

## **Health benefits and potential applications of Fish Protein hydrolysate**

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Fish protein hydrolysate (FPH) is a fishery product containing mixture of amino acids and peptides of various molecular size range. FPH is one of the most researched fishery product of the last decade. Edible portion or processing discards from finfish or shellfish with considerable quantity of protein can be used as a raw material for FPH preparation. The process involves acid/alkali hydrolysis or enzymatic hydrolysis. Enzymatic hydrolysis has the advantages of control over the process and the properties of the end product i.e FPH. Proteases from different sources like plant, animal, bacteria and fungi have been extensively researched for their uses in FPH production. FPH find its application in food, cosmetics, pharmaceutical, nutraceutical and functional food industry. Indian seafood industry generates a huge quantity of waste which are rich in protein can be effectively converted in to fish protein hydrolysate by an integrated approach for the higher return of seafood processor. On the other hand, this approach would help to alleviate the pollution issues created by seafood processing discards and also contribute to the improved utilization of available resources.

### **Waste generation in Indian Aquatic Food Industry**

Fish processing operations generate more than 60 % of the raw material. In developing country like India, these wastes are disposed or converted into animal feed, fish meal and fertilizer. This practice leads to underutilization of raw material and may affect the sustainable utilization of available resources. The disposal of fish processing waste is under strict regulations due to environmental issues and it adds to the operational cost of seafood industry (Elavarasan et al., 2016). Hence, the effective utilization of fish processing waste is gaining importance. There is no authenticated data on by-product generation from Indian fish processing sector. The amount of waste generated will vary with the size, style of product and species, nature of handling (machine/manual handling, skill of operating/handling person). The major wastes from crustaceans are shell waste which is utilized to some extent as a raw material in chitin industry. Wastes from fin fishes are containing considerable quantity of proteins which can be converted/recovered in to protein hydrolysates for improved utilization. From fish protein hydrolysate industry point of view, quantity of fin fish wastes / by-products is more important. Waste generation from major type of seafood products exported and the nature of waste generated is presented in Table 2.

## Global protein market

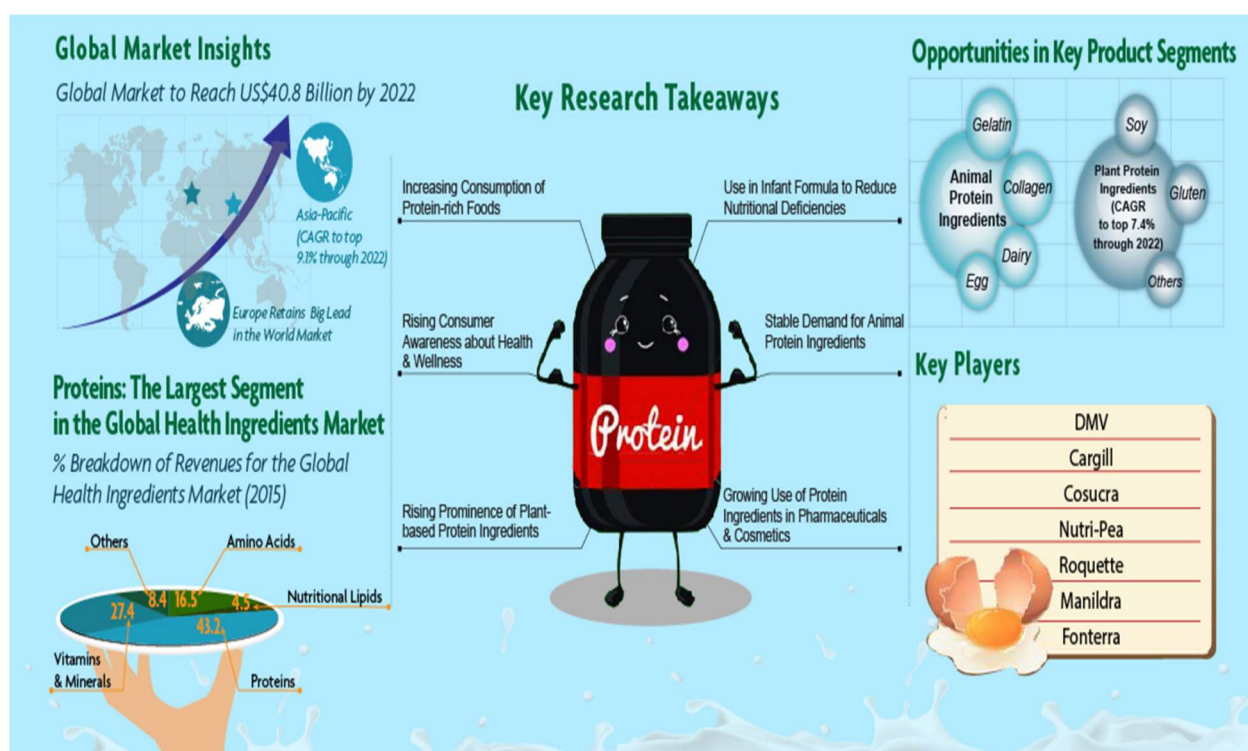
Global protein ingredient market analysis revealed that 43.2 % of revenues in the global health ingredients market contributed from protein (Fig. 1). Europe retains its big lead in the global protein market. Key products under animal protein ingredients include, gelatin, collagen, egg and dairy. There is a stable demand for animal protein ingredients. The global protein market is expected to reach the value of US \$ 40.88 Billion (2.7843618 trillion Indian rupees) by 2022. The use of protein ingredients in infant formula reduced protein deficiencies. The use of protein ingredients in pharmaceutical and cosmetic industry is increasing

