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A Review on the Properties and Applications of Fish Protein Hydrolysates

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Abstract

Seafood proteins have gained much attention for their nutritional richness and functionality. Peptides derived from seafoods have wide application possibilities in the form of nutritional supplements, functional food ingredients as well as improve the storage stability of food commodities facilitating superior quality products. This review presents an overview of the properties exhibited by seafood derived peptides and critically looks into the application possibilities of these derivatives in the food industry.

Keywords: Seafood, peptides, nutrition, functional, antioxidant, food application

Fish proteins, owing to its structural diversity as well as nutritional, functional and biological properties, can be effectively exploited for their recovery to different forms *viz.,* concentrates, isolates, hydrolysates, protein fractions like collagen, gelatin etc. Various authors have reported the identification of several bioactive compounds from fish proteins (Kim & Mendis, 2006; Nishioka et al., 2007). In this regard, both main raw material as well as protein rich seafood processing discards could be utilized by enzymatic conversion into protein hydrolysates which has immense application possibilities in food, nutraceutical and pharmaceutical sectors (Chalamaiah et al., 2012; He et al., 2013).

Protein hydrolysate,are proteins fragmented down into peptides of different sizes either chemically by using acids/bases or biologically using enzymes (Pasupuleti & Braun, 2010). These peptides hold numerous desirable properties like functional, health promoting bioactivities, making them eligible components in nutraceuticals and functional foods (Kristinsson, 2007; Harnedy & Fitz Gerald, 2012). Various fish species/ seafood substrate, proteolytic enzymes and process parameters *viz.,* pH, temperature, hydrolysis time and enzyme-substrate ratio facilitate the optimized production of fish protein hydrolysates (FPH) with tailor-made molecular structures and associated bioactive properties with nutritional or therapeutic interest (Guerard et al., 2002; Chabeaud et al., 2009).

Enzymatic hydrolysis

Enzymatic hydrolysis of proteinsis the most frequently opted and most promising compared to other processes as it results in products with high functionality and organoleptic characteristics to the food in relation to its nutritive value and intestinal absorption characteristics (Pasupuleti & Braun, 2010; Wisuthiphaet et al., 2015). Further this method requires mild hydrolytic conditions with comparatively small quantity of enzymes that can be deactivated easily. The short reaction time, ease of scalability and predictability also increases the process convenience. The use of enzymes does not destroy amino acids and resulting mixtures of peptides can be easily purified (Herpandi et al., 2011). An added advantage of utilizing enzymes for proteolysis is their enhanced ability for protein recovery from the substrate as well as their capability in selectively deriving bioactive compounds from complex raw materials (Rubio-Rodriguez et al., 2010).

Proteolytic enzymes from several sources *viz.,* animal, plant and microbial can be used for the hydrolysis process and areavailable commercially. The widely opted enzymes for deriving peptides from animal sources comprise pancreatin, trypsin and pepsin; plant based ones being papain and bromelain and of microbial origin include bacterial

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and fungal proteases (Pasupuleti & Braun, 2010; Hou et al., 2017). In common, the application conditions for these enzymes is that, they should be of food grade and those of microbial origin should be produced fromnon-pathogenic organisms (Bhaskar & Mahendrakar, 2008).

Factors influencing hydrolysis

Several factors influence the hydrolysis of food proteins *viz.,* the protein substrate used, the enzymesemployed, the physico-chemical settingsfor hydrolysis including enzyme- substrate ratio (E/S), reaction time, temperature as well as pH (Ren et al., 2008a; Benjakul et al., 2010). Generally, the substrate preferred as protein source for hydrolysate preparation should be of lean variety with minimum fat content. Use of fatty fishes for FPH calls for additional treatments to remove excess fat (Ritchie & Mackie, 1982) and hence increases the cost of production. A major role is played by the enzymes used for hydrolysis as they dictates the pattern of peptide bond cleavage. Proteases from diverse sources like animal, plantor microbial are generallyoptedto get a more selective proteolysisas they are peptide specific and hence assist in deriving desired products (Korhonen & Pihlanto, 2003). The physicochemical condition of the reaction determines the degree of hydrolysis as well as the molecular weight of the derived peptides which are contributors to the bioactive properties exhibited by the peptides (Ren et al., 2008b).

