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 formulating diets to boost and improve the coloration of many aquacultures farmed species, subsequently product quality, consumers' acceptance and market demand are increasing, and revenue generated. Moreover, the wide range of other physiological benefits of astaxanthin includes various improvements in survival, growth performance, reproductive capacity, stress tolerance, and disease resistance as well. Also, astaxanthin has some other applications like, it is an anticancerous agent, it can prevent diabetes and cardiovascular diseases and enhances nutritional qualities. 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. Some reseaches showed that astaxanthin had better bio activity than other carotenoid pigments [1]. One of the major drug administrative body USFDA(The United States Food and Drug Administration) has approved the application of carotenoid pigment astaxanthin in animal and fish feed as a source of colouring agent [2]. Also natural astaxanthin is recognized by The European Commission as a food dye. Since astaxanthin (3,3′-dihydroxy-4,4′-diketo-,′-carotene) occurs naturally in large amounts and was initially discovered in the marine environment, it is mostly found in the flesh of salmonids, the carapace of crustaceans, and other marine organisms [5,6]. The naturally occurring carotenoid pigment astaxanthin is generally biosynthesized in microalgae at the primary production level of the food chain. Following the consumption of microalgae by crustaceans, zooplankton, or insects, astaxanthin is bioaccumulated to higher trophic levels where it is then swallowed by fish and other aquatic organisms. Astaxanthin is derived from H. pluvialis or acquired from seafood for use as a nutritional supplement in case of humans and other animals also. Astaxanthin ingestion can either prevent or lower the risk of several diseases in both humans 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 of the consumer. Astaxanthin is increasingly being used as a nutritional supplement in meals, feeds, nutraceuticals, and medications. Thus, the manuscript presents a thorough, in-depth, and current analysis of the literature that sheds light on the astaxanthin source, extraction processes, storage stability, biological activities, medical benefits in the treatment and prevention of various diseases, and commercial uses for mankind.

2. STRUCTURE OF ASTAXANTHIN

Astaxanthin is a pigment containing carbon, hydrogen and oxygen atoms ($C_{40}H_{52}O_4$) with a molar mass of 596.84 g/mol (Figure 1). Two terminal rings connected by a polyene chain make up astaxanthin. This molecule has two asymmetric carbons located at the 3, 3′ positions of the β-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

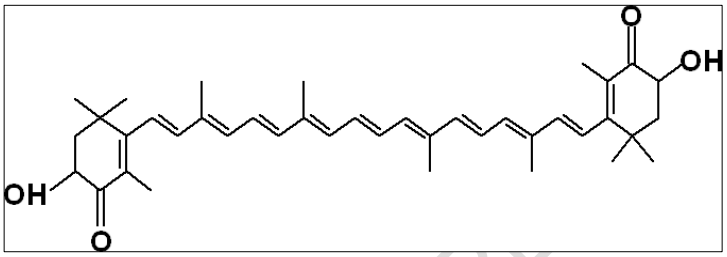


Fig.1. Planner structure of astaxanthin [7].

natural sources, exists in stereoisomers, geometric isomers, free and esterified forms among which the most prevalent stereoisomers in nature are (3S, 3′S) and (3R, 3′R). While yeast Xanthophyllomyces dendrorhous creates the (3R, 3′R) isomer, Haematococcus synthesises the (3S, 3′S) isomer. [8]. There are three isomers of synthetic astaxanthin are present: (3S, 3′S), (3R, 3′S), and (3R, 3′R). While the predominant astaxanthin stereoisomer in wild Atlantic salmon is (3S, 3′S), the Antarctic krill (*Euphausia superba*) contains (3R, 3′R), which is the principal stereoisomer primarily contains esterified form. [9]. Astaxanthin content (µg/g) in Antarctic krill, Copepod, Red yeast and Crab shell is shown in Figure 2.

