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Astaxanthin: A Review of its Chemistry and Applications

I. HIGUERA-CIAPARA, L. FÉLIX-VALENZUELA, and F. M. GOYCOOLEA

Centro de Investigación en Alimentación y Desarrollo, A.C., P.O. Box 1735. Hermosillo, Sonora. México. 83000

Astaxanthin is a carotenoid widely used in salmonid and crustacean aquaculture to provide the pink color characteristic of that species. This application has been well documented for over two decades and is currently the major market driver for the pigment. Additionally, astaxanthin also plays a key role as an intermediary in reproductive processes. 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 an unusual antioxidant activity which has caused a surge in the nutraceutical market for the encapsulated product. Also, health benefits such as cardiovascular disease prevention, immune system boosting, bioactivity against* Helycobacter pylori, *and cataract prevention, have been associated with astaxanthin consumption. Research on the health benefits of astaxanthin is very recent and has mostly been performed* in vitro *or at the pre-clinical level with humans. This paper reviews the current available evidence regarding astaxanthin chemistry and its potential beneficial effects in humans.*

Keywords astaxanthin, health benefits, carotenoids

INTRODUCTION

Astaxanthin (AX) is a pigment that belongs to the family of the xanthophylls, the oxygenated derivatives of carotenoids whose synthesis in plants derives from lycopene. AX is one of the main pigments included in crustacean, salmonids, and other farmed fish feeds. 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. The use of AX in the aquaculture industry is important from the standpoint of pigmentation and consumer appeal but also as an essential nutritional component for adequate growth and reproduction. In addition to its effect on color, one of the most important properties of AX is its antioxidant properties which has been reported to surpass those of β-carotene or even α-tocopherol (Miki, 1991). Due to its outstanding antioxidant activity AX has been attributed with extraordinary potential for protecting the organism against a wide range of ailments such as cardiovascular problems, different types of cancer and some diseases of the immunological system. This has stirred great interest in AX and prompted numerous research studies concerning its potential benefits to humans and animals. 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 of this unusual carotenoid underlining 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 (Goodwin, 1980). The structure of carotenoids is derived from lycopene (Figure 1). The majority are hydrocarbons of 40 carbon atoms which contain two terminal ring systems joined by a chain of conjugated double bonds or poliene system (Urich, 1994). Two groups have been singled out as the most important: the carotenes which are composed of only carbon and hydrogen; and the xanthophylls which are oxygenated derivatives. In the latter, oxygen can be present as OH groups (as in zeaxanthin), or as oxi-groups (as in canthaxanthin); or in a combination of both (as in AX). (Figure 1).

The poliene system gives carotenoids its distinctive molecular structure, their chemical properties and their light-absorption

Address correspondence to I. Higuera-Ciapara, Centro de Investigación en Alimentación y Desarrollo. -A.C. Carretera a la Victorial Km 0.6. AP 1735 Hermosillo, Sonora 83000 Mexico. E-mail: higuera@cascabel.ciad.mx

