Fish Protein Hydrolysates: A potential additive in foods

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Fig 1a. Fish protein hydrolysate solution

b. Fish protein hydrolysate powder

Introduction

The significance of fish as a quality source of protein with a balanced profile of amino acids, is well proven and hence is recommended by WHO as a major source of essential amino acids (Usydus et al., 2009). Fish protein hydrolyzates are derived by digestion of fish protein either enzymatically or chemically and is considered as an effective approach towards utilization of unrecognized fish biomass into edible protein products, instead of its utility as animal feed or fertilizer. Peptides derived by the enzymatic hydrolysis of fish proteins with well defined molecular weight ranges, tailor-made for superior functionalities and interesting bioactivities are in high demand (He et al., 2013). They also maintain a high essential amino acid content and better digestibility. Based on the extent of hydrolysis that the parent protein undergoes, the properties exhibited by the hydrolysate vary. Hydrolysates with low degree of hydrolysis exhibit better functional properties whereas a high degree of hydrolysis results in higher bioactive peptides of low molecular weight. More over protein recovery is a major factor of consideration during conversion of protein to its hydrolysate form from an economic point of view. Use of different fish species/ substrate, proteolytic enzymes and adequate control of the process parameters such as temperature, pH, time and enzyme-substrate ratio facilitate the optimized production of FPH with desirable molecular structures and bioactive properties with therapeutic or nutritional interest. Bioactive peptides isolated from various fish protein hydrolysates have shown numerous bioactivities such as anti-hypertensive, anti-thrombotic, immunomodulatory, anti-cancer, anti-coagulant, anti-diabetic, anti-obesity, hypocholesterolemic effect, anti-microbial, anti-oxidative and calcium binding activities. Further, fish protein hydrolysates contain higher protein content of 60% to 90% which demonstrates its potential use as protein supplements for human nutrition. Hence, FPH could successfully replace functional compounds such as sodium caseinate and BSA used in food formulations. The numerous essential and unique properties they possess viz., water holding and oil binding capacities, solubility, gelation ability, foaming and emulsification ability etc. make them potential candidate as functional ingredients for application in different foods like cereal products, seafoods, meat products etc.

Nutritional composition of fish protein hydrolysates

The chemical composition of fish protein hydrolysates is significantfrom the nutritionalviewpointfor better human well-being. Of the various components, as the name implies, protein is the major constituent of interest in fish protein hydrolysate.Studies have reported a protein content in the range of 60% to 90% of total composition (Choi et al., 2009; Parvathy et al., 2016). Protein in the raw material are selectively extracted by hydrolysis and the unwanted insoluble fractions are removed by centrifugation during the process.Removal of lipids also occur during the centrifugation process together with other insoluble fraction resulting in a fat content of below 5 %, for most of the fish protein hydrolysates. Reports suggest a moisture below 10% for different fish protein hydrolysateswhich is on account of the higher temperatures employed during drying process of hydrolysate solution for better storage stability. The ash content of fish protein hydrolysates ranged between 0.45% to 27% of whole composition with suggestionthat theapplication of acid or base for pH adjustment of medium to berelated to the high ash content in some hydrolysates.

Hydrolysis of proteins derives a mixture of free amino acids and short chain peptides with superior nutritional profile widening its applicability innutraceuticals or functional foods. The amino acid profile of fish protein hydrolysatessignifies the nutritional value as well as influences the other properties. Inherent and external factors like raw material composition, enzymes and hydrolytic parameters influence the aminoacid profile of the derived fish protein hydrolysates (Klompong et al., 2009). Previous studies report aspartic and glutamic acid to be higher in most of the fish protein hydrolysates.

Similar to fish muscle hydrolysates, head, skin and visceral hydrolysates were also reported to contain all the essential and non-essential amino acids which also signifies the applicability of waste utilization for high value products.

Functional properties

Functional properties are those physicochemical properties accountable for the behavior of protein in food systems during different stages like storage, processing, preparation and consumption. These attributes in turn influences the quality and organoleptic attributes in food and therefore areparticularly important when used as ingredients in food products. Functional properties are related to protein structure viz., the sequence and composition of amino acids, molecular weights, conformation and the net charge distributed on the molecule. Enzymatic hydrolysis generates a mixture of free aminoacids, di-, tri- and oligopeptides, increases the number of polar groups and hydrolysatesolubilities thereby modifying the functionalities and bioavailability (Kristinsson and Rasco, 2000).