**Table 2. Seafood products in the export market from India and their respective waste**

Major Products for Export	Major Waste	Major Compounds
Shrimp based products	Carapace (head) Telson (tail) Rostrum Antennae Appendages Eggs Cook juice	Chitin <b>Protein</b> Pigments
Frozen Squid (Whole Cleaned, Fillet, Rings)	head behind the tentacles Visceral mass Beak Ink sac and ink Squid pen Skin membrane	<b>Protein</b> Enzymes Melanin Chitin
Cuttlefish (Whole cleaned, deskinned)	head behind the tentacles Visceral mass hard Beak Ink sac and ink Cuttle bone Skin membrane	<b>Protein</b> Enzymes Melanin Chitin
Lobster Meat	Lobster shell Appendages	Chitin
Pasteurized crab meat	Crab shell	Chitin Pigments
Fish products (fillet, surimi)	Head Frame/bone Skin Scales Gills	<b>Proteins</b> Lipids Enzymes Minerals

	Fins Visceral mass Wash water	
Frozen clam/Mussels	Shells Shuck water	Calcium oxide <b>Protein</b>

continuously. Global protein ingredient market share is moderately consolidated with DuPont, Bunge, ADM, Cargill, and Mead Johnson being the major industry players. Raw material availability in China and India influence industry players to shift manufacturing base in the region. The protein supplements market in India is growing at 6%, and is currently valued at Rs. 252 crores annually.



**Fig. 1 Global protein ingredient market** (Source: Global Industry Analysts. Inc, accessed on 21-January 2017, URL: <http://www.strategyr.com/MCP-1233.asp>)

### Fish protein hydrolysate

Fish protein hydrolysate is a product prepared from proteins sourced from fish meat/fish processing by products via enzymatic or chemical process. Enzymatically produced hydrolysates are widely accepted which contain mixture of peptides of varying sizes and free amino acids.

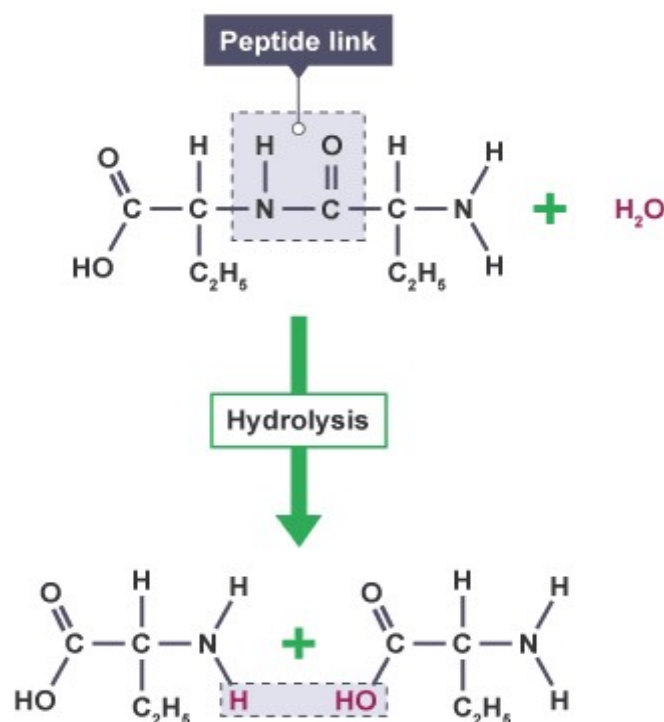
### Process for production of protein hydrolysate

