# 13. Protein derivatives and Nutraceutical products from secondary raw material of aquatic origin-An Industrial Perspective

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## Introduction

Fish and shellfish are highly nutritious and fishery products are delicious. There is a growing awareness on the health benefits of consuming fish and the demand for fish is ever increasing. On the other hand, fish is highly perishable compared to meat from land animals due to near neutral post mortem pH, low glycogen reservoir, low connective tissue content and high moisture content. Immediately after harvesting of fish (immediately after death), it undergoes various bio-chemical and microbiological changes which lead to spoilage. Hence, fish is essentially processed and preserved to make the fish available in edible condition. As a result of processing, a greater portion of raw material is discarded as waste which is biochemically equivalent to edible portion.

## Secondary raw material

Aquatic food processing discards are now called as secondary raw material because of their potential for the production of high value products. For any country, to develop a systematic way to utilize or to set up an industry, the information on amount of waste generated would be

the first aspect to be searched. Unfortunately, even in well developed countries, the data on waste generation from fish processing sector is not available, due to the complexity in obtaining such information. The available data are derived from the information on export quantity. However, it is essential to have information part wise, as many of the high value ingredients are derived from the specific parts. The properties of derived high value products depend on the parts from which they are derived. For example, the properties of gelatin from fish skin, scale and bone is different.

**Factors influencing the amount of waste generated**

Fish processing sector generate two types of waste i.e. solid waste and liquid waste. Often the effluents undergo various treatments prior to discharging. Most often, the environmental issues are emerging when these discards are not properly handled/disposed particularly the solid waste creates the problem. The amount of waste generated from fish and shellfish depends on certain inherent aspects and processing related *parameters.*

*Fish related parameters*

- ✓ Species
- ✓ Size/Age group
- ✓ Biological nature (size of head, length of intestine, shorter fins etc.,)
- ✓ Body shape (Cylindrical, flat etc)

*Process related parameters*

- ✓ Style of dressing
- ✓ Style of product

- ✓ Skill of handling person
- ✓ Skill on handling the machines involved and their design
- ✓ Intended use
- ✓ Quality of raw material

Obtaining the information on waste generation is quite difficult with reference to above parameters. Hence, generating a data base for the commercially important processed fish is essential and highly useful for any nation which aims in industrial development in this sector.

**Quantification of Secondary raw material of aquatic origin from India**

During the financial year 2015-16, India has exported 9, 45, 892 MT of Seafood worth US$ 4.7 Billion (Rs. 30,420.83 crores). The quantity of export is roughly less than 1% of Indian total fish production. Today, the Indian seafood are tasted in 106 countries in the world and major markets are SE Asia, EU, USA, Japan, China and Middle East. India secured the position as a largest exporter of shrimp to USA, the 2$^{nd}$ largest exporter of shrimps to Europe and the 4$^{th}$ largest exporter of shrimps to Japan. The demand for Indian seafood products across the global consumers is increasing and the phase of Indian seafood business changes day by day. The resource and infrastructure of the Indian seafood industry has witnessed a tremendous growth in the recent past. India has well established processing capacity of 23,000 M.T with 506 processing plants, out of which over 62% of them are EU approved plants. Almost every plant has put in place HACCP and other Quality control system on par with the best in the world to ensure highest quality output.

**Table 1. Amount of waste generated (%) during industrial processing of seafood**

| Products | Waste Generated (%; w/w) |
|---|---|
| Shrimp products | 50 |
| Fish fillets | 65 |
| Fish steaks | 30 |
| Whole and gutted fish | 10 |
| Surimi | 70 |
| Cuttle fish rings | 50 |
| Cuttle fish whole | 30 |
| Cuttle fish fillets | 50 |
| Squids whole cleaned | 20 |
| Squid tubes | 50 |
| Squid rings | 55 |

In the present article, for estimating the approximate raw material could have been used and waste could have been generated in the processing industry, the waste percentage was considered conservatively. The presented value of waste generation is only from industrial processing sector and excluded the waste generation during house hold preparations. Hence the countries estimate for the fish by product generation will be definitely pretty higher than the represented figure.

