidative stress and lipid peroxidation [63].

Furthermore, oxidants have been directly linked to the stimulation of inflammation genes in endothelial cells [64]. Similarly, ROS have been attributed an aggravating role in the inflammation that accompanies asthma [26] and exercise-induced muscle damage [65]. Astaxanthin was found to reduce induced swelling of rat paw, that vitamin E did not reduce [28]. Macedo *et al.* [66] showed that astaxanthin significantly reduced the production of pro-inflammatory cytokines, such as tumor necrosis factor-a and interleukin-6 in lipopolysaccharide stimulated neutrophils.

The results also showed that astaxanthin improved neutrophil phagocytic and microbicidal capacity and reduced superoxide anion and hydrogen peroxide production, which appeared to be mediated by calcium released from intracellular storages and nitric oxide

production, indicating a beneficial effect of astaxanthin on human neutrophils function . Immune cells are particularly sensitive to oxidative stress due to a high percentage of polyunsaturated fatty acids in their plasma membranes and generally produce more oxidative products. Park *et al*., [67] studied the possible immune-enhancing, antioxidative, and anti-inflammatory activity of astaxanthin in young healthy adult female human subjects, and showed that astaxanthin could decrease a DNA oxidative damage biomarker and inflammation, and enhance immune response. The immunomodulatory, antioxidative, and anti-inflammatory activity of astaxanthin would likely influence the etiology of cancer and inflammatory diseases [67].

The anti-inflammatory activity of astaxanthin may also have a role in the prevention or treatment of asthma. It was reported that that ginkgolide B, astaxanthin, or their combination could suppress activation of T cells from asthma patients [68]. In the recent experiment,Haines *et al.,* [69] showed that the asthmatic animals fed astaxanthin, Ginkgo biloba extract and vitamin C alone or in combination exhibited significantly lower bronchoalveolar lavage fluid inflammatory cell numbers and enhancement of lung tissue content of cAMP and cGMP, and the efficacy was equal to or better than ibuprofen, a widely used nonsteroidal anti-inflammatory drug.

More recently, dietary astaxanthin was found to help fight symptoms of ulcer disease from *Helicobacter pylori*. Astaxanthin reduced symptoms of gastric inflammation and was also associated with shifts in the inflammation response [70]. Although it could be assumed that the antioxidant properties of astaxanthin explains its anti-inflammatory activity, further studies are needed to better understand the specific mode of action of astaxanthin in fighting inflammation.

Astaxanthin and Heart Health: High blood levels of LDL-cholesterol (the 'bad' cholesterol) are associated with an increased risk of atherosclerosis. However, HDL blood levels are inversely correlated with coronary heart disease and are indicative of protection against atherosclerosis. The risk of developing arteriosclerosis in humans correlates positively with the cholesterol content bound to Low Density Lipoprotein (LDL) or "bad cholesterol" [71].

Many studies have documented that high levels of LDL are related to prevalence of cardiovascular diseases such as angina pectoris, myocardial infarction, and brain thrombosis [72]. Inhibition of oxidation of LDL has been postulated as a likely mechanism through which antioxidants could prevent the development of arteriosclerosis. Several studies have looked at carotenoids, mainly *β*-carotene and canthaxanthin, as inhibitors of LDL oxidation [73, 74]. Astaxanthin is carried by VLDL, LDL and HDL in the human blood. An in vitro test and a study with human subjects ingesting daily dosages as low as 3.6 mg astaxanthin per day for two consecutive weeks demonstrated that astaxanthin protects LDL-cholesterol against induced in vitro oxidation [75].

In an animal model study, astaxanthin supplementation led to an increase in blood levels of HDL [76], the form of blood cholesterol inversely correlated with coronary heart disease. Thus, astaxanthin could benefit heart health by modifying blood levels of LDL and HDL cholesterol.Finally, astaxanthin could also be beneficial to heart health by reducing inflammation presumably associated with the development of coronary heart disease [77]. Lipid and macrophage infiltration is closely associated with early plaque development [78]. It was found that astaxanthin significantly reduced the macrophage infiltration in the lesions, and lowered the occurrence of macrophage apoptosis and plaque ruptures, indicating that astaxanthin might improve plaque stability in the atherosclerotic setting [78] by increasing adiponectin.

Another study showed that astaxanthin could suppress the scavenger receptors upregulation, matrix metalloproteinases activation, and pro-inflammatory cytokines expression in macrophages, indicating that astaxanthin is effective to regulate the macrophage atherogenesis-related functions [29]. Hussein *et al*. [79] found that oral administration of astaxanthin for 14 days significantly lowered the arterial blood pressure in spontaneously hypertensive rats but not in normotensive Wistar Kyoto strain, and the long-term administration of astaxanthin for 5wk could also delay the incidence of stroke in the stroke prone spontaneously hypertensive rats.