Protein hydrolysates from fish processing discards can be prepared using four different process namely acid process, alkali process, enzymatic process and microbial fermentation.

The basic mechanism, effect of different factors and advantages and disadvantages of each methodology is discussed below.

### **Acid hydrolysis process**

Hydrolysis (from Greek *hydro-*, meaning "water", and *lysis*, meaning "to unbind") usually means the cleavage of chemical bonds by the addition of water. This is opposite to condensation reaction in which two molecules join together into a larger one and eject a water molecule. Thus hydrolysis adds water to break down, whereas condensation builds up by removing water. When the nucleus loving agent attack the carbon present in the carbonyl group of the peptide (amide bond) bond, the hydrolysis takes place. In an aqueous base, hydroxyl ions are more nucleophilic than polar agents such as water molecules. In acids, the carbonyl group becomes protonated, and this leads to a much easier nucleophilic attack. Acid hydrolysis of proteins used more commonly than alkali hydrolysis. The hydrolysis process improves the nutritive value and functional properties of food proteins. Acid hydrolysis process is a harsh and quite hard to control. Extensively acid hydrolysed proteins used as flavor and taste enhancers in processed meat, crackers, and soup mixes.



**Fig. 2 Hydrolysis of peptide bond**

Complete hydrolysis of fish protein using hydrochloric acid, or in some cases sulfuric acids have been achieved at high temperature, and often high pressure. After hydrolysis, the solution is neutralized to pH 6.0 to 7.0, concentrated and further dried. The end product (hydrolysate) is highly soluble. Total hydrolysis of fish protein substrate can be achieved in 18 h at 118°C in 6 M HCl. The major disadvantages are high salt formation due to neutralization which in-turn affect the palatability and the functionality and another drawback is the destruction of tryptophan, which is an essential amino acid. The acid

hydrolysis is also widely utilized to convert underutilized and secondary raw material from fish into fertilizer due to the low production cost and resulting extensive hydrolysis.

### ***Alkali hydrolysis process***

In alkaline hydrolysis process of fish protein, large water-soluble polypeptides are rapidly cleaved and further degradation in to simple compounds at a slower rate. For alkali hydrolysis process, sodium hydroxide is primarily used. Limited alkali treatment is employed in the food industry to recover and solubilize wide range of proteins as the extensive alkali hydrolysis impair the functionality and nutritive value. The small-scale batch process developed to hydrolyze the fish protein concentrates for improving the functionality has employed a high pH of 12.5 at 95°C for 20 min. racemization of amino acids, loss of cysteine and formation of unusual amino acids are the notable deleterious effects reported to occur during alkaline treatment of protein. The products formed may also inhibit the hydrolysis reaction and slow down the process.

### ***Enzymatic process***

Enzymatic hydrolysis of fishery by products use either autolysis process or by adding exogenous protein. Autolysis process involves incubating ground fishery waste at optimum reaction conditions of endogenous enzymes and also uses the fish visceral waste (Kristinsson and Resco, 2000). The endogenous enzymes trigger the breaking down of biomolecules to smaller peptides through autolysis process. The autolysis is usually conducted at neutral or slightly alkaline pH, exploiting the presence of serine protease of intestine in alkaline or the carboxyl pro-tease of gastric juice in acidic pH.

