

Marine Nutraceuticals from seafood waste

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Introduction

The marine ecosystem is still an underexploited reservoir of several bioactive compounds, having significant therapeutic and prophylactic role against a number of common lifestyle diseases. With the growing public consciousness of the health benefits of fish and seafood in general, the health food platform is now to set for the development of mainstream nutraceutical formulations. The current nutraceutical industry is familiar with a small number of marine-based nutraceuticals. Fish oil (mainly omega-3 polyunsaturated fatty acids), algal oil, shark liver oil and squalene, chondroitin salts, collagen, gelatin, collagen peptide, chitin, chitosan as well as their monomers and oligomers, peptides and related compounds, vitamins (A, particularly its precursor β -carotene, D and E), seaweed (macroalgae) and its components, protein hydrolysates and other products have become a topic of great interest for both pharmaceutical and health food industries.

It is estimated that fish processing waste after filleting accounts for approximately 75% of the total fish weight. About 30% of the total fish weight remains as waste in the form of skins and bones during preparation of fish fillets. Bio conversion of these wastes is an environmental friendly and profitable option for the utilisation of fish waste. Some viable options for generating wealth from waste through nutraceutical products are discussed in this chapter.

Options and opportunities

Generally two different methods, mass transformation and sorting, have been developed to improve the economic value of fish wastes. Mass transformation involves the conversion of fish waste into a single product. Typical examples of transformed fish waste include fishmeal, fish oil, fertilisers, and hydrolysates such as protein hydrolysate. Alternatively, sorting involves utilising various fish body parts such as bones, guts, and fins separately to enhance their economic value. For example, sorting enables the production of specialised products such as liver oil, gelatine, omega-3, protein containing sports food and drinks, calcium, cosmetics, and pharmaceuticals. Wider acceptance and adoption of both methods could lead to significant reductions in wastes going to landfill and reduce the damaging impact of fish wastes on the environment.

Fish protein hydrolysate: Fish protein hydrolysates are obtained by the controlled hydrolysis of fish protein either by employing acid, alkali or commercially available proteolytic enzymes. Hydrolysates find application as milk replace and food flavouring. Enzymes like papain, ficin, trypsin, bromelain and pancreatin are used for hydrolysis. The process consists of chopping, mincing, cooking and cooling to the desired temperature, hydrolysis, sieving, pasteurizing the liquid, concentrating and drying (by vacuum or spray drying). The fish protein hydrolysate have desirable functional properties with potential applications as emulsifiers and binder agents; and can be used in place of dairy based and plant based protein hydrolysates as well as protein powders currently available in market place (Binsi et al., 2016). The yield of hydrolysate is a critical parameter which decides the economics of operation. The yield is primarily dependent on factors such as enzyme-substrate ratio, temperature, pH, hydrolysis period, enzyme used etc.

The peptides formed by the hydrolysis of fish proteins are proven to have bioactive properties like antihypertensive, antithrombotic, immune modulatory and antioxidative properties. Also, they are good source of nutritional and functional properties. A variety of nutraceuticals from FPH are commercially produced and are available in international markets. Oyster peptide extract developed by ICAR-CIFT possessed antioxidant and anti-inflammatory activities. Similarly, hydrolysate made from squilla meat effectively reduced oil absorption in breaded and battered products, when incorporated in the batter mix.

In the industrial process of preparation of hydrolysates enzyme hydrolysis process is followed. Papain, bromelain, pepsin, ficin and trypsin are used for hydrolysis. Most hydrolysates are bitter in taste. Hence flavouring agents like cocoa, malt and sugar are used during the fortification in food preparation to mask the bitter taste. Protein hydrolysate has special application in sports medicine because its consumption allows amino acids to be absorbed by the body more rapidly than intact proteins, thus maximizing nutrient delivery to muscle tissues. Bioactive peptides are generally short peptides (3–20 amino acids) derived from proteins that can exert biological activities over and above their expected nutritional value. From a nutritional perspective, these peptides are more bioavailable than proteins or free amino acids and at the same time, less allergenic than their native proteins. Apart from their nutritional benefits, bioactive peptides exhibit a wide range of physiological functions including antihypertensive, antioxidative, opioid agonistic, anticancer immunomodulatory, antiproliferative, antimicrobial, prebiotic, mineral binding, antithrombotic, hypolipidemic and

hypocholesterolemic effects. These beneficial properties of fish protein hydrolysates may be due to the unique combination or high proportions of certain amino acids such as arginine and taurine with low levels of branched-chain amino acids found in fish meat.