**Table 2. Approximate estimation of fish by products generated in the processing industry**

| Product | Quantity (ton) | Approximated waste percentage | Raw material quantity (ton)[1] | Quantity of waste generated (ton)[2] |
|---|---|---|---|---|
| Frozen shrimp | 373866 | 40% | 623110 | 249244 |
| Frozen fin fish | 228749 | 50% | 457498 | 228749 |
| Frozen cuttlefish | 65596 | 50% | 131192 | 65596 |
| frozen squid | 81769 | 50% | 163538 | 81769 |
| Dried items | 43320 | 20% | 54150 | 10830 |
| Live items | 5493 | 00% | 5493 | 0 |
| Chilled items | 33150 | 20% | 41437.5 | 8287.5 |
| Others | 113949 | 10% | 126610 | 12661 |
| Total | 945892 | | 1603028.5 | 657136.5 |

[1, 2]The presented values are approximate estimation, not the actual figures.

**Protein content in secondary raw material**

The discards from fish/shellfish contain protein in the range of 9-27% depends on the waste parts. The tissue proteins for example the meat from head and filleting frames contains major muscle protein fractions like myosin, actin, troponin, tropomyosin etc. The skin, scale and bone contains the protein namely collagen (an integral protein moiety of connective tissues). Shrimp shell waste contains carotenoproteins.

**Table 3. Protein content in major fish waste parts**

| | Waste Parts | Protein (%) |
|---|---|---|
| **1.** | Head | 11-13 |
| **2.** | Back-bone/ frame | 10-15 |
| **3.** | Cut-offs | 12-22 |
| **4.** | Skin | 8-12 |
| **5.** | Milt | 14-27 |
| **6.** | Viscera | 9-23 |
| **7.** | Shrimp head waste | 9-14% |

**(Source:** Rustard, 2007**)**

## Table 4. Categorization of seafood discards

| Based on the site | Based on physical state of waste | | Based on the aquatic animal | Based on the richness of bio-chemical constituent | Based on the complexity |
|---|---|---|---|---|---|
| | Solid waste | Liquid waste (effluents) | | | |
| • On board waste<br>• Industrial waste<br>• Landing center waste<br>• Retail waste<br>• Waste from domestic preparation | • Dark meat<br>• Head<br>• Skin<br>• Scale<br>• Fins<br>• Frames<br>• Visceral mass (including Air bladder and liver)<br>• Gills<br>• Crab shells<br>• Shrimp head and shells<br>• Cuttle fish bone<br>• Squid pen<br>• Ink sac<br>• Cuttle fish skin<br>• Shells from oyster, mussels and clams | • Effluents consist of blood, slime, mucus, wash off (Processing units effluents and peeling shed effluents)<br>• Surimi wash water | • Fin fish waste<br>• Shellfish waste<br>• Crustacean waste<br>• Cephalopods waste<br>• Mollusk waste | • Waste rich in protein<br>• Waste rich in lipid<br>• Waste rich in minerals<br>• Waste with special molecules | • Simple waste (Scale, skin, shrimp cuticle)<br>• Complex waste (Head waste, visceral waste, shrimp head, Squid and cuttlefish waste) |

**Protein derivatives and nutraceutical products from secondary raw material**

1. **Fish protein hydrolysate and peptide**

Fish protein hydrolyzates (FPH) are derived from fish meat or fish processing waste by enzymatic hydrolysis using protease enzymes. Fish protein hydrolyzates are rich in proteins and bioactive peptide. It has been reported that bioactive peptides from fish protein hydrolysates found to have anticancer, anti-coagulant, anti-diabetic, anti-obesity, hypocholesterolemic effect, antimicrobial, antioxidative, antihypertensive, antithrombotic and calcium binding activities. Fish protein hydrolysates (FPH) has wide application in food which includes cereal products, simulated fish and meat products, beverages, soups, gravies, breads, cakes, desserts and crackers etc. Functional and nutraceutical properties of fish protein hydrolysate are depends on the amino acid composition and bioactive peptides. Properties of fish protein hydrolysates are influenced by various factors which includes, type and quality of raw material, hydrolysis condition and enzyme used. Fish protein hydrolysates (FPH) also used as microbiological growth media, aquaculture and poultry feed ingredients.

2. **Fish protein isolate (FPI)**

Fish protein isolate is prepared from fish muscle by the pH-shift process (either acid or alkali solubilization). It is a form of concentrated myofibrillar proteins (i.e., myosin and actin) with high nutritional. FPI also prepared from fish meat and fish processing waste. Fish protein isolate can be used as a fish protein ingredient for the production of

functional fishery products. It is also used as a partial or whole in mince and surimi formulation.