Nutritional properties

Protein is the major component of interest in fish protein hydrolysate and previous studies have reported about 60 - 90% of proteins of the whole composition (Choi et al., 2009; Khantaphant et al., 2011; Parvathy et al., 2016). This richness in protein fraction is accounted tothe protein solubilization during hydrolysis process as well as the exclusion of undesirable unsolvable solid matter through centrifugation (Chalamaiah et al., 2010). This further validates the potential use of FPH as protein supplements for human nutrition. Previous studies on peptides reported a fat content of below 5% from diverse seafood substrates (Bhaskar et al., 2008; Ovissipour et al., 2009) which is attributed to the process of centrifugation facilitating the removal of lipids along with insoluble fractions. Low moisture content of below 10% was reported in most of the studies on protein hydrolysates from various seafoods (Parvathy et al., 2016; Bhingarde, 2017). Thiscan be related to the nature of sample as well as to the higher temperatures employed during the drying process. Further, low moisture content facilitates better handling as well as storage stability to the hydrolysates. Values ranging from 0.45 - 27% of total composition were reported for ash content of fish protein hydrolysates (Yin et al., 2010; Mazorra-Manzano et al., 2012). These wide disparities must be due to the differences in the adopted hydrolysis processes including the usage of added acid or base for pH adjustment of the medium (Gbogouri et al., 2004; Dong et al., 2005; Choi et al., 2009). Numerous studies have been carriedout by researchers on the proximate content of protein hydrolysate from different seafood sources (Raftani Amiri et al., 2016; Bhingarde, 2017; Parvathy et al., 2018).

Hydrolysis of food proteins derives peptides composed of a mixture of amino acids and short chain peptides that exhibitnumerous beneficial properties facilitating their use as nutraceuticals or functional foods. Santos et al. (2011) opined that amino acid profileof fish protein hydrolysates is important because of its nutritional significance as well as its influence on the functional as well as bioactive properties. In seafoods, among the different body parts, fish muscle is the most extensively studied and reported one (Nakajima et al., 2009; Klompong et al., 2009). Numerous studies have been carried out by researchers on the amino acid profile of protein hydrolysate (Raftani Amiri et al., 2016, Tejpal et al., 2017). Reports suggest variations in the amino acid composition of FPH with regard to several factors:raw material used, enzyme source and hydrolysis conditions (Klompong et al., 2009; Chalamaiah et al., 2012). Aminoacids *viz.,* aspartic acid and glutamic acid were quantified to be in higher amounts in most of the reported studies (Parvathy et al., 2016; Wisuthiphaet et al., 2016). Similar to hydrolysates derived from main raw material, hydrolysates from other body parts *viz.,* head, viscera, skin etc. were also reported to have a balanced profile of amino acids (Gimenez et al., 2009; Ovissipour et al., 2009; Yin et al., 2010).

Functional properties

Functional properties, one of the major characteristics of protein hydrolysates, are those physicochemical attributes that influence the behavior of proteins in food systems during the course of storage, processing, preparation as well as utilization (Phillips et al., 1994). As they are influential with regard to the ultimate quality and organoleptic attributes in food, these properties areto be considered vital, especiallywhen used as food ingredients. These properties are associated to the protein structure *viz.,* the amino acid sequence as well as itscomposition, molecular weight and size of peptide, conformation and the net charge associated with the molecule (Casarinet al., 2008). Hydrolysis of protein generates a mixture of peptides and free aminoacids, increasing the number of polar groups and hydrolysate solubilities thereby modifying the functionalities and bioavailability (Adler-Nissen, 1986; Kristinsson & Rasco, 2000). Reports suggest the functional properties of peptidesto belinked to the concentration in the diet and to the protein source (Elaziz et al., 2017). Extensive studies have been carried out on the functional properties of FPH from different seafoods, indicating fish-derived bioactive peptides to have superior functional properties in comparison to other sources (Taheri et al., 2013) (Table 1). Elavarasan (2014) reported that FPH showed enhanced functional properties, in comparison to un-hydrolyzed fish protein, or other commercial food-grade stuffs. The important functional characteristics of FPH include solubility, foaming and emulsifying properties and fat absorption capacity (Motoki & Kumazawa, 2000).

Solubility

Solubility can be defined as thequantity of protein that goes into the solution under defined hydrolytic conditions. This property is regarded as the most important one and an excellent indicator of protein functionalities, as it influences the otherattributes like emulsifying and foaming properties (Mahmoud, 1994). Myofibrillar proteins from the parent source have solubilityissuesin water over a wide pH range. Enzymatic hydrolysis is promisive in this regard with improved solubility in wider pH range for the derived peptides, facilitating itssuitability for ready application in formulated food systems (Venugopal & Shahidi, 1994; Geirsdottir et al., 2011). Hydrophobic and ionic interactions are regarded as the key factors influencing the protein solubility, former promotingthe protein-protein interactions resulting in decreased solubility whereas latterfacilitate protein-water interactions leading to increased solubility (Adler-Nissen, 1986).