a) Licopene

b) β-carotene

c) Zeaxanthin

d) Canthaxanthin

e) Astaxanthin
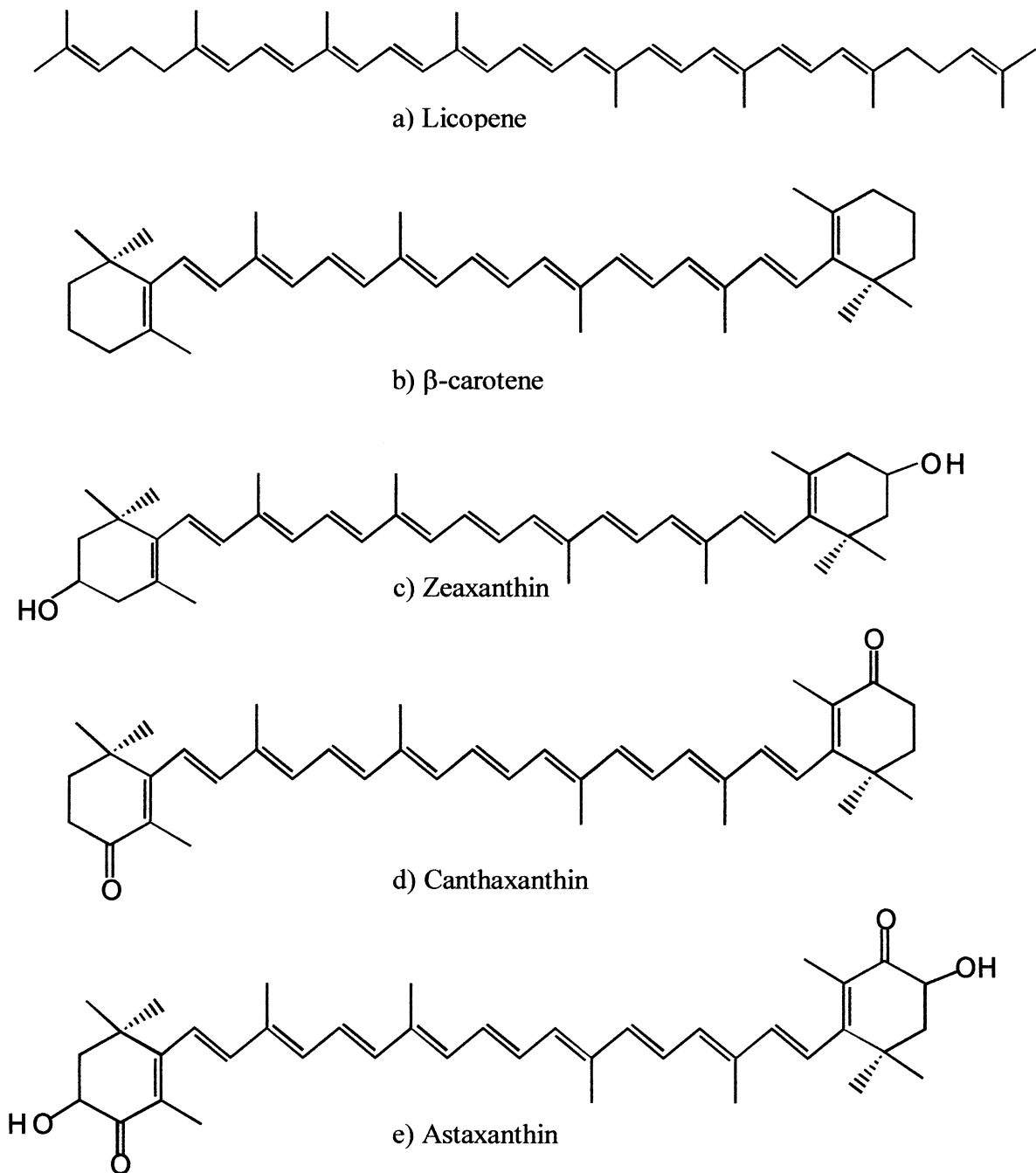
Figure 1 Chemical structure of some carotenoids. Source: Urich, 1994.

characteristics. Each double bond from the poliene chain may exist in two configurations; as geometric isomers *cis* or *trans*. *Cis*-isomers are thermodynamically less stable than the *trans* isomers. Most carotenoids found in nature are predominantly all *trans* isomers (Britton, 1995). 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) (Turujman et al., 1997) (Figure 2). From all these isomers, the 3S, 3′S is the most abundant in nature (Parajo et al., 1996). Synthetic AX consists of a racemic mixture of the two enantiomers and the meso form (Turujman et al., 1997). Three types of optical isomers can be found in crustacea (Cortés, 1993).

