

## Review Article

# Benefits of carotenoid Astaxanthin: A review

### ABSTRACT

Astaxanthin is a high value keto-carotenoid pigment renowned for its commercial application in various industries such as aquaculture, food, cosmetic, nutraceutical and pharmaceutical sectors. It is commonly employed in salmonid and crustacean aquaculture to give the pink hue that these species are known for. Scientific literature reviews have persistently demonstrated the instrumental role of astaxanthin in targeting several animal health conditions. Most importantly, the profound effect on pigmentation, where astaxanthin is frequently utilized as an additive in formulated diets to boost and improve the coloration of many aquacultures farmed species, subsequently product quality, consumers' acceptance and market demand are increased, and revenue generated. Moreover, the wide range of other physiological benefits of astaxanthin include various improvements in survival, growth performance, reproductive capacity, stress tolerance, and disease resistance as well. Astaxanthin, can be used as a nutritional supplement, antioxidant and anticancer agent, prevents diabetes and cardiovascular diseases too. Astaxanthin products are used for commercial applications in the dosage forms as tablets, capsules, syrups, oils, soft gels, creams, biomass and granulated powders. Astaxanthin patent applications are available in food, feed and nutraceutical applications. This manuscript basically reviews the current available evidence regarding biological sources of astaxanthin, extraction procedures, stability, biological activity, health benefits, and commercial uses.

*Keywords: Astaxanthin, Extraction, Health benefits, Shrimp shell, Biochemistry*

### 1. INTRODUCTION

Astaxanthin is a xanthophyll carotenoid, a red fat-soluble pigment without pro-Vitamin A activity in human body, although some of the studies reported that astaxanthin has more potent biological activity than other carotenoids [1]. The United States Food and Drug Administration (USFDA) has approved the use of astaxanthin as food colorant in animal and fish feed [2]. The European Commission considers natural astaxanthin as a food dye. *Haematococcus pluvialis*, a green microalga, accumulates high astaxanthin content under stress conditions such as high salinity, nitrogen deficiency, high temperature and light. Astaxanthin (3,3'-dihydroxy-4,4'-diketo- $\beta,\beta'$ -carotene) [3], an oxidized form of  $\beta$ -carotene, being widely distributed in nature and largely discovered in the marine environment, is abundant in the flesh of salmonids, carapace of many crustaceans (e.g. shrimp, crabs, lobsters and crayfish) and also in other marine organisms such as microbes [4] and microalgae [5,6]. Naturally, the carotenoid pigment astaxanthin is primarily biosynthesized in microalgae within the food chain at the primary production level. Microalgae are then consumed by crustaceans, zooplankton or insects that bioaccumulate the astaxanthin to the higher trophic levels when ingested by fish and other aquatic animals. For dietary supplement in humans and animals, astaxanthin is obtained from seafood or extracted from

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*H. pluvialis*. The consumption of astaxanthin can prevent or reduce risk of various disorders in human and animals. This carotenoid pigment is best known as an essential aquacultural feed additive for imparting the pinkish-red coloration to the flesh of salmon, trout, ornamental fish, shrimp, lobsters and crayfish resulting in a better quality and acceptance to the consumer. Astaxanthin is increasingly being used as a nutritional supplement in meals, feeds, nutraceuticals, and medications. Thus, the manuscript depicts a detailed, thorough, in-depth, and up-to-date review of literatures enlightening the source, extraction methods, storage stability, biological activities, health benefits in prevention and treatment of various diseases, and commercial applications of astaxanthin for mankind.

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## 2. STRUCTURE OF ASTAXANTHIN

Astaxanthin is a member of the xanthophylls containing carbon, hydrogen and oxygen atoms ( $C_{40}H_{52}O_4$ ) with a molar mass of 596.84 g/mol (Figure 1). Astaxanthin consists of two terminal rings joined by a polyene chain. This molecule has two asymmetric carbons located at the 3, 3' positions of the  $\beta$ -ionone ring with hydroxyl group (-OH) on either end of the molecule. While one hydroxyl group reacts with a fatty acid, it forms mono-ester; whereas when both hydroxyl groups react with fatty acids it results di-ester. Astaxanthin, available in