Fat Absorption Capacity

The ability of peptides to bind fat/oil is amajor characteristics that influences the product's palatability. This functional characteristic is essential especially for meat and confectionery industry. The fat absorption mechanism is mainly attributed to the physical entrapment of oil which is in turn linked to its bulk density and hydrophobicity. Protein hydrolysates develop this hydrophobicity upon undergoing hydrolysis wherein the parent chain is cleaved exposing the buried hydrophobic groups (Kristinsson & Rasco, 2000). However, excessive hydrolysis compromises structural integrity of the protein molecule resulting in itsnetwork degradation affecting its ability to entrap oil and hence not favorable as far as this property is concerned (Wasswa et al., 2007). Studies have suggested hydrolysates from seafood substrates to exhibit superior fat absorptionability in comparison to commercial food-grade ones demonstrating its application possibilities as oil binders in processed commercial food commodities.

Emulsifying properties

Food protein hydrolysates offer some desirable physical attributes when at the air–water interface. They arewell suited for the production of emulsions and foams with previous proved studies indicating their potential as good emulsifiers. This ison account of their enhanced amphiphilic nature with more hydrophilic and hydrophobic groups exposed enabling their orientation at the oil-water interface for more effectual adsorption (Klompong et al., 2007). Studies have reported that protein hydrolysates should have atleast twenty amino acid residues to possess good emulsifying properties (Kristinsson & Rasco, 2000). The three major attributes that brings desirable emulsifying properties in protein hydrolysates are their rapid interfacial adsorption ability, ability for effective unfolding and reorientation at an interface and ability to interact with the neighboring molecules at interface facilitating the formation of a strong cohesive, viscoelastic film capable of withstanding thermal and mechanical motions (Philips, 1981; Damodaran, 1996). However, the degree of hydrolysis need to be carefully monitored, as excessive hydrolysis can lead to decreased emulsifying capacity of hydrolysates (Kristinsson & Rasco, 2000; Gbogouri et al., 2004; Klompong et al., 2007).

Foaming properties

Foams are defined as a colloid in which particles of a gas are dispersed throughout a liquid (Green et al., 2013). Food peptides reveal great structural

Table 1. Recent studies on functional properties of fish protein hydrolysates

Functional properties	Fish species	References
Solubility	Blue whiting	Geirsdottir et al., 2011
	Skip jack tuna	Liu et al., 2015
	Common carp	Saputra & Nurhayati, 2016
	Alaska Pollock	Liu et al., 2018
	Yellowfin tuna	Parvathy et al., 2018a
	Mackerel tuna	Parvathy et al., 2018b
	Asian swamp eel	Halim & Sarbon, 2020
Fat Absorption Capacity	Tilapia	Foh et al., 2011
	Blue whiting	Geirsdottir et al., 2011
	Yellowtail King fish	He et al., 2015
	Mackerel Tuna	Parvathy et al., 2016
	Cuttle fish	Raftani Amiri et al., 2016
	Alaska Pollock	Liu et al., 2018
	Finstripe goatfish	Akhila et al., 2019
	Asian swamp eel	Halim & Sarbon, 2020
Emulsifying properties	Tilapia	Foh et al., 2011
	Skip jack tuna	Liu et al., 2015
	Sardine and small-spotted catshark	Garcia-Moreno et al., 2016
	Pony fish	Johnrose et al., 2016
	Cuttle fish	Raftani Amiri et al., 2016
	Tilapia	Tejpal et al., 2017
	Alaska Pollock	Liu et al., 2018
	Starry triggerfish	Sripokar et al., 2019
	Finstripe goatfish	Akhila et al., 2019
	Asian swamp eel	Halim & Sarbon, 2020
Foaming properties	Rainbow trout	Taheri et al., 2013
	Pony fish	Johnrose et al., 2016
	Mackerel Tuna	Parvathy et al., 2016
	Cuttle fish	Raftani Amiri et al., 2016
	Tilapia	Tejpal et al., 2017
	Alaska Pollock	Liu et al., 2018
	Starry triggerfish	Sripokar et al., 2019
	Finstripe goatfish	Akhila et al., 2019
	Asian swamp eel	Halim & Sarbon, 2020
Sensory properties	Salmon	Kouakou et al., 2014; Steinsholm et al.,2020
	Nile tilapia	Yarnpakdee et al., 2015
	Atlantic Salmon	Aspevik et al., 2016
	Yellowfin tuna	Parvathy et al., 2018a
	Mackerel tuna	Parvathy et al., 2018b
	Cod	Steinsholm et al., 2020

