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Review Article

Fish waste Utilization with Reference to Fish Protein Hydrolysate – A Review

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Abstract

Aquaculture and capture fisheries have contributed to the global economy for decades, supporting livelihood as well as nutritionall status. The sector has been growing substantially that more than 60% by-products as waste are generated which includes skin, head, frames, fins and viscera. With an increasing population with limited resource and food supply, the need for utilizing the discarded waste have added interest in many processing industries. This review discusses on hydrolysis of un-utilised fish and fish waste which are rich source of nutrient that can impart stability in food products, possess noble pharmaceuticals and neutraceuticals significance to health. Fish protein hydrolysate has attracted much attention as an excellent source of bioactive peptides imparting antioxidant, antihypertensive, immunomodulatory and antimicrobial properties with a good balance of essential amino acids. Above all, the review also reveals the present status and future potential of utilizing fish waste into economically and sustainable products for human consumption

Keywords: Fish waste, fish protein hydrolysate, antioxidant activity, functional properties

Introduction

In the last three decades, the fishery sector has witnessed tremendous growth, with capture fisheries production increasing from 69 million tons to 90 million tons and world aquaculture production from 5 million to 80 million tons (FAO, 2018). In 2013, fish accounted for about 17% of the global population intake of animal protein and 6.7% of all protein consumed. The intake of fish protein exceeds 50% in many unless developed countries. Human consumption of fish has increased from 67% (<40 million tonnes) in the 1960s to 87% (>150 million tonnes) in 2016 (FAO, 2018). An estimate of one billion people, directly or indirectly are involved in the fishing industry for livelihood (Oosterveer, 2008).

Out of the total fish catch, 27% is unutilised or lost between landing and consumption due to low value discards, storage problems and spoilage (FAO, 2018). These unutilised fish are simply dumped causing mass pollution problem and threat to the environment or are used to generate low market value products like fish meal, animal feed and fertilizers. An estimate of 567474 tonnes of unutilised fish waste was generated from fish processing industries of India alone during 2015-16 (Rajeswari et al., 2018). The maximum of these wastes was produced from processing of shrimps followed by finfishes and cephalopods. On the context of environmental pollution, waste from fish processing is of great concern today. In India, it was observed that the waste generated from processing of finfish and shellfish was highest recorded from Gujarat (30.51%), Maharastra (23%) and Kerala (17.5%) (Zynudheen, 2012).

Several studies have been made to utilize underutilized fish and by-products for the production of commercially valuable products (Kristinsson & Rasco, 2000; Gao et al., 2006; Klompong et al.,

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2007; Sarmadi & Ismail, 2010). Though, many techniques are employed to produce fish meal, fish ensilage, fish oil, fish glue, fish essence, fish calcium, fish collagen and fish hydrolysate, there is little to no prospect for human utilisation as food or as an additive in the food industry. Enzymatic hydrolysis of protein can yield a by-product which is found to be rich in nutrients and bioactive compounds with pharmaceutical and industrial application as food (Kristinsson & Rasco, 2000; Ðliþytë et al., 2005; de Castro & Sato, 2014; de Castro et al., 2015). Fish protein hydrolysate is a concentrated and purified form of protein developed by cleavage of molecular bonds through biological processes (Rutherfurd & Gilani, 2009). This reviews trend to focus on the research of fish protein hydrolysate, its potential applications in food and pharmaceuticals.

Composition of fish waste

Fish processing generally involves size grading, identification, de-scaling and removal of carcass, fins and viscera which are further made into fillets, steaks or canned. Fish contain 15-30% protein, 0-25% fat and 50-80% moisture (Murray & Burt, 1969; Ghaedian et al., 1998). Solid waste of fish post-processing consists of head, tails, skin, gut, fins and frames. Around 30-50% of the meat is usually left along the ribs and backbone during filleting. Nearly 4-5% of skin, 21-25% of head, and 24-34% of bones are unutilized during whole fish filleting accounting for more than 45% (Ghaly et al., 2013). These byproducts can be an important source of proteins and amino acids, collagen and gelatin, oil and several enzymes (Sutcliffe & Disney, 1977; Esteban et al., 2007). Fish wastes contain approximately 58% proteins, 19% fat and minerals. In addition, monosaturated acids, palmitic acid and oleic acid are abundant in fish waste constituting about 22%.

