PREPARATION CHARACTERIZATION AND APPLICATION OF PROTEIN ISOLATES

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Consumers' demand for healthy food products is increasing worldwide. Development of functional foods involves incorporation of specific compounds (or ingredients) with demonstrated health benefits (Hamer, Owen, & Kloek, 2005). In addition to the importance of the health effects; sensory attributes such as taste, texture, and flavor as well as convenience remain crucial factors for consumers. Fish and fishery products play a major role in human health due to presence of essential amino acids, fatty acids, vitamins and minerals. In the fish industry, processing of raw fish into food products generates large quantities of by-products (heads, skin, bones, frames, scales etc.) that contain proteins and lipids. If these proteins and lipids are recovered, it could be a source of nutrients for humans; and therefore, could be used in the development of food products destined for human consumption. These problems can be overcome by using Isoelectric solubilization/precipitation (ISP) method or pH shift technology which allows the separation of proteins and lipids. Isoelectric solubilization/precipitation (ISP) is a pH-shift process that induces water solubility of meat proteins. While proteins are dissolved, they are separated from lipids and other insoluble materials such as skin, bones, scales, etc.

Recovery of proteins from fish and fish processing by-products using isoelectric solubilization/precipitation

In fish muscle homogenates, myofibrillar proteins are present as aggregates that are held together by weak protein–protein hydrophobic interactions (Undeland, Kelleher, Hultin, McClements, & Thongraung, 2003). The solubility of fish muscle proteins can be "turned" on or off by providing conditions that either favor or disfavor protein solubility, respectively. When acid is added to a solution, it dissociates yielding hydronium ions (H_3O+). Protonation of negatively charged side chains on glutamyl or aspartyl residues results in an increased net positive surface charge. Similarly, when base (− OH) is added to a solution, deprotonation of side chains on tyrosyl, tryptophanyl, cysteinyl, lysyl, argininyl or histidinyl residues contributes to an increased net negative surface charge. When the charge equilibrium is reached

and a protein solution attains homeostasis, the final status of a protein surface electrostatic charge at a given pH is referred to as the net charge. The accumulation of a net positive or negative charge induces protein–protein electrostatic repulsion and an increased hydrodynamic volume due to expansion and swelling (Kristinsson, Theodore, Demir, & Ingadottir, 2005; Undeland et al., 2003). As proteins assume more positive or negative net charge, they gradually start electrostatic interactions with water (i.e., protein–water interactions). Due to increased protein– water interactions, the protein–protein hydrophobic interactions decrease. Therefore, as the protein molecules become more polar (charged), more water associates on and around the protein surface and proteins become water soluble. However, it is possible to adjust the pH of a protein solution so that the number of negative charges on the protein's surface is equal to the number of positive charges, and therefore, the protein molecule assumes a zero net electrostatic charge. The pH at which the net electrostatic charge of a protein is equal to zero is called the isoelectric point (pI). The pI is very specific for different proteins, and isoelectric focusing is often used to pinpoint the pI. The iso electric solubilization and precipitation (ISP) can be universally applied to recover muscle proteins from animal processing by-products, including fish. Fish protein isolates (FPIs) have thus far been recovered using ISP in a batch mode at the laboratory scale (Choi & Park, 2002; Kim, Park, & Choi, 2003; Kristinsson & Hultin, 2003; Undeland Kelleher, & Hultin, 2002) and pilot scale (Mireles DeWitt, Nabors, & Kleinholz, 2007). ISP processing has been applied to beef, chicken and fish processing by-products (Chen & Jaczynski, 2007a; Mireles DeWitt, Gomez, & James, 2002; Tahergorabi, Beamer, Matak, & Jaczynski, 2011).

Protein isolate from fish meat

Fish protein isolate is a concentrated protein mainly consists of myofibrillar proteins (eg. actin and myosin). Fish protein isolate (FPI) is prepared from fish meat and discards different kinds of raw materials by the pH-shift technology. The process of protein recovery by pH-shift was proposed by Hultin and Kelleher (1999). In the pH-shift process, proteins of the muscle tissue are first solubilized at either acid (pH below 1.5-3.0) or alkali pH (near 10.5-13.0) and centrifuged. Then, the top lipid layer and sedimentation (insoluble impurities such as bones and skin) at the bottom are discarded. The middle layer of protein solution is collected and precipitated by adjusting the pH to a value near the isoelectric point (5.2-5.5) Then it was centrifuged and precipitate is collected which is called protein isolate (Kristinsson et al. 2005).

