Astaxanthin: structural and functional aspects[1]

Astaxantina: aspectos estruturais e funcionais

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ABSTRACT

Astaxanthin, a carotenoid belonging to the xanthophyll class, has stirred great interest due to its antioxidant capacity and its possible role in reducing the risk of some diseases. Astaxanthin occurs naturally in microalgae, such as *Haematococcus pluvialis* and the yeast *Phaffia rhodozyma,* and has also been considered to be the major carotenoid in salmon and crustaceans. Shrimp processing waste, which is generally discarded, is also an important source of astaxanthin. The antioxidant activity of astaxanthin has been observed to modulate biological functions related to lipid peroxidation, having beneficial effects on chronic diseases such as cardiovascular disease, macular degeneration and cancer. Researches have shown that both astaxanthin obtained from natural sources and its synthetic counterpart produce satisfactory effects, but studies in humans are limited to natural sources. There is no established nutritional recommendation regarding astaxanthin daily intake but most studies reported beneficial results from a daily intake of 4mg. Thus, this review discusses some aspects of the carotenoid astaxanthin, highlighting its chemical structure and antioxidant activity, and some studies that report its use in humans.

Indexing terms: Antioxidants. Astaxanthin. Carotenoids. Chronic diseases.

RESUMO

A astaxantina, carotenóide pertencente à classe das xantofilas, tem despertado grande interesse devido à sua capacidade antioxidante e possível papel na redução de risco de algumas doenças. A astaxantina pode ser encontrada naturalmente em microalgas como Haematococcus pluvialis e na levedura Phaffia rhodozyma como também tem sido considerada principal carotenóide em salmão e crustáceos. Os resíduos do processamento de camarão, geralmente descartados, são também importante fonte de astaxantina. A atividade antioxidante da astaxantina tem demonstrado importante função na modulação de funções biológicas relacionadas à peroxidação lipídica, desempenhando efeitos benéficos em doenças crônicas como doenças

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cardiovasculares, *degeneração macular e câncer. Pesquisas têm demonstrado efeitos satisfatórios da astaxantina obtida de fontes naturais assim como da obtida sinteticamente, porém os estudos em humanos se limitam à utilização de fontes naturais. Não há recomendação nutricional estabelecida para a ingestão diária de 4mg de astaxantina, mas muitos estudos relatam resultados benéficos com a ingestão diária média de 4mg. Assim, a presente revisão discute alguns aspectos do carotenóide astaxantina, com destaque para sua estrutura química e atividade antioxidante, mostrando também alguns estudos que relatam seu uso em humanos.*

Termos de indexação: *Antioxidantes. Astaxantina. Carotenóides. Doenças crônicas.*

INTRODUCTION

Astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione) is an important colorant in the salmonid and crustacean aquaculture feed industry[1,2] and, in many countries, it is also used as a dietary supplement[3,4]. By belonging to the class of carotenoids called xanthophylls, astaxanthin shares many of the metabolic and physiological activities attributed to carotenoids; however, astaxanthin has other peculiar chemical properties owing to its molecular structure[5]. The presence of hydroxyl and carbonyl functional groups in ketocarotenoids, like astaxanthin, makes them excellent antioxidants[5,6]. The high antioxidant power of astaxanthin has shown beneficial effects on various diseases related to oxidative damage, such as hypertension[7], obesity[8], macular degeneration[9] and cancer[10,11].

Astaxanthin is naturally present in seafood, such as salmon[12], shrimp, and lobster[2,13]; in the microalgae *Haematococcus pluvialis* (*H. pluvialis*)[4,14,15]; and in the yeast *Xanthophyllomyces dendrorhous* (former *Phaffia rhodozyma*)[16,17]. Nowadays, a large proportion of commercial astaxanthin is produced synthetically[18]. This synthesis starts with a C-9 unit, ketoisophorone, which is obtained from petroleum feedstocks[19,20]. However, the growing demand for natural feeds and the high cost of synthetic pigments have led to the search of natural sources of astaxanthin, such as microalgae, yeasts and crustacean by-products[13-15,17,21,22].

