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Review Article

Functional Proteins from Seafood Processing Discards and their Applications

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Abstract

Commercial processing of seafood results in enormous amounts of solid discards, offal or by-products. These discards, on dry weight basis, contain up to 50% proteins, consisting mostly of myofibrillar proteins, collagen, enzymes, and soluble nitrogenous compounds. This article briefly discusses techniques for isolation of these proteins, which include mechanical deboning of fish frames, iso-electric pH solubilization precipitation of proteins, solubilization based on weak acid-induced gelation, protein dispersions and treatment by proteolytic enzymes. The article further briefly points out nutritional and functional properties of the isolated proteins as well as their co-products such as enzymes, protein hydrolyzates and peptides. Potentials of these products in food and nutraceutical applications are also briefly pointed out. Efforts in this direction can facilitate total utilization of seafood production and also can greatly alleviate seafood-borne environmental hazards.

Keywords: *Functional Proteins; Seafood; Protein Dispersions*

Introduction

Proteins are essential for: healthy life, which are required for various functions in the human body such as muscle growth, weight management, support of immune system among others. A person needs to consume **0.8 grams** of protein per kilogram of body weight. The U.S. 2015 - 2020 dietary guidelines recommend consumption of 46g and 54g proteins by adult female and male, respectively [1]. Awareness on the importance of proteins in health management has encouraged food and pharmaceutical industries to develop novel and alternate sources of proteins for uses as supplements and nutraceuticals [2]. Commercial processing of foods including of both agricultural and animal origin including seafood annually generates about 1.3 billion tons of wholesome and edible parts as waste, which are rich in proteins and also other nutrients [3]. The current annual production of seafood including aquaculture is about 170 million metric tonnes [4]. Centralized commercial seafood processing operations intended for international trade generate voluminous amounts of discards, which include heads, filleting frames, intestines, tails, skin, bone, shells and other items. Discards from finfish generally constitute 25 to 50% of the raw material, and comprises of entrails, heads, skeletal frames, skin, scales and viscera. Canning operations of fish such as tuna result in as much as 60% solid wastes consisting of muscle, dark flesh, head, bone, and skin. Processing discards of crustacean shellfish are composed of the cephalothorax, carapace, tail, and shell, which constitute as high as 50% of the shellfish. More than 50,000 metric tons of lobster processing wastes are generated annually, which account for 50 - 70% of the starting harvest. These seafood discards constitute proteins as high as 58% (and about 19% fat) on dry weight basis [5]. It has been estimated that annual seafood discards in the US represent loss of 208 billion g of proteins, which can meet the total yearly requirement of protein for 10.1 million men [6]. Besides, the discards are also rich in polyunsaturated fatty acids (PUFA), carotenoids, minerals, vitamins, squalene, glycosaminoglycans and others having various health benefits [7]. There is immense scope for isolation of these proteins and also other compounds for uses as functional ingredients, nutraceuticals, and pharmaceuticals for a wide range of commercial applications that can also reduce seafood associated environmental problems.

Proteins from seafood discards can be isolated as concentrates, hydrolysates and peptides for their various uses including as functional ingredients and nutraceuticals. Recent advances in biotechnology offer environmental friendly strategies for isolation of proteins and also other biomolecules from food processing discards. These processes include biotransformation of macromolecules using enzymes and microorganisms, followed by isolation of the products by techniques such as subcritical and supercritical extractions, ultrafiltration, microwave- and ultrasound-assisted recovery processes and membrane separation [8]. This article briefly discusses some recent methods for the isolation of proteins from seafood discards and their potential uses.