Protein

Fish frames contain substantial amounts of muscle, which are composed of structural myofibrillar and sarcoplasmic proteins. They also include certain amino acids like lysine, valine and phenylalanine (Hayes & Flower, 2013). These proteins and amino acids can be extracted by enzymatic hydrolysis rather than being discarded as waste (Venugopal et al., 1996). Proteins derived from fish have a superior balance of dietary vital amino acids when matched to other animal protein sources (Yanez et al., 1976; Friedman, 1996).

Lipid

Fish waste also contains adequate amount of lipid. This fraction of waste contains omega-3, squalane, phospholipid, cholesterol and fat-soluble vitamins (Rai et al., 2010). Polyunsaturated fatty acid extracted using wet rendering method is particularly a major for utilization directed towards the food and nutraceutical industries. Arvanitoyannis & Kassaveti (2008) reported the production of biodiesel from fish contributing a new study in the energy sector. Fish protein hydrolysate, which on the other hand requires a final product with low lipid content can thus yield lipid as a by-product in its production process. Several studies involve the de-fattening of raw material before the hydrolysis process, which potentially could add a major advantage to the production process.

Bioactive peptides

Proteins from the fish muscle contain a number of peptides which have many bioactivities such as antihypertensive, antithrombotic, immune modulatory and antioxidative properties (Choi et al., 2000). The bioactive peptides derived from the fish muscle have anticoagulant and antiplatelet properties (Je et al., 2004).

Collagen and gelatin

Fish skin and bones are an excellent source of collagen and gelatin which are used in food, cosmetic and biomedical industries. The collagen and gelatin extracted from fish skin eliminate risks of mad cow disease or bovine spongiform encephalopathy (BSE) unlike from bovine sources. Gelatin also has a unique sequence of glycine-proline-alanine which imparts antioxidative property of gelatin (Byun et al., 2005; Kim & Mendis, 2006). The collagen and gelatin extracted from fish is also gaining interest among researchers in alteration to mammalian collagen for implication in food and pharmaceutical products (Karim & Bhat, 2009).

Enzymes

The internal organs of the fish are a rich source of enzymes. The enzymes include pepsin, trypsin, chymotrypsin and collagenase which possess enhanced catalytic properties, better efficiency at lower temperatures, lower sensitivity to substrate concentrations and greater stability in a wide range of pH (Byun et al., 2005; Kim & Mendis, 2006; Zhou

et al., 2011). Several endogenous enzymes are also identified to being used to hydrolyse proteins (Vannabun et al., 2014).

Minerals

Mineral content in waste fish is generally higher due to higher percentage of bones, scales and head. Fish bones contain 60-70% minerals including calcium, phosphorous and hydroxyapatite (Kim & Mendis, 2006). Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ is often used in medical and dental applications as a bone graft material (Larsen et al., 2000). Wang et al. (2012) and Chi et al. (2014) demineralized the raw material in order to decrease the ash content of the final hydrolysed product.

Fish protein hydrolysate

The technology of FPH has been considered as an effective way to utilize the underutilized or by-catch fish. These fishes have a poor consumer preference and acceptability and hence low commercial value due to small size, appearance, taste and flavour. FPH could help minimize these waste by-products through application of correct and standardized operational procedures.

The enzymatic processing of various biopolymers in food (such as polysaccharides, proteins and pectin) can improve the physical, chemical and organoleptic properties of the final product. The enzymatic extraction of protein is carried out under controlled pH conditions without degrading their nutritional qualities for easy acceptance in the food industry and for a wide range of applications in variety of food industries in numerous forms, such as milk replacers, flavour enhancers in confectionery products, stabilizers of beverages, protein supplements, animal food and microbial media (Shahidi et al., 1995). Most of these diets are composed of peptides and are rich in amino acids. FPH has acquiring reputation owing to an array of potential bioactive properties including antioxidant, antimicrobial, immune modulatory, neuroactive, antihypertensive, and mineral or hormone regulating abilities.

The enzymes used in the food industry for the preparation of fish protein hydrolysate are mostly carbohydrases, proteases and lipases. Alcalase, an endoprotease prepared from *Bacillus* spp, is widely accepted for the production of fish protein hydrolysate with high solubility and digestibility. However, technical aspect for enhancing the taste and flavour of crustacean and fish hydrolysated protein is limited.