This review discusses some aspects of the chemical structure of astaxanthin and its antioxidant function, sources and possible role in reducing the risk of some diseases. The articles were searched in databases, such as MedLine, PubMed, Lilacs, SciELO and FSTA, using the following keywords: astaxanthin, xanthophylls, carotenoids, antioxidants. To be included in the review, the articles had to be experimental researches, clinical trials or reviews containing relevant information to the theme.

Chemical structure of astaxanthin

Carotenoids can be divided into two groups based on the chemical elements they contain in their molecules: carotenes, which only contain carbon and hydrogen; and xanthophylls, which also contain oxygen. In the xanthophylls, oxygen may be present as hydroxyl groups, carbonyl groups or as a combination of both, as seen in astaxanthin[22]. The presence of hydroxyl (OH) and carbonyl (C=O) in each ionone ring (Figure 1) explains some of the features of astaxanthin, such as the ability to be esterified, a more polar nature and a high antioxidant capacity[5].

Astaxanthin is derived from β-carotene by 3-hydroxilation and 4-ketolation at both ionone end groups. These reactions are catalyzed by β-carotene hydroxylase and β-carotene ketolase, respectively. Hydroxylation is widespread in higher

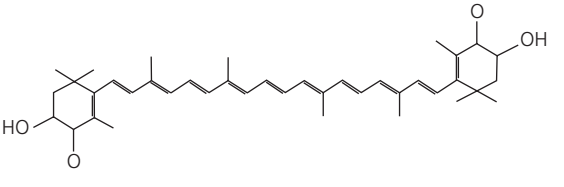

Figure 1. Free astaxanthin.

Note: Source: Ogawa *et al.*[23] (authorized by the author).

plants, but ketolation is restricted to a few bacteria, fungi, and some unicellular green algae[18].

The polyene system (conjugated double bonds) gives carotenoids their unique molecular structure, chemical properties and light-absorbing characteristics. Each double polyene bond can exist in two configurations: as *cis* or *trans*-geometric isomers. Most carotenoids found in nature are *trans* isomers. Thermodynamically, the all-*trans*-astaxanthin is more stable than other *cis* isomers[24] but they may be isomerized from one form to another when exposed to light, heat, acid or metal ions[5]. Due to the presence of two stereogenic carbon atoms at the C3 and C3' position, there are three stereoisomers for astaxanthin: a pair of enantiomers (3R,3'R- and 3S,3'S-astaxanthin) and an optically inactive mesoform (3R,3'S-astaxanthin). In nature, 3S,3'S-astaxanthin is the most abundant isomer and different organisms produce astaxanthin in different stereoisomeric ratios[3,16]. Synthetic astaxanthin has a stereoisomeric ratio of 1:2:1 for the 3R,3'R, 3R,3'S and 3S,3'S isomers, respectively[3,12,16]. The presence of stereoisomer by-products, in addition to the naturally occurring 3S,3'S, may have an inhibitory effect on the biological activity of astaxanthin, and synthetic astaxanthin may be contaminated by other reaction by-products or intermediates[18]. The obtainment of enantiopure compounds for the development of pharmaceutical products requires chirality to be introduced at a very early stage of its synthesis and maintained throughout a scalable, reproducible, and economically viable manufacturing process[19].

Depending on its origin, astaxanthin may be esterified with different fatty acids, such as palmitic, oleic, stearic or linoleic acid; it may also be free, with non-esterified hydroxyl groups, but this makes it considerably unstable and particularly susceptible to oxidation[6]; or it may complex with proteins (carotenoid proteins) or lipoproteins (carotenoid-lipoproteins). Synthetic astaxanthin is non-esterified, whereas astaxanthin in algae is always esterified[25]. On the other hand, crustaceans contain a mixture of the three aforementioned forms[22].