Depending on their origin, AX can be found in association with other compounds. It may be sterified in one or both hydroxyl groups with different fatty acids such as palmitic, oleic, estearic, or linoleic: it may also be found free, that is,
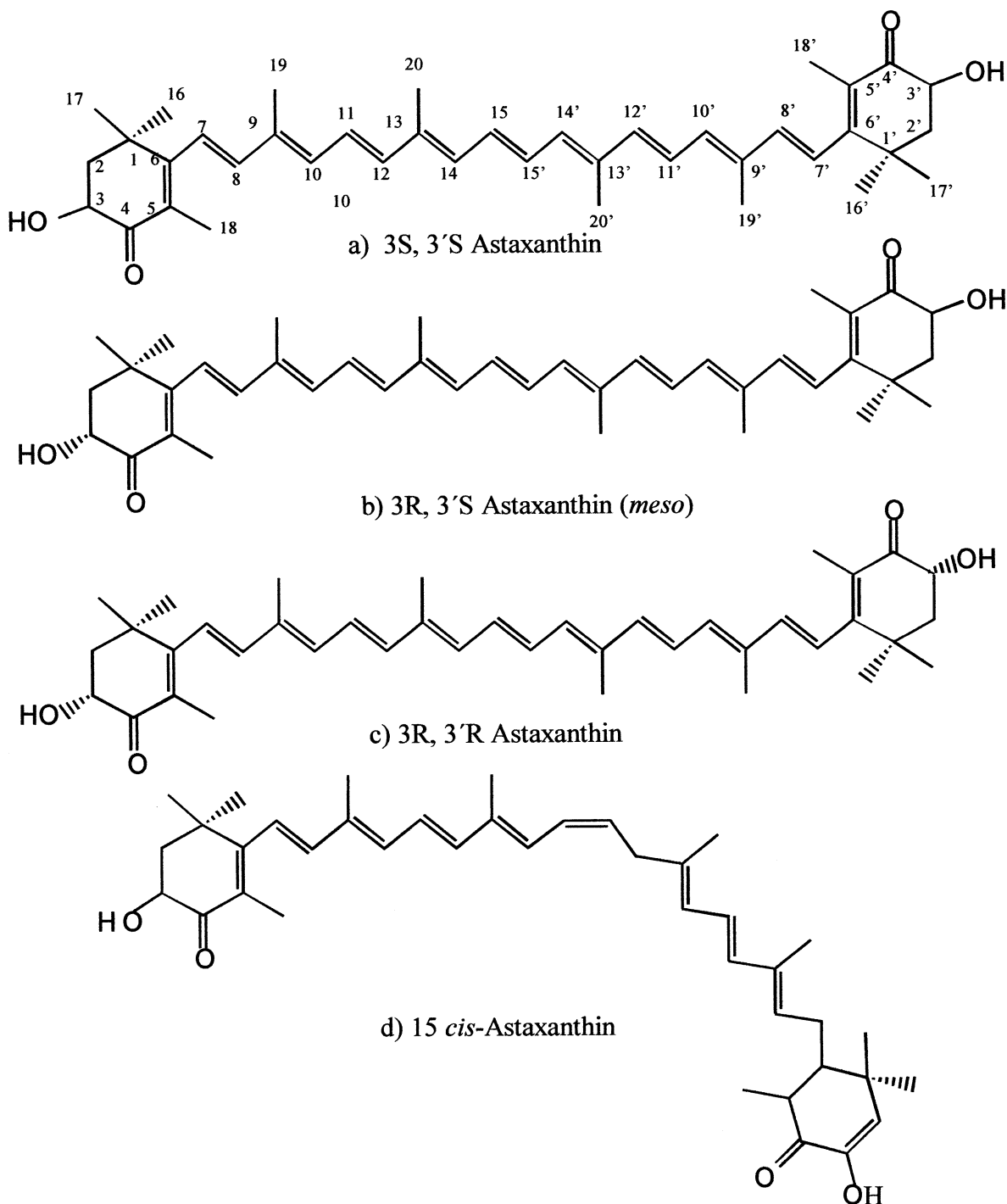
Figure 2 Astaxanthin configurational isomers (a–c) and a geometric *cis* isomer (d). Source: Turujman et al., 1997; Osterlie et al., 1999.

with the hydroxyl groups without sterification; or else, forming a chemical complex with proteins (carotenoproteins) or lipoproteins (carotenolipoproteins). Synthetic AX is not sterified, while found in algae is always sterified (Johnson and An, 1991; Yuan et al., 1997). Crustacean AX on the other hand, is a mixture of the three forms previously described (Arango, 1996).

SOURCES OF AX

Synthetic AX

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. Since 1990, Roche began a large scale production of synthetic AX and practically fulfilled the world market for the pigment, estimated at 150–200 million dollars. 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 Johnson and An (1991), Nelis and De Leenheer (1991), and Parajo et al. (1996). Several small companies have been founded (Igene, Aquasearch, and Cyanotech) and are trying to compete with Roche by offering AX from natural sources. However, so far, these products only take up a very small fraction of the market due to their limited production (McCoy, 1999).

Microalgae

Numerous research reports exist concerning the study of microalgae, particularly *Haematococcus pluvialis* with the aim of optimizing the AX production processes. The main focus of these efforts has been the assessment of various factors and conditions which affect algae growth and the production of AX (Kakizono et al., 1992; Kobayashi et al., 1992, 1993; Harker et al., 1995, 1996; Fabregas et al., 1998, 2000; Gong and Chen 1998; Boussiba et al., 1999; Zhang et al., 1999; Hata et al., 2001; Orosa et al., 2001; and Choi et al., 2002). The recent advances in photobioreactor technology has been a fundamental tool to achieve commercial feasibility in the production of AX from microalgae (Olaizola, 2000) as it has allowed the development of culture methods with AX concentration varying from 1.5 to 3% on a dry weight basis (Lorenz and Cysewsky, 2000). The production system consists of microalgae cultivation in large ponds under controlled conditions, followed by processing to break down the cell wall to increase the bioavailability of the carotenoid (Cyanotech, 2000) since the intact spores present low digestibility (Sommer et al., 1991). The biomass is finally dried to obtain a fine powder of reddish color. Several AX products currently marketed are derived from *H. pluvialis* microalgae and are being manufactured with the method previously described. These products may contain between 1.5 and 2.0% of AX and are utilized as pigments and nutrient for aquatic animals and also in the poultry industry for the pigmentation of broilers and egg yolk (Cyanotech, 2000).