Research has been done in various species to find out the nutritive essence of FPH, some of them using whole fish which include *Barbus carnaticus, Rastrelliger kanagurta* (Sen et al., 1962); *Barbus dubious* (Sripathy et al., 1963); *Cynoglossus* sp., *Johnius* sp., *Nemipterus japonicus, Platycephalus macracanthus, Saurida tumbil, Sphyraena* sp.,*Tachysurus* sp., *Thrissocles* sp., *Trichiurus* sp. (Thankamma et al., 1979) using papain; *Thrissocles* sp., *Harpodon nehereus, Johnius dissumieri, Oreochromis mossambicus* (Venugopal & Lewis, 1981); Dhoma (*Johnius dussumieri*) (Warrier et al., 1996); Yellow stripe trevally (*Selaroides leptolepis*) (Klompong et al., 2007) using alcalase and flavourzyme.

Preparation of protein hydrolysates from fish skin waste has also been widely reported. These include Amur sturgeon (Nikoo et al., 2015) using alcalase and flavourzyme; Grass carp (*Ctenopharyngodon idella*) (Wasswa et al., 2007) using alcalase; *Rachycentron canadum* (Yang et al., 2008) using bromelain, papain, pancreatin and trypsin enzymes; *Lutjanus vitta* (Khantaphant et al., 2011) using proteases from pyloric caeca of brownstripe red snapper; Tilapia (Yang et al., 2009) using Type I collagen from calf skin; Sole (Gimenez et al., 2009) using alcalase; *Theragra chalcogramma* (Zhuang et al., 2009) using trypsin and flavourzyme; *Priacanthus macracanthus* (Phanturat et al., 2010) using Pyloric caeca from big eye snapper, trypsin, alcalase and neutrase; North Atlantic lean fish (Picot et al., 2010) using industrial bacterial endopeptidase; Channel catfish (*Ictalurus punctatus*) (Yin et al., 2010) using pepsin and trypsin; *Oreochromis niloticus* (Ngo et al., 2010) using alcalase, pronase E, trypsin and pepsin; *Aphanopus carbo* (Batista et al., 2010) using protamex; *Raja kenojei* (Lee et al., 2011) using alcalase, α-chymotrypsin, neutrase, pepsin, papain, and trypsin; *Magalaspis cordyla* and *Otolithes ruber* (Kumar et al., 2012) using pepsin, trypsin and α-chymotrypsin; Bluefin leatherjacket (*Navodon septentrionalis*) (Chi et al., 2014) trypsin, flavourzyme, neutrase, papain, alcalase and pepsin.

In addition to these, shellfishes have also been used for the preparation of protein hydrolysate. Lobster (*Panulirus* spp.) (Vieira et al.,1995) using papain, pepsin and fungal protease; *Corbicula fluminea* (Tsai et al., 2006) using protamex and flavorzyme; Hard clam (*Meretrix lusoria*) (Tsai et al., 2008) using protamex; Blue mussel (*Mytilus edulis*) (Wang et al.,

2012) using pepsin, papain, neutrase and alcalase; scallop (*Patinopecten yessoensis*), abalone (*Haliotis discus hannai Ino*) (Zhou et al., 2012) using protease from *Bacillus subtilis*; *Mytilus coruscus* (Kim et al., 2013a) using flavourzyme, neutrase, protamex, alcalase, papain, pepsin, a-chymotrypsin, trypsin; *Crassostrea talienwhanensis* (Wang et al., 2014) using subtilisin; *Saccostrea cucullata* (Umayaparvathi et al., 2014) using protease from *Bacillus cereus*; *Mytilus edulis* (Park et al., 2016) using pepsin; *Perna canaliculus* (Vijaykrishnaraj et al., 2016) using pepsin, trypsin, α-chymotrypsin and pancreatin.

Proteolytic enzymes such as alcalase, α-chymotrypsin, neutrase, papain, pepsin, trypsin, pancreatin, flavourzyme, bromelain, pronase E, protamex, orientase, thermolysin, validase, protease A amano, protease N amano and cryotin F derived from plant, animal and microbial sources have been successfully tested for the production of antioxidative peptides from fish protein sources (Wu et al., 2003; Ren et al., 2008; Samaranayaka & Li-Chan, 2008; Raghavan & Kristinsson, 2008; Nakajima et al., 2009; Batista et al., 2010; Ngo et al., 2010). Besides the choice of a suitable proteolytic enzyme, the physicochemical conditions of the process such as temperature, time and pH for optimal activity of enzyme and hydrolysis time are vital in the production of antioxidative protein hydrolysates or peptides with desirable functional properties (Samaranayaka & Li-Chan, 2011).