Subsequently, [79] showed that astaxanthin might modulate the blood fluidity in hypertension, and the antihypertensive effects of astaxanthin might be exerted through mechanisms including normalization of the sensitivity of the adrenoceptor sympathetic pathway, particularly a-adrenoceptors, and by restoration of the vascular tone through attenuation of the angiotensin II- and reactive oxygen species-induced vasoconstriction. These results indicated that astaxanthin could exert beneficial effects in protection against hypertension and stroke [14, 79]. In addition, it was shown that astaxanthin lowered blood pressure and lessened the activity of the renin-angiotensin system in Zucker Fatty Rats, indicating that the renin-angiotensin system was involved in the ability of astaxanthin to lower blood pressure [80].

Astaxanthin and Cellular Health: In the mitochondria, multiple oxidative chain reactions generate the energy needed by the cell but produce large amounts of free radicals that need to be neutralized to maintain proper mitocondrial function. It is hypothesized that the cumulative oxidative damage to mitochondria is the main culprit for the senescence of cells, which in turn is responsible for aging [81]. The efficacy of astaxanthin in preventing in vitro peroxidation of mitochondria of rat liver cells can be as high as 100 times that of vitaminE [28]. This highlights the unique capacity of astaxanthin in helping to preserver mitochondrial functions and its unique potential in the fight against aging.

Astaxanthin's superior role in protecting cellular membranes is believed to derive from its ability to protect both the inner part and external surface of membranes against oxidation (a result of the moieties of its polyene chain and terminal rings as well as of rigidifying membranes and modifying their permeability) [31, 82, 9]. Antioxidants, carotenoids in particular,are not only essential to cellular health because they help protect cellular components against oxidative damage but also because they have a role in regulating gene expression and in inducing cell-to-cell communications [83, 84].

Recently, astaxanthin was reported to have a role in regulating CYP genes in rat hepatocytes, although it did not seem to have that effect in human hepatocytes [85]. Also carotenoids are active inducers of communication between cells at the cell-gap junctions (the water-filled pores in the cell membranes that permit cell to-cell communications needed to modulate cell growth and, in particular, to limit expansion of cancerous cells) [84]. Thus, it is hypothesized that carotenoids affect DNA regulating RNA responsible for gap-junction communications and that this role in cell-gap junctions communications might explain some of the anti-cancer activities of astaxanthin.

Anti-cancer properties of Astaxanthin: Several studies have demonstrated the anti-cancer activity of astaxanthin in mammals. A case-control study with a large cohort involving ten countries showed that higher plasma concentrations of some individual carotenoids, retinol, and a-tocopherol were associated with reduced risk of gastric cancer [86]. There are two different classes of chemopreventive agents, retinoids/provitamin A carotenoids and the nonprovitamin carotenoids, which may act through separate mechanisms [87].

Increasing evidence has shown that carotenoids possess potent cancer chemopreventive properties independent of their antioxidant activity or their potential for conversion to retinoids [88]. Some of carotenoids showed more potent anticarcinogenic activity than b-carotene and might be more useful for cancer prevention [89]. It had been reported that in individuals at high risk for developing lung cancer as a consequence of smoking and/or asbestos exposure, b-carotene failed to demonstrate protection, and even was found to induce lung pathology [90], suggesting that the use of carotenoids without pro-vitamin A activity such as astaxanthin might provide protection and avoid the toxicity associated with retinoids [87].

Chew and Park [91] had suggested that although astaxanthin, canthaxanthin, and b-carotene inhibited tumor growth, astaxanthin showed the highest anti-tumor activity. Growth-inhibitory effects of astaxanthin have been reported in different tumor cells, including colon, oral fibrosarcoma, breast, prostate cancer cells, and embryonic fibroblasts [88]. Daubrawa *et al*. [92] compared the effects of canthaxanthin and astaxanthin on gap junctional intercellular communication, which is important for homeostasis, growth control, and de velopment of cells, in primary human skin fibroblasts, and found that astaxanthin was a strong suppressor of gap junctional intercellular

communication and affected channel function by changing the phosphorylation pattern of connexin43. However, in contrast to astaxanthin, canthaxanthin and other carotenoids could stimulate gap junctional intercellular communication and enhance connexin 43 expression in cell culture.Briviba *et al*., [93] compared the subcellular localization of astaxanthin and b-carotene in cultured HT29 human colon adenocarcinoma cells. The results showed that astaxanthin was effectively taken up by the cells and localized mostly in the cytoplasm, and cells incubated with b-carotene showed about a 50-fold lower cellular amount of b-carotene.