On the other hand, other algal species have been proposed as sources of AX but so far without much success as compared to the species previously described. Gouveia et al. (1996, 2002) shown that *Chlorella vulgaris* is efficient for pigmentation purposes with the same magnitude of synthetic pigments. More recently, a group of researchers has shown interest in the identification, extraction, and purification of carotenoids from the microalgae *Chlorococcum sp* (Li and Chen, 2001; Ma and Chen, 2001; Zhang and Lee, 2001; Yuan et al., 2002). *Chlorococcum* seems to be a promising source of AX as well as other carotenoids such as canthaxanthin and adonixanthin.

The interest shown by the aquaculture industry for natural sources of AX has been growing as a result of the increasing demand for fish fed with natural pigments (Guerin and Hosokawa, 2001). In general, the microbial sources of carotenoids are comparable to synthetic sources as far as pigmentation is concerned (Choubert and Heinrich, 1993; Gouveia et al., 1996, 2002; Bowen et al., 2002; Gomes et al., 2002). However, it is worth noting that some authors suggest that sterified AX sourced from algae could be twice as effective as synthetic AX for the pigmentation of red seabream (Guerin and Hosokawa, 2001) in addition to providing a better growth rate in *Penaeus monodon* larvae (Darachai et al., 1999).

Yeast

For more than two decades, the red yeast *Phaffia rhodozyma* has been widely studied due to its capacity in producing AX. The scientific literature is very abundant in reports on this microorganism. Many of these reports have been focused on the effect of different nutrients or carbon sources in the culture media on the production of yeast biomass and AX (Kesava et al., 1998; Parajo et al., 1998a; Chan and Ho, 1999; Ramirez et al., 2000; An, 2001; Flores-Cotera and Sanchez, 2001). Other authors have been most interested in optimizing the conditions which favor larger AX yields (Parajo et al., 1998b; Vazquez and Martin, 1998; Ramirez et al., 2001) or in assays testing salmonid pigmentation with diets containing *Phaffia*, with a similar efficiency to that achieved using synthetic AX (Gentles and Haard, 1991; Whyte and Sherry, 2001). Other researchers have concentrated on the utilization of genetically-improved strains of the same yeast to increase AX yields (An et al., 1989; Adrio et al., 1993; Calo et al., 1995; Fang and Chiou, 1996; An, 1997). Currently the yeast is marketed in a fine powder form as a natural source of AX, protein, and other nutrients and utilized as an ingredient in salmonid feed. It is manufactured by natural fermentation in a carefully controlled environment thus effectively obtaining a product with a high percentage of free AX (8,000 μg/g) (Igene, 2003).

Crustacean Byproducts

Crustacean byproducts are generated during processing operations of recovering or conditioning of the edible portion of crabs, shrimp, and lobster. Generally, these byproducts are made up of mineral salts (15–35%), proteins (25–50%), chitin (25–35%), lipids, and pigments (Lee and Peniston, 1982). The carotenoid pigments contained therein have been thoroughly studied and quantified (Kelley and Harmon, 1972; Meyers and Bligh, 1981; Mandeville, 1991; Shahidi and Synowiecki, 1991; Olsen and Jacobsen, 1995; Gonzalez-Gallegos et al., 1997). The carotenoid content in shrimp and crab byproducts varies

Table 1 Carotenoid contents in various sources of crustaceon biowastes

Source	Total astaxanthin (mg/100g)	Astaxanthin (%)			Others carotenoids	Reference
		Free	Monoester	Diester		
Shrimp (*P. borealis*)	14.77	3.95	19.72	74.29	zeaxanthin	Shahidi and Synowiecki, 1991
Shrimp (*P. borealis*)	4.97[a]	8	22.5	69.5	—	Torrisen et al., 1981
Shrimp (*P. borealis*)	3.09[a]	5.6	18.5	75.9	—	Guillou et al., 1995
Crawfish (*P. clarkii*)	15.3	40.3	49.4		astacene	Meyers and Bligh, 1981
Backs snow crab (*Ch. Opilio*)	11.96	21.16	5.11	56.57	lutein, zeaxanthin, astacene	Shahidi and Synowiecki, 1991

[a]mg/100g wet basis.

between 119 and 148 μg/g. AX is mainly found free or sterified with fatty acids. These byproducts may also contain small quantities of lutein, zeaxanthin and astacene (Shahidi and Botta, 1994) Table 1.