Astaxanthin sources

In the aquatic environment, microalgae synthesize astaxanthin. They are then eaten by zooplankton, insects or crustaceans, which, in turn, are eaten by fish, thereby providing them with their color[22]. The use of renewable sources of astaxanthin is of increasing economic interest as an alternative to its synthetic production[26]. The yeast *Xanthophyllomyces dendrorhous (Phaffia rhodozyma)* and the microalgae *Haematococcus pluvialis* (*H. pluvialis)* are known as the main microorganisms capable of synthesizing astaxanthin[27]. A number of studies have been carried out to determine the best conditions to synthesize and extract astaxanthin from these microorganisms[28-30]. *H. pluvialis* accumulates higher amounts of ketocarotenoids in cytoplasmic lipid vesicles and has been reported to be the richest source of natural astaxanthin[14], reaching 9.2mg/g cell[27].

Astaxanthin has been cited as the main carotenoid in fish, such as salmon and trout, as well as in most crustaceans. Turujman *et al*.[12] determined the astaxanthin content of wild salmon and found 4.45mg/100g in wild sockeye and 0.61mg/100g in Atlantic salmon. In cultured Atlantic salmon (*Salmo salar*), astaxanthin level is determined by their diet. Bjerkeng *et al*.[1] found higher astaxanthin levels in salmon supplemented with *Phaffia rhodozyma* (0.26 mg/100g) than those fed synthetic astaxanthin (0.20mg/100g). A study conducted to investigate the different sources of astaxanthin in red porgy skin (*Pagrus pagrus*) found higher astaxanthin levels in the skin of fish fed *H. pluvialis* (4.89mg/100g) than in the skin of fish fed synthetic astaxanthin (2.91mg/100g). The authors suggested that the ability of *H. pluvialis*, which contains esterified astaxanthin, to pigment the skin of red porgy more efficiently may be explained by the higher intestinal solubility

and easier incorporation of astaxanthin esters into mixed micelles when compared with synthetic, unesterified astaxanthin[31].

Shrimps are another important dietary source of astaxanthin. Yanar *et al*.[32] found an astaxanthin content of 1.41mg/100g in the muscle portion of wild *Penaeus semisulcatus* and 1.69mg/100g in the *Metapenaues monoceros* shrimp. Cultured *Litopenaeus vannamei* shrimp, fed a commercial diet, contained 2.24mg of astaxanthin/100g[33]. Niamnuy *et al*.[34] found 6.16mg of astaxanthin/100g in dried, wild *Penaeus indicus* (*P. indicus*) shrimp. Shrimp pigments are mainly located in the cephalothorax, abdominal epidermal layer and abdominal exoskeleton[2]; thus, most data available on the astaxanthin content of shrimp regard processed wastes. In wastes of fresh shrimps (cephalothorax and shells), astaxanthin levels range from 4.79mg/100g in *P. indicus*[13] to 9.17mg/100g in *Xiphopenaeus kroyeri* [35].

Astaxanthin extraction from crustacean wastes would imply in larges quantities of this by-product. Various alternative methods have been suggested to solve this problem, such as silage, which consists of treating crustacean wastes with organic or inorganic acids[26], and astaxanthin extraction with vegetable or fish oils, which can be directly incorporated into feeds[36].

Several companies in the United States, Europe and Japan sell astaxanthin supplements for humans, obtained mainly from *H. pluvialis* extract[15,37]. The amount of astaxanthin in these supplements range from 4 to 20mg[15,37,38]. According to the levels reported by Turujman *et al*.[12] and Bjerkeng *et al*.[1], a person would need to consume 600 to 2000g of wild or cultured salmon, respectively, to obtain 4mg of astaxanthin. On the other hand, one would have to consume roughly 260g of the shrimp species reported by Yanar *et al*.[32], but if the shells were also consumed, astaxanthin intake would be even higher.

