Sources and Bioactivities of Astaxanthin

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Article history: Received 20 April 2012, Received in revised form 8 May 2012, Accepted 9 May 2012, Published 10 May 2012.

Abstract: Astaxanthin is a pinkish-orange carotenoid, and mainly produced by microalgae. Astaxanthin is also found in shrimp, lobster, salmon and feathers of some birds including flamingos and quail, which accumulate astaxanthin from the food chain. Astaxanthin has attracted wide attention because of its considerable bioactivities, such as antioxidant and free radical scavenging, anti-inflammation, cardiovascular health protection, anticancer, and neuroprotection activities, and protective effects on diabetes and ultraviolet radiation damages. In this review, we provide an up-to-date coverage of astaxanthin with reference to source, separation and analytical methods, and bioactivities, and special attention is paid to its bioactivities.

Keywords: astaxanthin; source; bioactivity; anticancer; antioxidant; determination.

1. Introduction

Astaxanthin is one of the main pigments belonging to the family of the xanthophylls, and found in living organisms particularly in the marine environment where it is present in microalgae, plankton, krill and seafood. It is also present in yeast, fungi, complex plants and the feathers of some birds including flamingos and quail, and gives salmon, trout, and crustaceans such as shrimp and lobster their distinctive reddish coloration (Hussein et al., 2006). Various astaxanthin stereoisomers are found in nature, which differ in the configuration of the two hydroxyl groups on the molecule (Fig. 1). The 3S, 3'S stereoisomer is the main form found in H. pluvialis and in wild salmon (Turujman, 1997). Astaxanthin contains two oxygenated groups on each ring structure, which is responsible for its enhanced antioxidant features (Guerin et al., 2003). Astaxanthin is closely related to other well-known
carotenoids, such as β-carotene, zeaxanthin and lutein, thus they share many of the metabolic and physiological functions attributed to carotenoids. The presence of the hydroxyl and keto endings on each ionone ring, explains some unique features, such as the ability to be esterified, a higher antioxidant activity and a more polar configuration than other carotenoids (Martin et al., 2003). Astaxanthin has a wide range of biological activities, including antioxidant, anti-inflammatory, anticancer and cardioprotective activities (Anderson, 2001; Choi et al., 2011; Kurashiga et al., 1990; Nakao et al., 2010; Ohgami et al., 2003). This review provides an up-to-date coverage of astaxanthin in regard to source, separation and analytical methods, and bioactivities, and special attention is paid to its biological activities.

Figure 1. The chemical structures of astaxanthin.

2. Sources of Astaxanthin

Although mammals and most fish are unable to convert other dietary carotenoids into astaxanthin, crustaceans (such as shrimp and some fish species including koi carp) have a limited capacity to convert closely related dietary carotenoids into astaxanthin. It is also present in the feathers
of some birds including flamingos and quail (Martin et al., 2003). In addition, 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* (Yuan et al., 2002). Although astaxanthin can be synthesized by plants, bacteria, a few fungi and green algae, the green microalgae *H. pluvialis* is considered to have the highest capacity to accumulate astaxanthin in reported sources (Boussiba et al., 1999; Boussiba, 2000; Martin et al., 2003).

Astaxanthin has three stereoisomers of (3-\( R \), 3′-\( R \)), (3-\( R \), 3′-\( S \)) and (3-\( S \), 3′-\( S \)). Disodium disuccinate astaxanthin (DDA) is a synthetic astaxanthin containing a mixture of all three stereoisomers, in the proportions 1:2:1. DDA was manufactured by Cardax Pharmaceuticals (Lauffer et al., 2005). DDA is no longer available but the same company now produces the second synthetic astaxanthin compound, HeptaxHeptax/XanCor, CDX-085. The company claims that it is developed for thrombotic protection, triglyceride reduction, metabolic syndrome, and inflammatory liver disease. In addition, it has increased water dispersibility and enhanced bioavailability (Khan et al., 2010).