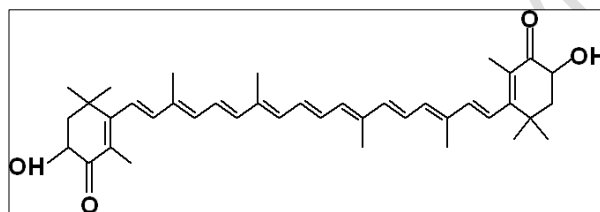
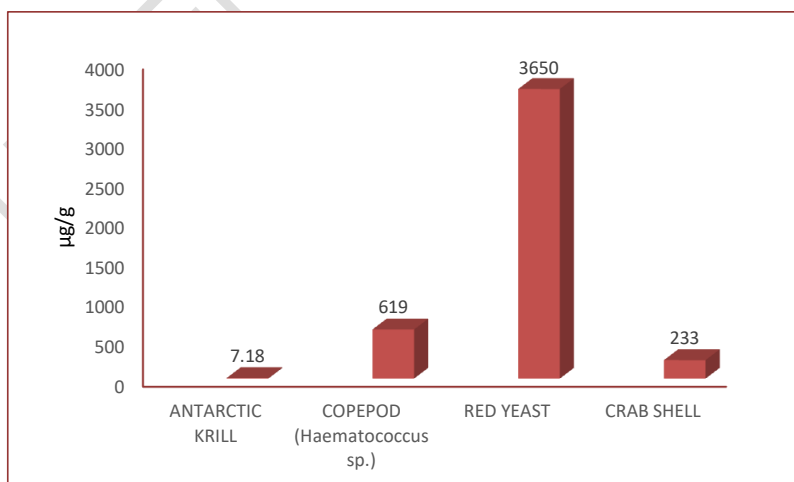


Fig.1. Planner structure of astaxanthin [7].

natural sources, exists in stereoisomers, geometric isomers, free and esterified forms among which the stereoisomers (3S, 3'S) and (3R, 3'R) are most abundant in nature. *Haematococcus* biosynthesizes the (3S, 3'S)-isomer whereas yeast *Xanthophyllomyces dendrorhous* produces (3R, 3'R)-isomer [8]. Synthetic astaxanthin comprises of isomers (3S, 3'S) (3R, 3'S) and (3R, 3'R). The primary stereoisomer of astaxanthin found in the Antarctic krill *Euphausia superba* is (3R, 3'R) containing mainly esterified form, whereas in wild Atlantic salmon, (3S, 3'S) occurs as the free form [9]. Astaxanthin content ( $\mu\text{g/g}$ ) in Antarctic krill, Copepod, Red yeast and Crab shell is shown in Figure 2.

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**Fig.2. Astaxanthin content (µg/g) in Antarctic krill, Copepod, Red yeast and Crab shell [10,11,12,13].**

### 3. SOURCES OF ASTAXANTHIN

The primary natural sources of astaxanthin are relatively simple microorganisms such as copepods, crab shell and yeast (Fig. 2). Not a single animal can biochemically synthesize it, but animals do accumulate astaxanthin in their tissues through the consumption of astaxanthin-containing organisms for attractive coloration. In marine environments, astaxanthin-rich algae are a food for zooplankton which is ingested by fish (e.g., salmonids) and exoskeleton bearing creatures (e.g., crabs, crayfish, lobsters, krill and shrimp); thus, astaxanthin is bioaccumulated and biomagnified subsequently at the higher trophic level (Table 2 & Fig. 3).

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**Table 1. Microbial sources of Astaxanthin**

Class	Species	Astaxanthin (%) On a dry wt. basis	References
1. Chlorophyceae	<i>Chlorococcum sp.</i>	0.2 - 0.57	[14]
	<i>C. zofingiensis</i>	0.68 – 0.71	[15]
	<i>H. pluvialis</i>	4 - 7.72	[16]
2. Florideophyceae	<i>Catenella repens</i>	0.02	[17]
3. Alphaproteobacteria	<i>Argobacterium auranticum</i>	0.01	[18,19]
	<i>Paracoccus sp.</i>	2.2	
4. Tremellomycetes	<i>Xanthophyllomyces dendrohous</i>	0.41 – 0.97	[20, 21]

Fish Species	Astaxanthin (mg/kg flesh)
1. Atlantic salmon ( <i>Salmo salar</i> )	3-10
2. Chinook salmon ( <i>Oncorhynchus tshawytscha</i> )	5.4
3. Chum salmon ( <i>Oncorhynchus keta</i> )	3-5
4. Coho salmon ( <i>Oncorhynchus kisutch</i> )	10-21
5. Masu salmon ( <i>Oncorhynchus masou</i> )	4.6