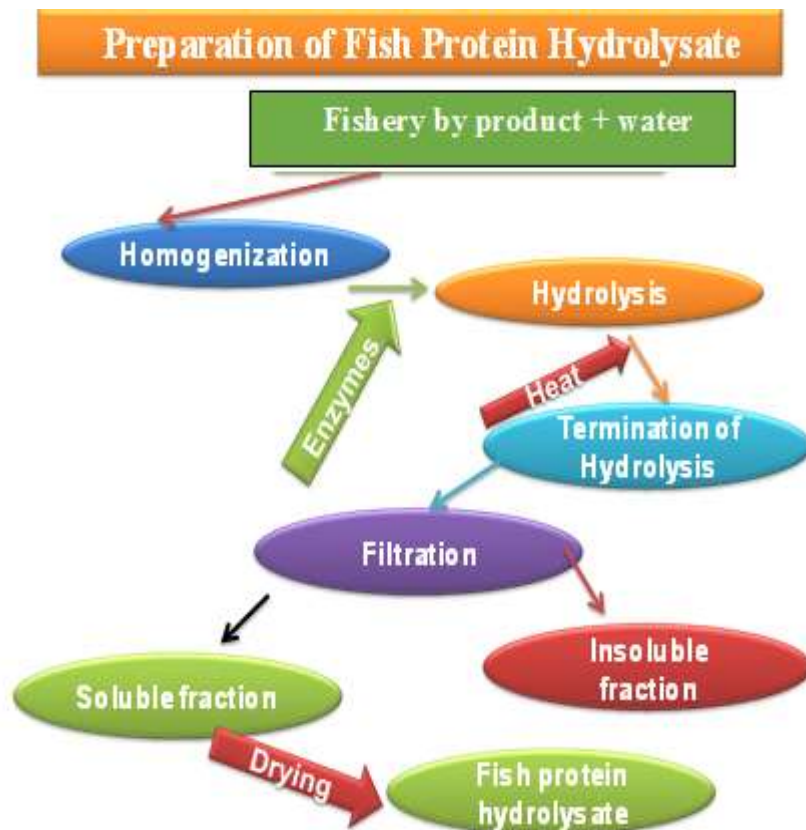
Proteolysis is the enzymatic hydrolysis of the amide bond in peptides and proteins. The enzymes are exploited to perform desired functions in processing and analysis and to facilitate the conversions of raw materials into high quality more desirable foodstuffs. Enzymes used in the food industry and research are predominantly hydrolases. Proteolytic enzymes are economically the most important group of enzymes and their use is well established in the food industry. Use of proteases in the preparation of fish protein hydrolysates has received a wide attention among the researchers as it is more economical and ease of process control. The nature of enzymes, substrate and hydrolysis will determine the properties of (Fish Protein Hydrolysate) FPH. The general flow line for the production of FPH by use of enzymes is depicted in Fig 1. The process involves homogenization of fish meat or fish waste with addition of water. The homogenate is brought to the optimum temperature and pH. The hydrolysis is initiated by the addition of enzyme at desired concentration. After a particular duration of incubation, the hydrolysis is terminated by applying heat or by adjusting the pH. The soluble fraction after removing the unhydrolysed portion is concentrated by freeze drying / oven drying / spray drying. The dried protein powder is referred as protein hydrolysate.

1. In order to produce the FPH with different and desired properties, it is important to know the mechanism of protein hydrolysis. Some proteases preferentially catalyze the hydrolysis of bonds adjacent to a particular amino acid residue, while some are less specific. The catalysis by proteases occurs primarily as three consecutive reactions (Krsitinsson and Rasco, 2000): the formation of complex between the original peptide chain and the enzyme referred as the Michaelis complex
2. cleavage of the peptide bond to liberate one of the two peptides
3. nucleophilic attack on the remains of the complex to split off the other peptide and to reconstitute the free enzyme

The hydrolysis of peptide bonds leads to an increase in the numbers of ionizable groups ( $\text{NH}_3^+$  and  $\text{COO}^-$ ), with a concomitant increase in hydrophobicity and net charge, decrease in molecular size of the polypeptide chain, and an alteration of the molecular structure leading to the exposure of the buried hydrophobic residues to the aqueous environment. Upon addition of enzyme to the proteins, the enzyme-substrate complex will be formed. This complex is referred as Michaelis complex which may dissociate back to reactant substrate and free enzyme, or to free enzyme and product molecules (Adler-Nissen, 1986). The generally accepted mechanism for proteases indicates that the second step that is dissociation of enzyme substrate complex into free enzyme and product is the rate-determining step, which determines the overall rate of reaction.

