# Protein and protein derivatives from aquatic food processing waste

**Elavarasan, K.**

*By-Products Section, Fishing Processing Division, ICAR- CIFT, Cochin*

elafishes@gmail.com

## Introduction

I ’ ario, a large portion of the global population very much aware of health benefits one can achieve through consumption of aquatic food products. Particularly, fish and shellfish are highly nutritious and delicious. The demand for fish is ever increasing. On the other hand, aquatic animals like fish and shellfish are 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. In this chapter, the potentiality of fish waste as secondary raw materials, protein richness, waste generation during industrial processing and technology availability in producing protein and derivatives with ICAR-Central Institute of Fisheries Technology (ICAR-CIFT, Cochin) is briefed.

## 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 (organs). 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 are 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 – A case study**

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 an installed processing capacity of 23,000 M.T with 506 state-of-the-art 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. 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) |

**Table 4. 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**)**

**Handling of secondary raw material**

Considering the importance of secondary raw material generated in seafood processing industry, the hygienic handling of raw material to be given due importance. Without proper utilization of secondary raw material, sustainability in fish processing sector will be impossible. The following points may be followed to maintain the quality based on the intended use.

- Collection of waste
- Sorting of waste parts wise and based on quality
- Washing in chilled water/chlorinated water
- Packing in a suitable packaging material
- Preservation based on the intended use (Chilling, freezing, salting and drying or any other chemical treatment)

**Proteins from secondary raw material and the possible industrial products**

Fish processing discards are rich in fish muscle proteins (Myosin, actin troponin, tropomyosin etc.), connective tissue proteins (Collagen and its derivative gelatin), fish enzymes, hemoproteins and carotenoproteins. The relevant industrial products which exploit the above mentioned proteins are fish protein concentrate, surimi from frame meat, fish meal, shrimp head meal, squid meal, dried fish scale and dried fish skin.

**Table 5. The protein components from secondary raw material and the relevant possible industrial products**

| Proteins from secondary raw material | Protein rich industrial products from secondary raw material |
|---|---|
| • Fish muscle proteins (Myosin, actin troponin, tropomyosin)<br>• Collagen<br>• Gelatin<br>• Fish enzymes<br>• Hemoproteins<br>• Carotenoproteins | • Fish protein concentrate/fish protein powder<br>• Surimi<br>• Fish meal<br>• Shrimp head meal<br>• Clam meal<br>• Squid meal<br>• Dried fish scale<br>• Dried fish skin |

**Fish protein concentrate**

Fish protein powder (FPP) is a dried fish product, meant for human consumption, in which the protein is more concentrated than in the original fish flesh. Different methods for the separation of meat from fish are employed, such as washing meat with water for two to 3 cycles and concentrating, solubilization of muscle by pH adjustment and iso-electric precipitation, solvent extraction to method to remove the fat, cooking and drying, and a combination of various methods. The raw material such as fish filleting frames, head waste, tuna red meat and belly flaps can be used to produce fish protein concentrate

Earlier studies conducted on rat have shown that fish proteins have greater cholesterol lowering ability (Ammu et al., 1989) and can protect the animal against lipid peroxidation. Fish protein reduces serum cholesterol, triglycerides and free fatty acids and increases the proportion of HDL cholesterol. In general, protein supplements claims to help weight loss and muscle building. Fish protein supplement have shown beneficial effects on blood levels of glucose and LDL-cholesterol as well as glucose tolerance and nutritional composition of body in overweight adults (Vikoren et al., 2013). In another study, dietary scallop protein completely prevented high-fat, high-sucrose-induced obesity whilst maintaining content of lean body mass and improving the lipid profile of plasma in male C57BL/6J mice (Tastesan et al., 2014).