Functional properties are important when the fish protein hydrolysates interact with other components of food such as oil and water.

The important functional properties of FPH include solubility, water absorption capacity, emulsifying properties, foaming properties and oil binding capacity.

Solubility

Protein solubility is referred to as the quantity of protein that goes into the solution under identified settings andis one of the key functional property as this in turn influences other functional attributes like emulsifying and foaming properties. Hence an excellent solubility index widens the protein functionalities and its potential applications.

Fish myofibrillar proteins in its intact or native form lacks solubility in a wide pH range while enzymatic hydrolysis of these proteins increases its solubility index. Factors like hydrophobic and ionic interactions impact the solubility characteristics of proteins.

Hydrophobic interactions promote protein-protein interactions which decreases their solubility whereas ionic interactions support protein-water interactions favoring the solubility characteristics. Fish protein hydrolysate, being soluble in a varied range of ionic strengths and pH makes it promising to be used in a variety of seafood products for enhanced properties.

Extent of hydrolysis is related to the protein solubility with longer hydrolysisperiod leading to a high degree of hydrolysis resulting in protein solutions with smaller molecular weights of higher solubility. It is also thought that an increase in hydrophilic polar groups lead to an increased water-solubility.

Oil Binding Capacity

Oil binding capacity is a key attribute which influences the product palatability and hence is a major characteristics considered in meat and confectionery industries. This propertyis correlated with surface hydrophobicity and protein hydrolysates develops this hydrophobicity on account of the hydrolysis which cleaves the protein chain resulting in the exposure of more internal hydrophobic groups (Kristinsson and Rasco, 2000). The fat absorption property is ascribed mostly to the physical entrapment of the oil and this inturn is influenced directly by physical attributes like bulk density of the protein sample.

Emulsifying properties

Emulsions are thermodynamically unstable systems as a result of the large positive energy at the interface of the two liquids(Comas et al.,2006). Emulsifiers are able to form a protective coatingaround the oil droplets leading to prevention of coalescence phenomenon.The emulsifying properties of FPH are directly connected to their surface properties. Fish protein hydrolysates, on account of their enhanced amphiphilic nature, act as good emulsifier.

Protein hydrolysis results in exposure of more hydrophilic and hydrophobic groups facilitating their orientation at the oil–water interface enabling effective adsorption(Klomponget al., 2007). Studies suggest protein hydrolysatesto have at least 20 residues to possess good emulsifying capacity.

As these properties are generally inversely related to the extent of hydrolysis, careful monitoring of hydrolysis is important for desired emulsifying properties.

Foaming properties

Food foams comprise of dispersed air droplets which are enveloped by a continuous liquid phase with a soluble surfactant that depress the surface as well as interfacial tension of the liquid (Kinsella, 1976). Similar to emulsifying properties, foaming properties also rely on protein's surface properties. Hydrophobicity have a significant correlation to foaming formation and comparable to other functional properties, these attributes are also related to the degree of hydrolysis. Foaming properties are usually expressed as foaming capacity and foam stability. Foaming capacity is referred to as the ability of protein to form foams also described as overrun,which is the excess amount of foam produced on whipping a protein solution in comparison to the initial protein solution volume. Foam stability is measured as the decrease in volume of the whipped protein solution within a specific period.

Sensory properties

Sensory properties of a commodity is of paramount importance for itseffective adaptation in the food sector (Kristinnson and Rasco, 2000). Though enzymatic hydrolysis of proteins is essential for enhancing the required functional properties, it has the drawback of bitterness promotionwhich is a major hindrance with respect to the utilization and commercialization of bioactive protein hydrolysates (Kim and Wijesekara, 2010).One of the generally accepted mechanism of bitterness is the role played by hydrophobic amino acids of peptides. During hydrolysis of protein theburied hydrophobic peptides are exposed which results in bitterness detection by readily interacting with the taste buds. Various techniques have been advocated to reduce or mask bitterness in protein hydrolysates of which strict control of the hydrolysis processby termination at a low degree of hydrolysis isone of the effective method for improved functional as well as sensory properties.