Fish collagen/gelatin/collagen peptides: Collagen is the major structural protein in the connective tissue. Collagen extracted from fishes can be used in cosmetics, foods, biomedical applications etc. CIFT has developed the method for the preparation of absorbable surgical sutures from fish gut. Gelatin is the hydrolysed form of collagen with applications in development of bio degradable packaging, food and pharmaceuticals. Both collagen and gelatin are high molecular weight proteins of approximately 300 kDa, hence a considerable proportion is unavailable to human body for biological functions. Consequently, in recent years, much attention has been paid to the development of small molecular weight peptides from the native collagen with improved biological activities. This can be achieved by the process of hydrolysis in which the native collagen/gelatin molecules are cleaved to small fragments of less than 5 kDa. Currently, collagen peptides are being incorporated in a wide array of food products including protein bars, cereal bars, protein drinks, smoothies, yogurts, cold desserts, soups, cured meats etc. Nowadays, collagen/gelatin peptides have gained increasing attention as these peptides exhibit various biological activities such as antioxidant, anti-hypertensive, anti-human immunodeficiency virus, anti-proliferative, anticoagulant, calcium-binding, anti-obesity, anti-diabetic activities and postponement of age-related diseases. ICAR-Central Institute of Fisheries Technology (Cochin, India) has standardised a protocol for the extraction of collagen peptide from fish scale and bone. Further a nutritional mix based on collagen peptides was developed with a protein content of 78%. The product is mainly intended for middle aged and old people, ladies and sports-persons who needs a regular supply of collagen for healthy joints and bones. It may also be beneficial for patients suffering from osteoporosis and long-term-nursing home residents where there is a possibility of development of pressure ulcers.



Collagen peptide from fish scale and Nutritional mix formulated by CIFT

Chitins: The shrimp processing industry in India churns out more than 2 lakh tones of head and shell waste per annum, which can be economically converted to chitin and its derivatives. Chitin is the most abundant polymer next to cellulose. It is a linear polymer of N acetyl-D-glucosamine. Glucosamine hydrochloride can be produced from chitin by hydrolysis. Glucosamine hydrochloride and sulphate are at present marketed as food supplement for the treatment of osteoarthritis. It also possesses other beneficial actions in wound healing and skin moisturization. The deacetylated chitin is known as chitosan. Chitin and chitosan have various applications in agriculture such as in germination of seeds and enhanced protection against pathogenic organisms in plants and suppress them in soil to induce chitinase activity and protenase inhibition, antiviral activity, in micro encapsulation fertilizers and insecticides. The delivery of drugs and the interactions with living tissues seem to be the major topics of current research on chitosan. Other areas of interest are the antimicrobial action, nerve regeneration, cartilage and bone regeneration, skin and bone substitutes, oral delivery for wound healing etc. Carboxy methylation of chitosan imparts water-solubility to chitosan. ICAR-CIFT has recently standardised the methodology for production of chitin, glucosamine hydrochloride, chitosan and carboxymethyl chitosan. Similarly, collagen-chitosan film from fish waste, developed by CIFT has wide applications in wound dressing and dental surgery. The antioxidant chitosan derivative developed by CIFT recently was found to be useful in microencapsulating vitamins and β carotene, so as to give a novel delivery system. Similarly, a biocompatible and biodegradable wound healing formulation, composed of microencapsulated curcumin and hydrogel composite (Succinyl chitosan-fish collagen-poly ethylene glycol) developed at ICAR-CIFT, showed significantly enhanced rate of collagen deposition and hydroxyproline content in wound tissue on 14th day of post wounding as

compared to control and standard. Apart from that, free radical mediated grafting of gallic acid, ferulic acid, vanillic acid and coumaric acid onto chitosan were optimised. All the derivatives showed good antioxidant and antimicrobial activities.