The potential utilization of shrimp, krill, crab, and langostilla byproducts to induce pigmentation of cultured fish has been tested (Coral et al., 1997). Byproducts generally contain less than 1000 μg/g of AX. This would imply the incorporation of large quantities of byproducts as feed ingredients (10–25%) in order to attain an efficient pigmentation process. A means of processing is through the transformation of this biomass into meal. However, the drying methods which depend on heat application are not suitable because of the high susceptibility of carotenoids to oxidative degradation under such thermal processing conditions (Olsen and Jacobsen, 1995). An additional disadvantage is the high ash and chitin content which significantly decrease the digestibility by fish and severely limit the rate of byproduct addition to the formulations (Guillou et al., 1995; Gouveia et al., 1996; Lorenz, 1998b). In order to avoid this problem various alternative methods have been suggested so as to process crustacean byproducts. One such methods is silage, which consists of treating byproducts with organic or inorganic acids in order to protect them from bacterial decomposition and ease pigment recovery (Torrisen et al., 1981; Chen and Meyers, 1983; Gillou et al., 1995). During this treatment, calcium salts are partially dissolved at the low pH (4–5) due to acid addition; this results in AX increase in the solid fraction and a higher digestibility (Torrisen et al., 1981). Alternatively, the pigments have also been extracted with the use of vegetable or fish oils (Chen and Meyers, 1982a, 1982b; Meyers and Chen, 1985; Omara-Alwala et al., 1985; Coral et al., 1997) which can be incorporated directly as feed ingredients. Similarly, the concurrent recovery of proteins and pigments in a stable complex form (carotenoprotein) has also been demonstrated to be feasible and to provide an excellent source of pigments and aminoacids (Simpson and Haard, 1985; Manu-Tawiah and Haard, 1987; Simpson et al., 1992). The carotenoprotein complexes from crustacea provide a bluish-brown coloring. When these compounds are denatured by heat, AX is exposed and develops the typical reddish-orange color expected by consumers.

AX IN AQUACULTURE

Salmonid and crustacean coloring is perceived as a key quality attribute by consumers. The reddish-orange color characteristic of such organisms originate in the carotenoids obtained from their feeds which are deposited in their skin, muscle, exoskeleton, and gonads either in their original chemical form or in a modified state depending on the species (Meyers and Chen, 1982). The predominant carotenoid in most crustacea and salmonids is AX (Yamada et al., 1990; Shahidi and Synowiecki, 1991; Gentles and Haard, 1991). For instance, from the total carotenoids in crustacean exoskeleton, AX comprises 84–99%, while in the internal organs it represents 70–96% (Tanaka et al., 1976). In the aquatic environment, the microalgae biosynthesize AX which are consumed by zooplankton, insects, or crustacea, and later it is ingested by fish, thereby getting the natural coloration (Lorenz, 1998a). Farmed fish and crustacea do not have access to natural sources of AX, hence the total AX intake must be derived from their feed.

The use of AX and/or canthaxantin (Figure 1) as pigmenting agents in aquaculture species has been well documented through many scientific publications for more than two decades (Meyers and Chen, 1982; Torrisen, 1989; Yamada et al., 1990; No and Storebakken, 1991; Putnam, 1991; Storebakken and No, 1992; Smith et al., 1992; Choubert and Heinrich, 1993; Coral et al., 1998; Lorenz, 1998a; Gouveia et al., 2002; Bowen et al., 2002). Currently, the synthetic form of both pigments represents the most important source for fish and crustacean farming operations. AX is available under the commercial brand name Carophyll Pink[TM] and canthaxanthin as Carophyll Red.[TM] Both of these trademarks are owned by Hoffman-LaRoche. In spite of the fact that canthaxanthin provides a fairly good pigmentation, AX is widely preferred over it due to the higher color intensity attained with similar concentrations (Storebakken and No, 1992). Additionally, AX is deposited in muscles more efficiently probably due to a better absorption in the digestive tract (Torrisen, 1989). It has also been reported that when a combination of both carotenoids is used, a better pigmentation is obtained than when using either pigment separately (Torrisen, 1989; Bell et al., 1998). However, in a more recent study of Buttle et al.