6. Pink salmon ( <i>Oncorhynchus gorbuscha</i> )	4-7
7. Rainbow trout ( <i>Oncorhynchus mykiss</i> )	24
8. Sockeye salmon ( <i>Oncorhynchus nerka</i> )	26-38

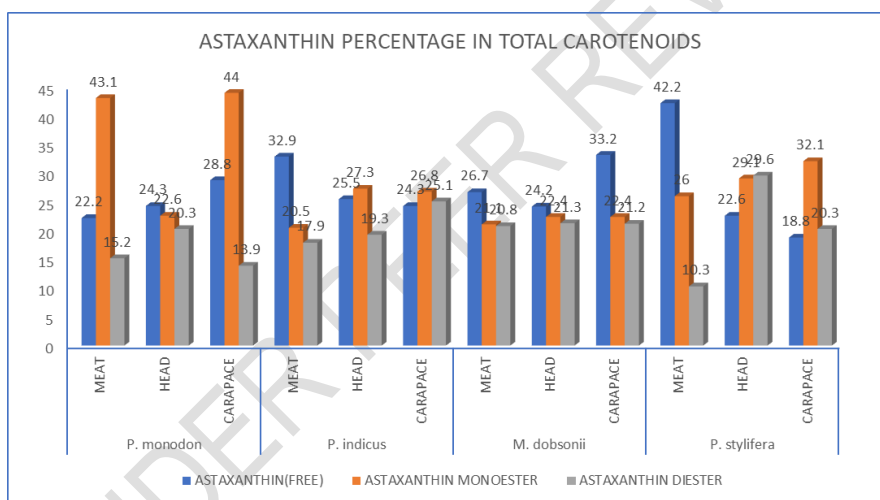
**Table 2. Astaxanthin content of salmonids [22]**

**Fig.3. Astaxanthin content (%) of shrimps (Adapted from [23])**

#### 4. EXTRACTION OF ASTAXANTHIN

##### 4.1 Extraction of astaxanthin from *H. pluvialis*

Astaxanthin is accumulated predominantly in encysted cells of *H. pluvialis* up to 3-4% on a dry weight basis under unfavourable environmental conditions [24-26]. Intact astaxanthin-rich hematocysts (aplanospores) are characterized by thick and resistant cell walls which require mechanical disruption prior to use in order to take full advantage of its bioavailability [27-29]. Many different techniques have been developed over the years to disrupt *H. pluvialis* cells. Destruction of cell wall is usually achieved through physical or mechanical pretreatment and more specifically bead milling and expeller pressing [30-34]. A bead miller



consists of a disruption or milling chamber loaded with tiny grinding beads (e.g., ceramic, glass and steel) that are agitated at high speeds resulting in multiple collisions. The dried biomass is fed in these chambers, and cells are disrupted in the bead collision zones by compaction and shear forces. The algal biomass must be quickly dehydrated to avoid quality degradation or spoiling through freeze-drying (lyophilization), spray-drying, drum-drying and sun-drying [35-38]. Expeller pressing employs squeezing force alongside high pressure to rupture tough cell walls. Then the algal biomass must be quickly dehydrated to avoid quality degradation or spoiling through freeze-drying (lyophilization), spray-drying, drum-drying and sun-drying [39].

Astaxanthin is a lipid soluble (lipophilic) pigment which can be dissolved in oils and organic solvents. Various techniques have been adopted to extract the astaxanthin from *H. pluvialis* utilizing acids, organic solvents and edible oils (Table 4) [40,41]. Sarada *et al.* [40] evaluated the extractability of astaxanthin from *H. pluvialis* with different acid treatments at 70°C and discovered that hydrochloric acid treatment facilitated 86–94% recovery of the pigment without affecting its ester profile (Figure 4).