The difference of astaxanthin and b-carotene distribution in cells of intestinal origin suggested that the possible defense against reactive molecules by carotenoids in these cells might also be different [93]. Astaxanthin protected mice from carcinogenesis of the urinary bladder by reducing the incidence of chemically induced bladder carcinoma [94]. Rats fed a carcinogen but supplemented with astaxanthin had a significantly lower incidence of different types of cancerous growths in their mouths than rats fed only the carcinogen. The protective effect of astaxanthin was even more pronounced than that of b-carotene [95]. Furthermore, a significant decrease in the incidence of induced colon cancer in those rats fed astaxanthin versus those administered only the carcinogen was found [95].

Dietary astaxanthin is also effective in fighting mammary cancer by reducing growth of induced mammary tumors by 50%, more so than b-carotene and canthaxanthin [96]. Astaxanthin inhibits the enzyme 5-a-reductase responsible for prostate growth and astaxanthin supplementation was proposed as a method to fight benign prostate hyperplasia and prostate cancer [97].

More recently, astaxanthin supplementation in rats was found to inhibit the stress-induced suppression of tumor-fighting natural killer cells [98]. As noted earlier, astaxanthin's anti-cancer activity might be related to the carotenoids' role in cell communications at gap junctions, which might be involved with slowing cancercell growth [84] the induction of xenobiotic-metabolizing enzymes [99] or by modulating immune responses against tumor cells [100].

Astaxanthin in detoxification and liver function: The liver is a complex organ in which intense catabolism and anabolism take place. Liver functions include active oxidation of lipids to produce energy, detoxification of contaminants, and destruction of pathogenic bacteria, viruses and of dead red blood cells. These functions can lead to significant release of free radicals and oxidation byproducts and therefore it is important to have mechanisms that protect liver cells against oxidative damage. Astaxanthin is much more effective than vitamin E at protecting mitochondria from rat liver cells against lipid peroxidation [28].

Astaxanthin also induces xenobiotic metabolizing enzymes in rat liver, a process that could help prevent carcinogenesis [101]. Astaxanthin can induce xenobiotic metabolizing enzymes in the lung and kidney [99]. Recently,Bhuvaneswari *et al*. [54] evaluated the effects of astaxanthin in obese mice fed a high fat plus high fructose diet, and showed that astaxanthin restricted weight gain, promoted insulin sensitivity, and prevented liver injury by decreasing cytochrome P 4502E1, myeloperoxidase,and nitro-oxidative stress, and improving the antioxidant status. In addition, lipid deposition and increased transforming growth factor-b expression induced by the high calorie diet were also abolished by astaxanthin [54]. These studies indicated that astaxanthin might be of value in preventing obesity, metabolic syndrome, and liver disease arising from insulin resistance/obesity in affluent societies

Astaxanthin and Neurodegenerative Diseases: The nervous system is rich in both unsaturated fats (which are prone to oxidation) and iron (which has strong prooxidative properties). These,together with the intense metabolic aerobic activity and rich irrigation with blood vessels found in tissues of the nervous system, make tissues particularly susceptible to oxidative damage [102]. There is substantial evidence that oxidative stress is a causative or at least ancillary factor in the pathogenesis of major neurodegenerative diseases (Alzheimer's, Huntington's, Parkinson's and amyotrophic lateral sclerosis, ALS) and that diets high in antioxidants offer the potential to lower the associated risks [103, 104, 10]. In a scientific study with rats fed natural astaxanthin [40] demonstrated that astaxanthin can cross the blood brain barrier in mammals and can extend its antioxidant benefits

beyond that barrier. Liu *et al*., [106] demonstrated that astaxanthin could prevent docosahexaenoic acid hydroperoxide or 6-hydroxydopamine-induced neuronal apoptosis, mitochondrial abnormalities, and intracellular reactive oxygen species generation in SH-SY5Y cells. Chan *et al.,* [107] also showed that astaxanthin likely enhanced cell and mitochondrial membrane stability. These studies suggested that astaxanthin had the protective effects on a neurodegenerative disease, dependent on its antioxidant potential and mitochondria protection, and might be a promising neuroprotective therapeutic agent for oxidative stress-associated neurodegeneration such as Parkinson's disease [6, 106, 107].

It has been found that astaxanthin could reduce ischemia induced free radical damage, apoptosis, neurodegeneration and cerebral infarction in brain tissue through the inhibition of oxidative stress, reduction of glutamate release, and antiapoptosis, and might be clinically useful for patients vulnerable or prone to ischemic events [108].

CONCLUSION: The unique potential of astaxanthin to be used as the nutritional component in treatment or prevention strategies against several health problems caused by oxidative stress, UV-light photooxidation or inflammation, cancers and other pathological conditions has generated great interest in clinical trials and increased production of astaxanthin at commercial levels.