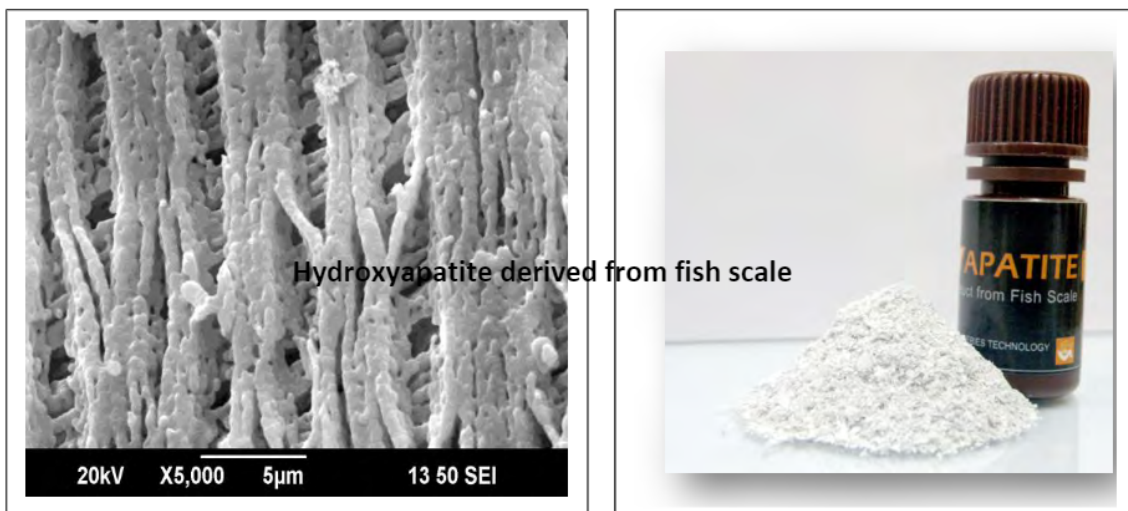
Fish calcium: In marine ecosystem, there is a large amount of calcium, mainly in the form of calcium carbonate and calcium phosphate, distributed as skeletal elements of teleosts, exoskeletal elements of molluscs or as coral deposits. Every year a considerable amount of total fish catch is discarded as processing left overs and these include trimmings, fins, frames, heads, skin and viscera. The bone fraction, which comprises approximately 15-20% of the total body weight of fish has high calcium content. Calcium and phosphorus comprise about 2% (20 g/kg dry weight) of the whole fish. Generally, fatty fish have lower ash levels compared to lean species. The filleting wastes of tuna and other bigger fishes are very good sources for calcium when the quantity of calcium is concerned. Also, the bone structure differs between species since a large number of teleosts have acellular bone (bone without enclosed osteocytes). Cellular bones are confined to only a few fish groups, e.g. Salmonidae. The higher surface to volume ratio in acellular fish bone is likely to increase the calcium availability compared to cellular bone. The ash content is highest in lean fish species with acellular bones. Apart from that exoskeleton of mollusks and coral deposits are excellent source of calcium. However, the calcium forms these deposits are mainly in the form of calcium carbonate. Central Institute of Fisheries Technology, Cochin has optimised the process to extract from fish bone which is mainly treated as processing discards during filleting operation of larger fishes, viz tuna, carps etc. The calcium powder was supplemented with vitamin D which is known to enhance absorption and bioavailability of calcium in the body. *In vivo* studies conducted at CIFT in albino rats have shown that fish calcium powder supplemented with vitamin D has improved the absorption and bioavailability.