### *Fish Collagen*

Collagen is a structural protein found mainly in the skin and bones of all animals. Collagen is the most abundant protein originating from the animal source, comprising approximately 30% of total animal protei I α-chains which are intertwined to form a triple-helix. It is present in the connective tissue matrix that makes the framework of skin, bones and joints, cornea, blood ducts, and the placenta. There are many types of collagen, but 90% ' -I collagen. It is found to be rich in amino acids such as glycine, valine, alanine, proline and hydroxyproline (Burghagen, 1999). Glycine constitutes one third of the total amino acid content of collagen followed by hydroxyproline and proline, which account for another one-third. Owing to this structural uniqueness of collagen molecule, there is increasing interest for the direct consumption of collagen in the form of their easily digestible derivatives. Worldwide, this interest has been taken-up by the nutraceutical industry, especially in developing countries.

Currently, collagen is used in many pharmaceutical and cosmetic products, due to its structural role and better compatibility with human body. It is commonly used in the cosmetic industry for the production of skin lotions as it forms a superior protective film to soothen and hydrate the skin. Such potential of collagen has tremendous bearing on anti-aging treatment. Apart from that, collagen has a wide range of applications in the field of cosmetic and burn surgery, especially as dermal fillers in the reconstruction of skin and bone. Collagen gels have potential clinical importance in the preparation of 'artificial skin' used in treating major wounds. Injectable collagen hydrogels have been successfully used for soft-tissue augmentation, drug delivery carriers and hard-tissue augmentation. Microfibrous collagen sheets are used as promising drug carriers for the treatment of cancer. It is also an essential component in diverse orthopedic and dental treatments. Further, collagen is recently projected as a joint mobility supplement.

### *Fish Gelatin*

Gelatin is a soluble polypeptide obtained by denaturing the insoluble collagen. Procedures to derive gelatin involve the breakdown of cross-linkages existing between the polypeptide chains of collagen along with some amount of breakage of intra-polypeptide chain bonds. Tissues that contain collagen are subjected to mild degradative processes, i.e., treatment using alkali or acid followed or accompanied by heating in the presence of water, the systematic fibrous structure of collagen is broken down irreversibly and gelatin is obtained. It is the only protein based food material that gels and melts reversibly below the human body temperature (37°C). Gelatin possesses unique and outstanding functional properties and can be obtained in reasonable cost, make it one of the most widely used food and pharmaceutical ingredient.

Fish skins and bones can be utilized to produce gelatin, thus contributing to solve the problems of waste disposal with the advantage of value addition. The main drawback of the fish gelatins are the gels based on them tend to be less stable and have inferior rheological properties compared to mammalian gelatins. It may be noted that fish gelatin has its own unique

, -flavor and off-odor than a commercial pork gelatin, which offer new opportunities to product developers.

***Fish enzymes***

Fish visceral waste can serve as a source of large amount of enzymes which have potential applications in different sector starting from laundry application to pharmaceutical applications (Simpson and Haard, 1987). The nature of fish visceral enzymes is different from the enzymes found in the digestive system of terrestrial animals. Hence, they can be exploited for certain distinct applications. Fish pepsins can act even at low temperature and higher pH optimum than the pepsins from terrestrial source. Moreover, fish pepsins do not undergo autolysis at low pH (Raa, 1990). The differences in the properties of pepsins from fish and other sources could be attributed to the difference in the sequence and composition of aminoacids (Gildberg and 0verbj, 1990). Fish enzymes can be used as processing aids in the following applications

- Protein hydrolysates production
- In production of caviar from a variety of fish species
- for removal of squid skin
- for cleaning of scallop
- for descaling of fish
- coagulation of milk
- Cheese production

**Hemoproteins**

Hemoproteins are complex proteins, composed of a protein molecule and a non-protein compound (prosthetic group). Hemoglobin and myoglobin belongs to the category of hemoproteins involves in transport of oxygen in the blood and tissues of animals, respectively. The heme portion can be recovered from blood as well as muscles discards. The recovered material may be used iron supplement or as a chemical substrate for production of the cooked cured-meat pigment. During the production of hydrolyzates from meat, hemin could be recovered as by-product.

**Carotenoproteins**

Carotenoproteins and carotenoids are other classes of compounds found in the flesh and skin of fishes and in the exoskeleton of shellfish. They are not synthesized in their body. They are acquired through their food chain (Haard, 1992). Similar to hemoproteins, Carotenoids are also composed of a protein moiety and a non-protein prosthetic group. Isolation of carotenoproteins and carotenoids from shellfish processing discards has been reported (Long and Haard, 1988). Inclusion of caratenoids pigments in feed formulations of some of the aquacultured fishes and ornamental fishes shows the importance of these compounds in industrial applications (Shahidi et al., 1993).

