

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 *Helicobacter 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 polyene 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 polyene system gives carotenoids its distinctive molecu-

Address correspondence to I. Higuera-Ciapara, Centro de Investigación en Alimentación y Desarrollo. -A.C. Carretera a la Victorial Km 0.6. AP 1735

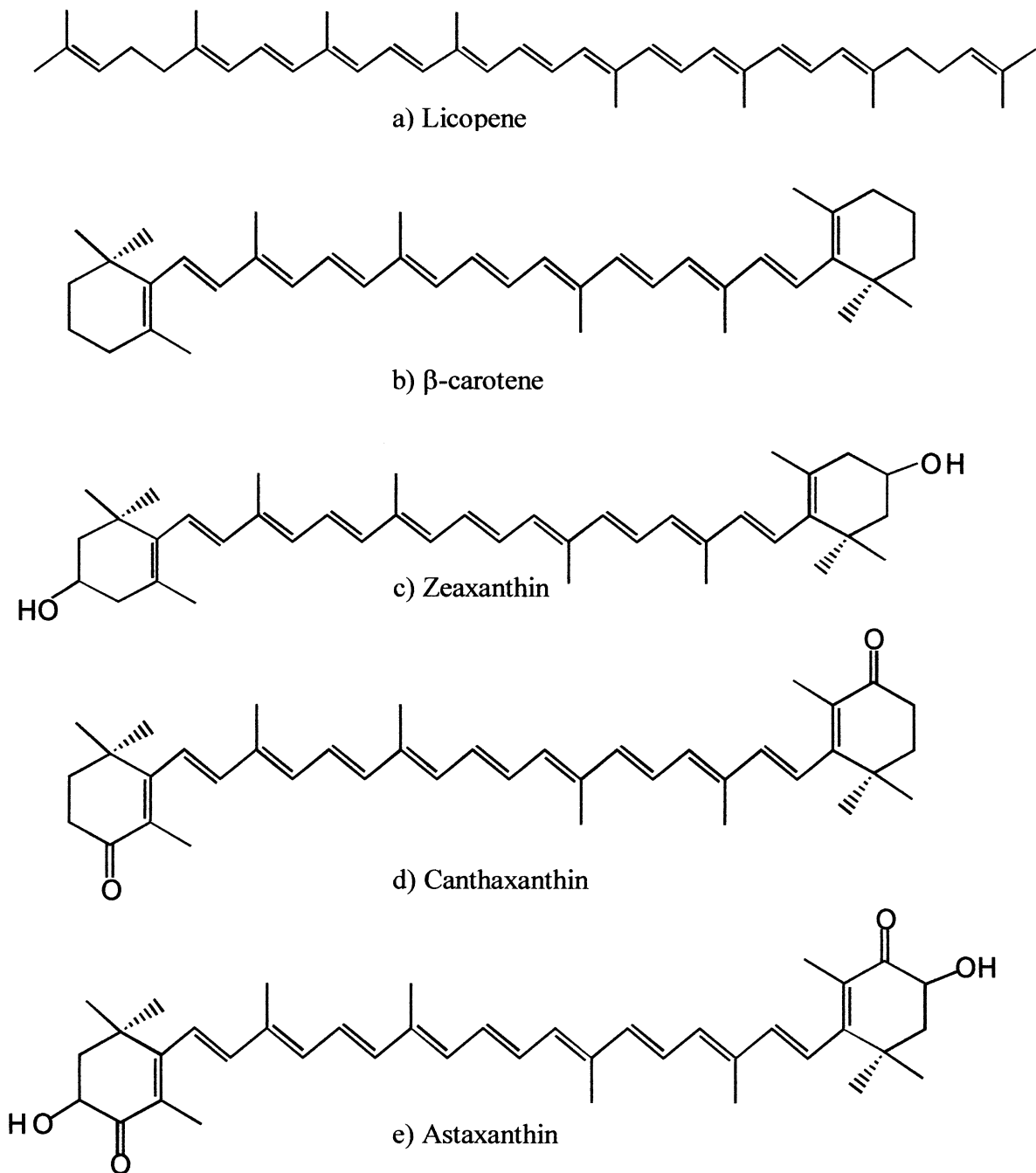


Figure 1 Chemical structure of some carotenoids. Source: Ulrich, 1994.

characteristics. Each double bond from the polyene 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 esterified in one or both hydroxyl groups with different fatty acids such as palmitic, oleic, stearic, or linoleic; it may also be found free, that is,