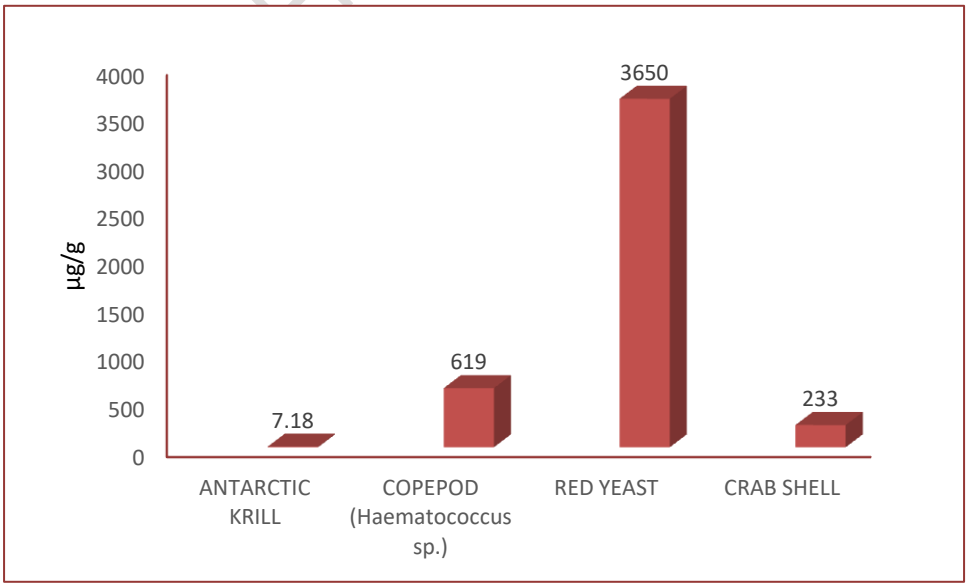


Fig.2. Astaxanthin content (µg/g) in Antarctic krill, Copepod, Red yeast and Crab shell [10,11,12,13].

3. SOURCES OF ASTAXANTHIN

The primary natural sources of astaxanthin are relatively simple microorganisms such as copepods, crab shell and yeast (Fig. 2). By ingesting astaxanthin containing creatures, animals do assemble astaxanthin in their tissues for beautiful colour, despite not being able to biochemically produce it. Salmonids and other fish, as well as organisms with exoskeletons like crabs, crayfish, lobsters, krill, and shrimp, consume zooplankton, which is fed on astaxanthin-rich algae in marine habitats; thus, astaxanthin is bioaccumulated and biomagnified subsequently at the higher trophic level (Table 2 & Fig. 3).

Table 1. Microbial sources of Astaxanthin

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

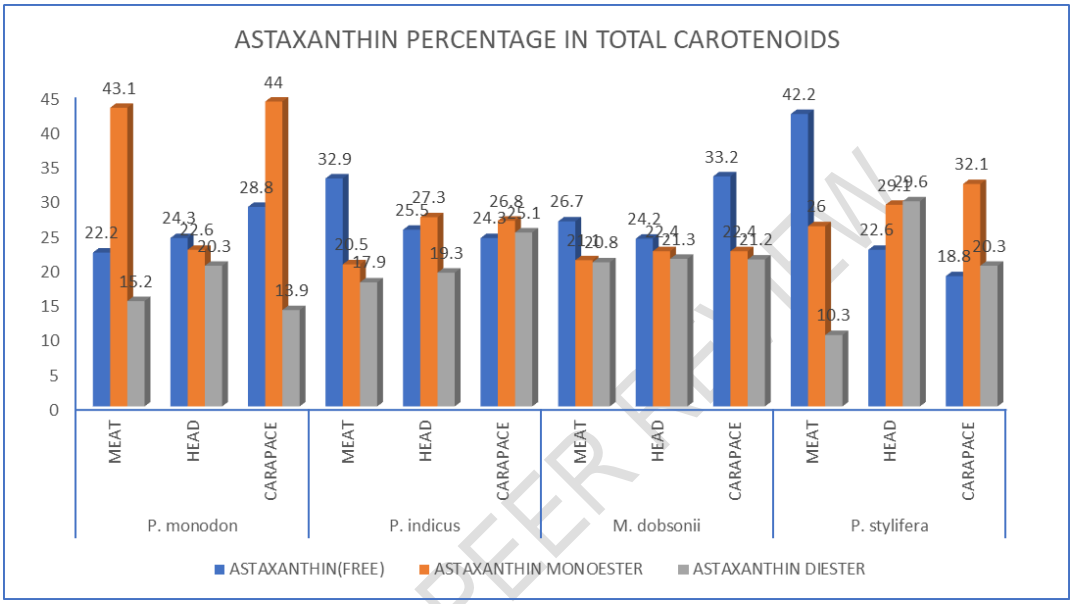
Table 2. Astaxanthin content of salmonids [22]