Methods to recover proteins from seafood processing discards

The most successful attempt in the recovery of functionally active fish proteins came with the development of surimi, a Japanese name for concentrate of stabilized fish myofibrillar proteins. In order to make surimi, fish or their filleting frames is initially subjected to mechanical deboning to remove skin and bone. The soluble components of the mince are then removed by repeated chilled water washing of the mince giving concentrate of myofibrillar proteins. Incorporation of cryoprotectants such as polyphosphate protects the surimi proteins against denaturation and associated loss of functional properties during frozen storage. Alaska Pollock, Pacific hake, Pacific cod, arrow tooth flounder, blue whiting, hoki and threadfin bream are some of the low-fat fish used for surimi production. Because of its excellent gel-forming abilities, surimi is ideal for fabrication of restructured food items such as imitation products, which resemble seafood such as crabmeat, shrimp, lobster, and scallop [9].

Isoelectric solubilization precipitation

Isoelectric solubilization precipitation (ISP), also known as the 'pH shift process', was developed by Hultin's group for the recovery of myofibrillar proteins as fish protein isolate (FPI) [10]. The process, as shown in figure 1, involves initial homogenization of underutilized fish or fish meat/processing discards with either dilute acid (pH 2.5 to 3.5) or alkali (pH 10.8 to 11.5). The treatment dissolves sarcoplasmic and myofibrillar proteins, while insoluble impurities such as bone, skin, oil and membranes are removed. Up to 90% of the dissolved proteins are precipitated by adjusting the pH of the solution to isoelectric pH (pH 5.2 to 6.0) of the proteins. The treatment helps simultaneous separation of inedible parts such as shells, membranes, bones, scales, and skin and also lipids [10]. The ISP process has been applied for protein recovery from several fish and shellfish sources and their processing discards. The finfish species include mackerel, catfish frames. rockfish, Pacific whiting, rainbow trout, Atlantic croaker, bullhead catfish, common carp, tilapia frames, monkfish, herring, salmon, cod and herring, big-eye snapper, bighead carp, silver carp, yellow fin tuna roe, lantern fish, and also several underutilized fish. Shellfish items include crab, mussel and krill and also their shell and head discards. For the ISP process, alkali treatment is generally preferable, which can yield up to 95% of proteins from the raw material. Typically, sodium hydroxide is used for alkali extraction. Replacing sodium hydroxide with calcium hydroxide helps recovery of proteins low-in sodium, but rich in calcium. Denaturation and enzymatic degradation of proteins are avoided when the pH-shift process is ideally performed at 10°C or below. The proteins are concentrated by centrifugation or filtration. Grossly, the integrity of myofibrillar proteins was maintained during the process [11].

Weak-acid induced solubilisation of fish proteins

Another method for recovery of proteins from fish meat makes use of their ability to undergo gelation under mild acidic conditions, when the affinity of myofibrillar proteins to water increases significantly. The process consists of washing of fish mince with chilled (0 - 5°C) water as in the case of surimi preparation. The washed mince is homogenized in equal amount of fresh chilled water. To the viscous