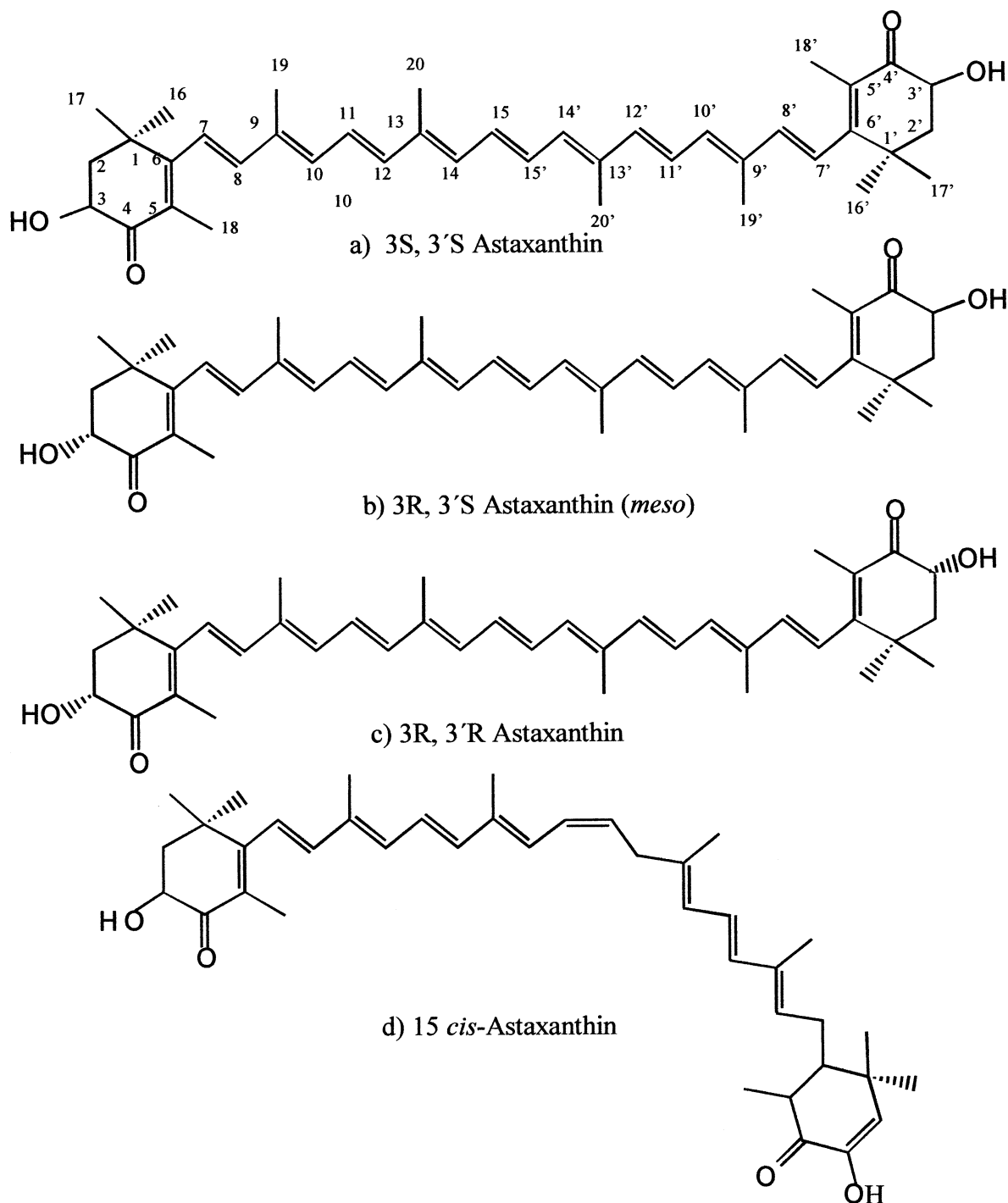


Figure 2 Astaxanthin configurational isomers (a–c) and a geometric *cis* isomer (d). Source: Turujman et al., 1997; Osterlie et al., 1999.

with the hydroxyl groups without esterification; or else, forming a chemical complex with proteins (carotenoproteins) or lipoproteins (carotenolipoproteins). Synthetic AX is not esterified, while found in algae is always esterified (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; Mo 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 $\mu\text{g/g}$) (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 Blich, 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 crustacean biowastes

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

^amg/100g wet basis.

between 119 and 148 $\mu\text{g/g}$. 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 $\mu\text{g/g}$ 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 (Torrison 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 (Torrison 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 canthaxanthin (Figure 1) as pigments in aquaculture species has been well documented through many scientific publications for more than two decades (Meyers and Chen, 1982; Torrison, 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 PinkTM and canthaxanthin as Carophyll RedTM. 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 (Torrison, 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 (Torrison, 1989; Bell et al., 1998). However, in a more recent study of Ruttle et al.

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.