Protein isolate from fish / shrimp head waste

The shrimp head homogenate is mixed with distilled water at different ratio (1:2 to 1:4 w/v). Then, pH (9.43) is adjusted with 1M NaOH. Then it is centrifuged and supernatant is collected. After that pH is adjusted to 4.5 using 1M HCl to precipitate the protein in the supernatant. The precipitated proteins are collected by centrifuging at 10,000 rpm for 15 min at 4° C followed by decantation of supernatant. The precipitants are resuspended in distilled water and the pH is adjusted to 7 using 1M NaOH. Then the precipitate is collected by centrifugation at 10,000 rpm for 15 min at 4° C. The precipitate can be washed thrice to remove the salts using distilled water for further application.

Characterization of protein isolate:

Moisture

The AOAC method is used to measure the moisture of the samples. Calculation of the moisture was done according to the following formula;

$$Moisture\ (\%) = \frac{Pre - dry\ weight\ (g) - After\ dry\ weight}{Pre - dry\ weight\ (g)} x100$$

Pressure Induced Drip

Pressure Induced Drip is an important factor to estimate the water content of FPI and the quality of the protein in the product. 50 g of the test sample is transferred to a circular cylinder with an inner diameter of 35 mm and 120-150 mm in length, made of stainless steel and a perforated plate with holes 1 mm in diameter in the bottom. Pressure is applied with a 1 kg cylindrical rod 34 mm in diameter and left for 20 minutes. The weight of the dripped liquid is measured and the percentage of the weight of the test sample is calculated.

Objectionable matter

The term “objectionable matter” is used here means skin, small bones and any objectionable matter other than fish muscle. In this method 10 g of the test sample is spread to the thickness of 1 mm or less, and the number of visible objectionable matters more than 2 mm in diameter is noted.

Whiteness

The colour and whiteness of protein isolate gel is another important factor and it can measured by using colour [L*(lightness), a* (red-green colours) and b* (yellow-blue colours)] analyzer. Whiteness, as an index for the general appearance of surimi gel, can be calculated as:

Whiteness = L* - 3b*.

Determination of water holding capacity by measuring expressible moisture

Water holding capacity of protein isolate is easily determined by measuring expressible moisture of cooked isolate gel. A small amount of test sample (around 2 g) is placed between filter papers and pressed using texture analyzer under a fixed pressure (10 kg/cm²). The expressible water is calculated according to the following formula to the first decimal place:

$$Expressible\ moisture\ (\%) = \frac{Pre-pressed\ weight\ (g) - After-pressed\ weight}{Pre-pressed\ weight\ (g)} x100$$

Determination of functional properties

Solubility

Protein sample (10 mg ml^{-1}) in distilled water at neutral pH is vortexed for 30 min at room temperature and centrifuged at 7500 g for 15 min. Protein contents in the supernatant is determined by Kjeldahl method and protein solubility is calculated as follows (Morr et al., 1985).

$$Protein\ solubility\ (\%) = \frac{Total\ protein\ content\ in\ supernant}{Total\ protein\ content\ in\ sample} x100$$

Foaming properties

Foaming capacity and stability of fish protein isolate is determined by the method described by Sathe and Salunkhe (1981). Protein solution (1.0 %) is homogenized (230 VAC T-25 digital Ultra-turrax, IKA, India) at a speed of 16,000 rpm for 2 min to entrap air and foaming capacity is determined instantly whereas foam stability after a time period of 3 minutes as:

Foaming capacity/stability (%) = [(A-B) / B] x100

Where A is the volume immediately after whipping (foam capacity) and after 3 min standing (foam stability); B is the volume before whipping.

Emulsifying properties

Emulsifying properties is determined according to the method of Pearce and Kinsella (1978). A pre-mix containing 1% protein solution and vegetable oil (3:1 (v/v)) is homogenized (230 VAC T-25 digital Ultra-turrax, IKA, India) for a period of 1 min at 20,000 rpm and an aliquot of the emulsion (50 µl) is carefully taken from the bottom of the container at 0 and 10 min after homogenization. Further it is mixed with 5 ml of 0.1% sodium dodecyl sulphate (SDS) solution and the absorbance measured at 500 nm (Lambda 25 UV/Vis, Perkin Elmer Life and Analytical Sciences, Singapore) immediately (A0) and 10 min (A10) after emulsion formation to evaluate the emulsifying activity index (EAI) and the emulsion stability index (ESI) as:

$$\text{EAI (m2 /g)} = \frac{2 \times 2.303 \times A_0}{0.25 \times \text{wt of protein}}$$

$$\text{ESI (min)} = A_{10} \times \Delta t / \Delta A$$

Where $\Delta A = A_0 - A_{10}$ and $\Delta t = 10$ min

Oil absorption capacity

OAC of sample is determined as the volume of edible oil held by a known quantity of the material as per the method of Shahidi et al., (1995). A 0.5 g sample with oil added (10 ml) is vortexed (Expo Hitech, India) for 30 sec following centrifugation (K-24A, Remi Instruments, Mumbai) at 2800 g for 25 min. The free oil is decanted and the OAC was determined by weight difference (g of oil per gram of sample).