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

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

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

4. EXTRACTION OF ASTAXANTHIN



4.1 Extraction of astaxanthin from *H. pluvialis*

Under unfavourable climatic conditions, astaxanthin accumulates mostly in the encysted cells of *H. pluvialis* up to 3–4% of the dry weight [24–26]. In order to fully benefit from astaxanthin's bioavailability, intact astaxanthin-rich hematocysts must be mechanically disrupted before usage because of their thick and resistant cell walls. [27-29]. Over the years, numerous methods have been created to disrupt H. pluvialis cells. Typically, physical or mechanical pretreatment—more specifically, bead milling and expeller pressing—is used to destroy cell walls [30-34]. A bead miller is a device that uses a disruption or milling chamber filled with tiny grinding beads (such as steel, glass, and ceramic) that are stirred up quickly and collide repeatedly. At these chambers, the dried biomass is fed, and compaction and shear forces cause cell disruption in the bead impact zones. To prevent quality deterioration or spoilage, the algal biomass must be dehydrated as soon as possible using techniques like freeze-drying (lyophilization), spray-drying, drum-drying, and sun-drying [35-38]. Expeller pressing causes strong cell walls to burst by applying pressure and force simultaneously. The algal biomass must next be swiftly dehydrated by freeze-drying (lyophilization), spray-drying, drum-drying, and sun-drying to prevent quality degradation or spoilage. [39].

A lipophilic pigment, astaxanthin can be digested in oils and organic solvents. Acids, organic solvents, and edible oils all have been used in a variety of ways to isolate astaxanthin from H. pluvialis (Table 4) [40,41]. Using several acid treatments at 70°C, Sarada et al. [40] assessed overall extraction efficiency of astaxanthin from H. pluvialis and reported that HCl treatment permitted 86-94 percent recovery of the pigment without changing its ester profile. (Figure 4).

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

Process Name	Procedure
1. Acid pre-treatment	First, 10 mg biomass was combined with 1 mL of HCl and incubated at 70°C for 2 minutes in a centrifugal tube. After the mixture had cooled, it was centrifuged for five minutes at 5000 rpm. It was then suspended in 1 mL of acetone after being rinsed twice in distilled water. The sample was collected after 20 minutes in the ice-water bath, and it was centrifuged by ultrasonography for 6 minutes at 4 degrees Celsius at 3500 rpm. In order to determine the HPLC of the extracted astaxanthin, the supernatants are then gathered. Nitrogen is used during the entire process, and light is avoided.
2. Solvents extraction	Binary organic solvents of hexane/isopropanol (6: 4) and 10 mg of the lyophilized organisms were added. After 20 minutes in an ice bath and an ultrasound-assisted auxiliary extraction, the sample was collected, centrifuged at 3500 rpm for 5 minutes at 4 C, then concentrated under vacuum. On a dry basis, the collected yield is computed. In order to determine the HPLC of the extracted astaxanthin, the supernatants are then gathered. Nitrogen is used during the entire process, and light is avoided.
3. Methanol extraction	A 15 ml screw-top dark glass vial containing 10 mg of biomass was continuously sonicated in an ice bath with 1 ml each of methanol and acetone for 5 minutes. Samples were first extracted using 1 ml of methanol in a 15 ml dark glass vial with a screw top, and then centrifuged at 3500 rpm for 5 minutes at 4 °C. T hen it was used for HPLC analysis of the extracted astaxanthin. 1 ml of acetone was then added to a glass vial in this step. Nitrogen is used during the entire process, and light is avoided.
4. Oil-Soy Extraction	Mix 2.5 g of cellular biomass with 20 ml of vegetable oil in a 250 ml flask (protected from light) and place on a hot plate with stirring at room temperature for 2 h. Further, it was filtered through cellulose (0.22 µm) to obtain an oil extract. Extractable astaxanthin using the supernatant was measured by HPLC.