The protection mechanism that are likely to be involved are the activation of thymocites, the expression of immune-related genes, the up-regulation of proteins involved in cell-to-cell communication, and the increase in membrane fluidity, decreased expression or production of inflammatory mediators and cytokines by suppressing the activation of nuclear factor-kB, decreased expression or production of transforming growth factor-b1, increased levels of circulating adiponectin and insulin sensitivity, decreased activity of the renin-angiotensin system. The important antioxidant and health promoting aspect of this unusual carotenoid in the living organism has generated great interest among behavioral ecologists and scientists.

The growing industry of nutraceutical has been focusing on safety aspect of this carotenoid supplementation, and commercial production .With in few years to come,it may be an important antioxidant supplement with good market potential if its production cost are optimized.

REFERENCES:

1 Lorenz R T, Cysewski G R : Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin.Trends Biotechnol 2000*;* 18: 160–167.

2 Johnson E A, An G H :Astaxanthin from microbial sources. Crit Rev Biotechnol 1991*;* 11: 297–326.

3 Miki. W: Biological functions and activities of animal carotenoids. Pure Appl. Chem. 1991;63(1):141-146.

4 Kurihara H, Koda H, Asami S, Kiso Y, Tanaka T: Contribution of the antioxidative property of astaxanthin to its protective effect on the promotion of cancer metastasis in mice treated with restraint stress. Life Science 2002;70: 2509–2520

5 Hussein G, Sankawa U, Goto H, Matsumoto K, Watanabe H: Astaxanthin, a carotenoid with potential in human health and nutrition. Journal of Nature Products 2006;69: 443–449.

6 Ikeda Y, Tsuji S, Satoh A, Ishikura M, Shirasawa T, Shimizu T: Protective effects of astaxanthin on 6-hydroxydopamine induced apoptosis in human neuroblastoma SH-SY5Y cells. Journal of Neurochemistry 2008; 107: 1730–1740.

7 Yuan J P, Chen F, Liu X, Li Z : Carotenoid composition in the green microalga *Chlorococcum*. Food Chem. 2002;76:319–325

8 Goodwin T W: Nature and distribution of carotenoids. Food Chemistry 1980 ; 5:3-13

9 Matsushita Y *et al*.: Antioxidant activity of polar carotenoids including astaxanthin-b-glucoside from marine bacterium on PC liposomes. Fish Sci 2000; 66:980–985

10 Shahidi, F, and Synowiecki, J: Isolation and Characterization of nutrients and value-added products from snow crab (*Chinoecetes opilio*) and shrimp (*Pandalus borealis*) processing discards. J. Agric. Food Chem 1991; 39:1527–1532

11 Palozza, P and Krinsky, N I:Astaxanthin and canthaxanthin are potent antioxidants in a membrane model. Arch. Biochem. Biophys 1992; 297:291-295.

12 Britton, G: Structure and properties of carotenoids in relation to function. FASEB J 1995; 9: 1551 –1558.

13 Higuera-Ciapara I, Fe´lix-Valenzuela,L , Goycoolea, F M: Astaxanthin: a review of its chemistry and applications. Crit. Rev. Food Sci. Nutr. 2006; 46: 185–196

14 Hussein G, Goto, H, Oda, S, Sankawa, U *et al.*,:Antihypertensive

1. potential and mechanism of action of astaxanthin: III. Antioxidant and histopathological effects in spontaneously hypertensive rats. Biol Pharm Bull 2006; 29:684–688.

15 Ranga Rao A, Raghunath Reddy, R L Baskaran, V Sarada R., Ravishankar G A: Characterization of microalgal carotenoids by mass spectrometry and their bioavailability and antioxidant properties elucidated in rat model. J. Agric. Food Chem. 2010; 58:8553–8559.

16 Peng J, Xiang W Z, Tang Q M., Sun N *et al*. :Comparative analysis of astaxanthin and its esters in the mutant E1 of *Haematococcus pluvialis* and other green algae by HPLC with a C30 column. Sci China Ser. C-Life Sci 2008; 51:1108–1115.

17 Turujman S A, Wamer W G, Wei R R et al. : Rapid liquid chromatographic method to distinguish wild salmon from

aquacultured salmon fed synthetic astaxanthin. Journal of AOAC International 1997; 3:622–632.

18 Parajo J C, Santos V, and Vazquez M:. Optimization of carotenoid production by *Phaffia rhodozyma* cells grown on xylose. Process Biochemistry 1998a; 33:181–187.