Enzymatic hydrolysis gives high yield and good quality final product and unlike other commercial methods, control of characteristics in flavour, texture and functional properties through variation of enzyme reactions is possible. Selecting the right type of enzyme for a given raw material can deliberately yield desired results. The main drawback with the enzymatic hydrolysis of seafood is the formation of bitter flavour. Now, with emerging enzyme systems with endoprotease and exopeptidase, bitterness can be minimized (Stanley, 1981; Tchorbanov et al., 2011; CLEGG & McMillan, 1974). As more commercial high-performance enzymes are developing, highly acceptable seafood flavours can be mass produced through hydrolysis of various seafood processing by-products.

Hydrolysis conditions

Many studies have shown the optimization of hydrolysis conditions such as the temperature, pH, hydrolysis time, and enzyme-substrate ratio (Hoyle & Merritt, 1994; Baek & Cadwallader, 1995; Martin & Porter, 1995; Shahidi et al., 1995; Vieira et al., 1995). The higher the enzyme concentration and longer the time of hydrolysis, higher will be the degree of hydrolysis. The optimum temperature and pH of the enzyme could be obtained from the manufacturers. However, due to the difference of substrate and the purpose of hydrolysis, the optimum enzyme concentration for the desired degree of hydrolysis (DH) has to be determined. Hence, bioactive peptides derived by enzymatic process needs to be accurately monitored to ensure the reproducibility of protein hydrolysate.

Antioxidant activity of fish protein hydrolysate

Studies have shown that protein hydrolysates have antioxidative properties and may help in preventing peroxidative damage in food as well as the physiological systems. Certain amino acids and peptides, which are found in muscle foods, have been studied to have excellent antioxidative properties (Karel et al., 1966; Chan et al., 1994). Shahidi et al. (1995) suggested that FPH from capelin effectively reduced lipid oxidation by up to 60.4% when added to ground pork. Wu et al. (2003) showed that transitional peptides more effectively increased the lag time before oxidation when added to a linoleic acid emulsion peroxidation system, compared to small molecular weight peptides. Jeon et al. (1999) also demonstrated that different FPH fractions representing different molecular weight ranges have different antioxidative activities. Another broad study by Kristinsson et al. (2005) on the potential antioxidative mechanisms of FPH made from catfish muscle protein isolate was found to have good metal chelating ability, again higher with increasing DH and on the contrast, the reducing power decreased as DH increased. Many of these studies employ methodologies where metal ions are the pro-oxidants. Chuang et al. (2000) demonstrated that FPH from mackerel had the ability to decrease haemoglobin-mediated lipid oxidation in a linoleic acid system. It is to mention that the possibility of antioxidative activity, is not necessarily due to the peptides produced during the hydrolysis. A number of compounds in the fish muscle which have a good antioxidative effect could have been reflected in the FPH preparations. Undeland et al. (2003) demonstrated that 'press juice' of fish muscle has a strong antioxidative activity on haemoglobin-mediated oxidation in a washed fish muscle matrix.

Protein hydrolysates from prawn (Suetsuna, 2000), tuna cooking juice (Jao & Ko, 2002), yellowfin sole frame (Jun et al., 2004), Alaska Pollack frame (Je et al., 2005), herring (Sathivel et al., 2003), mackerel (*Scomber austriasicus)* (Wu et al., 2003), Capelin (Amarowicz & Shahidi, 1997), *Sphyrna lewini* muscle (Wang et al., 2012), conger eel muscle (Ranathunga et al., 2006), Blue mussel (*Mytilus edulis*) (Wang et al., 2012), Catla (*Catla catla*) (Elavarasan et al., 2012), Skate (*Okamejeikenojei*) skin (Ngo et al., 2014), Alaska pollock skin (Guo et al., 2013), *Ruditapes philippinarum* (Kim et al., 2013b), round scad muscle (Jiang et al., 2014), Monkfish muscle (Chi et al., 2014), whitemouth croaker muscle and its by-product (da Rosa Zavareze et al., 2014), leatherjacket (*Navodon septentrionalis*) (Chi et al., 2015) and grass carp muscle (Ren et al., 2008) have been found to possess antioxidant properties.

Functional properties of fish protein hydrolysate

Fish protein hydrolysates have potential application as functional ingredients in different foods because they possess important and unique properties such as water holding capacity, oil absorption capacity, protein solubility, gelling activity, foaming capacity and emulsification ability (Chalamaiah et al., 2010).