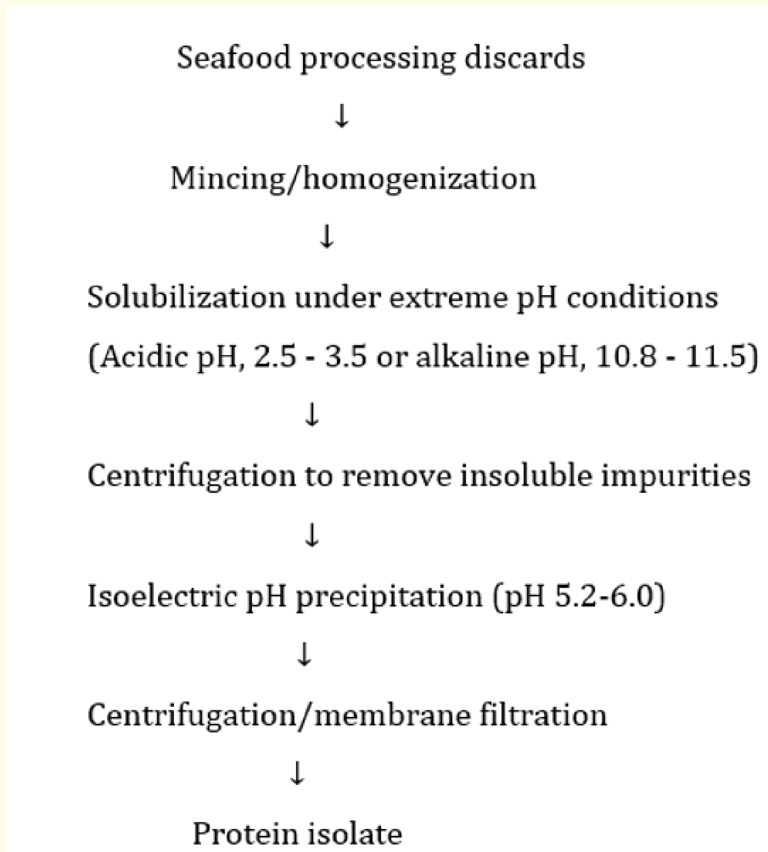

Figure 1: *Recovery of proteins from seafood discards by isoelectric solubilization precipitation.*

homogenate, weak acid, such as acetic acid, is added drop-wise while gently stirring to lower the pH to 3.5 to 4.0. Acidification induces gelation of the proteins associated with decrease in viscosity of the homogenate. During gelation the proteins in solution undergo partial unfolding, followed by irreversible aggregation of unfolded myosin to form a three-dimensional structure, incorporating water in the gel matrix. Gelation-associated drop in viscosity is enhanced when the acidified homogenate is warmed to about 45°C. The proteins are bound to water in the free-flowing dispersion, and are therefore highly stable against thermal precipitation, even at temperatures as high as 100°C. Making use of this phenomenon, thermo-stable dispersions have been prepared from fish species including Atlantic herring, Atlantic mackerel, threadfin bream and shark, among others. In the case of capelin, the proteins are totally solubilized after washing alone, without the need for reduction of pH by organic acids [12]. Shark muscle structural proteins behave differently in this respect. Lowering pH by weak acid of washed collagen-free shark meat homogenate in water results in gelation of the proteins, which is associated with significant rise in viscosity, resulting in a hard mass that can have valuable uses [13].

Enzymatic extraction of proteins from seafood discards

Proteolytic enzymes, under optimal treatment conditions, can extract proteins from seafood processing discards. The treatment results in recovery of proteins as hydrolyzates. Commercial proteolytic enzymes from various sources include those from microorganisms (such as alcalase, flavourzyme, protamex), animal (collagenase, proteinase, serine-protease, neutrase, trypsin), and plants (papain, bromelain, ficin). The ideal treatment conditions are: incubation temperature, 35 - 37°C; enzyme to substrate ratio, 1 to 50; and duration of incubation up to 24 hrs. The hydrolyzed protein fraction is recovered by filtration and concentrated generally by spray drying. The degree of hydrolysis determines functional properties of the hydrolyzate, such as solubility, water-holding capacity, emulsification and foam-forming abilities. Enzymatic digestion of proteins, either *in vitro* or in the human digestive system, leads to formation of peptides from proteins including those from marine sources. Bioactive peptides are specific protein fragments that have positive impact on consumer health. Such peptides have been produced from food proteins including those from seafood such as salmon, oyster, squids, sea urchin, shrimp, snow crab, oysters, mussels, clams, scallop, jellyfishes, prawns, sea cucumbers and sea squirts [14,15]. Another approach for protein recovery involves fermentation using lactic acid bacteria. The fermented liquor is rich in hydrolyzed proteins with a degree of hydrolysis, above 50% [16].