19 Johnson E A, and An Gil-Hwan: Astaxanthin from microbial sources.CRC Crit. Rev. Biotechnol 1991; 11:297–326.

20 Nelis H J and De Leenheer A P: Microbial sources of carotenoid pigments used in foods and feeds. Journal of Applied Bacteriology 1991; 70:181–191.

21 Bohn T: Bioavailability of non-provitamin A carotenoids. Curr. Nutr. Food Sci. 2008; 4:240–258.

22 Sugawara T,Yamashita K, Asa A, Nagao A *et al*.: Esterification of xanthophylls by human intestinal Caco-2 cells. Arch. Biochem. Biophys 2009; 483: 205–212.

23 Hussein G, Sankawa U, Goto H, Matsumoto K,Watanabe H: Astaxanthin, a carotenoid with potential in human health and nutrition. J. Nat. Prod. 2006; 69: 443–449.

24 Shimidzu N *et al*.: Carotenoids as singlet oxygen quenchers in marine organisms. Fish. Sci. 1996; 62: 134–137.

25 O'Connor I and O'Brien N: Modulation of UVA light-induced oxidative stress by beta-carotene, lutein and astaxanthin in cultured fibroblasts. J. Dermatol. Sci. 1998;16: 226–230.

26 Greene L: Asthma and oxidant stress: Nutritional, environmental, and genetic risk factors. J. Am. Coll. Nutr. 1995;14: 317–324.

27 Cross C E, Halliwell B, Borish E T, Pryor W A, Ames B N, Saul R L, McCord J M, and Harman D: Oxygen radicals and human disease. Ann. Intern. Med. 1987; 107:526.

28 Kurashige M, Okimasu E, Inoue M and Utsumi K: Inhibition of oxidative injury of biological membranes by astaxanthin. Physiol. Chem. Pys. & Med. NMR 1991;22:27-38

29 Kishimoto Y, Tani M, Uto-Kondo H, Iizuka M *et al*.: Astaxanthin suppresses scavenger receptor expression and matrix metalloproteinase activity in macrophages. Eur. J. Nutr. 2010; 49: 119–126.

30 Martin H D, Jager C, Ruck C, Schmidt M: Anti- and prooxidant properties of carotenoids. J. Prakt. Chem. 1999;341:302–308.

31 Goto S et al.: Efficient radical trapping at the surface and inside the phospholipid membrane is responsible for highly potent antiperoxidative activity of the carotenoid astaxanthin. Biochim. Biophys Acta 2001;1512: 251–258

32 Nishigaki I, Rajendran P, Venugopal R, Ekambaram G *et al.:* Cytoprotective role of astaxanthin against glycated protein/iron chelate-induced toxicity in human umbilical vein endothelial cells. Phytother. Res. 2010; 24: 54–59

33 Papas A M: Antioxidant Status, Diet, Nutrition, and Health, CRC Press 1999.

34 Domínguez-Bocanegra A R, Ponce-Noyola T and Torres-Muñoz J A: Astaxanthin production by Phaffia rhodozyma and *Haematococcus pluvialis*: a comparative study. Applied Microbiology and Biotechnology 2007; 75(4): 783-7.

35 Matsumu Y, Ananthaswamy H N: Toxic effects of ultraviolet radiation on the skin. Toxicology and Applied Pharmacology 2004; 195 (3): 298–308.

36 Winkler B S, Boulton M E, Gottsch J D, Sternberg P: Oxidative damage and age-related macular degeneration. Mol Vis 1999; 3:32-35.

37 Jacques, P: The potential preventive effects of vitamins for cataract and age-related macular degeneration. Int. J. Vitam. Nutr. Res. 1999; 69: 198–205.

38 Lyle B J et al.: Antioxidant intake and risk of incident agerelated nuclear cataracts in the Beaver Dam Eye Study. Am. J. Epidemiol 1999;149: 801–809.

39 Landrum J T *et al*.: Analysis of zeaxanthin distribution within individual human retinas. Methods Enzymol 1999; 299: 457–467.

40 Tso M.O.M. and Lam T-T: Method of Retarding and Ameliorating Central Nervous System and Eye Damage. U.S. Patent 1996; #5527533.

41 Nagaki Y, Mihara M, Tsukahara H, Ono S: The supplementation effect of astaxanthin on accommodation and asthenopia. J. Clin. Ther. Med. 2006; 22: 41–54.

42 Nagaki Y, Mihara M, Takahashi J, Kitamura A *et al.:* The effect of astaxanthin on retinal capillary blood flow in normal volunteers. J. Clin. Ther. Med. 2005; 21: 537–542.

43 Izumi-Nagai K, Nagai N, Ohgami K, Satofuka S *et al*.: Inhibition of choroidal neovascularization with an anti-inflammatory carotenoid astaxanthin. Invest. Ophthalmol. Vis. Sci. 2008; 49: 1679–1685.