**Protein derivatives from secondary raw material**

**Fish protein hydrolysates (Bioactive peptides)**

Apart from being highly nutritious, fish muscle proteins can be made use for preparing fish protein hydrolysates which comprises of bioactive peptides with valuable nutraceutical and pharmaceutical potentials. Fish protein hydrolysates (FPH) are the mixture of amino acids and peptides obtained by digesting proteins from fish meat or fish processing waste with proteases.The size of these peptides may range from 2 to 20 amino acid residues with the molecular masses of $<6000$ Da and are highly bioactive. The food derived peptides can be used as functional food ingredients or as nutraceuticals to benefit the human health and prevent disease. In this context, large pharmaceutical companies are more interested to invest in bioactive peptide research to open therapeutic prospects.

**Application of fish protein hydrolysates**

**Nutritional application**

The proximate composition of fish protein hydrolysate would vary with the raw material (head, bone, skin, viscera), type of process, type of drying, extent of hydrolysis and any other pre-treatment of raw material. The chemical composition of food materials has an important role on human health in supply of essential nutrients for maintaining prosperous health. Chemical composition of fish protein hydrolysates is important in nutrition perspective of human health.

Table 6. Proximate composition of fish protein hydrolysate

| Waste Parts | Protein (%) |
|---|---|
| Moisture | < 10 % |
| Protein | 60-90 % |
| Fat | <5 % |
| Ash | 0.45-27% |

**(Source; Chalamaiah et al., 2010)**

Amino acid composition of protein hydrolysates from different raw material produced using different enzyme source under different hydrolysis conditions expected to have variation. In general, required essential amino acids are abundant in FPH with richness in glutamic and aspartic acid content. FPH do also have non-essential amino acids. Presence of aromatic amino acid in fish frame protein hydrolysates has been reported. Studies have clearly shown that FPH from fish meat/fish waste could be an ideal source of essential amino acids (Chalamaiah et al., 2010).

**Nutraceutical applications**

There are fish protein hydrolysate products/peptides specifically marketed as health supplements in developed countries. These products are proven to have specific health role other than the nutritional benefit. Protein hyd5rolysates or peptides present in the hydrolysate have demonstrated to have antioxidant, anti-obesity, immune modulation, anti-coagulation, anti-microbial, anticancer and antihypertension etc. (Elavarasan et al., 2014; and Elavarasan et al., 2016).

**Table 7. Commercially marketed fish protein hdyrolysate products as Nutraceuticals**

| Product brand name | Particulars | Nutraceutical applications | Country |
|---|---|---|---|
| PROTIZEN® | Produced by enzymatic hydrolysis of white fish proteins | I “ ” supplement to fight against stress and its symptoms (weight disorders<comma> work pressure<comma> sleep troubles<comma> concentration difficulties and mood troubles). | UK |
| Amizate® | Produced from Atlantic salmon fish proteins by autolysis | ( ’ muscle anabolism and metabolic recovery). | North America |
| Nutripeptin® | Manufactured by enzymatic hydrolysis of Cod fish fillet/muscle protein | It helps in the blood glucose stabilization<comma> and weight management. | UK and USA |
| Seacure® | Prepared by hydrolyzing deep ocean white fish proteins | Dietary supplement helps to support the cells in the gastrointestinal tract and regulate bowel functions. | US and Canada |
| Vasotensin® | Produced from Bonito (*Sarda orientalis*) by thermolysin hydrolysis | It supports healthy vascular function for optimal blood flow and healthy blood pressure levels. | US and Japan |
| LIQUAMEN® | Prepared from *Molva molva* by autolysis | Dietary supplement that helps in reducing oxidative stress<comma> lowering glycemic index and anti-stress. | UK |
| Stabilium® 200 | Prepared from *Molva dypterygia* by autolysis | ’ stress and provides nutritional support for memory and cognitive function. | UK |
| PEPTACE® | Produced from Bonito (*Sarda orientalis*) by thermolysin hydrolysis | It lowers the blood pressure by inhibiting ACE enzyme. | US and Japan |
| SEAGEST® | Prepared by hydrolyzing deep | It supports the structure of the intestinal lining and promotes intestinal health. | US |