Metabolic engineering in higher plants is potentially one of the most important tools for the production of astaxanthin. The recent cloning and characterization of β-carotene ketolase genes in conjunction with the development of effective co-transformation strategies that allow easy co-integration of multiple transgenes in target plants provided essential resources and tools to produce ketocarotenoids *in planta* by genetic engineering. Transgenic expression of β-carotene ketolase from *H. pluvialis* in the cyanobacterium *Synechococcus* PCC7942, which normally accumulates β-carotene and zeaxanthin, generated significant levels of astaxanthin and provided the first evidence of genetic modification of a plant-type carotenoid biosynthesis pathway[18]. However, despite the reported successes in generating transgenic plants with altered ketocarotenoid composition, relatively little is known about how the pathway is regulated and the subject is currently an area of active research.

Antioxidant function of astaxanthin

The first role established for animal carotenoids was that of a vitamin A precursor. However, owing to the presence of oxygenated groups in their terminal rings, most of the xanthophylls do not have the structural requirements to exercise the activity of vitamin A, which likely explains the fact that their great importance to human health has not received due recognition[21]. On the other hand, xanthophylls behave as excellent antioxidants by capturing singlet oxygen, reactive oxygen species and free radicals derived from cellular metabolic processes or environmental pollutants[39]. In order to be an effective antioxidant, a molecule such as a carotenoid would have to eliminate these radicals either by reacting with them to yield harmless products or by disrupting free-radical chain reactions [24].

The concentrations of carotenoids in mammalian tissues are generally much lower than those used to demonstrate antioxidant activity in model systems. To act as an antioxidant *in vivo*, the carotenoid would need to be incorporated

into the tissues in the correct location and at a suitable concentration relative to the oxidizing agent and the molecule that is to be protected. Carotenoids are commonly located in membranes where they constitute an integral part of the complex membrane structure. They can be incorporated into systems such as the liposome phospholipid bilayers at defined concentrations, but their orientation within the bilayer depends on its structure[24]. Mcnulty *et al*.[40] measured the effects of various carotenoids on the rates of lipid peroxidation in membranes enriched with polyunsaturated fatty acids. Apolar carotenoids, such as lycopene and β-carotene, disordered the membrane bilayer and showed a pro-oxidant effect. On the other hand, astaxanthin reduced lipid peroxidation by 40% while preserving membrane structure. Liang *et al*.[41] showed that incorporation of astaxanthin decreases the fluidity of the membrane measured by fluorescence anisotropy, which may further hamper diffusion and bimolecular radical reactions, increasing antioxidant efficiency.

The scientific literature describes antioxidant effects for natural and synthetic astaxanthin. Santocomo *et al.*[42] found that the synthetic carotenoids lutein, zeaxanthin and astaxanthin were capable of protecting the DNA of neuroblastoma cells exposed to reactive nitrogen species, such as S-nitrosoglutathione monoethyl ester. In a study with human dermal fibroblasts exposed to moderate doses of UVA, synthetic astaxanthin exhibited a pronounced photoprotective effect. In comparison with irradiated control cells, the formation of thiobarbituric acid reactive substances (TBARS) decreased to approximately 70%[43].

A feeding experiment was carried out by Tejera *et al*.[31] to determine the influence of different astaxanthin sources on the pigmentation and lipid peroxide levels of red porgy skin (*Pagrus pagrus*). The diets included a basal diet, without astaxanthin; diets containing 25 or 50mg/kg of natural astaxanthin from *H. pluvialis* (NatuRose™); a basal diet plus 12% of frozen shrimp; and diets with 25 or 50mg/kg of synthetic astaxanthin (Carophyll Pink®). The results showed that the levels of lipid peroxides in the skin of red porgy fed the diets containing natural astaxanthin from *H. pluvialis* or frozen shrimp were lower than those of fish fed the basal diet without astaxanthin. The synthetic astaxanthin-fed group also had lower levels of lipid peroxides than those fed the basal diet, but the values were not significantly different.