Calcium extracted from Tuna bone

Hydroxyapatite (HAp): Hydroxyapatite is the major mineral component of bone tissue and teeth, with the chemical formula of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. The composition Hap derives from

biological sources differs from that of synthetic hydroxyapatite, due to the presence of several ionic substitutions in the lattice, such as CO_3 , F, Mg^{2+} and Na^+ . It is a member of the calcium phosphate group with 1.67 stoichiometric of Ca/P ratio. It is one of the few materials, classified as a bioactive biomaterial that supports bone in growth and osseointegration when used in orthopedic, dental and maxillofacial applications. Fish bone and scale is a rich source of hydroxyapatite. The hydroxyapatite content of fish skeleton may vary between 40-60%. Generally, very high heat treatment is used for extraction of HAp from bone and this temperature gives a higher strength to HAp structure. The high temperature also burns away any organic molecules such as collagen protein. Hydroxyapatite, found in fish is chemically similar to mineral components of bone and hard tissues in mammals. Approximately, 65-70% of the fish bone is composed of inorganic substances. Almost all these inorganic substances are hydroxyapatite composed of calcium, phosphorous, oxygen and hydrogen.



Squalene: Squalene is a highly unsaturated hydrocarbon present in the liver oil of certain species of deep sea sharks mainly *Centrophorus* and *Squalidae* spp. The liver oil of these species contain high percentage of squalene (90%) which can be isolated and purified and can be used as a dietary supplement. It belongs to a class of antioxidant molecules called isoprenoids. Squalene is found to be a proficient chemo preventive agent against lung metastasis in mice bearing lung carcinoma. Squalene revives damaged body cells and aids to revitalize cell generation. Its chief attribute is the protection of cells from oxidation reactions. Squalene assists to clean, purify, and detoxify the blood from toxins, facilitating systemic circulation. It purifies the gastrointestinal tract and kidneys, causes better bowel

movement and urination. Squalene helps in regulating the female menstrual cycle and also improves irregular and abnormal cycles.

Taurine: Taurine is a sulfur-containing non-protein amino acid (2-aminoethanesulfonic acid), with multiple functions like neurotransmission, cell volume regulation, stabilization of cell membranes and in the transport of ions such as calcium, sodium, potassium and magnesium. Taurine is one of the most abundant amino acids in the brain, retina, muscle tissue, and organs throughout the body, and taurine deficiency is associated with cardiomyopathy, retinal and tapetum degeneration, renal dysfunction, immune deficiency, muscle atrophy, developmental abnormalities, premature aging, and impaired reproduction. It can be synthesized from methionine and cysteine with the help of vit B6. The importance of taurine in biological system

stem, cardiac function, and cholesterol metabolism. The osmoregulatory role of taurine in facilitating the passage of sodium, potassium, calcium and magnesium ions into and out of cells, thereby stabilizing the structural and functional integrity of cell membranes was well discussed in earlier reports. It is involved in detoxification of xenobiotics and also essentially required for efficient fat absorption and solubilisation. Taurine has a protective effect on the tissue damage that results from oxygen free radicals in mercury induced toxicity. It plays a crucial role in prenatal and infant development. Epidemiological studies have shown that increased taurine intake is associated with diminished risk of hypertension. The deficiency of taurine does not impose immediate health issues, however long-term deprivation can affect a multitude of metabolic pathways. It is a key ingredient of bile and has a major role in the maintenance of normal gastrointestinal development and functions. Taurine is found in greater concentrations in all animal products. Meat, breast milk, dairy products, and fish are good sources of taurine. Shell fish contain higher concentration of taurine compared to that of fin fish. Zhao et al. (1998) determined the taurine concentration of a variety of common marine fish species and reported the highest content in crustacean and molluscs, ranging from 300-800 mg per 100 g meat. Apart from that red algae is considered as a good edible source of taurine. A possible beneficial action of taurine apoptosis is proposed. Eventhough, the cellular and biochemical mechanisms mediating the actions of taurine are not fully revealed, mounting evidences suggest that taurine might be a

key functional ingredient for use as a nutritional supplement to protect against oxidative stress, neurodegenerative diseases, atherosclerosis and hypertension.