Enzymatic hydrolysis of proteins is a complex process because of several peptide bonds and their specific accessibility to enzymatic reactions. The specificity of enzymes is not the only factor that affects the peptide profile of the final product and factors such as temperature and pH play an important role. The temperature and pH can greatly affect the enzyme reaction kinetics and their effect is different for each enzyme. Generally, there is an optimum combination of both pH and temperature, where the enzyme is most active. Temperature and pH extremes deactivate the enzymes by denaturing them.

The factors involved in hydrolysis of proteins are most important both in terms of kinetics and nature of the end product. The most important factors influencing the properties of FPH are nature of substrate, nature of protease and degree of hydrolysis (DH) and drying method.



**Fig. 3 General scheme used for enzymatic hydrolysis of fishery by-product**

### **Fermentation process**

Although several protein hydrolysates have been produced from fishery by-products sources using exogenous proteases, few studies have been conducted on the production of protein hydrolysate using microbial fermentation. Fermentative hydrolysis of fishery by-products for the production of bioactive peptides is an emerging process that provides a natural technology. It uses natural or added microorganisms (starter cultures). During the growth of the organism, they hydrolyze the nutrients particularly carbohydrate and proteins. The extracellular microbial proteases hydrolyze the proteins into peptides of varied molecular size. Depend upon the microorganism species used for fermentation, the property and quality of protein hydrolysate differs.

**Table 4: Comparison of different processes used for protein hydrolysate production**

Processing method	Advantages	Disadvantages
Chemical process (acid and alkali)	High protein recovery Short processing time Low processing cost	Absence of homogeneous hydrolysates Bitterness Poor functionalities High salt content Metal corrosion of equipment Reaction is hard to control Form toxic substances like lysino-alanine Form D-amino acids, which are not absorbed by humans
Enzymatic process	Less bitterness of final hydrolysates Maintain functions and nutritive value of final hydrolysates Low salt content in final products Produce homogeneous hydrolysates	High processing cost Long processing time

### Protein hydrolysates from different fish processing –by products

Different wastes generated during fish processing like head, skin, roe, frame waste and bone have been used to produce the hydrolysate. Alternatively, the proteins isolated from the waste parts can also be used for this purpose. The protein content in different fish waste parts are presented in Table 5. Most of the studies have been carried out with reference to the hydrolysis process and their bioactive and functional properties.

**Table 5. 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



Scientific studies have been reported for the preparation of fish protein hydrolysate from fish head, viscera, roe, skin, frame and bone. Most of these studies have focused on their antioxidant properties and various antioxidant peptide molecules have been isolated and characterized. Chalamiah et al. (2012) has exhaustively reviewed the protein hydrolysates from various parts of fish waste. Fish head is a major fishery waste contains gills, Eyes, head frame and shoulder muscle. It is difficult to recover the protein due to its structural complexity. Enzymatic process will solubilize the protein by converting into peptide forms then facilitate easy recovery of proteins. Protein hydrolysates from fish head by-product waste have been prepared from various species. The major protein present in fish head is collagen. Hence the peptides generated will have usually the sequence from collagen which are known for their anti-arthritis and anti-obesity properties. Fish skin is again a rich source of collagen. Attempts have been made to produce the hydrolysate either directly from the fish skin or after isolating the collagen or gelatin. Fish liver is an another by product usually goes for oil and meal production. Tuna liver has been used to prepare the hydrolysate using protamex, flavorzyme, alcalase and neutrase. Fish viscera is also a potential source of protein that can serve as a raw material for the preparation of protein hydrolysates. It is expected that visceral waste protein hydrolysate may exhibit unique properties. In recent times, many attempts have been performed for the utilization of fish visceral waste for protein hydrolysates production (Batista et al., 2010). Fish roe contains considerable amount of protein. In order to utilize this underutilized protein source from fish roe, protein hydrolysates have been prepared. For example, roe protein hydrolysate from *Cirrhinus mrigala* using alcalase and papain has been reported. Fish bone, which is separated after removal of muscle proteins on the frame, is another valuable source in identifying health-promoting components. The organic component of fish bone, which accounts for 30% of the material, is made out of collagen. Therefore, fish bone is considered as a source for protein hydrolysate particularly collagen peptides and gelatin hydrolysates.