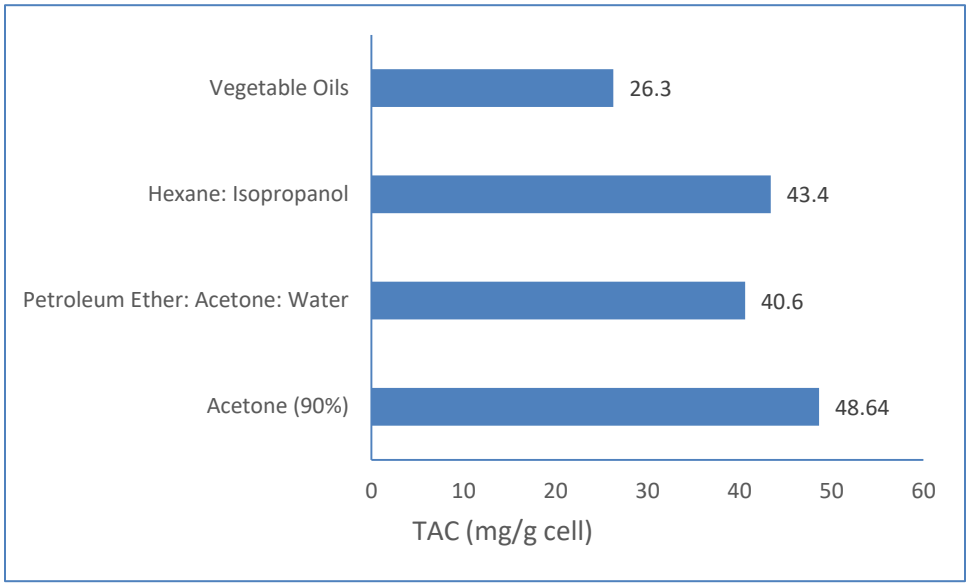


Fig. 4. Effect of different extraction methods on total astaxanthin content (TAC) of extracts from *H. pluvialis* cells [40,41].