44 Wu T H, Liao J H, Hou W C, Huang F Y *et al.:* Astaxanthin protects against oxidative stress and calciuminduced porcine lens protein degradation. J. Agric. Food Chem. 2006; 54: 2418–2423.

45 Liao J H, Chen C S, Maher T J, Liu C Y *et al*.: Astaxanthin interacts with selenite and attenuates selenite-induced cataractogenesis. Chem. Res. Toxicol. 2009;22: 518–525.

46 Nakajima Y, Inokuchi Y, Shimazawa M, Otsubo, K *et al*.: Astaxanthin, a dietary carotenoid, protects retinal cells against oxidative stress *in vitro* and in mice *in vivo*. J. Pharm. Pharmacol 2008; 60: 1365–1374.

47 Cort A, Ozturk, Akpinar N, Unal D M *et al*.: Suppressive effect of astaxanthin on retinal injury induced by elevated intraocular pressure. Regul. Toxicol. Pharmacol. 2010; 58: 121–130.

48 Leite M F, de Lima A, Massuyama M, Otton R: *In vivo* astaxanthin treatment partially prevents antioxidant alterations in dental pulp from alloxan-induced diabetic rats. Int. Endod. J. 2010; 43: 959–967.

49 Uchiyama K, Naito Y, Hasegawa G, Nakamura N *et al*.: Astaxanthin protects b-cells against glucose toxicity in diabetic db/db mice. Redox Rep. 2002; 7: 290–293.

50 Otton R, Marin D P, Bolin A P, Santos R D *et al*.: Astaxanthin ameliorates the redox imbalance in lymphocytes of experimental diabetic rats. Chem. Biol. Interact. 2010; 186: 306–315.

51 Nakano M, Onodera A, Saito E, Tanabe M *et al*.: Effect of astaxanthin in combination with alpha-tocopherol or ascorbic acid against oxidative damage in diabetic ODS rats. J. Nutr. Sci. Vitaminol. (Tokyo) 2008; 54: 329–334.

52 Nishigaki I, Rajendran P, Venugopal R, Ekambaram G *et al*.: Cytoprotective role of astaxanthin against glycated protein/iron chelate-induced toxicity in human umbilical vein endothelial cells. Phytother. Res. 2010; 24:54–59.

53 Hussein G, Nakagawa T, Goto H, Shimada Y *et al.:* Astaxanthin ameliorates features of metabolic syndrome in SHR/NDmcr-*cp*. Life Sci. 2007; 80: 522–529.

54 Bhuvaneswari S, Arunkumar E, Viswanathan P, Anuradha C V, Astaxanthin restricts weight gain, promotes insulin sensitivity and curtails fatty liver disease in mice fed an obesity-promoting diet. Process Biochem. 2010; 45:1406–1414.

55 Fuchs J: Potentials and limitations of the natural antioxidants RRR-alpha- tocopherol, L-ascorbic acid and beta-carotene in cutaneous photoprotection.Free Radic. Biol. Med. 1998; 25:848–873.

56 Lee J *et al*: Carotenoid supplementation reduces erythema in human skin after simulated solar radiation exposure. Proc. Soc. Exp. Biol. Med. 2000; 223: 170–174.

57 Stahl W *et al*.: Carotenoids and carotenoids plus vitamin E protect against ultraviolet light–induced erythema in humans. Am.J. Clin. Nutr. 2000;71: 795–798.

58 Torissen O J *et al*. Pigmentation of salmonids carotenoid deposition and metabolism. CRC Crit. Rev. Aquat. Sci. 1989; 1: 209–225.

59 Savoure N *et al*.: Vitamin A status and metabolism of cutaneous polyamines in the hairless mouse after UV irradiation: action of b-carotene and astaxanthin. Int. J. Vitam. Nutr. Res. 1995; 65: 79–86.

60 Lyons N M, O'Brien N: Modulatory effects of analgal extract containing astaxanthin on UVA-irradiated cells in culture. J. Dermatol. Sci. 2002; 30: 73–84.

61 Camera E, Mastrofrancesco A, Fabbri C, Daubrawa F *etal.:* Astaxanthin, canthaxanthin and b-carotene differently affect UVA-induced oxidative damage and expression of oxidative stress-responsive enzymes. Exp. Dermatol. 2009; 18: 222–231.

62 Suganuma K, Nakajima H, Ohtsuki M, Imokawa G: Astaxanthin attenuates the UVA-induced up-regulation of matrix metalloproteinase-1 and skin fibroblast elastase in human dermal fibroblasts. J. Dermatol. Sci. 2010; 58:136–142.