Isolations of collagen and gelatin

Collagen plays an important role in the maintenance of the structural integrity of cells. The protein represents up to 70% of dry weight of skin and about 30% of total proteins in most organisms. Collagen structure is characterized by three polypeptide (three α-chains) fibrils with glycine (Gly) as the predominant amino acid. The polypeptide chains predominantly have a sequence of Gly-X-Y unit, where X is mostly proline and Y is hydroxyproline. About 29 types of collagen have been identified in animal tissues, which differ considerably in their amino acid composition, sequence of amino acids, and hence in structural and functional properties. Seafood processing by-products are rich sources of type-I collagen. During fish processing operations such as skinning and filleting, the removal of collagen-containing materials can be up to 30% of the fish. Extraction of collagen involves pre-treatment with dilute alkali to remove the non-collagenous proteins and pigments from the discards, which is followed by demineralization and removal of fat. Collagen can then be isolated using mild acids (usually 0.5M acetic acid). Collagen can also be isolated by enzymes such as pepsin, trypsin, or collagenase. Gelatin is a soluble protein obtained by mild heating of collagen suspended in hot water at near 45°C [17,18].

Properties of protein isolates and their co-products

Unlike surimi which is a concentrate of fish myofibrillar proteins fish protein isolates (FPIs) prepared by the ISP process are refined concentrate of myofibrillar proteins, particularly myosin and actomyosin, which also contain sarcoplasmic proteins. FPIs have protein contents of at least 65% and have fat contents below 2% (w/w). FPIs retain their properties for at least 6 months when stored at 5°C but loses rapidly at 30°C For longer storage life, it is ideal to store the powder under anaerobic conditions, preferably under frozen conditions in presence of cryoprotectants to prevent loss of protein quality. Seafood proteins are considered nutritionally equivalent or slightly superior to meat proteins and also to casein. Most seafood proteins show a digestibility above 90% releasing peptides and essential amino acids (EAAs). Shrimp muscle proteins have excellent digestibility and amino acid composition with good contents of essential amino acids [19]. Proteins isolated from shrimp shells and heads may retain the excellent nutritional properties of the native shellfish proteins. Nutritional value of the proteins is not significantly lost by conventional processing treatments [20]. Due to mild extraction conditions, the FPIs, like native fish proteins, retain nutritive value in terms of their amino acid composition, contents of EAAs, and susceptibility to enzymatic digestion. FPI from krill had higher contents of EAAs than whole krill proteins. FPIs from cod, herring and salmon and krill can meet the FAO/WHO/UNU recommendations for EAAs for adults and infants. Cod proteins, rich in arginine, glycine and taurine, can improve resolution of inflammation in the muscle. Besides their significant nutritive values, FPIs also possess valuable functional properties such as water holding capacity, gelation, emulsification and foaming capacities and ability to interact with other food constituents. Gelation helps incorporation of suitable food ingredients in the gel matrices to improve texture and flavor of prepared products.

Applications of isolated proteins and their co-products

Food product development

Protein isolates have immense scope for various applications. One important use is for the development of restructured food products. Restructuring involves conversion of food portions into products that resemble conventional or popular food items in their appearance, texture and flavor [21]. Surimi technology has been, perhaps, the most successful restructuring process for the development of imitation foods, which resemble shrimp, crab sticks, lobster tails, etc [9]. Apart from surimi, fish mince isolated by mechanical deboning of discards such as filleting frames can be directly used for products such as sausages, noodles, and preformed fish products such as meat balls, patties, fermented products and extruded products [22,23]. FPIs, because of their functional properties, have potentials as a binder, dispersing agent, and emulsifier in restructured food products. Uses of FPIs blended with surimi can improve hardness, cohesiveness, and whiteness of restructured fish products. Shark meat gel prepared by mild acid-induced gelation can be raw material for edible restructured products [13].