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. Using 90% acetone	10 ml of 90% acetone was used to break 1 g of moist shrimp shell waste. Whatman filter paper was used to filter the extract. A colourless filtrate was obtained after the sample was once again proceeded with the same process using new solvent (3 times). 9.4 ml of 0.73 percent NaCl was added to the pooled extracts in a separate conical flask, and everything was thoroughly mixed. The epiphase was then thoroughly mixed and collected. The lower phase was mixed thoroughly with an equivalent volume of water before the epiphase was extracted.
2. Using Petroleum Ether, acetone and water	A combination of 10 ml of petroleum ether, acetone, and water was used to extract 1 g of moist shrimp shell at a ratio of 15:75:10. Whatman filter paper was used to filter the extract. The material was extracted again using freshly prepared solvent, and filtered until it was colourless The mixed extracts were obtained in a separate conical flask, and 9.4 ml of a 0.73 % NaCl solution and 12.5 ml of petroleum ether were then added. The extract was then vigorously stirred and epiphase was collected. The lower phase was properly mixed with an equivalent amount of water before the epiphase was recovered. To evaporate petroleum ether, the mixed epiphases were kept in a water bath at 60° C.
3. Using Hexane, Isopropanol	Use 10 ml of hexane: isopropanol to pulverise 1 g of moist shrimp shell at a ratio of 3:2. It was done using Whatman No. 42 filter paper to prepare the extract. Until a colourless filtrate was achieved, samples were repeatedly extracted and filtered with fresh solvent. Hexane: isopropanol (3:2 v/v) mixed extracts were separated using an equal amount of a 1 percent (w/v) NaCl solution. The epi phase was obtained by collecting it and anhydrizing it over anhydrous sodium sulphate. The residue was then evaporated to dryness in vacuo and dissolved in 5 ml of hexane.
4. Using different Vegetable Oils (Coconut Oil, Palm Oil, Sunflower Oil)	1 g of moist shrimp shell was mixed with 10 ml of vegetable oil until a colorless sample was obtained. The ratio of oil: waste used for vegetable oil extraction was 2:1 for wet samples and 4:1 for dry samples. The solvent was removed in vacuo and redissolved in 5 ml of hexane. The antioxidant butylhydroxytoluene (BHT) was added at 0.05% (w/v) and heated at 70° C. for 150 minutes.

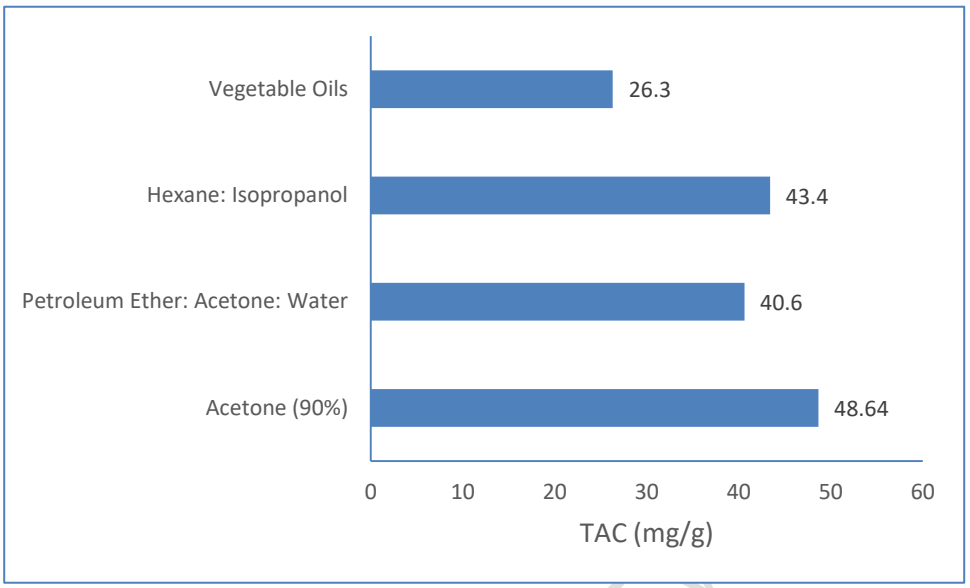


Figure 5: Effect of the different extraction methods by using different solvents on total astaxanthin content (TAC) of extracts from shrimp shell waste [41,43,44]

BIOCHEMISTRY OF ASTAXANTHIN

Conjugated double bonds, hydroxyl group and keto groups all are present in astaxanthin. It possesses hydrophilic and lipophilic characteristics [45]. The red hue is a result of the compound's conjugated double bonds, that operate as a potent antioxidant [46]. Due to its ability to bind cell membranes from the inside to the outside, astaxanthin demonstrated more biological activity than other antioxidants [47].