63 Aghdassi E and Allard J P: Breath alkanes as a marker of oxidative stress in different clinical conditions. Free Radic. Biol. Med. 2000; 28:880–886.

64 Aw T Y: Molecular and cellular responses to oxidative stress and changes in oxidation-reduction imbalance in the intestine. Am.J. Clin. Nutr. 1999; 70: 557–565.

65 Dekkers J et al.: The role of antioxidant vitamins and enzymes in the prevention of exercise-induced muscle damage. Sports Med. 1996; 21: 213–238.

66 Macedo R C, Bolin A P, Marin D P, Otton R: Astaxanthin addition improves human neutrophils function: *in vitro* study. Eur. J. Nutr. 2010; DOI: 10.1007/s00394-010- 0103-1.

67 Park J S, Chyun J H, Kim Y K, Line L L, Chew B P: Astaxanthin decreased oxidative stress and inflammation and enhanced immune response in humans. Nutr. Metab. 2010; 7: 18-22.

68 Mahmoud F F, Haines D D, Abul H T, Abal A T *et al*.: *In vitro* effects of astaxanthin combined with ginkgolide B on T lymphocyte activation in peripheral blood mononuclear cells from asthmatic subjects. J. Pharmacol. Sci. 2004; 94: 129–136.

69 Haines D D, Varga B., Bak I, Juhasz B *et al*.: Summative interaction between astaxanthin, *Ginkgo biloba* extract (EGb761) and vitamin C in suppression of respiratory inflammation: a comparison with ibuprofen. Phytother. Res. 2010; DOI: 10.1002/ptr.3160.

70 Bennedsen M et al.: Treatment of H. pylori infected mice with antioxidant astaxanthin reduces gastric inflammation, bacterial load and modulates cytokine release by splenocytes. Immunol. Lett. 1999; 70:185–189.

71 Golstein J L and Brown M S: Low DL pathway and its relation to atherosclerosis. Annu. Rev. Biochem. 1977; 46:897–930.

72 Frei B: Cardiovascular disease and nutrient antioxidants: role of low-density lipoprotein oxidation. Crit. Rev. Food Sci. Nutr. 1995; 35:83–98.

73 Carpenter K L H, Van Der Veen C, Hird R *et al*.: The carotenoids *β*-carotene, canthaxanthin and zeaxanthin inhibit macrophage mediated LDL oxidation. FEBS Letters. 1997; 401:262–266.

74 Kritchevsky S B: b-Carotene, carotenoids and the prevention of coronary heart disease. J. Nutr. 1999; 129: 5–8.

75 Miki W, Hosoda K, Kondo K, and Itakura H: Astaxanthin-containing drink. Patent application number 10155459. 1998; Japanese Patent Office. Publication date 16 June 1998.

76 Murillo E Efecto hipercolesterole´mico de la cantaxantina y la astaxantina en ratas. Arch. Latinoam. Nutr. 1992; 42: 409–413.

77 Tracy R P Inflammation markers and coronary heart disease. Curr. Opin. Lipidol. 1999;10: 435–441

78 Li W, Hellsten A, Jacobsson L S, Blomqvist H M *et al*.: Alpha-tocopherol and astaxanthin decrease macrophage infiltration, apoptosis and vulnerability in atheroma of hyperlipidaemic rabbits. J. Mol. Cell. Cardiol. 2004; 37:969–978.

79 Hussein G, Nakamura M, Zhao Q, Iguchi T *et al*.: Antihypertensive and neuroprotective effects of astaxanthin in experimental animals. Biol. Pharm. Bull. 2005; 28: 47–52.

80 Preuss H G, Echard B, Bagchi D, Perricone N V, Yamashita E: Astaxanthin lowers blood pressure and lessens the activity of the renin-angiotensin system in Zucker Fatty Rats. J. Funct. Foods 2009; 1: 13–22.

81 Gershon D: The mitochondrial theory of aging: Is the culprit a faulty disposal system rather than indigenous mitochondrial alterations? Exp. Gerontol. 1999;34: 613–619

82 Barros M P *et al*: Astaxanthin and peridinin inhibit oxidative damage in Fe2 þ -loaded liposomes: Scavenging oxyradicals or changing membrane permeability? Biochem. Biophys. Res. Commun 2001; 288: 225–232.

83 Allen R G and Tresini M: Oxidative stress and gene regulation. Free Radic. Biol. Med. 2000;28: 463–499.

84 Bertram J S: Carotenoids and gene regulation. Nutr. Rev. 1999; 57: 182–191.

85 Kistler A *et al*.: Metabolism and CYP-inducer properties of astaxanthin in man and primary human hepatocytes. Arch. Toxicol. 2002;75: 665–675.