### 3. Extraction and Determination of Astaxanthin

Choi et al. (2007) developed an efficient method for extraction of astaxanthin from the red yeast *Xanthophyllomyces dendrorhous*. Firstly, the yeast culture suspension was treated with microwaves to destroy the cell walls and microbodies. Secondly, the yeast was dried, and astaxanthin pigment was extracted using ethanol, methanol, acetone, or a mixture of the three as the extraction solvent. A frequency of 2,450 MHz, an output of 500 watts, and irradiation time of 60 s were the most optimum conditions for yeast cell wall destruction. Furthermore, optimal pigment extraction occurred when using a cell density of 10 g/L at 30 °C over 24 h, with a 10% volume of ethanol (Choi et al., 2007). Moreover, Kim et al. (2011) introduced the extraction and determination of astaxanthin in the research of identification of an emulsifier and conditions for preparing stable nanoemulsions containing the antioxidant astaxanthin. The apparatus includes a chiller, a high-pressure pump for CO\(_2\), a heating chamber, an extraction vessel, a back-pressure regulator, a number of collection vials and a wet gas meter. Samples containing 200 g of astaxanthin were loaded into extractor with glass bead. The pressure in the extractor was controlled by a needle valve just prior to the separator and the flow rate by the metering pump. The pressure ranged from 200 to 350 bars. The extractor temperature ranged from 50 to 70 °C. The total flow rate of CO\(_2\) was measured by a dry gas meter (Kim et al., 2011).

Astaxanthin in the extract could be analyzed by a high-performance liquid chromatography Agilent 1200 series, equipped with a diode array detector. The extract solutions were diluted with dichloromethane/methanol (1:3, v/v) and injected through the auto sample and separated with a reversed-phase C18-YMCA carotenoid column (250 × 4.6 mm, 5 μm) at 25 °C. Gradient elution was performed. The detection wavelength was kept at 474 nm. The astaxanthin concentration in the extract was estimated on the basis of peak area (Kim et al., 2011). In addition, a highly selective, convenient and precise method was developed for the determination of astaxanthin in *Haematococcus pluvialis* (Liu et al., 2011). Astaxanthin coexisting with chlorophyll and β-carotene could be analyzed by first-
order derivative spectrophotometry. The results showed that when detected at 432 nm, the interfering substances could be eliminated. The dynamic linear range was 2.0 - 8.0 µg/mL, with a correlation coefficient of 0.9916. The detection threshold was 0.41 µg/mL. The relative standard deviation (RSD) for the determination of astaxanthin was in the range of 0.01 - 0.06%. The results of recovery test were 98.1 - 108.0%. The statistical analysis between first-order derivative spectrophotometry and HPLC by T-testing did not exceed their critical values, revealing no significant differences between these two methods. It was proved that first-order derivative spectrophotometry is a rapid and convenient method for the determination of astaxanthin in *H. pluvialis* that can eliminate the negative effect resulting from the coexistence of astaxanthin with chlorophyll and β-carotene.

4. Bioactivities of Astaxanthin

4.1. Antioxidant Activity

Carotenoids are potent biological antioxidants that can absorb the excited energy of singlet oxygen onto the carotenoid chain, leading to the degradation of the carotenoid molecule but preventing other molecules or tissues from being damaged (Beutner et al., 2001; Mortensen et al., 1997). They can also prevent the chain reaction production of free radicals initiated by the degradation of polyunsaturated fatty acids, which can dramatically accelerate the degradation of lipid membranes (Palozza et al., 1992). In some cases, astaxanthin has up to several-fold stronger free radical antioxidant activity than vitamin E and β-carotene (Kurashiga et al., 1990). 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 pylori* infection, aging and age-related diseases, or the promotion of the immune response, liver function and heart, eye, joint and prostate health (Martin et al., 2003).

Choi et al. (2011) investigated the effects of astaxanthin on oxidative stress in overweight and obese adults. This study revealed that supplemental astaxanthin for 3 weeks improved oxidative stress biomarkers by suppressing lipid peroxidation and stimulating the activity of the antioxidant defense system. In addition, Kim et al. (2011) showed that astaxanthin supplementation might prevent oxidative damage in smokers by suppressing lipid peroxidation and stimulating the activity of the antioxidant system in smokers. Nakagawa et al. (2011) conducted a randomized, double-blind, placebo-controlled human trial to assess the efficacy of 12 weeks astaxanthin supplementation (6 or 12 mg/d) on both astaxanthin and phospholipid hydroperoxides levels in the erythrocytes of thirty middle-aged and senior subjects. The results indicated that erythrocyte astaxanthin concentrations were higher in both the 6 and 12 mg astaxanthin groups than in the placebo group. In contrast, erythrocyte phospholipid hydroperoxides concentrations were lower in the astaxanthin groups than in the placebo group. Therefore, astaxanthin supplementation results in improved erythrocyte antioxidant status and decreased phospholipid hydroperoxides levels, which may contribute to the prevention of dementia.