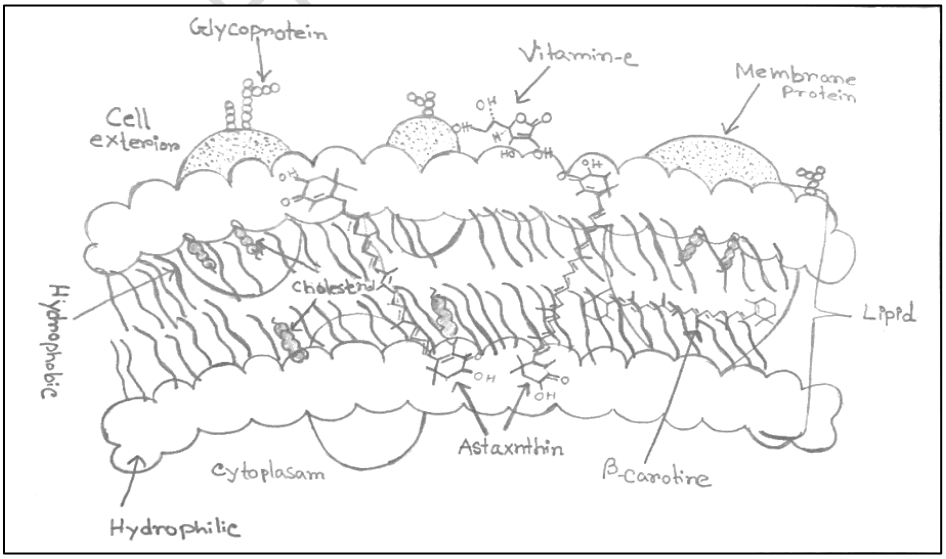


Fig. 6. position of astaxanthin in the cell membrane

6. USES AND BENEFITS OF ASTAXANTHIN IN AQUATIC ANIMALS

6.1 Use in reproduction

Astaxanthin is crucial towards the breeding as well as the cultivation of different aquaculture species. There are numerous proofs that indicate astaxanthin having a major effect on aquatic species' reproductive health, egg production, and egg quality [48–53]. In several fish farming facilities, efforts have always been made to enhance the quality of the eggs and larvae while neglecting the quality of the sperm. Therefore, it is imperative to comprehend the nutritional needs of farm animals since food availability directly affects a number of reproductive physiologic processes. Previous research have shown how dietary astaxanthin supplementation affects the performance of diverse aquatic species' reproductive systems and brood stocks. [51,52].

6.2 Use in growth parameters

Depending on production volume and cultivation techniques, feed often accounts for more than 60% of the overall hatchery management costs in aquaculture operations. In order to reduce production costs, it is crucial to produce feeds with nutrient components that support the growth and survival of the farmed species. The growth and survival (or both) of farmed fish and crustaceans during aquaculture techniques were positively correlated with dietary astaxanthin supplementation, according to a growing number of quantitative research publications. [54,55].

6.3 Prevent diseases

The rise of contagious diseases in intensive farming, particularly in the early phases of production, constitutes a substantial drawback or leading threat that has a considerable influence on the global economy. Farm animals are routinely subjected to physical stressors in high-density aquaculture operations, including grading, shipping, handling, vaccination, crowding and confinement, or any other physical disturbance that could be very stressful and immune-depressive. These unfavourable elements could disrupt the delicate equilibrium that aquatic creatures and their habitats must maintain, leading to stress reactions [56]. Excessive stress contributes to physiological malfunction, slower growth, immunosuppression, heightened susceptibility to pathogenic incursions, and even death. Therefore, it is crucial in aquaculture research to lessen unfavourable conditions that can cause a lot of stress and damage the host organism. Although farmed prawns and shrimp have been shown to exhibit extraordinary stress tolerance and disease resistance when compared to growth performance and survival, significant research efforts have been focused on reducing stress and improving immunity in aquacultured crustaceans and fish utilising astaxanthin in their diet.