| | | | |
|---|---|---|---|
| | ocean white fish proteins | | |
| MOLVAL® | Produced from North Atlantic fish *Molva molva* by enzymatic hydrolysis | Dietary supplement recommended for cholesterol equilibrium<comma> stress control and promotes good cardiovascular health. | UK |

**(Source: Chalamaiah et al., 2010)**

**Fish protein hydrolysate as a functional ingredient**

Fish protein hydrolysates are soluble in wide range of pH which is an ideal characteristic helps to use in wide range of products. Protein hydrolysates have improved water holding, oil binding, emulsifying and foaming properties. However, the key factor which determine the functional properties is degree of hydrolysis. In general, extensive hydrolysis leads to loss of functionality. There is a critical degree of hydrolysis at which protein hydrolysates should be prepared with reference to particular function to be used as a functional ingredient (Elavarasan et al., 2016; Gajanan et al., 2017).

**Fish protein hydrolysate as a feed ingredient and other applications**

Fish protein hydrolysates (FPHs) have been used in aquaculture feeds in order to enhance the growth and survival of fish. Studies have shown that FPH has boosted the growth performance and immunological status of many culture species. The amino acid composition and the peptides present in hydrolysate are responsible for the improved growth and immunological status. FPH is also being used as a source of protein in poultry feed formulation and in pet animal foods. Other applications include FPH as a plant booster, ingredient in microbiological media and as a cryo-protectant in fish mince/surimi.

***Collagen peptide/gelatin hydrolysate***

‘ ’, ‘ ’

‘ ’ are high molecular weight proteins of approximately 300 kDa, it is difficult for digestion and hence becomes unavailable to human body for their 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. The hydrolysis process leads to formation of fragmenting from the collagen of about 300 kDa to small peptides having an average molecular weight of less than 5 kDa. The visible consequence of this hydrolytic transformation is the complete dissolution of resultant peptide mixture in cold water, which further widens the application prospects of collagen peptide.

Small peptides are desirable for nutraceutical and pharmaceutical applications, whereas large peptides are desirable for the functional modification of food products. Standardisation of collagen production technology is a stepping stone in the nutraceutical and health food industry. From a nutritional perspective, peptides are more bioavailable than proteins or free amino acids and at the same time, less allergenic than their native proteins (Otani et al., 1990). Apart from that collagen peptides are shown to promote the absorption of vitamins and minerals. Hence, recently combined formulations of collagen peptide with minerals and vitamins are coming up in the market. Apart from their nutritional benefits, bioactive collagen peptides possess a wide range of physiological functions including antihypertensive, antioxidative, anticancer, immunomodulatory, antimicrobial, mineral binding, antithrombotic and hypocholesterolemic effects (Gomez-Guillen et al., 2011). Enzymatically hydrolyzed collagen have shown better biological activities compared to the peptides derived from fish muscle protein with antioxidants and antihypertensive agents.

**Table 8. The protein derivatives from secondary raw material and the possible industrial products**

| **Protein derivatives from secondary raw material** | **Protein derivatives based industrial products from secondary raw material** |
|---|---|
| • Fish protein hydrolysate<br>• Collagen peptides<br>• Gelatin hydrolysate | • Fish silage<br>• Flavorings<br>• Collagen peptides<br>• Gelatin hydrolysate<br>• Fish protein hydrolysate<br>• Shrimp protein hydrolysate<br>• Fish waste paste<br>• Cuttlefish and squid by-products paste |

## Conclusion

Globally, the aquatic food waste (secondary raw material) has been identified as source of high value functional ingredients. On the other hand current exploitation of aquatic food waste is happening as high volume low value products for example fish silage, fish meal, squid meal, shrimp head meal etc. The major high value protein based product from fish waste is collagen and its derivatives. The way the fish waste utilized in India needs a rattled shift in order to realize the full potential of seafood processing waste generated in India.