In a study of the antioxidant defense mechanism, carnitine palmitoyl transferase 1, acetyl-CoA carboxylase beta, and acyl-CoA oxidase mRNA abundance were significantly increased by astaxanthin supplementation, suggesting the TG-lowering effect of astaxanthin may be due to increased fatty acid
beta-oxidation in the liver (Yang et al., 2011). Expression of the nuclear factor E2 related factor 2-responsive endogenous antioxidant gene was also induced with concomitantly lower glutathione disulfide levels in the livers of ASTX-fed apoE(-/-) mice compared to controls (Yang et al., 2011).

4.2. Anti-inflammation Effects

Choi et al. (2008) showed that astaxanthin could exert its anti-inflammatory actions by inhibiting the expression of inducible nitric oxide synthase and cyclooxygenase-2 and the production of nitric oxide in lipopolysaccharide-stimulated BV2 microglial cells. This inhibitory effect of astaxanthin on the production of nitric oxide has important implications for the development of anti-inflammatory drugs for chronic inflammatory diseases such as sepsis, rheumatoid arthritis, atherosclerosis, inflammatory bowel disease, and brain inflammatory diseases (Choi et al., 2008; Lee et al., 2003). Ohgami et al. (2003) investigated the effects of astaxanthin on lipopolysaccharide-induced inflammation in vitro and in vivo. The results showed that the anti-inflammatory effect of 100 mg/kg astaxanthin was as strong as that of 10 mg/kg prednisolone. Astaxanthin also decreased production of NO, activity of inducible nitric oxide synthase (NOS), and production of PGE2 and TNF-α in RAW 264.7 (a mouse macrophage cell line cells) in vitro in a dose-dependent manner.

4.3. Anti-cancer Properties of Astaxanthin

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. Furthermore, a significant decrease in the incidence of induced colon cancer in those rats fed astaxanthin versus those administered only the carcinogen was found (Tanaka et al., 1995). Dietary astaxanthin is also effective in fighting mammary cancer by reducing growth of induced mammary tumors by 50% more than β-carotene and canthaxanthin (Chew et al., 1999). Astaxanthin inhibits the enzyme 5-α-reductase responsible for prostate growth, and astaxanthin supplementation was proposed as a method to fight benign prostate hyperplasia and prostate cancer (Anderson, 2001). More recently, astaxanthin supplementation in rats was found to inhibit the stress-induced suppression of tumor-fighting natural killer cells (Kurahara et al., 2002). Chew and Park (2002) had suggested that although astaxanthin, canthaxanthin, and β-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 (Palozza et al., 2009).

4.4. Astaxanthin and the Immune Response

Park et al. (2011) reported the role of astaxanthin played in modulating immune responses in cats. The results indicated that dietary astaxanthin enhanced delayed-type hypersensitivity response to both the specific (vaccine) and nonspecific (concanavalin A) antigens. In addition, cats fed astaxanthin had heightened peripheral blood mononuclear cell (PBMC) proliferation and natural killer cell cytotoxic activity. Moreover, dietary astaxanthin increased concentrations of plasma IgG and IgM. Therefore, dietary astaxanthin heightened cell-mediated and humoral immune responses in cats. In
addition, Chew et al. (2010) investigated the effects of astaxanthin on immune response in dogs. The results showed that dietary astaxanthin increased concentrations of IgG and B cell population. Plasma concentrations of C reactive protein were lower in astaxanthin-fed dogs. Dietary astaxanthin heightened cell-mediated and humoral immune response and reduced DNA damage and inflammation in dogs.