6.4 Use for pigmentation

Aquaculture feed additives to improve the typical pinkish-red skin or meat coloration of aquatic species are perhaps astaxanthin's biggest potential use. The astaxanthin that is routinely added to their artificial diets and absorbed and deposited in rather high amounts causes skin and muscle coloration. From a commercial standpoint, maintaining natural pigmentation is crucial since it is closely linked to how customers perceive and interpret products, making it a crucial quality criterion before actual consumption that, in turn, drives up demand and product prices. The steadily growing aquaculture sector has created an insatiable demand for the carotenoid pigment since colour strongly influences customer preference and market demand for farmed species. Koi carp (Cyprinus carpio), kissing

gourami (Helostoma temminckii), and other aquatic species have been the subject of studies examining the impact of dietary astaxanthin on the skin and flesh coloration of aquatic animals. [58,59].

7. USE OF ASTAXANTHIN IN FEED FORMULATION

The steadily growing aquaculture sector has created an insatiable demand for the carotenoid pigment since colour strongly influences customer preference and market demand for farmed species. Koi carp (Cyprinus carpio), kissing gourami (Helostoma temminckii), and other aquatic species have been the subject of studies examining the impact of dietary astaxanthin on the skin and flesh coloration of aquatic animals..

The single most crucial factor in the efficient use of intracellular astaxanthin during milling appears to be the disintegration or disruption of microalgal cells [33, 64]. As a result, it has little effect on astaxanthin's stability [65]. However, grinding machinery, residence duration, and heat production all have a significant role in astaxanthin degradation. In order to ensure a consistent nutrient content in each fish pellet as part of the formulation, feed mixing is essential. However, mixing could introduce air into the mixture, which would lead to unfavourable carotene oxidation. To cope with air exposure and prevent air from entering the mixture, use a vacuum mixer. Alternately, it has been shown that the addition of secondary antioxidants (BHT and BHA) is effective in enhancing the oxidative stability of dietary carotenoids during feed processing. [66].

Extrusion aims to reduce nutritional breakdown in food while increasing the digestibility of starch and protein [67-69]. Additionally, it is verified that the formulation is adjusted to generate floating or sinking pellets. While retention values in extruded feed ranged from 86 % to 94 %, astaxanthin was relatively constant across extrusion, with an average retention of 86 % [65]. However, the stability of carotenoid pigments is most likely to be impacted by extrusion technology, which uses high amounts of heat, moisture, pressure, and mechanical shear. According to Storebakken et al. [71], the composition of astaxanthin during the manufacturing of extruded fish feed with a recovery range of 90-99 percent was not significantly impacted by the extruder temperatures of 102, 121, and 137°C. By improving the efficiency of nutrient consumption, pelletizing is the most popular process for formulating pellets [72,73]. To prevent the loss of pigment, the excess moisture is evaporated using a vacuum drying method (60–80°C temperature; a shelf-stable residual moisture of 10%). Additionally, a post-liquid coating application of fat or oil may lessen the chance of cooling immediately after potentially harming heat-sensitive carotenoids [74].

8. STABILITY OF ASTAXANTHIN

Due to astaxanthin's inherent chemical instability, it's bioavailability has faced significant difficulties, which has limited its use as a functional food ingredient. This compels the market to think about fresh ideas for improving storage, stability, economic and efficient utilization of astaxanthin. Review of literatures reveals that, astaxanthin was stable at 70^0-90^0C 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. After 63 days of storage, only 10% of the astaxanthin dried at 180/110°C and kept at -21°C in nitrogen had degraded. At 4°C and 25°C, astaxanthin's storage stability was improved in a complex solution of hydroxypropyl—cyclodextrin and water. The optimal storage conditions for dry astaxanthin, according to Gouveia and Empis [72], were under vacuum and nitrogen atmosphere in the dark, with high retention levels of more than 90% even after 18 months of storage. Astaxanthin stability was investigated using microencapsulation with polymeric nanospheres, emulsions and β-cyclodextrin [75-77]. Encapsulating homogenised astaxanthin-enriched H. pluvialis cells in a stiff polymer network of chitosan

increased their durability when stored at -18°C in a nitrogen environment for 24 weeks with only an 8% of pigment degradation. [78].