**3. Fish gelatin**

Gelatin is a protein obtained by the thermal denaturation of collagen present in connective tissue of animal skin and bone. Gelatin can be prepared from the skin, scale and bone of fish. Gelatin contains inimitable proteins as like fish muscle proteins and they are rich in glycine, alanine, valine and proline. Properties of gelatin are influenced by different factors such as amino acid composition, molecular weight distribution and hydrophobicity etc. Gelatin has wide application in food, pharmaceutical, cosmetic, and photographic applications. Gelatin derived from mammalian source is commercially available. Fish gelatin has been found to be a potential alternative to mammalian gelatin. Fish gelatin has its own characteristic properties like better release of a product's aroma and flavor with less inherent off-flavor and off-odor than a commercial pork gelatin, which offer new opportunities to food processing Industry.

**4. Collagen**

Collagen is one of the major structural proteins present in skin, connective tissues of animals. Fish skin, scale, fins and bones are considered as potential source for production of fish collagen. Collagen from marine sources is soluble in salt solutions, dilute acids and in acid buffers. The amino acid composition and functional properties of collagen depends on the processing conditions of the raw materials and method of collagen extraction. Collagen has wider application in

pharmaceutical, cosmetic and nutraceutical Industry. Due to its nutraceutical effects for human health, marine collagen are marketed as dietary supplements, capsules, powder drink mixes and syrups etc.

5. **Collagen peptide**

Collagen peptide is a hydrolysed form of collagen or gelatin with a molecular weight in the range of 1-5kDa and has great potential in pharmaceutical, health food, nutraceutical and cosmetic industry. Fish derived collagen peptides have been found to have noticeable antioxidant amino acids such as histidine, methionine, cysteine, tyrosine and phenylalanine. Collagen peptide as a food supplement increases levels of collagen in the skin supports the smooth functioning of cartilages and increases the thickness and strength of hair. Recently collagen peptide is being utilized in food industry in confectionery, dairy, bakery, meat processing, beverages etc.

6. **Fish calcium**

During fish processing, fins and bones are generated as waste which rich in calcium. Fish bone contains an appropriate balance of calcium and phosphorous hence it can be used as a dietary supplement and development of calcium fortified food products. It has been reported that insufficient supply of calcium and vitamin D leads to risk of osteoporosis in human. Hence, dietary supplement containing these nutrients getting more popular in preventing osteoporosis. The daily intake of 1.2 g of calcium as recommended by USFDA may prevent or reduce osteoporosis.

7. **Hydroxyapatite:** Hydroxyapatite [$Ca_{10}$ $(PO_4)_6OH_2$], a mineral

primarily found in bones, teeth and cartilage tissues in animals and human, as well as aquatic organism. Fish bone, scale found to be a potential source of extraction of hydroxyapatite. Alkaline hydrolysis or calcination method is commonly used for extraction of hydroxyapatite from fish bone and scale. Hydroxyapatite has wide application inthe field of tissue engineering for novel bone and cartilage replacement due its chemical composition closer to natural bone. It has been also found application in dentistry. Hydroxyapatite from fish has good biocompatibity in comparison to synthetic one.

8. **Fish oil**

Fish oil is an excellent source of omega- 3 fatty acids especially EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid).Omega -3 fatty acids are found to have numerous benefits for human health which includes reduction of joint pain, prevention of rheumatoid arthritis, cancer, psoriasis, reduction of risk of coronary heart disease, vision improvement and brain developments. Currently, omega-3 fatty acids fortified food getting popular due to its various human health benefits. The US Food and Drug Administration (FDA) has also recommended fish and algal oils for food fortification. Due to its unsaturated nature fish oils are susceptible to oxidation and it can be reduced by addition of antioxidant or preferably microencapsulation. Recently, microencapsulated fish oil has wide application in various food products which includes infant foods, health drink, milk based products, juices, pastas, bakery products etc. Apart from these fish oils also marketed as capsule and powder form. Recommended level

of EPA and DHA intake suggested by world health organization (WHO) and American Heart association is 0.7g/day.0.5-1.0g/day respectively. In addition to consumption of fish , intake of fish oil fortified food will meet the daily requirement of omega-3 fatty acids.