## References


1. Rustad T. Physical and chemical properties of protein seafood by-products. Maximising the value of marine by-products. 2007:3-21.
2. Ammu, K., Jose Stephen, and K. Devadasan. "Influence of dietary proteins on cholesterol levels in albino rats." (1989). Fishery Technology 26 (2):125-130
3. Vikøren, L. A., Nygard, O. K., Lied, E., Rostrup, E., Gudbrandsen, O. A. 2013. A randomised study on the effects of fish protein supplement on glucose tolerance, lipids and body composition in overweight adults. British Journal of Nutrition, 109: 648–657.
4. Tastesen, H. S., Keenan, A. H., Madsen, L., Kristiansen, K., & Liaset, B. (2014). Scallop protein with endogenous high taurine and glycine content prevents high-fat, high-sucrose-induced obesity and improves plasma lipid profile in male C57BL/6J mice. *Amino Acids*, *46*(7), 1659–1671.
5. Burghagen. 1999. Collagen. H.D. Belitz, W. Grosch (Eds.), Food chemistry (2nd ed.), Springer, Berlin, pp. 540–547.
6. Simpson, B. K., and Haard, N. F. 1987. "Cold-adapted Enzymes from Fish." Pp. 495-527. **In** *Food Biotechnology,* edited by D. Knorr, New York: Marcel Dekker.
7. RAA, j. 1990. "Biotechnology in Aquaculture and the Fish Processing Industry: A Success Story in Norway." Pp. 509-524. In *Advances in Fisheries Technology and Biotechnology for Increased Profitability,* edited by M. N. Voigt and j. R. Botta. Lancaster, PA: Technomic Publishing Co.
8. Gildberg, A.R. and Overbø, K., 1990. Purification and characterization of pancreatic elastase from Atlantic cod (Gadus morhua). *Comparative biochemistry and physiology. B, Comparative biochemistry*, *97*(4), pp.775-782.

9. Haard, N.F., 1992. Biochemistry and chemistry of color and color change in seafoods. *Advances in seafood biochemistry*, pp.305-360.
10. Long, A. and Haard, N.F., 1988. The effect of carotenoid-protein association on pigmentation and growth rates of rainbow trout (Salmo gairdneri). In *Proceedings of the Aquaculture International Congress* (pp. 99-101).
11. Shahidi, F., Synowiecki, J. and Penney, R.W., 1993. Pigmentation of Artic char (Salvelinus alpinus) by dietary carotenoids. *Journal of Aquatic Food Product Technology*, *2*(1), pp.99-115.
12. Chalamaiah M, Hemalatha R, Jyothirmayi T. Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. Food Chemistry. 2012 Dec 15;135(4):3020-38.
13. Elavarasan K, Naveen Kumar V, Shamasundar BA. Antioxidant and functional properties of fish protein hydrolysates from fresh water carp (Catla catla) as influenced by the nature of enzyme. Journal of Food Processing and Preservation. 2014 Jun 1;38(3):1207-14.
14. Elavarasan K, Shamasundar BA, Badii F, Howell N. Angiotensin I-converting enzyme (ACE) inhibitory activity and structural properties of oven-and freeze-dried protein hydrolysate from fresh water fish (Cirrhinus mrigala). Food chemistry. 2016 Sep 1; 206:210-6.
15. Gajanan PG, Elavarasan K, Shamasundar BA. Bioactive and functional properties of protein hydrolysates from fish frame processing waste using plant proteases. Environmental Science and Pollution Research. 2016 Dec 1;23(24):24901-11.
16. Otani H., Dong X.Y. and Hosono A. 1990. Preparation of low immunogenic peptide fragments from milk casein. *Milchwissenschaft*, *45*:217-220.
17. Gòmez-Guillèn MC., Turnay J., Fernández-Diaz MD., Ulmo N., Lizarbe M.A. and Montero P. 2002. Structural and physical properties of gelatin extracted from different marine species: a comparative study. *Food Hydrocolloids*, 16:25–34.

……………………..