9. EFFECTS OF ASTAXANTHIN ON HUMAN HEALTH

9.1 Antioxidant Property

Oxidation can be suppressed by antioxidants. Reactive oxygen species and free radicals start oxidative damage (ROS). These highly reactive chemicals are created by an organism's typical aerobic metabolism. Through chain reactions, excessively oxidative molecules can interact with proteins, lipids, and DNA, causing DNA damage and a variety of diseases [1]. Endogenous and exogenous antioxidants, like carotenoids with double bonds and polyene chains, can prevent this oxidative destruction by absorbing singlet oxygen and scavenging radicals to stop chain reactions. Carotenoids' biological advantages may derive from their antioxidant characteristics, which are linked to their chemical and physical associations with cell membranes. When compared to different carotenoids like lutein, lycopene, alpha- and beta-carotene, astaxanthin exhibited more antioxidant activity, according to research [79].

9.2 Prevents lipid peroxidation

Based on its unique chemical structure, astaxanthin can remain both inside and outside of the cell membrane. Compared to β-carotene and Vitamin C, which can be found inside the lipid bilayer, it offers superior protection [1]. It protects against oxidative damage through a number of ways, including quenching singlet oxygen, scavenging radicals to stop chain reactions, preserving membrane structure by preventing lipid peroxidation, boosting immune system performance, and controlling gene expression. Rats given ethanol to produce gastric ulcers and skin cancer displayed 80% anti-lipid peroxidation action with astaxanthin and its esters. [1,81].

9.3 Anti-Diabetic Activity

People with diabetes mellitus typically have very high levels of oxidative stress. Due to the malfunctioning of pancreatic beta-cells and tissue damage in patients, it is brought on by hyperglycemia. Astaxanthin may reduce oxidative stress induced by hyperglycemia in pancreatic β-cells while simultaneously improving glucose and serum insulin levels [82].

9.4 Prevent heart diseases

Strong anti-oxidant astaxanthin has anti-inflammatory effects in both humans and animals. Astaxanthin functions as a powerful therapeutic agent against the pathophysiological characteristics of atherosclerotic cardiovascular disease known as oxidative stress and inflammation [83].

9.5 Anticancerous agent

The precise dose of antioxidants may be useful for rapid recognition of many degenerative disorders During regular aerobic metabolism, superoxide, hydrogen peroxide, and hydroxyl radical are produced. Singlet oxygen is formed by photochemical processes, whereas lipid peroxidation produces peroxyl radicals. Through the oxidation of DNA, proteins, and lipids, these oxidizing agents contribute to aging and degenerative disorders including cancer and atherosclerosis [84].

9.6 Effect on immune system

Cells of the immune system are extremely vulnerable to damage by free radicals. Poly unsaturated fatty acids are found in the cell membrane. Being an antioxidant, astaxanthin provides resistance against free radical deterioration to maintain immune system defenses. There are studies on astaxanthin's impact on immunity in lab animals, but

there aren't any for humans. In a mouse model, astaxanthin had more immunomodulatory effects than β-carotene [85].

9.7 Health benifits

According to literature reviews, astaxanthin may have benefits for your health by preventing and treating conditions like cancer, chronic inflammatory diseases, metabolic syndrome, diabetes, diabetic nephropathy, cardiovascular diseases, gastrointestinal disorders, liver disorders, neurodegenerative disorders, eye disorders, skin disorders, exercise-induced fatigue, and male infertility [86]. Currently, astaxanthin may be referred to as "a medical food" [1]. 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 related to the practical application, mechanism of action, and effectiveness of astaxanthin derived from Haematococcus species.

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 used 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.

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