According to Gross & Lokcwood[44], naturally occurring carotenoids, as well as synthetic carotenoid derivatives, are excellent physical quenchers of singlet oxygen, but their low solubility in water would be a limiting factor. These authors demonstrated that a carotenoid derivative, the disodium disuccinate derivative of synthetic astaxanthin (*Cardax*™), exhibits high water dispersibility and cardioprotective effect in Sprague Dawley rats.

Astaxanthin and chronic diseases

The potent antioxidant activity of astaxanthin has been related to various biological functions, shown both in animal and clinical trials. Astaxanthin has promising applications for human health and nutrition[6,10,11,45]. Several studies have associated carotenoid intake with lower cancer incidence. In the specific case of astaxanthin, its action in chemically-induced neoplasms has been demonstrated[10]. Kurihara *et al*.[11] showed that an oral administration of 1mg/kg/day of astaxanthin for 14 days significantly reduced hepatic metastasis in rats, suggesting that it has an important role in enhancing the immunological response by inhibiting stress-induced lipid peroxidation. Astaxanthin has also been reported to protect against the toxic effects of some anticancer drugs. In a recent study, free astaxanthin showed chemoprotective potential in rats treated with cyclophosphamide[46].

The antioxidant potential of astaxanthin has also been related to obesity. Ikeuchi *et al*.[8] studied the effect of 30mg/kg *H. pluvialis* astaxanthin in obese rats fed a high-fat diet. The

results showed that astaxanthin inhibited weight gain, reduced liver weight, hepatic triglycerides as well as triglycerides and plasma cholesterol. Aoi *et al*.[47] found that *H. pluvialis* astaxanthin added to the diet of rats for 4 weeks accelerated the use of lipids during exercise, leading to better physical performance and reduced body fat. These observations demonstrate that the antioxidant effect of astaxanthin can modify muscular metabolism, resulting in improved muscular function during exercise.

Astaxanthin from *H. pluvialis* has also been shown to reduce blood pressure[7,48,49]. Hussein *et al*.[48] suggest that 5mg/kg/day of dietary astaxanthin given to hypertensive rats for 7 weeks modulates blood fluidity in hypertension and that its antihypertensive effect may be due to mechanisms that include the normalization of adrenoceptor sensitivity and the restoration of vascular tonus by attenuating the vasoconstriction induced by reactive oxygen species and angiotensin II. The administration of a higher dose (50 mg/kg/day) for a period of 22 weeks in obese rats reduced not only blood pressure but also other symptoms of the metabolic syndrome. Fasting glucose levels also decreased, insulin sensitivity increased, HDL levels increased and plasma triglyceride and non-esterified fatty acid levels decreased[50].

Oxidative stress and inflammation are implicated in several different manifestations of cardiovascular disease. They are partly generated from the overproduction of reactive oxygen and nitrogen species that activate transcriptional messengers, contributing to endothelial dysfunction, the initiation and progression of atherosclerosis, irreversible damage after ischemic reperfusion and arrhythmia. Preclinical studies of the xanthophyll carotenoid astaxanthin and its derivatives demonstrate that they have anti-inflammatory properties and potential efficacy in the setting of ischemia-reperfusion and reduce lipid peroxidation and rethrombosis after thrombolysis[51].

Curek *et al*.[52] observed that 5mg/kg/day of astaxanthin given to rats for 14 days decreased hepatocellular injury following ischemia/reperfusion damage and suggested that the mechanisms of action may include antioxidant protection against oxidative injury. Tripathi & Jena[53] showed that astaxanthin intervention (25mg/kg/day) ameliorates cyclophosphamide-induced oxidative stress, DNA damage and early hepatocarcinogenesis in rats. These authors reported for the first time that the protective effect of astaxanthin is mediated by the upregulation of the antioxidant response element and nuclear E_2-related factor 2 (Nrf2-ARE pathway).