Selection of the most suitable enzyme is also a prevalent methodology for bitterness reduction, as different enzymes have different aminoacid preferences. Bitterness generation is also correlated to the production of oxidation products during hydrolysis process and subsequent storage. A few recommendedapproaches for bitterness reduction includes the use of activated carbon to treat hydrolysatesfor absorption of bitter peptides, use of extracting solvents and by plastein reaction,a reverse hydrolysis process.

Masking is yet another effective technique to reduce the bitterness of peptides and is achieved by incorporationof additives or molecules. Masking additives promotes conformational alterations of the peptides and introduction of sweet tastes that cover the bitterness.

Antioxidant activity

Lipid oxidation is a major concern in the food industry as itresults information of undesirable off-flavors, off-odors, undesirable colors, taste deterioration as well as development of potentially lethal reaction products. Further diseases like cancer, coronary diseases and Alzheimer's are also reported to occur partially on account of oxidation/free radical reactions in the human system. Control of lipid oxidation in foods is effective by reducing the interaction with metal ions as well as by minimizing exposure to light and oxygen by means of proper packaging as well as by antioxidant incorporation. Fish protein hydrolysate is well established for its antioxidant properties on account for the bioactive peptides they possess. Bougatef et al.(2010) reported superior antioxidant property for peptides with amino acid residues ranging from 2-20 having amolecular mass of less than 6 kDa. These peptides remain inactive within the native protein sequence and is cleaved and released upon enzymatic hydrolysis.

Applications of Fish Protein Hydrolysates

Fish protein hydrolysates are source of specific aminoacids for dietic formulations which are readily absorbed and exploited for numerous metabolic processes (Nesse et al., 2011). Theyfind potential application as functional ingredients in different foods such as geriatric products, high energy food products, weight reductionsupplements, therapeutic or enteric diets, hypoallergic infant formulas etc.They also holdseveralsignificant and unique functional and antioxidant properties facilitating their potential application in food systems (Chalamaiah et al., 2010). On account of this, they have been successfully tested as emulsifiers, foaming agents, dispersants etc for incorporation into diverse food products like cereal based stuffs, meat and seafoods, confectionaries etc. for providing desirable characteristics to the product as well as to improve their storage stability. Reportsalso suggesttheir applicability as a potential cryoprotectant for quality maintenance of frozen fishery products. Similarly protein hydrolysates are well proven for their applicability as antioxidant in food systems (Parvathy et al., 2018).Moreover, FPH is also found to be effective in reducing the fat uptake in deep fried battered products and for water retention in fish fillets by replacing the commercially used water retention additive. A number of studies have been carried out at ICAR-Central Institute of Fisheries Technology on the application of fish protein hydrolysates in foods (Fig.1). Hence the application potential of FPH is immense opening wide market demand in the food and allied industry.

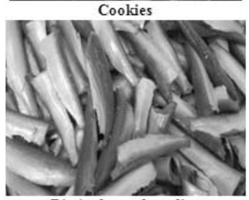
Conclusion : Future research should focus on upscaling of the laboratory outcomes to facilitate industrial production of fish protein hydrolysate. The main barrier behind upscaling of enzymatic hydrolysate is its low protein recovery, high cost, more production time requirement and sensory unacceptability due to bitterness. Hence more research need to be focused on the process modifications so as to reduce the cost of production by process optimization of enzymatic hydrolytic conditions emphasizing attributes like protein recovery, functionalities and sensory attributes. Effective addressal of these research gaps can facilitate commercialization of fish protein hydrolysatesfor its applicability as functional ingredients infood and nutritional supplements.

Table 1. Food Applications of Fish Protein Hydrolysate

Properties	Mode of action	Food systems
Solubility	Protein solvation	Beverages
Water absorption and binding	Hydrogen bonding of water; Entrapment of water (no drip)	Meat, cakes,breads,sausages, boiled foods
Viscosity	Thickening; water binding	Soups, gravies
Emulsification	Formation and stabilization of fat emulsions	Sausages, soups, bologna, cakes, mayonnaise and protein spreads
Fat absorption	Binding of free fat	Meats,sausages, crackers,doughnuts, spreads, deep fried products
Antioxidant	Delay the fat oxidation	Fish fillets, sausage, hamburgers, fish oil
Cryoprotection	Reduces protein denaturation	Fish mince and surimi


Cookies


Fish oil encapsulate


Dip in dressed sardine


Mayonnaise


Extruded snack

Fig 2. Application of FPH in different foods