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**Table 4. Extraction of astaxanthin by using acids, organic solvents and edible oils [40,41]**

Process Name	Procedure
<p>1. Hydrochloric acid pretreatment followed by acetone extraction (HCl-ACE)</p>	<p>HCl-ACE extraction procedures included two steps. Firstly, ten milligrams lyophilized biomass was treated with 1 mL of HCl in a centrifugal tube at 70°C for 2 min. The sample was cooled and centrifuged at 5000 rpm for 5 min. Secondly:</p> <p>The HCl-treated sample was washed twice by distilled water and resuspended in 1 mL acetone. The mixture was ultrasonically extracted in an ice-water bath for 20 min and then centrifuged at 3500 rpm at 4°C for 6 min.</p> <p style="text-align: center;">↓</p> <p>The supernatants were used for HPLC estimation of extractable astaxanthin. All the steps were carried out in light protection and filled with nitrogen.</p>
<p>2. Hexane/isopropanol (6 : 4, v/v) mixture solvents extraction (HEX-IPA)</p>	<p>HEX-IPA binary solvents extraction method consists of transferring 10 mg of the lyophilized organisms into 2 mL of hexane/isopropanol (6: 4, v/v) binary organic solvents for 20 min in an ice-water bath temperature and ultrasonically assistant extraction.</p> <p style="text-align: center;">↓</p> <p>The mixture of cell biomass, extract, and solvent was separated by means of centrifugation at 3500 rpm at 4°C for 5 min, followed by concentration under vacuum.</p> <p style="text-align: center;">↓</p> <p>The extraction yield was calculated in dry basis and expressed in % (w/w- dry basis). The supernatants were used for HPLC estimation of extractable astaxanthin. All the steps were carried out in light protection and filled with nitrogen.</p>
<p>3. Methanol extraction followed by acetone extraction (MET-ACE, 2-step extraction)</p>	<p>10 mg biomass was weighed into a 15 mL screw top amber glass vial and ultrasonically extracted in an ice-water bath with 1 mL methanol and acetone for 5 min in sequential order.</p> <p style="text-align: center;">↓</p> <p>First extraction step, the sample was extracted with 1 mL methanol in 15 mL screw top amber glass vial and centrifuged at 3500 rpm at 4°C for 5 min.</p> <p style="text-align: center;">↓</p> <p>Second step, 1 mL acetone was added to glass vial and extracts were combined for 2-step extraction and used for HPLC estimation of extractable astaxanthin. All the steps were carried out in light protection and filled with nitrogen.</p>
<p>4. Oil-Soy Extraction</p>	<p>2.5 g cell biomass mixed with 20 mL vegetable oil in a 250 mL flask (light protected), submitted to hot plates with 2 h agitation period at room temperature.</p> <p style="text-align: center;">↓</p>

Further, the oil extracts were recovered by cellulose filtration (0.22  $\mu\text{m}$ ). The supernatants were used for HPLC estimation of extractable astaxanthin.

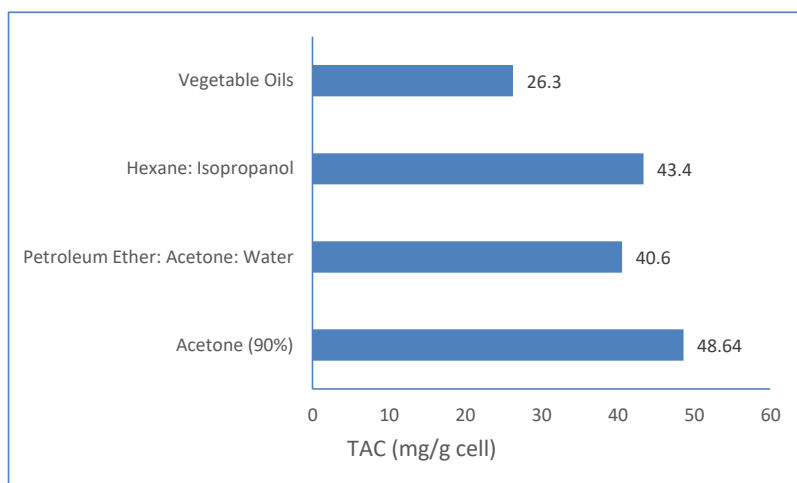


Fig. 4. Effect of different extraction methods on total astaxanthin content (TAC) of extracts from *H. pluvialis* cells [40,41].