The xanthophylls lutein and zeaxanthin are the predominant carotenoids in the macular pigment of the human retina, and their retinal concentration is related to age-related macular degeneration. Individuals suffering from age-related ophthalmologic diseases have a lower density of xanthophylls in the retina, and the levels of zeaxanthin and dietary lutein seem to be inversely related to the risk of retinal diseases and cataracts. Although astaxanthin has never been isolated from the human eye, its structure is very similar to that of lutein and zeaxanthin and seems to be related to protection against ultraviolet light[9]. Parisi *et al*.[45] found that patients with macular degeneration who once received daily 4 mg doses of astaxanthin associated with other antioxidants (vitamin C and E, zinc, copper, lutein and zeaxanthin) for 12 months had improved retinal function. Despite the long-term supplementation with astaxanthin, adverse effects were not reported in this study.

A preliminary clinical evaluation of the toxicity and efficacy of an astaxanthin-rich *H. pluvialis* extract was conducted by Satoh *et al*.[15] with 127 healthy adults that received a single, daily dose of 4, 8 or 20mg of astaxanthin for 4 weeks. Blood pressure and other parameters were collected before and after 4 weeks of supplementation. A significant decrease in systolic blood pressure and fasting blood glucose was observed in the subjects that ingested 4mg of astaxanthin. No significant differences were noted from baseline to end treatment for the other parameters.

There were no adverse effects or changes in the biochemical parameters of the supplemented groups.

A randomized, double-blind, placebo-controlled, 8-week trial designed to determine the safety of astaxanthin from *H. pluvialis* in 35 individuals showed that healthy adults can safely consume 6mg of astaxanthin per day[37]. In a study with 32 healthy male subjects, a single dose of 40mg of *H. pluvialis* astaxanthin was well tolerated[38].

Stewart *et al*.[4] gave an astaxanthin-rich biomass of *H. pluvialis* to rats to assess the possible side effects from consuming approximately 500mg of astaxanthin/kg/day. The authors found no adverse effects from high astaxanthin intake on their blood or biochemical parameters, such as albumin, globulin, creatinine, alkaline phosphatase, alanine and aspartate aminotransferase.

Studies showing the effects of high dosages of astaxanthin in humans are limited. Adverse effects following the oral administration of 100mg of astaxanthin were not reported by subjects participating in studies that examined the appearance of astaxanthin isomers in the plasma[54,55]. However, these studies were conducted with only three adult male volunteers and a single dose. There are no articles in the scientific literature reporting adverse effects of astaxanthin administration. Further investigations are needed to establish safe astaxanthin doses for humans and the effects of this carotenoid after long-term consumption.

FINAL CONSIDERATIONS

Astaxanthin is a member of a group known as xanthophylls, or oxygenated carotenoids. The presence of hydroxyl and carbonyl moieties on each ionone ring explains some of its functions. The main natural sources of astaxanthin are the microalgae *Haematococcus pluvialis,* the yeast *Phaffia rhodozyma*, some fish, as well as most crustaceans and their by-products.

The chemical structure of astaxanthin makes it an excellent antioxidant and a promising compound for human health and nutrition applications. It presents anti-cancer, anti-cardiovascular disease and anti-ocular degeneration activities. Several studies demonstrate the beneficial effects from natural astaxanthin supplementation. However, despite the considerable number of studies on the physiological functions of astaxanthin *in vitro* or in animal models, it is extremely important to continue the research with humans to determine the optimal daily intake of this carotenoid. Even though high doses have been found to be harmless, most studies suggest that beneficial effects can be achieved with a daily astaxanthin intake of 4mg. The determination of the astaxanthin content of some food products, such as salmon and crustaceans consuming different diets and from different regions, would be essential to establish the amount of astaxanthin contained in a healthy diet.

With the future perspectives in mind, the metabolic engineering of higher plants using cloned genes is possibly one of the most powerful tools for the production of astaxanthin for industrial and health applications.

CONTRIBUTORS

L.M.J. SEABRA and L.F.C. PEDROSA contributed to drafting and revising it critically.

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