4.5. Astaxanthin and Neurodegenerative Diseases

It had been demonstrated that astaxanthin can cross the blood brain barrier in mammals and can extend its antioxidant benefits beyond that barrier. Astaxanthin, is therefore an excellent candidate for testing in Alzheimer’s disease and other neurological diseases. Liu et al. (2009) 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. Chang et al. (2010) recently found that astaxanthin possesses an amazingly potent protective effect against the damaging effects elicited by β-amyloid peptide 25–35 in PC12 cells, and might be used as a very potential neuron protectant and a potent anti-Alzheimer’s disease adjuvant therapy, particularly in its early stage. In addition, Abadie-Guedes et al. (2008) demonstrated that astaxanthin could antagonize the ethanol-induced facilitation of cortical spreading depression propagation in the young adult rat brain and its antioxidant properties might be involved in such effects. Lu et al. (2010) reported that astaxanthin prevented cerebral ischemic injury induced by 2 h middle cerebral artery occlusion (MCAO) and 24 h reperfusion in rats. Pretreatment of astaxanthin intragastrically twice at 5 h and 1 h prior to ischemia dramatically diminished infarct volume and improved neurological deficit in a dose-dependent manner. Nissl staining showed that the neuronal injury was significantly improved by pretreatment of astaxanthin at 80 mg/kg.

4.6. Curing Cardiovascular Disease

Experimental studies in several species using an ischaemia-reperfusion myocardial model demonstrated that astaxanthin protects the myocardium when administered both orally or intravenously prior to the induction of the ischaemic event. Cardiovascular clinical trials are warranted based on the physicochemical and antioxidant properties, the safety profile and preliminary experimental cardiovascular studies of astaxanthin (Martin et al., 2003). A recent study showed that astaxanthin could increase heart mitochondrial membrane potential and contractility index dose dependently and tend to decrease plasma interleukin-1α, tumor necrosis factor-α, and serum amyloid A concentrations in BALB/c mice, supporting the possible effect of astaxanthin for cardiac protection (Nakao et al., 2010). Moreover, the effects of astaxanthin on blood pressure were assessed in spontaneously hypertensive rats (SHR). There was a significant reduction in blood pressure after 14-days of oral astaxanthin administration whereas this did not occur in normotensive Wistar Kyoto rats. Astaxanthin administered orally for five-weeks in stroke prone SHR also resulted in a significant BP reduction. Oral astaxanthin also enhanced nitric oxide induced vascular relaxation in the rat aortas (Hussein et al., 2005). In experiments in SHR, oral astaxanthin significantly decreased nitric oxide end products, which indicated that it might exert its blood pressure effects via this pathway. Moreover, the studies
using the SHR aorta and coronary arteries demonstrated that astaxanthin reduced the wall/lumen ratio in coronary arteries and decreased elastin bands in the aorta (Hussein et al., 2006). This suggests that astaxanthin may beneficially mediate atherosclerotic CVD processes. In addition, a series of two experiments were reported in the one article, one using the synthetic astaxanthin (CDX-085) and the other using free astaxanthin. The authors concluded that the results supported the potential of astaxanthin as a potential therapy to prevent thrombosis associated with cardiovascular disease (Jyonouchi et al., 1994).

4.7. Gastro-protective Effects

Studies both in vivo and in vitro have shown that astaxanthin is not only a free radical scavenger but also shows antimicrobial activity against H. Pylori (Akyo, 2002). Moreover, the studies had revealed that the ethanol-induced gastric damage was mediated by the generation of free radicals (Nashikawa et al., 2005). Kim et al. (2005) found that the oral administration of astaxanthin had significant protection against ethanol-induced gastric lesion in rats and could inhibit elevation of the lipid peroxide level in gastric mucosa.

4.8. Hepatoprotective Effects

A study showed that astaxanthin could obstruct the increase of glutamate-oxalacetate transaminase and glutamate-pyruvate transaminase activities and thiobarbituric acid reactive substances in response to CCl₄ while causing an increase in glutathione levels and superoxide dismutase activities in the CCl₄-treated rat liver (Kang et al., 2001). A recent study also showed that astaxanthin could attenuate the adverse effect of CCl₄ and protect hepatocytes (Kim et al., 2009). These studies suggested that astaxanthin protected liver damage induced by CCl₄ by inhibiting lipid peroxidation and stimulating the cellular antioxidant system (Kang et al., 2001; Kim et al., 2009) and modulating the inflammatory process.

4.9. Effects on Diabetes

Oxidative stress induced by hyperglycemia possibly causes the dysfunction of pancreatic b-cells and various forms of tissue damage in patients with diabetes mellitus (Uchiyama et al., 2002). 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 (Uchiyama et al., 2002).