#### 4.2 Extraction of astaxanthin from shrimp shell waste

Table 5. Extraction of astaxanthin from shrimp shell waste by using different solvents [42]

Solvents	Procedure
1. Extraction of Astaxanthin with Acetone (90%)	<p>1g of wet shrimp shell waste was ground using 10 ml of acetone.</p> <p style="text-align: center;">↓</p> <p>The extract was filtered using Whatman filter paper. The sample was repeatedly extracted and filtered with the fresh solvent until the colorless filtrate (3 times) was gained.</p> <p style="text-align: center;">↓</p> <p>The pooled extract was collected in a separated conical flask and 9.4 ml of 0.73% NaCl were added and mixed well.</p> <p style="text-align: center;">↓</p> <p>After thorough mixing the epiphase was collected. An equal amount of water was added to the lower phase, mixed well and then the epiphase was collected.</p>
2. Extraction with Petroleum Ether:	<p>1g of wet shrimp shell waste was extracted using 10ml of petroleum ether: acetone: water in the ratio (15:75:10 v/v/v).</p> <p style="text-align: center;">↓</p>

Acetone: Water

(15:75:10 v/v/v)

The extract was filtered using whatmann filter paper no. 42. The sample was repeatedly extracted using fresh solvent and filtered until the filtrate became colourless.



The pooled extract was collected in a separated conical flask and 12.5 ml of petroleum ether (BP 40-60 °C) and 9.4 ml of 0.73% NaCl were added.



After thorough mixing the epiphase was collected. An equal amount of water was added to the lower phase, mixed well and then the epiphase was collected. The pooled epiphase was kept in water bath at 60 °C for the evaporation of petroleum ether.

3. Extraction with Hexane: Isopropanol (3:2 v/v)

1g of wet shrimp shell waste was ground using 10 ml of hexane: isopropanol (3:2).



The extract was filtered using whatman no. 42 filter paper. The sample was repeatedly extracted and filtered with the fresh solvent until the colourless filtrate was obtained.



The pooled extracted with hexane: isopropanol (3:2 v/v) was separated with equal volume of 1% (w/v) NaCl solution.



The epiphase was collected and dehydrated with anhydrous sodium sulphate, and then evaporated to dryness under vacuum, and the residue was dissolved in 5 ml of hexane.

4. Extraction with Different Vegetable Oils (Coconut Oil, Palm Oil, Sunflower Oil)

1g of wet shrimp shell waste was mixed with 10 ml of vegetable oil until the colourless sample was obtained. The ratio of oil: waste used in vegetable oil extraction was 2:1 for wet sample, and 4:1 for dry samples.



The solvent was removed under vacuum and re dissolved in 5 ml of hexane.



An antioxidant butyl hydroxy toluene (BHT) was added at 0.05% (w/v) and heated at 70 °C for 150 min



Centrifuged at 5000 rpm and the pigmented oil was recovered.

Petroleum Ether: Acetone: Water

40.6

Acetone (90%)

48.64

0 10 20 30 40 50 60

TAC (mg/g)

isopropanol

43.4

Figure 5: Effect of different extraction methods by using different solvents on total astaxanthin content (TAC) of extracts from shrimp shell waste [41,43,44]

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## BIOCHEMISTRY OF ASTAXANTHIN

Astaxanthin contains conjugated double bonds, hydroxyl and keto groups. It has both lipophilic and hydrophilic properties [45]. The red color is due to the conjugated double bonds at the center of the compound that act as a strong antioxidant by donating the electrons and reacting with free radicals to convert them to be more stable product and terminate free radical chain reaction in a wide variety of living organisms [46]. Astaxanthin showed better biological activity than other antioxidants [47], because of its linking property with cell membrane from inside to outside.

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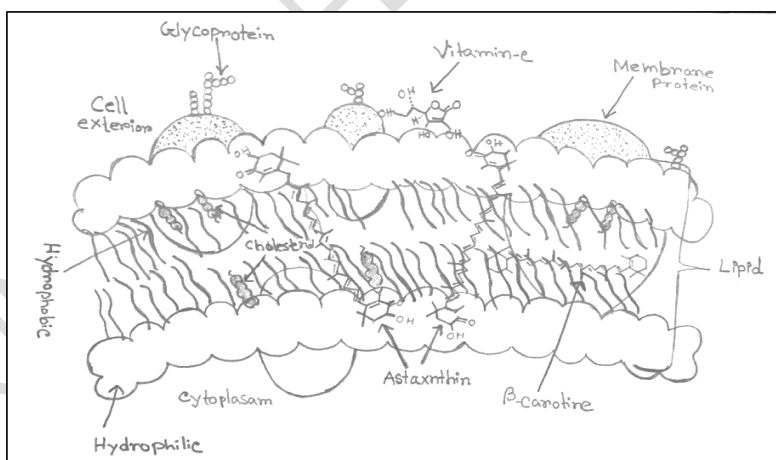