(2001) found that the absortion of these two pigments is species dependent. These authors found that canthaxantin is more readily deposited in the Atlantic salmon muscle (*Salmo salar*). Some researchers have geared their interest in studying the role of the optical and symmetry isomerism of AX on the absorption and distribution of these on the various tissues of salmonids. These studies have shown that the apparent coefficient of digestibility of the geometric *cis* isomers is lower than that of all *trans* ones, therefore they are not utilized to the same extent for muscle pigmentation. Moreover, *cis* isomers tend to preferentially accumulate in the liver, while *trans* ones do so on muscle and plasma (Bjerkeng et al., 1997; Bjerkeng, 2000). Also, studies undertaken on rainbow trout have shown that the distribution of R/S optical isomers found in faeces, blood, liver, and muscle resembled that of the overall content of the supplied diet (Osterlie et al., 1999). In spite of the fact that AX is widely used with the sole purpose of attaining a given pigmentation, it has many other important functions in fish related mainly to reproduction: acceleration of sexual maturity, increasing fertilization and egg survival, and a better embryo development (Putnam, 1991). It has also been demonstrated that AX improves liver function, it increases the defense potential against oxidative stress (Nakano et al., 1995) and has a significant influence on biodefense mechanisms (Amar et al., 2001). Similarly, several other physiological and nutritional studies have been performed in crustaceans, mainly on shrimp, which have suggested that AX increases tolerance to stress, improves the immune response, acts as an intracellular protectant, and has a substantial effect on larvae growth and survival (Gabaudan, 1996; Darachai et al., 1999). Chien et al., (2003) proposed that AX is a "semi-essential" nutrient for tiger shrimp (*Penaeus monodon*) because the presence of this compound can be critical to the animal when it is physiologically stressed due to environmental changes.

According to the above information, the use of AX in the aquaculture industry is important not only from the standpoint of pigmentation to increase consumer acceptance but also as a necessary nutrient for adequate growth and reproduction of commercially valuable species.

AX AS AN ANTIOXIDANT

Normal aerobic metabolism in organisms generates oxidative molecules, that is, free radicals (molecules with unpaired electrons) such as hydroxyls and peroxides, as well as reactive oxygen species (singlets) which are needed to sustain life processes. However, excess quantities of such compounds are dangerous due to their very high reactivity because they may react with various cellular components such as proteins, lipids, carbohydrates, and DNA (Di Mascio et al., 1991). This situation may cause oxidative damage through a chain reaction with devastating effects causing protein and lipid oxidation and DNA damage *in vivo*. This constant free radical attack against an organism is known as oxidative stress (Maher, 2000). Such damage has been associated with different diseases such as macular degeneration due to

the aging process, retinopathy, carcinogenesis, arteriosclerosis, and Alzheimer disease, among other ailments (Maher, 2000). In order to control and reduce oxidation, the human body generates its own enzymatic antioxidants such as super oxide dismutase, catalase, and peroxidase, as well as other molecules with antioxidant activity. However, in many cases, these compounds are not enough to provide suitable protection against oxidative stress. Many studies have shown that oxidation can also be inhibited by consuming proper quantities of antioxidants like vitamin E (Burton et al., 1982).

An antioxidant is a molecule which has the ability to remove free radicals from a system either by reacting with them to produce other innocuous compounds or disrupting the oxidation reactions (Britton, 1995). Water soluble dietary antioxidants include vitamin C, and lipophilic antioxidants include vitamin E (α-tocopherol) and carotenoids such as β-carotene and AX. β-carotene has been thoroughly studied, but lately AX has drawn more and more attention due to its multiple functions and its great antioxidant potential.

The potential effects of carotenoids on human health have been associated with their antioxidant properties. Persons who ingest a higher concentration of carotenoids have a lower risk of chronic diseases such as cardiovascular diseases, cataract development, macular degeneration, and some types of cancer (Ziegler, 1991; Mayne, 1996). Numerous studies have shown the antioxidant activity of antioxidants by quenching active oxygen species and free radicals *in vitro* and *in vivo* through well known mechanism (Burton and Ingold, 1984; Terao, 1989; Lee and Min, 1990; Di Mascio et al., 1991; Miki, 1991; Tsuchiya et al., 1992; Palozza and Krinsky, 1992; Kobayashi and Sakamoto, 1999; Rengel et al., 2000). However, antioxidants can also act as prooxidants, that is, substances that can induce oxidative stress. Recent reviews on the subject have summarized the available data and experimental evidence on the antioxidant/prooxidant activity of carotenoids in different lipid systems (Palozza, 1998; Haila, 1999; Young and Lowe, 2001).

Even when current knowledge of the mechanism by virtue of which carotenoids act as prooxidants is still controversial, a general mechanism has been described in which at high oxygen partial pressure, a carotenoid radical could react with oxygen to generate a carotenoid-peroxyl radical. This is an autoxidation process and such radical could act as a pro-oxidant by promoting oxidation of unsaturated lipids (Haila, 1999). Major factors involved in carotenoids prooxidant activity include oxygen partial pressure, carotenoid concentration, as well as the interaction with other antioxidant species, as reviewed by Palozza (1998). Thus, it has been demonstrated that the choice of experimental conditions in *in vitro* studies can greatly affect the antioxidant/prooxidant activity of these compounds (Haila, 1999).