Glucosaminoglycans: Glucosaminoglycans (GAGs) are linear polysaccharides with repeating sequences of disaccharides consisting of an amino sugar (*N*-acetylglucosamine, or *N*-acetylgalactosamine) and uronic acid (glucuronic acid or iduronic acid) or galactose. The major members of GAGs are hyaluronic acid or hyaluronan (HA), keratin sulfate (KS), chondroitin (CS), dermatan sulfate (DS), heparin and heparin sulfate (HS). HA is a high molecular weight molecule, typically with 2×10^7 Da and 2–25 μ , , short-chain molecules with of less than 50 kDa, more commonly 15–20 kDa. Hyaluronan lacks sulfate groups and is not covalently linked to protein, but the rest of the glycosaminoglycans are covalently linked to a protein core and contain sulfates at various positions. Dermatan sulphate is distinguished from chondroitin sulfate by the presence of iduronic acid. Keratan sulfates contains sulfated galactose and *N*-acetylglucosamine in place of uronic acids. GAGs are primarily considered as the components of various structural and connective tissues. Apart from the structural role, GAGs have been found to be associated with the regulation of a number of proteins, including chemokines, cytokines, defensins, growth factors, enzymes, proteins of the complement system and adhesion molecules. Apart from that, a few members like heparin possess anticoagulant, and anti-inflammatory properties. Dermatan sulfate (chondroitin sulfate B), also has a range of biological properties, although it has not yet been considered for therapeutic purposes. Marine heparin extracted from shrimp and sea squirt has proven anti-inflammatory properties.

Pigments- Astaxanthin, fucaxanthin, melanin etc. from different fish resources are found to have a variety of bioactive properties. The filleting discards of salmonids and the shell wastes of crustaceans contain significant amounts of carotenoid pigments such as astaxanthin and canthaxanthin. The protective role of carotenoids against the oxidative modification of LDL cholesterol could be explored by incorporating in health drinks. Carotenoids are also highly sought after as natural food colours. Cephalopod ink is another less tapped reservoir of a range of bioactives having therapeutic and curative values. It is an intermixture of black pigment melanin, glycosaminoglycans, proteins, lipids, and various minerals. Cephalopod ink has been reported to have anti-radiation activity, antitumor activity, immunomodulatory activity, procoagulant function and so on. The pigment melanin can be used both as a natural colorant

as well as antioxidant, in addition to a number of other therapeutic and prophylactic properties including anticancer, antihypertensive, Anti IDA etc.

Melanin : Cephalopods comprising mainly squids and cuttlefishes form an important resource of world oceans and their economic importance is growing exponentially. Consequently, cephalopods have emerged in recent years as an important component of the marine products, and are considered as a major delicacy in export markets. While several products (fillets, tubes, rings etc.) are made from cuttlefish, squid and octopus, considerable quantity, including the ink sac is disposed as waste. Interestingly, the cephalopod ink was identified as the most useful resource for the commercially important pigment melanin. Basically, squid ink is an intermixture of melanin, proteins, lipids, carbohydrates, glycosaminoglycans, various minerals etc. The predominant components are melanin and protein-polysaccharides complex. Each ink sac of sepia has ~1 g of melanin, and melanin constitutes ~15% of the total wet weight of ink with other proteins.

The basic structure of melanin comprises of covalently linked indole structure (Takaya and others 1994). Melanin performs a number of biological functions in the body, the main function being to protect the organism from harmful agents such as ultraviolet (UV) radiation; melanin is capable of dissipating over 99% of absorbed UV light. Besides, in the biological system, melanin plays a vital role in providing mechanical strength and protecting proteins from degradation. Numerous reports published in last thirty years reveal the therapeutic, prophylactic and curative value of cephalopod ink. The anti-ulcerogenic properties and anti-