## **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.

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 have been reported. Studies have clearly shown that FPH from fish meat/fish waste could be an ideal source of essential amino acids.

**Table 6. Proximate composition of fish protein hydrolysate**

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

### ***Nutraceutical applications***

There are fish protein hydrolysate products/peptides specifically marketed as health supplements in developed countries (Table 7). These products are proven to have specific health role other than the nutritional benefit. Protein hydrolysates 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; Elavarasan et al., 2016).

### ***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).

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

<b>Product brand name</b>	<b>Particulars</b>	<b>Nutraceutical applications</b>	<b>Country</b>
PROTIZEN®	Produced by enzymatic hydrolysis of white fish proteins	It is “mood food” and dietary supplement to fight against stress and its symptoms (weight disorders<comma> work pressure<comma> sleep troubles<comma> concentration difficulties and mood troubles).	UK

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Amizate®	Produced from Atlantic salmon fish proteins by autolysis	Sports nutrition (supports the body's 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 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 ( <i>Sarda orientalis</i> ) by thermolysin hydrolysis	It supports healthy vascular function for optimal blood flow and healthy blood pressure levels.	US and Japan
LIQUAMEN®	Prepared from <i>Molva molva</i> by autolysis	Dietary supplement that helps in reducing oxidative stress and lowering glycemic index and anti-stress.	UK
Stabilium® 200	Prepared from <i>Molva dypterygia</i> by autolysis	Supports the body's response to stress and provides nutritional support for memory and cognitive function.	UK
PEPTACE®	Produced from Bonito ( <i>Sarda orientalis</i> ) by thermolysin hydrolysis	It lowers the blood pressure by inhibiting ACE enzyme.	US and Japan
SEAGEST®	Prepared by hydrolyzing deep ocean white fish proteins	It supports the structure of the intestinal lining and promotes intestinal health.	US
MOLVAL®	Produced from North Atlantic fish <i>Molva molva</i> by	Dietary supplement recommended for cholesterol equilibrium and stress	UK

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enzymatic  
hydrolysis

control and promotes good  
cardiovascular health.

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### ***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.

### **Safety of protein hydrolysates in human nutrition**

In general, food business operator should ensure the safety of products. The safety aspects of any food ingredient need to be documented before release in the market. Protein hydrolysates can be considered as safe when they are hydrolysed from proteins having a history of safe for consumption and they are produced using proteases which are of food-grade and used common food-processing methods. The safety of fractions and bioactive peptides, derived from safe hydrolysates, should be evaluated by the manufacture before market introduction. A review of the safety assessment of the company by an external independent committee and subsequent approval by the competent authorities according to novel food procedures is essential when the source of protein and process is novel and under unusual high intake of amino acids. The decision tree to be followed before marketing the product is presented in Fig. 6.

### **Conclusion**

The fish processing industry in India generate huge protein rich material which is untapped and can be utilized by converting in to protein hydrolysate. Depends on the properties and chemical composition, further FPH finds application in various industries ranging from nutraceutical to plant growth boosting ingredient. Recent interest of FPH as nutraceutical compound/bioactive peptide demands hygienic handling and proper preservation of fish processing waste. However, safety of FPH when produced from fishery waste, economic feasibility and business case are remains unaddressed worldwide.