Information is not available on antioxidant/prooxidant mechanisms of carotenoids with structures different from β-carotene. As far as astaxanthin is concerned, only information accounting for its antioxidant activity is available. It has been reported that it has a antioxidant activity, as high as 10 times more than other

carotenoids such as zeaxanthin, lutein, canthaxantin, and β-carotene; and 100 times more that α-tocopherol. Thus, AX has been dubbed a "super vitamin E" (Miki, 1991). This property has caused great interest and a growing number of publications have appeared on the subject. Naguib (2000) measured the antioxidant activity of various carotenoids using a novel fluorometric assay procedure. These authors found that AX has a higher antioxidant activity than lutein, licopene, α and β-carotene, and α-tocopherol. In order to explain such high activity they propose that, depending on the solvent type, astaxanthin exists in an equilibrium, with the enol form of the ketone, thus the resulting dihydroxy conjugated polyene system possesses a hydrogen atom capable of breaking the free radical reaction in a similar way to that of α-tocopherol. Goto et al. (2001) reported that AX is twice more effective than β-carotene to inhibit the production of peroxides induced by ADP and Fe^{2+} in liposomes. Similarly, other studies have shown the superior antioxidant activity of AX in relation to other carotenoids (Terao, 1989; Lee and Min, 1990; Miki, 1991). The natural functions of carotenoids are determined by their physicochemical properties which depend on their molecular structure. Carotenoids react rapidly with free radicals and their reactivity depends on the length of the poliene system and the terminal rings (Lee and Min, 1990; Britton, 1995; Miller et al., 1996; Goto et al. 2001). Other authors have reported different findings. For instance, Mortensen et al. (1997) have proposed that the mechanism and rate of free radical scavenging is dependent on the nature of the free radicals rather than on the structure of the carotenoids. Thus, caution must be exercised when studying and comparing the antioxidant activity since results will be dependent on the experimental conditions set forth.

BENEFITS OF AX AS A HUMAN DIETARY SUPPLEMENT

Manufacturers of natural AX have long tried to penetrate the aquaculture market niche with very little or no success at all. In recent years, their attention has shifted towards another growing industry: the nutraceuticals market (McCoy, 1999). Currently there is a wide variety of AX products sold in health food stores in the form of nutritional supplements. Most of these products are manufactured from algae or yeast extracts. Due to their high antioxidant properties these supplements have been attributed with potential properties against many diseases. Thus, research on the actual benefits of AX as a dietary supplement is very recent and basically has thus far has been limited to *in vitro* assays or pre-clinical trials.

Anticancer Activity

Activity of carotenoids against cancer has been the focus of much attention due to the association between low levels of these compounds in the body and cancer prevalence. Several research groups have studied the effect of AX supplementation on various cancer types showing that oral administration of AX inhibits carcinogenesis in mice urinary bladder (Tanaka et al., 1994), in the oral cavity (Tanaka et al. 1995a) and rat colon (Tanaka et al., 1995b). This effect has been partially attributed to suppression of cell proliferation. Furthermore, Jyonouchi et al., (2000) found that when mice were inoculated with fibrosarcoma cells, the dietary administration of AX suppresses tumor growth and stimulates the immune response against the antigen which expresses the tumor. AX activity against breast cancer has also been studied in female mice. Chew et al. (1999) fed mice with a diet containing 0, 0.1% and 0.4% AX, β-carotene or canthaxanthin during three weeks before inoculating the mammary fat pad with tumor cells. Tumor growth inhibition by AX was shown to be dependent on the dose and more effective than the other two carotenoids tested. It has also been suggested that AX attenuates the liver metastasis induced by stress in mice thus promoting the immune response though the inhibition of lipid peroxidation (Kurihara et al., 2002). Kang et al. (2001) also reported that AX protects the rat liver from damage induced by CCl_4 through the inhibition of lipid peroxidation and the stimulation of the cell antioxidant system. Additionally, the effects of AX and other carotenoids on proliferation of human breast cancerous cells have also been studied. This study showed that β-carotene and lycopene are more effective than AX in inhibiting the proliferation of MCF-7 cell line *in vitro* (Li et al., 2002).

Prevention of Cardiovascular Diseases

The risk of developing arteriosclerosis in humans correlates positively with the cholesterol content bound to Low Density Lipoprotein (LDL) or "bad cholesterol" (Golstein and Brown, 1977). 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 (Maher, 2000). 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 (Carpenter et al., 1997). However such studies have produced conflicting results as some authors have suggested otherwise (Gaziano et al. 1995). With respect to AX, there has been very little research focused toward their ability to prevent coronary disease. Iwamoto et al. (2000) performed *in vivo* and *ex vivo* studies and their results suggest that AX inhibits the oxidation of LDL which presumably contributes to arteriosclerosis prevention. Miki et al. (1998) proposed the manufacture of a drink containing AX whose antioxidant action on LDL would be useful for the prevention of arteriosclerosis, ischemic heart disease or ischemic encephalopathy. While it is feasible that oxidation of LDL may be decreased by antioxidant consumption, more research is needed to establish the true effect on coronary heart disease (Jialal and Fuller, 1995).