Quality characteristics of protein Isolate

In general, protein isolate prepared by pH shift technology offer high quality protein consists of all essential amino acids. Protein isolate will have a protein content of 87–95%. Lipid and ash content ranged from 1–5%, 2–6%, respectively. Proximate composition of protein isolates varied depends on the raw material and process applied (acid or alkali solubilization). The nutritional quality of a protein source is determined based on the presence of all nine essential amino acids (EAAs) in adequate quantities to support human or animal health. ISP at basic (i.e., alkaline) pH allows recovery of FPIs with higher nutritional quality as assessed by a greater content of EAAs when compared to ISP at acidic pH (Chen, Nguyen, Semmens,

Beamer, & Jaczynski, 2007; Chen, Tou, & Jaczynski, 2009). The biological value (BV) of FPIs is higher than soybean protein concentrate and similar to milk protein (Bridges, Gigliotti, Altman, Jaczynski, & Tou, 2010; Gigliotti, Jaczynski, & Tou, 2008). Egg protein is commonly the reference protein due to its high nutritional quality, while lysine is often considered a limiting EAA. It needs to be emphasized that FPIs from fish processing byproducts have a similar concentration of lysine as the whole egg, and the concentration of lysine is even greater in the FPI from whole carp (gutted, but bone-in, skin-on) and whole krill (Chen et al., 2009; Taskaya, Chen, & Jaczynski, 2009). The protein isolate could be considered a source of high-quality complete protein.

Factors affecting quality of fish protein isolate

i) **Source of raw material:** Properties of fish protein isolate varies by types of fish or processing discard used and freshness of raw material

ii) **Process of homogenization:** The first step in preparation of fish protein isolate is homogenization. This process enhances the solubility of protein and recovery. Moreover, time given for homogenization also influence the yield and solubility of protein. Solubility of proteins also depends on pH, and/or temperature.

iii) **Ratio of fish to water:** FPI production involves homogenization of fish meat by using 10-20 parts of water (1:10 or 1:20). The ratio of fish to water influences the viscosity of protein solution. It has been reported that low viscosity is preferable to isolate insoluble material during centrifugation process.

iv) **Process Time and temperature:** Quality of protein isolate depends on time and temperature used for its production.

v) **Presence of hem proteins:** In case of dark muscle protein, presence of heme protein will affect the quality of fish protein isolate . Hence it should be removed from soluble fraction to reduce the fat oxidation.

vi) **Protein denaturation:** Denaturation of protein results loss of protein functionality. It can be overcome by control of temperature during processing

Fish protein isolate Vs Surimi

Fish protein isolate (FPI)	**Surimi**
Processing discards, under-utilized fish can be used for fish protein isolate production.	Fresh fish mince prepared from fillet or by using deboner is used for surimi production
pH meter, homogenizer and centrifuge is required	It requires high-cost instrument like filleting machine/ deboner, washing tank, strainer etc.
Water usage is less for fish protein isolate production (5-10lit/kg of isolate)	Water usage is high (25-30lit/ kg of Surimi production)
Less quantity effluent generated during process results less environmental pollution	Huge water uses during process generate large quantity of effluent for disposal
Production process involves acid and alkali usage. Hence its needs stronger safety requirement.	Production process does not involve chemical usage except sodium bicarbonate is used for gel strength improvement in dark meat process. Skilled person required for machineries operation
Yield of protein ranged from 70-90%	Yield of protein ranged from 55-70%
Fat is removed effectively during processing	Fat removal from fish mince depends on fish and number of washing cycle used
Cryoprotectant is required to improve the stability of fish protein isolate during frozen storage	Cryoprotectant is required to improve the stability of surimi during frozen storage
Fish protein isolate process is well developed. But fish protein isolate based products are under developmental stage.	Surimi process and surimi-based products are well established

Applications

- ✓ Fish protein isolate used as functional ingredients for preparation of ready to eat/ ready to cook value-added products
- ✓ Dried fish protein isolate powder can be used as protein supplements in food products.
- ✓ Fish protein isolate has an excellent functional property which results in wider applications in developing restructured products
- ✓ Unlike Surimi, FPI also used for production of seafood analogue products
- ✓ Fish protein isolate also used to develop nutraceutical products (eg. Fortification of FPI with omega -3 fatty acids)

Raw fish protein

Freeze dried fish protein

Restructured product from isolate

Fish protein isolate incorporated

Conclusion

The utilization of processing discards and fishes not suitable for human consumption is one of the biggest challenges in the seafood sector. These can be overcome by using Isoelectric solubilization/precipitation (ISP) method or pH shift technology to convert muscle tissue to edible protein and it can also serve as a substitute to surimi. However, production process of fish protein isolate is under developmental stage. If the production process and isolate based product is well established, fish protein isolate will serve as excellent protein source for human consumption. Moreover, it offers wider applications in functional food development.

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