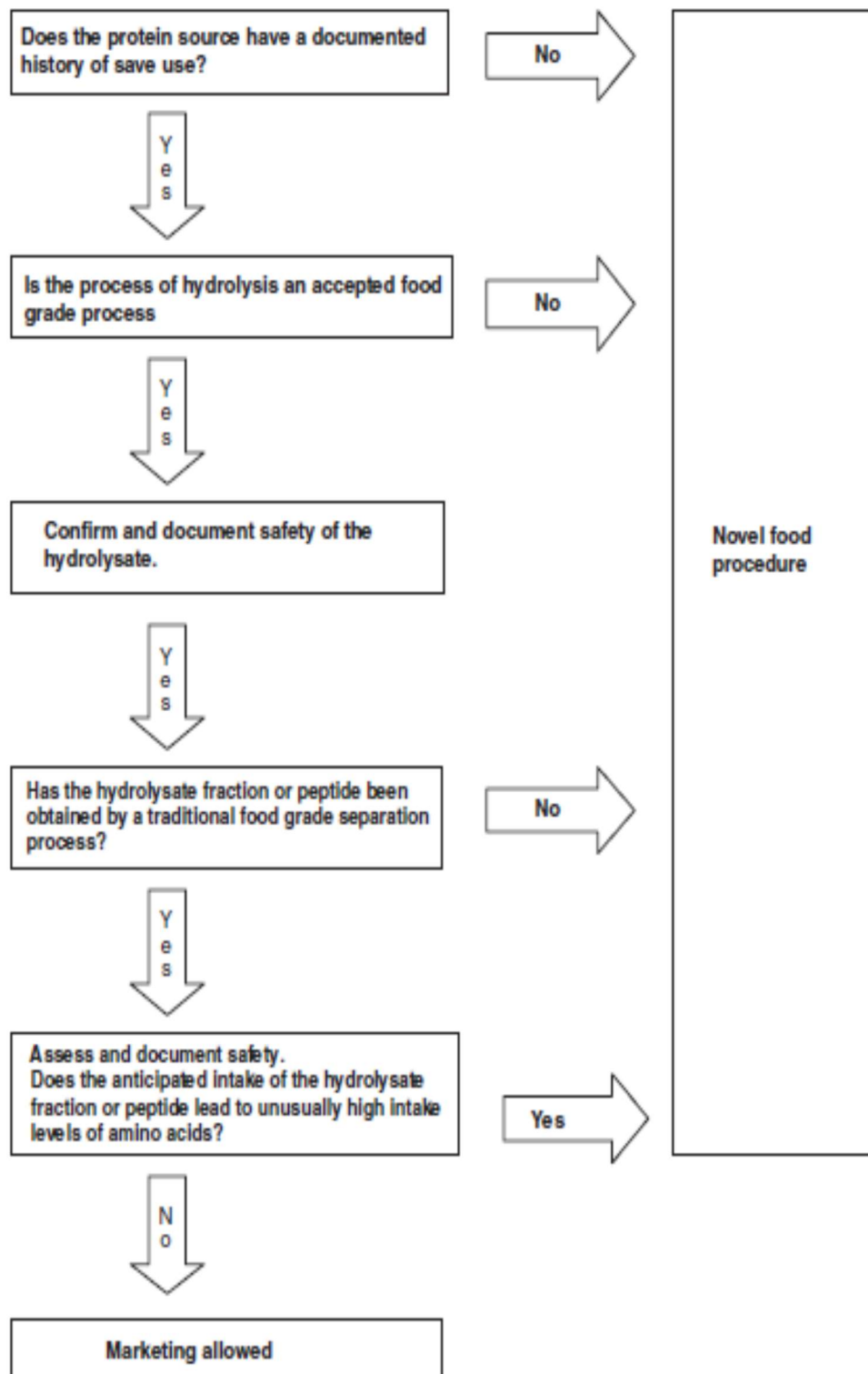


Fig. 6 Decision tree for protein hydrolysate product for marketing

### Suggested Readings

- FAO. 2016. The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome. 200 pp.
- Zynudheen, 2010. Utilisation of fish waste. In; Manual prepared for the Awareness-cum-Interactive workshop on 'Antibiotic, pesticide and insecticide residue contamination in Sea/Aqua foods' organized by the Visakhapatnam Research Centre of Central Institute of Fisheries Technology on 6<sup>th</sup> September, 2010 at Visakhapatnam, 96-109. URL: <http://hdl.handle.net/123456789/1146>
- Kristinsson HG, Rasco BA. Fish protein hydrolysates: production, biochemical, and functional properties. Critical reviews in food science and nutrition. 2000 Jan 1;40(1):43-81.
- Adler-Nissen, J., 1986. Enzymic Hydrolysis of Food Proteins, Elsevier Applied Science Publishers, Barking, UK.
- 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.
- Batista I, Ramos C, Coutinho J, Bandarra NM, Nunes ML. Characterization of protein hydrolysates and lipids obtained from black scabbardfish (*Aphanopus carbo*) by-products and antioxidative activity of the hydrolysates produced. Process Biochemistry. 2010 Jan 31;45(1):18-24.
- 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.
- 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.
- 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.