Other applications

Thermo-stable protein dispersions of fish structural proteins, prepared by making use of mild acid-induced gelation can be used for edible films and as coating to enhance the shelf life of chilled fishery products or as natural glazing for frozen fishery products to retard moisture loss, reduce lipid oxidation and discoloration during frozen storage [24-26]. Biodegradable edible film from myofibrillar protein of fish waste was developed. The process involves initial preparation of protein dispersion in water, which was subjected to gamma-irradiation at a dose of 10 kGy before casting. Examination of physical properties of the film showed that, as compared with control, the films prepared from irradiated proteins had better permeability, tensile strength and maximum elongation at break. The free sulfhydryl content was lowest in film from irradiated proteins, suggesting better interactions of the proteins in the irradiated samples [27].

Fish protein hydrolyzates can function as emulsifiers, and binding agents. FPIs can be logical vehicles for omega-3 polyunsaturated fatty acids, and other nutrients such as bioactive peptides, and dietary fiber. FPIs are ideal materials for making protein hydrolyzates and peptides. FPIs, fish protein hydrolyzates and peptides can be used as supplements and nutraceuticals in formulated foods. The pharmacological applications of peptides for their health benefits depend on their absorption and bioavailability in target tissues [14,15].

Seafood-derived collagen is favorable biomaterial for tissue engineering due to its excellent biocompatibility, biodegradability, cell adhesion properties and weak antigenicity. Applications of gelatin as whipping agent, stabilizer, emulsifier, adhesive, binder, coating and also as encapsulating agent is essentially based on its gel-forming properties. Fish gelatins could be substitutes to mammalian gelatins, since they have comparable properties such as bloom strength (gel strength equivalent, referred in the industry), viscosity, and solubility Gelatin can be carrier of drugs and bioactive compounds in pharmaceutical and food packaging applications. Collagen and gelatin have also interesting cosmeceutical applications [28]. Seafood enzymes can replace conventional seafood processing operations including isolation and modification of fish proteins and marine oils, production of bioactive peptides, acceleration of traditional fermentation, peeling and deveining of shellfish, scaling of finfish, removal of membranes from fish roe, extraction of flavors, shelf life extension, texture modification, removal of off-odors, and quality control either directly or as components of biosensors [11]. The beneficial applications of proteins recovered from seafood processing discards by ISP process are summarized in table 1.

- As binder, dispersing agent, and emulsifier in restructured foods such as sausage and nuggets.
- As protein supplement in low-protein foods such as bakery products.
- Peptides have promising applications as nutraceutical and functional food ingredients.
- As biomaterials in the form of nano- or microparticles, hydrogels, films, emulsions, and foams can be carriers of nutraceuticals such as omega-3 oil, dietary fiber and also drugs.

Table 1: *Some applications of proteins isolated by ISP process from seafood discard.*

Commercial prospects

Major encouragements for the isolation of FPIs by ISP process are availability of sufficient seafood processing discards as raw materials, high recovery rates, interesting functional properties of the isolated proteins and their potential applications. Since about 58% of the discards constitute proteins, and, further as high as 90% of proteins can be recovered by the ISP process, the treatment can reduce the discard volume at least by half leading towards better utilization of seafood production and also significant control of seafood-associated environmental hazards. Although commercial production of FPI by ISP process is sparse at present, some specific seafood protein products are commercially available. These include seafood protein extracts, krill extracts, enzymes such as fish trypsin, chymotrypsin, shrimp alkaline phosphatase, cold-active-bacterial chlamysin, lysozyme, and a cold tolerant protease, commercially known as Penzim from Atlantic cod. Many dietary supplements containing bioactive peptides have been developed. Gabolysat PC60 is a fish protein hydrolysate with anxiolytic properties commonly used as a nutritional supplement. Calcitonin, a peptide hormone useful for the treatment of osteoporosis, is currently isolated from salmon. Ziconotide, a peptide from a cone snail (*Conus magus*) having analgesic activity, is approved by the US FDA. Demand for collagen-derived ingredients for beauty and skin health, and sports nutrition is rising remarkably.

Conclusion

In conclusion, seafood processing discards can be interesting raw materials for nutritive proteins that can have interesting applications. Secondary processing of the discards for proteins and other nutrients can significantly ameliorate seafood associated environmental problems.

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