variability and greater potential as surface active molecules. Being smaller proteins, their relatively higher surface diffusing ability, combined with their enhanced amphiphilic nature, make them effective surface-active proteins. Studies have proved that even lower concentrations of protein hydrolysates are capable of effective saturation at the air–water interface to form strong and dynamic film with better stability. Generally, foaming properties are defined by foaming capacity and foam stability. Similar to other functional properties, strict control over the hydrolytic process is essential to obtain desirable foaming characteristics in the derived peptides for its effective food applications.

Sensory properties

Although enzymatic cleavage of proteins is desirable for enhanced functionality in protein hydrolysates, the disadvantage associated with this mechanism is the bitterness development, recognized as a main hindrance concerning its utilization and commercialization (Dauksas et al., 2004). The mechanism of bitterness has been documented to be linked with the presence of bitter peptides mostly comprising of hydrophobic amino acids. In addition, the native protein, the peptide sequences, the hydrolytic conditions, extent of hydrolysis, concentration and position of bitter taste residues, number of carbons on the 'R' group of branched chain amino acids and amino acidconformation are also influential in bitterness generation in peptides (Kim & Li-Chan, 2006). Protein hydrolysis leads to the exposure of buried hydrophobic peptides, which interacts readily with the taste buds leading to bitterness detection. However extensive hydrolysis leads to the liberation of free amino acids, decreasing the bitterness perception because hydrophobic peptides are far more bitter compared with a mixture of free amino acids (Adler-Nissen, 1979).

Many techniques have been suggested to reduce or mask bitterness in hydrolysates. Strict control of hydrolysis and termination at low degree of hydrolysis, choosing the most appropriate enzyme like use of exopeptidases (Cheung et al., 2015; Fu et al., 2019), treating hydrolysates with activated carbon (Bansal & Goyal, 2005; Aspevik, 2016), extracting bitter peptides with solvents (Dauksas et al., 2004), plastein reaction (Gong et al., 2015), masking by adding additives or molecules (Tamura et al., 1990; Aspevik et al., 2016) are a few among the reported options in this regard.

Antioxidative properties

On account of the potential health issues prevailing regarding food safety, there is growing interest to identify substitutes *viz.,* natural and safe sources of food anti oxidants for replacing the synthetic ones (Di Bernardini et al., 2011). Protein hydrolysates from seafoods is well established for its antioxidative attributes, accountable for the bioactive peptides they possess. These antioxidant fish peptides have wide potential for application as functional ingredients in food and nutraceutical sectors due to their health promoting effects. They are dormant within the sequence of parent protein and are released upon enzymatic cleavage, usually varying from two to twenty amino acid residues with the molecular mass of less than 6 kilo Dalton (Wang et al., 2008; Bougatef et al., 2010).

Earlier in 1995, Shahidi et al. reported the first scientific work on the antioxidant property of fish protein hydrolysates which was followed by numerous studies on this bioactivity from diverse seafood sources (Zhong et al., 2011; Elavarasan et al., 2014; Srikanya et al., 2018; Sripokar et al., 2019; Parvathy et al., 2019). Davalos et al. (2004) in their studies, reported that several amino acids, *viz.,* trptophan, tyrosine and methionine, followed by cysteine, histidine and phenyl alanine showed high antioxidant activity. It is well studied that aromatic amino acid residues easily contribute protons to the electron deficient radicals thereby directly participating in the radical scavenging process. Further, the antioxidative potential of histidine containing peptides has been reported, which is ascribed to the hydrogen donating, lipid peroxyl radical trapping, as well as metal chelating property (Rajapakse et al., 2005).

Identification as well as quantification of individual anti oxidants in a desired food complex is significant and hence appropriate techniqueis to be selected to measure the antioxidative efficiency of peptides (Najafian & Babji, 2012). Based on the chemical reactions involved, assays to assess antioxidant capacity are generally grouped into two *viz.,* methods based on hydrogen atom transfer reactions and those based on electron transfer reactions (Huang et al., 2005). A few commonly used antioxidant assays include DPPH radical scavenging activity, ferric reducing antioxidant power assay, ABTS radical scavenging activity, linoleic acid autoxidation inhibition activity, reducing power activity and ROS scavenging activity (Chalamaiah et al., 2012).