Fig. 6. Superior position of astaxanthin in the cell membrane

## 6. USES AND BENEFITS OF ASTAXANTHIN IN AQUATIC ANIMALS

### 6.1 Reproductive performance and egg quality

Astaxanthin plays a fundamental role not only in the cultivation but also in the breeding of diverse kinds of aquaculture species. To date, there exist many lines of evidence suggesting that astaxanthin confers a significant impact on reproductive performance, egg production and egg quality of aquatic animals [48-53]. In many aquaculture farms, there have always been attempts to improve egg and larval quality while disregarding sperm quality. Hence, it is absolutely necessary to understand the dietary requirement of farmed animals as nutrient availability directly influences various aspects of reproductive physiology. The influence of dietary astaxanthin supplementation on the reproduction and brood stock performance of different aquatic animals has been documented in earlier studies [51,52].

Comment [U16]: cultivation, but

## 6.2 Growth performance and survival

In most aquaculture operations, feed represents more than 60% of a total hatchery management cost depending on production scale and cultivation methods. Therefore, it is absolutely necessary to develop feeds with nutritional ingredients that promote growth and survival of the farmed species as a requisite to minimize production costs. Beneficial role of astaxanthin as a critical nutritive additive essential for excellent growth and survival has been investigated in various aquatic animals. An increasing number of quantitative research papers revealed significant positive correlations between dietary astaxanthin supplementation with the growth, survival, (or both) of farmed fish, and crustaceans during aquaculture practices [54,55].

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## 6.3 Stress tolerance and disease resistance

The emergence of infectious diseases in intensive farming, particularly during the early production stages, represents a major downside or leading threat contributing to significant economic impacts worldwide. High-density aquaculture operations frequently subject farmed animals to various physical stressors which involve grading, transport, handling, vaccination, crowding and confinement or any other forms of physical disturbance that could be extremely stressful and immune-depressive. These negative factors may disrupt the fine balance between aquatic animals and their surrounding environments triggering stress responses [56]. Excessive stress attributes to physiological dysfunction, reduction in growth rate, immunosuppression, susceptibility towards pathogenic invasions and even mortality. So, it is of utmost importance in aquaculture research to reduce adverse conditions that may induce considerable stress and weaken the host organism. Through the years, substantial research efforts have been directed towards relieving stress and boosting immunity of aquacultured crustaceans and fish using astaxanthin in their diet, although remarkable stress tolerance and disease resistance of farmed prawns and shrimps have been reported as compared to growth performance and survival.

Comment [U19]: The remarkable

## 6.4 Skin and flesh pigmentation

Perhaps, the greatest potential application of astaxanthin is aquaculture feed additive to enhance the typical pinkish-red skin or flesh coloration of aquatic animals including salmon, red sea bream, trout, ornamental fish, crayfish, lobster and shrimp. Skin and muscle pigmentation is due to the absorption and deposition of relatively large amounts of dietary astaxanthin which is frequently administered in their artificial diets. Maintenance of natural pigmentation is of utmost importance from a commercial perspective, being directly associated with the perception and subjective interpretation of consumers, as an important quality criterion prior to actual consumption which consequently commands a better demand and product market price. As, colour exerts a strong decisive role in consumers' preference and market demand of the farmed species, the progressive expansion of aquaculture industry has established an insatiable demand for the carotenoid pigment. Studies assessing the effect of dietary astaxanthin on the skin and flesh pigmentation of aquatic animals are Koi Carp (*Cyprinus carpio*), Kissing Gourami (*Helostoma temminckii*) etc. [58,59].

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## 7. ASTAXANTHIN IN FEED PROCESSING

Astaxanthin is widely known to possess great sensitivity to heat, intense light and oxidative conditions due to its highly unsaturated molecular structure [60-63]. The exposure of astaxanthin to such shocks during processing and storage of feed, may render the loss of its nutritive value and desirable biological properties. Thus, stability of astaxanthin must be ensured during aquaculture feed formulations for its maximal efficacy [57]. Feed manufacturing comprises of milling, mixing, extrusion, pelletizing and drying.