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Mimura et al. through a series of rat model studies. Later on, several researchers confirmed the effect of squid melanin on both phenylbutazone induced ulceration in gastric mucosa and secretion of gastric juice in rats. Apart from that, melanin has been reported to have radio-protective activity, antitumor activity, immunomodulatory activity, procoagulant function and so on. Natural melanin has been reported to have defense activity, protection function and metal chelating ability. It could participate in physiological and pathological activities in human body and even in the treatment of Acquired Immune Deficiency Syndrome (AIDS). A new generation photo-thermal dopamine-melanin colloidal nanospheres was developed by Liu et al. (2012) which could efficiently damage tumour cells at low power density and short duration, without damaging healthy tissues. Melanin also functions as photoprotective and chemoprotective pigment, protecting the body from damaging radiations, as observed at an

effective dose of 50 mg/kg body weight in mice model. Similarly, oral administration of melanin for protection against radiation was reported by Dadachova et al (2016). The protective activity of melanin is primarily attributed to the inhibition of radiation-induced hematopoietic damages. Several other physiological studies conducted on squid ink also revealed significant effects on granulopoiesis of hemopoiesis impaired mice induced by ^{60}Co γ cyclophosphamide, but has no effect on erythropoiesis. Melanin has been widely and conventionally used as an antioxidant and natural colorant in food formulation. The most interesting thing is that melanin can be used as food additives to prevent the rancidity caused by the presence of bacteria by quenching the bacterial quorum sensing. Squid melanin was reported to have hemopoietic function in Iron Deficiency Anaemic rats, which might be exploited as a safe, efficient new iron tonic. Deficiency of melanin is associated with disorders such as vitiligo and oculocutaneous albinism. Interestingly, melanin is thought to play a protective role against the age-associated and noise-induced hearing loss. Recently, the anti-ageing property of melanin was demonstrated in mice model, suggesting its use in nutraceutical formulations. Even though melanin is a part of normal human diet, research on dietary intake of melanin is not much explored.



Melanin from cuttlefish ink

Marine algae

Algae, in particular, are virtually fat and calorie-free, making them increasingly sought for commercial purposes. Macroalgae, *generally referred as seaweeds*, have been found to be good sources of dietary fiber and carotenoids with antioxidant activity and play important roles in the prevention of neurodegenerative diseases. Several bioactive compounds have been isolated from brown algae with different pharmacological activities such as cytotoxic, antitumor, nematocidal, antifungal, anti-inflammatory and antioxidant. Algins, carrageenans

and agar are examples of polysaccharides derived from algae that are widely used as thickeners and stabilizers in foods as well as for gels. Sulphated fucans, carrageenans and ulvans, have been known to act as modulators of coagulation as well as reveal antithrombotic, anti-inflammatory, antioxidant, anticancer and antidiabetic activities, among. Soluble polysaccharides from algae have tremendous potential as dietary fiber for human nutrition and are being evaluated as new possible prebiotic compounds. Microalgae are considered important producers of some highly bioactive compounds found in marine resources; they can be used to improve food nutritional profile due to their richness in PUFAs and pigments such as carotenoids and chlorophylls.

Challenges and way backwards

The key to successful seafood waste utilisation and management is to develop appropriate eco-friendly reprocessing technologies that can convert all the valuable components present in the waste into valuable products and reduce the amount of waste going to disposal route. However, there are many challenges that must be overcome to achieve this goal.

1. Consumer awareness and education is one such challenge. Without consumer acceptance of food waste reduction approaches, no sustainable eco-friendly food waste utilisation and management strategy can succeed. This demands proper extension efforts from the research and extension organizations.
2. Seafood sector is a poorly organised sector. Highly scattered nature of seafood processing operations (across domestic market and processing facilities) poses problems in collection and processing.
3. Seafoods are highly perishable in nature owing to its unique richness in terms of protein, peptides, enzymes and microbial flora. This quite often leads to the mass resistance from public in starting up a business venture in the vicinity.
4. Stringent legal and environmental restrictions from the regulatory bodies as seafood waste “ / ” j invest upon this resource
5. Inappropriate cold chain management from the source of generation to the point of conversion as the processors are least interested to invest further on discards

6. There is no baseline data on the availability and economics of production collected over the past years, which poses uncertainty about economics and market demand of secondary products
7. Lack of clear legal classification of secondary products in the international market is yet another major challenge to the investors
8. Lack of unified protocols for quality assurance (such as HACCP) for secondary products leads to frequent rejections from the buyers.

Suggested readings

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