Application potentials

The nutritional abundance linked to seafood proteins as well as their role as a main peptide source with multi functional activities is well established (Dhaval et al., 2016; Parvathy et al., 2019). This nutritional richness of proteins and their hydrolysates is basically associated with their essential amino acid profile while the nature of bioactive peptides determines the functionalities as well as bioactive properties (Saadi et al., 2015). In comparison to the parent protein, their biopeptides offer a lot of advantages *viz.,* relatively superior bioactivity, wide spectrum of therapeutic actions and are comparatively milder and safer. Fish protein hydrolysates have potential application as functional ingredients in different foods on account of the numerous important and unique properties that they possess *viz.,* water holding and oil absorption properties, protein solubility, gel forming ability, foaming and emulsification properties (Chalamaiah et al., 2010). Food application of fish protein hydrolysate accounts for nutritional enrichment as well as functional stabilization and studies have been reported in food systems like beverages and snack products (Sinha et al., 2007; Pacheco-Aguilar et al., 2008; Leksrisompong et al., 2012; Ismail & Sahibon, 2018). The water binding capability of hydrolysate results in hydrogen binding with food components facilitating water entrapment and finds suitability in foods like meat, sausages, breads, cakes, boiled foods etc (Ibarra et al., 2013). Incorporation of protein hydrolysate in soups and gravies on account of their viscous behaviour have been reported by Zhang et al. (2013). Similarly, the emulsifying property of protein hydrolysate facilitates formation and stabilization of fat emulsions in various products *viz.,* sausages, bologna, soups, cakes, protein spreads and mayonnaise (Sathivel et al., 2005b; Cavalheiro et al., 2014; Intarasirisawat et al., 2014; Parvathy et al., 2020). Incorporation of hydrolysate in various products *viz.,* meats, sausages, doughnuts, spreads, crackers, deep fried products exploring their fat absorption property were reported (Yu & Tan, 1990; He et al., 2015). The antioxidant property of fish protein hydrolysate has also been well demonstrated in various products *viz.,* fish muscle (Dekkers et al., 2011); fish fillets (Sathivel et al., 2008; Dey & Dora, 2014); fish sausage (Intarasirisawat et al., 2014); hamburgers (Bernardi et al., 2016); fish oil (Morales-Medina et al., 2016); dressed fish (Parvathy et al., 2018c); oil encapsulate (Parvathy et al., 2019) etc. Previous reports also suggest the powerful effect of FPH as a novel cryoprotectant to preserve the quality of frozen seafoods (Cheung et al., 2009). Studies were reported by Kittiphattanabawon (2012) in fishery product to reduce protein denaturation using protein hydrolysate. Sumaya-Martinez et al. (2005) suggested fish protein hydrolysate to be a good source of specific amino acids for dietic formulations. Studies have recommended fish peptides to be easily absorbed and utilized for various metabolic activities and hence are advised for nutritionally challenged individuals to meet their physiological needs (Clemente, 2000; Nesse et al., 2011). Protein hydrolysates also have wide application potential in sports nutrition as a highenergy supplement for enhanced muscle protein anabolism in healthy athletes (Manninen, 2009). However, for effective commercialization of FPH for applications in food or nutraceutical sector, prior comprehensive food safety tests need to be carried out. These include mainly histamine content test, microbiological test and allergy test which are necessary to ensure that the product meets the food safety standards (He et al., 2013).

Future Perspectives

Seafood derived peptides have proved to have its abundance with regard to the nutritional profile as well as bioactive properties playing a major role in human health and nutrition. The functional properties applicable to the food industry mainly include solubility, fat absorption capacity, foaming and emulsification properties. Other potent bioactivities include antioxidative, anti hypertensive, antiproliferative properties etc. However, the enzymatic hydrolytic process has still not extended beyond the laboratory outcomes to an industrial mode. More effective and optimized processing methods are mandatory for developing fish protein hydrolysate with superior functions in an economically feasible manner. The key research gaps include enzyme cost, low protein recovery, acceptability issues due to bitterness, high production time, unpredictability in production of desired molecular weight peptides with specific functions, lack of demonstration regarding applications in food and nutritional products etc. Further to support and commercialize FPH for food/ nutraceutical applications, relevant food safety tests need to be carried out. Successfully addressing these limitations can promise commercial expansion of fish protein hydrolysates as biofunctional ingredients for food and nutraceutical formulations.

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