AX Effect Against Helicobacter Pylori Infections

H. pylori is considered an important factor inducing acute gastritis, peptic ulcers, and stomach cancer in humans. The antibacterial action of AX has been shown in mice infected with this bacterium. When mice are fed with an AX rich diet, the gastric mucous inflammation is reduced as well as the load and colonization by the bacterium (Bennedsen et al., 1999; Wang et al., 2000). Thus, the development of products for therapeutic and prophylactic treatment of the mucous membrane of the gastrointestinal system caused by *H. pylori* has been proposed (Wadstron and Alejung, 2001). The mechanism of AX action to produce this effect is not known but it is suspected that its antioxidant properties play an important role in the protection of the hydrophobic lining of the mucous membrane making colonization by *H. pylori* much more difficult (Wadstron and Alejung, 2001). The use of AX could represent a new and attractive strategy for the treatment of *H. pylori* infections.

AX as a Booster and Modulator of the Immunological System

The group led by Jyonouchi et al. has performed the large majority of investigations regarding the potential activity of AX as a booster and modulator of the immunological system. AX increases the production of T-helper cell antibody and increases the number of antibody secretory cells from primed spleen cells (Jyonouchi et al., 1996). These authors also studied the effect of AX in the production of immunoglobulins *in vitro* by human blood cells and found that it increases the production of IgA, IgG, and IgM in response to T-dependent stimuli (Jyonouchi et al., 1995). Other studies performed *in vivo* using mice have shown the immunomodulating action of AX and other carotenoids for humoral responses to T-dependent antigens, and suggested that the supplementation with carotenoids may be useful to restore immune responses (Jyonouchi et al., 1994). In agreement with the above results, various foods and drinks with added AX have been prepared to increase the immune response mediated by T-lymphocytes and NK cells, to alleviate or prevent the decrease of immunological functions caused by stress (Asami et al., 2001). Due to its immunomodulating action, AX has also been utilized as a medication for the treatment of autoimmune diseases such as multiple sclerosis, rheumatoid arthritis and Crohn's disease (Lignell and Bottiger, 2001).

Additional Benefits

Ultraviolet radiation is a significant risk factor for skin cancer due to the activation of a chain reaction which generates peroxides and other free radicals from lipids. These molecules damage the cell structures like DNA thus increasing the risk for cancer development. As we discussed previously, AX is a potent antioxidant which stimulates and modulates de immune system. These effects are capable of preventing or delaying sunburns.

The ability of AX extracted from algae to protect against DNA damage by UV radiation has been shown in studies with cultured rat kidney fibroblasts (O'Connor and O'Brien, 1998) and human skin cells (Lyons and O'Brien, 2002). Various AX supplements consisting of injectable solutions, capsules, or topical creams have been manufactured for sunburn prevention from UV exposure (Lorenz, 2002).

Additional beneficial effects attributed to AX include anti-inflammatory activity (Uchiumi, 1990; Nakajima, 1995), anti-cataract prevention activity (Guyen et al., 1998), as a treatment against rheumatoid arthritis and also carpal tunnel syndrome (Lignell and Bottiger, 2001; Cyanotech, 2002).

The large majority of the studies to support the multiple potential benefits of AX have been performed with animal models. A few clinical trials have been performed with voluntary patients by the manufacturing companies. For instance, Cyanotech (2002) has performed extensive work on the preventative effects of AX on the development of rheumatoid arthritis and carpal tunnel syndrome. Safety studies of algae derived AX have also been performed with volunteers who were given a low dose (228 mg of algal meal equivalent to 3.85 mg AX) or a high dose (1140 mg of algal meal equivalent to 19.25 mg AX) during 29 consecutive days. According to the clinical tests performed on the patients, they did not present any disease or intoxication at these consumption levels. However, the recommended dose is 5 mg AX per day (250 mg of algal meal) (Mera Pharmaceuticals, 2003).

AX BENEFITS IN MAMMALS AND CHICKENS

Several studies have been done using AX esters in mammals to prove its effectiveness in the treatment of muscle diseases, for example, equine exertional rhabdomyolysis (Lignell, 2001) or to increase the production of breeding and production mammals (porcine, bovine, and ovine) (Lignell and Inborr, 2000). The administration of AX to layer hen diet increases fertility, improves the overall health status of these animals, and decreases chicken mortality. Egg production and the yellow coloration of yolks is also increased, while salmonella infections reduced dramatically probably due to a stronger membrane formation (Lignell et al., 1998). It also provides greater pigmentation to chicken meat, a desirable attribute to some consumers (Akiba et al., 2001).

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