4.10. Prevention of UV-Light Photooxidation

Astaxanthin has not been isolated in the human eye. However, an animal study (Tso et al., 1996) demonstrated that astaxanthin is capable of crossing the blood–brain barrier and, similar to lutein, will deposit in the retina of mammals. The retinal photoreceptors of rats fed astaxanthin were
less damaged by a UV-light injury and recovered faster than animals not fed astaxanthin. Therefore, it can be inferred that deposition of astaxanthin in the eye could provide superior protection against UV light and oxidation of retinal tissues pointing to the potential of astaxanthin for eye health maintenance. 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 β-carotene and lutein at preventing UV-light photooxidation of lipids (Oconnor et al., 1996).

4.11. Effects on Fertility

Eskenazi et al. (2005) suggested that a healthy diet with high intake of antioxidants might be an inexpensive and safe way to improve semen quality and fertility. In addition, Tripathi and Jena (2008) showed that astaxanthin treatment significantly improved the testes weight, sperm count, and sperm head morphology as compared with only cyclophosphamide-treated animals, indicating the chemoprotective potential of astaxanthin against cyclophosphamide induced germ cell toxicity in mice.

Some bioactivities of astaxanthin are summarized in Table 1. In view of the potential application in human health and nutrition, the United States Food and Drug Administration approved astaxanthin as a feed additive for use in the aquaculture industry in 1987, and in 1999 it was approved for use as a dietary supplement (Guerin et al., 2003). There is no established nutritional recommendation regarding astaxanthin daily intake but most studies reported beneficial results from a daily intake of 4 mg (Juca et al., 2010).

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<th>Bioactivities</th>
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<td>antioxidation</td>
<td>Beutner et al., 2001; Choi et al., 2011; Kim et al., 2011; Kurashiga et al., 1990; Martin et al., 2003; Mortensen et al., 1997; Nakagawa et al., 2011; Palozza et al., 1992; Yang et al., 2011</td>
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<td>anti-inflammation</td>
<td>Choi et al., 2008; Lee et al., 2003; Ohgami et al., 2003</td>
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<td>anti-cancer</td>
<td>Anderson, 2001; Chew et al., 1999; Chew and Park, 2002; Kurahara et al., 2002; Palozza et al., 2009; Tanaka et al., 1995</td>
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<td>immune response</td>
<td>Chew et al., 2010; Park et al., 2011</td>
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<td>neuroprotection</td>
<td>Abadie-Guedes et al., 2008; Chang et al., 2010; Liu et al., 2009; Lu et al., 2010</td>
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<td>cardiovascular protection</td>
<td>Hussein et al., 2005 &amp; 2006; Jyonouchi et al., 1994; Martin et al., 2003; Nakao et al., 2010</td>
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<td>gastro-protection</td>
<td>Akyo, 2002; Kim et al., 2005; Nashikawa et al., 2005</td>
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<td>hepatoprotection</td>
<td>Kang et al., 2001; Kim et al., 2009</td>
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5. Conclusions and Prospects

Astaxanthin is one of the main pigments belonging to the family of the xanthophylls, and has been found in crustaceans (such as shrimp and some fish species including koi carp), the feathers of some birds including flamingos and quail and several microorganisms including the microalgae *Haematococcus pluvialis, Chlorella zofingiensis* and so on. To extract the astaxanthin from the red yeast *Xanthophyllomyces dendrorhous*, the yeast culture suspension could be treated with microwaves to destroy the cell walls and microbodies, and then astaxanthin pigment was extracted using ethanol, methanol, acetone, or a mixture of the three as the extraction solvent. To analyze the content of astaxanthin, high-performance liquid chromatography with a diode array detector could be applied. The antioxidant properties of astaxanthin are believed to have a key role in several other properties such as the protection against UV-light photooxidation, inflammation, cancer, neurodegenerative diseases, cardiovascular diseases or the promotion of the immune response, liver function, eye health, diabetes, gastro-protective and so on. Therefore, astaxanthin has considerable potential and promising applications in human nutrition and health.

Acknowledgments

This research was supported by the Hundred-Talents Scheme of Chinese Academy of Sciences, and the Hundred-Talents Scheme of Sun Yat-Sen University.

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