The functional properties of proteins changes through physical and chemical treatments (pH, ionic strength, heat, mechanical shear) as well as enzymatic treatment (Nielsen, 1997). Hydrolysis potentially influences the molecular size, hydrophobicity and polar groups of the hydrolysate (Adler-Nissen, 1986; Kristinsson & Rasco, 2000). The characteristics of hydrolysate directly affect the functional properties and its uses in food as ingredients. However, a very high degree of hydrolysis can have negative effects on the functional properties (Kristinsson & Rasco, 2000).

Solubility

Solubility is essential for a protein to perform as a functional ingredient in food (Nielsen, 1997). Hydrolysate has excellent solubility at a high degree of hydrolysis (Gbogouri et al., 2004; Quaglia & Orban, 1987; Shahidi et al., 1995). Fish myofibrillar proteins tends to be less water solubility in a wide range of pH (Venugopal & Shahidi, 1994). Enzymatic breakdown of the protein gradually cleaves protein into smaller peptide units. High solubility of fish protein hydrolysate over a wide range of pH is a substantially useful characteristic for many food applications. The enhanced solubility may be due to the newly exposed ionizable amino and carboxyl groups of the amino acids that increase its hydrophilicity (Mahmoud, 1994). The solubility of protein hydrolysate may also increase due to the reduction of molecular weight and increase in the polar groups (Nielsen, 1997). The pH also influences the change in the weak acidic and basic side-chain groups with the lowest solubility at their isoelectric points (Gbogouri et al., 2004).

Furthermore, it influences the other functional properties, such as emulsifying and foaming properties (Kristinsson & Rasco, 2000; Gbogouri et al., 2004).

Emulsifying properties

Proteins are commonly used as a surfactant in foods to achieve emulsion properties (Nielsen, 1997). The number of polar groups and hydrophobicity increases with partial hydrolysis and improves the emulsifying properties of protein while the molecular weight decreases which enhance the interaction of oil-water interface due to the presence of hydrophobic and hydrophilic functional groups. Hydrolysates become surface active and thereby promote oil and water emulsions (Wilding et al., 1984). Emulsifying capacity and emulsifying stability are used to measure the ability of protein hydrolysate to form emulsions (Sathivel et al., 2003; Gbogouri et al., 2004).

Studies on several protein hydrolysates show that the emulsifying properties are affected by the degree of hydrolysis. According to Gbogouri et al. (2004), better emulsion stability is obtained at a lower degree of hydrolysis with salmon protein hydrolysate. Similarly, hydrolysate obtained from sardine had a greater emulsifying capacity and emulsion stability when the degree of hydrolysis was low (Quaglia & Orban, 1990). The emulsifying capacity increases significantly up to approximately 5% DH (Adler-Nissen, 1979; Kristinsson & Rasco, 2000; Kong et al., 2007). The emulsifying property is also influenced by the molecular size. An optimum molecular size or chain length of peptides will deliver good emulsifying properties (Adler-Nissen, 1979). The emulsifying capacity of protein hydrolysate is generally low at isoelectric pH (Gbogouri et al., 2004).

Foaming properties

The change in balance and conformation and altered hydrophobicity compared to the native molecule are produced by the digestion of protein. Reduction in molecular weight will make them more flexible, forming a stable interfacial layer and increasing the rate of diffusion to the interface, improving the foaming properties (Wilde & Clark, 1996). Protein hydrolysate with smaller peptide chain has poor foam stability (Liceaga-Gesualdo & Li-Chan, 1999).

The insensitivity of protein hydrolysate to change in pH is an advantage. The effect of pH on the foaming properties particularly foam stability is markedly less with highest at an isoelectric point (Kinsella, 1981). Sanchez & Patino (2005) showed that there is a higher rate of diffusion with an increase in protein concentration. Shahidi et al. (1995) reported good foaming properties for capelin protein hydrolysates prepared by Alcalase at a low degree of hydrolysis.

Conclusion

The future research on FPH is furthermost important considering its significance and a welcoming global expanding market. There is potential for FPH to be produced and marketed as functional food ingredients. Further research is needed on laboratory-tested processes for commercial applications in food based on improved understanding of the mechanisms of enzymatic hydrolysis. But at present other applications such as plant nutrients, fertilizers, and animal feeds might be more feasible. Advanced research at molecular level could promote the utilisation of the fish waste on different aspects of biofunctional and pharmaceutical applications of FPH.

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