**9. Enzymes**

Fish visceral waste contains rich sources of enzymes, which have potential applications in different sector includes food, biomolecule extraction, descaling of fish, stain removal and pharmaceutical applications. It has been reported that fish visceral waste contain rich source of proteolytic enzymes namely, pepsin, trypsin, chymotrypsin and collagenases. Enzyme extracted from marine sources has found application in Fish curing and fermentation, hydrolyzed products production, pigment extraction, wastewater treatment and meat tenderizing. These enzymes also used as a component of biosensor for rapid assessment of fish quality.

10. **Fish roe**

Fish roe is an imperative underutilized by-product having essential amino acids and fatty acids. They are best natural source of Vitamin D. It has been found that a single teaspoon of fish eggs contains almost 17,000 IU of vitamin D. Besides that, they also contain zinc, vitamin K2, and vitamin A. They have ample amount of poly unsaturated fatty acids (omega-3 fatty acids) which help in functional of brain and its health. Fish eggs are considered as potential sources of lectins. Lectins, a glycoprotein has the ability to bind with carbohydrates and it can serve as potential antibiotics. Fish eggs also

help in pacing up the body metabolism and are considered helpful in reducing inflammation of the body tissues. Even some studies suggest significant role of fish roe to help patients with hepatitis or cirrhosis.

11**. Chitin and chitosan:**. Chitin is derived from crustacean shell waste by acid and alkali treatment. Chitin is chemically called as N-acetyl-Dglucosamine (N-acetyl-2-amino-2-deoxy-D-glucopyranose). Chitosan is derived from by deacetylation process. Chitosan is composed mainly of 2-amino-2-deoxy-β-D-glucose (glucosamine). Chitosan and its oligomer has wider nutraceutical application which includes; wound healing, drug delivery system, hemostatic, lipid absorption reduction, antigastritis agent, hypocholesterolemic effect etc.. Potential food applications of chitosan includes beverage clarification, texture controlling agent, emulsifying agent, color stabilizer, dietary fiber, edible film- antioxidant and antimicrobial substances.

**12. Glucosamine**

D-glucosamine (2-amino-2-deoxy-D-glucose) is an amino sugar naturally found in human body and crustacean shells. It is derived from chitin by acid hydrolysis. After hydrolysis, excess acid will be distilled under vacuum and crude glucosamine hydrochloride can be obtained and it will be diluted with water and clarified with activated charcoal and to be filtered. Addition of methanol/alcohol can separate glucosamine hydrochloride from the solution. Glucosamine is an amino monosaccharide acting as a preferred

substrate for the constitution of glucosamonoglycan chain. It also acts substrate for the production of proteoglycan which gives hydrophilicity to the cartilage thus beneficial in treatment of osteoarthritis. It has anticancer, anti-inflammatory and antibacterial effects. Glucosamine has wide application for the treatment of osteoarthritis, knee pain, and back pain. It is commercially marketed as dietary supplement in the form of n-acetyl glucosamine or glucosamine hydrochloride.

13. **Carboxymethyl chitosan:** Carboxymethyl chitosan is considered as a very promising ingredient for making feed, foliar spray and insecticide formulations, as it is soluble at neutral pH and exhibit superior functionalities. The unique properties of Carboxymethyl chitosan such as high viscosity, large hydrodynamic volume, gel-forming capabilities, and antibacterial properties, besides its excellent solubility at neutral pH, make it an attractive option for its use in processing and preservation of wet and dry commodities. It has enhanced biological and physicochemical properties compared to chitosan and is a promising candidate for biomedical applications such as drug carriers, antimicrobial material, gene delivery systems and tissue regeneration devices.

14. **Astaxanthin**

Astaxanthin, a ketocarotenoid pigment naturally found in crustaceans and represent 74 and 98% of the total pigments. It is chemically called 3,3-dihydroxy-β, β-carotene-4,4-dione. It has very good antioxidant effect and enhances the activity of other antioxidant

such as vitamin E and Vitamin C. It has found wide application in food, feed, pharmaceutical and cosmetic products. It is also used as dietary supplement with very potent antioxidant effect for human health. In pharmaceutical, it is used for treatment of various diseases such as cancer, Alzheimer's disease, Parkinson's disease, brain attack and high cholesterol.

**Conclusion**

During fish processing, fish bone, fins, skin, scale and visceral waste are generated. They are rich proteins and other bioactive compounds which has wide application in food, pharmaceutical and cosmetic products. Hence, effective utilization of fish processing discards will more helpful for production of high value products such as chitosan, glucosamine, gelatin, collagen, hydrolysate, and omega-3 fatty acids etc. Further, it also reduce the environment pollution.