During milling, the disintegration or disruption of microalgal cells appears to be the single most important attribute in effective utilization of intracellular astaxanthin [33, 64]. Therefore, it has no significant impact on the stability of astaxanthin [65]. However, degradation of astaxanthin during milling is heavily dependent on equipment used, residence time and heat production. Feed mixing is important to ensure uniform distribution of nutrients resulting a homogenous nutrient content in each fish pellet as the formulation. However, mixing may incorporate air into the blend causing undesirable oxidation of carotenoids. Using a vacuum mixer is a good way to deal with air exposure, thus eliminating air entry into the mixture. Alternatively, the inclusion of secondary antioxidants (BHT and BHA) has been demonstrated to be efficacious in improving the oxidative stability of dietary carotenoids during feed processing [66].

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Extrusion is aiming at improving the digestibility of starch (gelatinization) and protein while minimizing the degradation of food nutrients [67-69]. Moreover, formula adjustment is ensured to produce floating or sinking pellets. Astaxanthin was fairly stable through extrusion with an average retention of 86% [65]; whereas, retention values ranging from 86% to 94% was documented in extruded feed. Nevertheless, extrusion technology involves high levels of heat, moisture, pressure and mechanical shear that are most likely to influence the stability of carotenoid pigments. Storebakken *et al.* [71] reported that a range of extruder temperatures (102, 121 and 137°C) had little influence on the composition of astaxanthin during extruded fish feed production with a recovery range of 90–99%. Pelletizing is the most frequently used method for producing pellets by increasing the efficiency of the nutrient utilization. Carotenoid content in a refrigerated feed formulation (4°C) was also found to be unaffected during palletization without steam [72,73]. A vacuum drying process (60–80°C temperature) is employed to evaporate the excess moisture (a shelf-stable residual moisture <10%) to reduce the loss of pigment [65]. Moreover, a post-liquid application of fat or oil via coating may avoid the risk of damaging heat-sensitive carotenoids [74] followed by subsequent cooling.

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Comment [U28]: extruding

## 8. STORAGE AND STABILITY OF ASTAXANTHIN

The bioavailability of astaxanthin has suffered a great challenge due to its intrinsic chemical instability which hampered its application as a functional food ingredient. This forces the market to consider new strategies to improve storage, stability, economic and efficient utilization of astaxanthin. Review of literatures reveal that, astaxanthin was stable at 70<sup>o</sup>-90<sup>o</sup>C in rice bran, gingelly (sesame), and palm oil with a retention of 84-90% of retention of astaxanthin content which can be used in food, pharmaceutical and nutraceutical applications, whereas astaxanthin content was reduced at 120 and 150 °C (Ranga Rao *et al.*, 2007). Anarjan and Tan (2013) reported that degradation of astaxanthin was significantly higher in skimmed milk than orange juice. Degradation of astaxanthin was as low as 10% in biomass dried at 180/110°C and stored at -21°C in nitrogen after nine weeks of storage. The storage stability of astaxanthin was enhanced at 4°C and 25°C in a complex mixture of hydroxypropyl-β-cyclodextrin and water. Gouveia and Empis [72] reported that the best storage conditions for astaxanthin dry biomass were under vacuum and nitrogen atmosphere in the dark with high retention >90% even after 18 months of storage. Astaxanthin stability was investigated using microencapsulation with polymeric nanospheres, emulsions and β-cyclodextrin [75-77]. The stability of astaxanthin-enriched *H. pluvialis* cells (homogenized) was enhanced by encapsulation into rigid polymeric matrix of chitosan and

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stored under nitrogen atmosphere at -18°C with less loss of pigment (8%) after 24 weeks [78].

Comment [U30]: a nitrogen

## 9. BIOLOGICAL ACTIVITIES OF ASTAXANTHIN AND ITS HEALTH BENEFITS

### 9.1 Antioxidant Effects

Antioxidant can inhibit oxidation. Oxidative damage is initiated by free radicals and reactive oxygen species (ROS). These molecules have very high reactivity and are produced by normal aerobic metabolism in organisms. Excess oxidative molecules may react with proteins, lipids and DNA through chain reactions, resulting in protein and lipid oxidation and DNA damage leading to various disorders [1]. This oxidative degradation can be inhibited by endogenous and exogenous antioxidants such as carotenoids containing polyene chain and long conjugated double bonds, which carry out antioxidant activities by quenching singlet oxygen and scavenging radicals to terminate chain reactions. The biological benefits of carotenoids may be due to their antioxidant properties attributed to their physical and chemical interactions with cell membranes. Astaxanthin had higher antioxidant activity when compared to various carotenoids such as lutein, lycopene,  $\alpha$ -carotene and  $\beta$ -carotene reported [79].