86 Jenab M, Riboli E, Ferrari P, Friesen M *et al*.: Plasma and dietary carotenoid, retinol and tocopherol levels and the risk of gastric adenocarcinomas in the European prospective investigation into cancer and nutrition. Br.J. Cancer 2006; 95: 406–415.

87 Bertram J S,Vine A L: Cancer prevention by retinoids and carotenoids: independent action on a common target. Biochim. Biophys. Acta 2005; 1740:170–178.

88 Palozza P, Torelli C, Boninsegna A, Simone R *et al*.: Growth-inhibitory effects of the astaxanthin-rich alga *Haematococcus pluvialis* in human colon cancer cells.Cancer Lett. 2009; 283: 108–117.

89 Nishino H, Murakoshi M, Ii T, Takemura M *et al*.: Carotenoids in cancer chemoprevention. Cancer Metast. Rev. 2002; 21: 257–264.

90 Bertram J S: Induction of connexin 43 by carotenoids: functional consequences. Arch. Biochem. Biophys. 2004; 430: 120–126.

91 Chew B P, Park J S: Carotenoid action on the immune response. J. Nutr. 2004; 134: 257S–261S.

92 Daubrawa F, Sies H, Stahl W: Astaxanthin diminishes gap junctional intercellular communication in primary human fibroblasts. J. Nutr. 2005;135:2507–2511.

93 Briviba K, Bornemann R, Lemmer U: Visualization of astaxanthin localization in HT29 human colon adenocarcinoma cells by combined confocal resonance Raman and fluorescence microspectroscopy. Mol. Nutr. Food Res. 2006; 50:991–995.

94 Tanaka T, Morishita Y, Suzui M, Kojima T, Okomura A and Mori H: Chemoprevention of mouse urinary bladder carcinogenesis by the naturally occurring carotenoid astaxanthin. Carcinogenesis 1994b; 15:15-19.

95 Tanaka T *et al*.: Chemoprevention of rat oral carcinogenesis by naturally occurring xanthophylls, astaxanthin and canthaxanthin.Cancer Res. 1995; 55: 4059–4064

96 Chew B P *et al*.: A comparison of the anticancer activities of dietary b-carotene, canthaxanthin and astaxanthin in mice in vivo. Anticancer Res. 1999;19: 1849–1854.

97 Anderson M: Method of Inhibiting 5-a Reductase with Astaxanthin to Prevent and Treat Begnign Prostate

Hyperplasia(BPH) and Prostate Cancer in Human Males. US Patent #6277417, 2001.

98 Kurihara H *et al*.: Contribution of the antioxidative property of astaxanthin to its protecti ve effect on the promotion of cancer metastasis in mice treated with restraint stress. Life Sci 2002; 70: 2509–2520.

99 Jewell C. and O'Brien N: Effect of dietary supplementation with carotenoids on xenobiotic metabolizing enzymes in the liver, lung, kidney and small intestine of the rat. Br. J. Nutr. 1999; 81: 235–242.

100 Jyonouchi H *et al*: Antitumor activity of astaxanthin and its mode of action. Nutr. Cancer 2000; 36: 59–65.

101 Gradelet S *et al*: Dietary carotenoids inhibit aflatoxin B1-induced liver preneoplastic foci and DNA damage in the rat: role of the modulation of aflatoxin B1 metabolism. Carcinogenesis 1998;19:403–411.

102 Facchinetti F *et al*: Free radicals as mediators of neuronal injury. Cell. Mol. Neurobiol. 1998;18: 667–682.

103 Borlongan C *et al*.: Free radical damage and oxidative stress in Huntington's disease. J. Fla. Med. Assoc. 1996; 83: 335–341.

104 de Rijk M *et al*.: Dietary antioxidants and Parkinson disease. The Rotterdam Study. Arch. Neurol 1997; 54: 762–765.

105 Ferrante R *et al*.: Evidence of increased oxidative damage in both sporadic and familial amyotrophic lateral sclerosis. J. Neurochem 1997; 69: 2064–2074.

106 Liu X B, Shibata T, Hisaka S, Osawa T: Astaxanthin inhibits reactive oxygen species-mediated cellular toxicity in dopaminergic SH-SY5Y cells *via* mitochondria-targeted protective mechanism. Brain Res. 2009;1254: 18–27.

107 Chan K C, Mong M C, Yin A C, Antioxidative and anti-inflammatory neuroprotective effects of astaxanthin and canthaxanthin in nerve growth factor differentiated PC12 cells. J. Food Sci. 2009; 74: H225–H231.

108 Shen H, Kuo C C, Chou J, Delvolve A *et al.:* Astaxanthin reduces ischemic brain injury in adult rats. FASEB J. 2009; 23:1958–1968.

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