### 9.2 Anti-Lipid Peroxidation Activity

Astaxanthin has a unique molecular structure which enables it to stay both in and outside the cell membrane. It gives better protection than  $\beta$ -carotene and Vitamin C which can be positioned inside the lipid bilayer [1]. It serves as a safeguard against oxidative damage by various mechanisms, like quenching of singlet oxygen; scavenging of radicals to prevent chain reactions; preservation of membrane structure by inhibiting lipid peroxidation; enhancement of immune system function and regulation of gene expression. Astaxanthin and its esters showed 80% anti-lipid peroxidation activity in ethanol induced gastric ulcer rats and skin cancer rats [1,81].

Comment [U31]: the quenching

### 9.3 Anti-Diabetic Activity

Generally, oxidative stress levels are very high in diabetes mellitus patients. It is induced by hyperglycemia, due to the dysfunction of pancreatic  $\beta$ -cells and tissue damage in patients. Astaxanthin could reduce the oxidative stress caused by hyperglycemia in pancreatic  $\beta$ -cells and also improve glucose and serum insulin level [82].

Comment [U32]: the pancreatic

### 9.4 Cardiovascular Disease Prevention

Astaxanthin is a potent antioxidant with anti-inflammatory activity against both animal and human. Oxidative stress and inflammation are pathophysiological features of atherosclerotic cardiovascular disease against which astaxanthin acts as a potential therapeutic agent [83].

### 9.5 Anticancer Activity

The specific antioxidant dose may be helpful for the early detection of various degenerative disorders. Reactive oxygen species such as superoxide, hydrogen peroxide and hydroxyl radical are generated during normal aerobic metabolism. Singlet oxygen is generated by photochemical events, whereas, peroxy radicals are produced by lipid peroxidation. These oxidants contribute to aging and degenerative diseases such as cancer and atherosclerosis through oxidation of DNA, proteins and lipids. The new astaxanthin prodrug CDX-085 spreads among lipoproteins after being given orally reduces total cholesterol and triglyceride levels in mice, indicating that it might be used in human trials [84].

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### 9.6 Immuno-Modulation

Immune system cells are very sensitive to free radical damage. The cell membrane contains poly unsaturated fatty acids (PUFA). Antioxidants, particularly astaxanthin, offer protection against free radical damage to preserve immune-system defenses. There are reports on astaxanthin and its effect on immunity in animals under laboratory conditions; however, clinical research is lacking in human. Astaxanthin showed higher immuno-modulating effects in mouse model as compared to  $\beta$ -carotene [85].

### 9.7 Health promotional effects of natural astaxanthin

Literature reviews have shown that astaxanthin has potential health-promoting effects in the prevention and treatment of various diseases, such as cancers, chronic inflammatory diseases, metabolic syndrome, diabetes, diabetic nephropathy, cardiovascular diseases, gastrointestinal diseases, liver diseases, neurodegenerative diseases, eye diseases, skin diseases, exercise-induced fatigue, male infertility [86]. Astaxanthin may be called as 'a medical food' presently [1]. Although, there are literatures on effect of astaxanthin on immunity in animals under laboratory conditions; prominent research gap is existing regarding clinical research on humans, literatures are scanty too. Thus, there is a huge scope of future research in medical science regarding practical application, mode of action and efficacy of astaxanthin obtained from *Haematococcus sp.*

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### 10. CONCLUSION

Currently, astaxanthin might be considered "a medicinal food." Although there are publications on how astaxanthin affects immunity in lab animals, there is a significant research deficit in the area of human clinical studies, and those publications are also few. As a result, there is a vast area for future study in the field of medicine about the use, mechanism, and effectiveness of astaxanthin. Astaxanthin ingestion can either prevent or lower the risk of a number of illnesses in both humans and animals. The pinkish-red colour of the flesh of salmon, trout, ornamental fish, shrimp, lobsters, and crayfish is imparted by this carotenoid pigment, which is well recognized as an important aquaculture feed addition. This results in greater quality and customer acceptability. In foods, feeds, nutraceuticals, and medicines, astaxanthin usage as a nutritional supplement has been expanding quickly. Future studies should concentrate on how astaxanthin esters affect various biological processes and how they are used in pharmaceutical and nutraceutical products.

Comment [U36]: used

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