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Preparation of fish peptide powder through enzymatic hydrolysis of white croaker (*Otolithoides pama*)

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Abstract: The present study was conducted to prepare Fish peptide powder (FPP) through enzymatic hydrolysis of low-cost fish, white croaker (*Otolithoides pama*) and to determine its nutritional and functional properties. As source of extracellular protease, three types of enzymes *viz.* purified papain, papaya peel and ginger were used in triplicates and these enzymes were found to be very effective to break the fish muscle protein down. Highest protein concentration (89.12%) was found in peptides using purified papain followed by using papaya peel (87.46%) and ginger (76.04%). Solubility, foam expansion and emulsifying activity index (EAI) were determined to analyze its functional properties. Purified papain showed the highest solubility (84%) followed by 80% and 72% in FPP using papaya peel and ginger respectively. Three sample concentrations (0.1%, 0.5% and 1%) were used in triplicates to determine foam expansion percentage and EAI. The stability of foam was increased and EAI was decreased with increasing concentrations of samples. The study concluded that papaya peel would be a source of low-cost protease enzyme as an alternative of purified papain as the protein percentage did not vary significantly.

Keywords: fish peptides; enzymatic hydrolysis; protease; papaya; white croaker

1. Introduction

Protein-energy malnutrition is a major cause of morbidity and mortality in children of developing countries (Müller *et al.*, 2005). Providing a diet containing adequate macro- and micro-nutrients is the mainstay when it comes to feed the children with adequate nutrition. However, the problem remains that poor family cannot easily take well-balanced protein because of high cost of animal food which include well-balanced protein. While 'fish and rice' used to be a traditional diet in Bangladesh, fish in many cases is no longer affordable for the poor. Current practices of fishing and marketing through cold chains make fish expensive delicacies for underprivileged people. Using a new biotechnological way, it is possible to process fish into fish peptides so that it can be used as an inexpensive protein source for low-income group of the country.

During fishing in the Bay of Bengal different types of fish are caught of which some of them are not economically important. Therefore this low-cost fish can effectively be utilized to prepare fish peptide. Although, the coastal farmers earn their livelihood mainly by fishing and fish drying activities in Bangladesh, the practice of manufacturing different value added products such as fish peptide powder would be a good potential income source for them. As the production of fish peptides is a promising sector throughout the world, preparing this from low valued fish will trigger the economy of our country. This powder form product will be more preferable food item rather than the available ones, for its ease to use as well as fulfilling the nutraceutical requirements. Moreover, another important aspect of this product is that it does not require any special preservation measures.

Fish peptide powder, also known as fish surimi peptide is a food grade product prepared through enzymatic hydrolysis of white fish muscle (Aristotelis *et al.*, 2011). It can also be prepared from shrimp using shrimp muscle (Suetsuna, 2000). As the product consists of all kinds of amino acids including nine essential amino acids; it can be used as an attractive alternative source of protein and amino acids in the diet of all age group. It has several health benefits for human health as it improves lipid metabolism, boosts weight loss and controls blood glucose resulting controlled blood pressure (Bragadottir *et al.*, 2007). In addition, it has anti-aging and anti-carcinogenic properties (Guha *et al.*, 2013).

Considering all these into account, the present study was designed and focused on preparing fish peptide powder from low-cost fish to determine its nutritional profile, storage condition and acceptability.

2. Materials and Methods

2.1. Preparation of fish peptide powder

The study was started from April, 2016 to March, 2017 at Nutrition and Fish Processing Laboratory of Faculty of Fisheries in Chittagong Veterinary and Animal Sciences University, Bangladesh.

2.2. Collection of low-cost fish species

As a low-cost white fish i.e. White croaker (*Otolithoides argenteus*), weighing 350 g in average was collected from local fish landing center, Chittagong in iced condition and transported to the laboratory immediately. The collected fish was thoroughly washed, decapitated, gutted and filleted in laboratory. Fatty belly portion of the fish was discarded during processing.

2.3. Surimi preparation

The muscle part was scooped by spatula from the fillet. Collected muscle was washed under running tap water for 3 times for removal of exudates and residual contents followed by sieving through a fine meshed net for removal of excess water. Mincing of fish muscle was done using sharp knife. Then the intermuscular bones were separated with bone remover and the surimi was placed in ice in a beaker.

2.4. Inclusion of enzymes

In the present study three types of enzymes were used for hydrolysis of fish muscle which is enriched with protease enzymes i.e. purified papain enzymes, papaya peel (Khairuddin *et al.*, 2016) and ginger peel (Nafi *et al.*, 2013). Enzyme concentrations were added in fish muscle at 5% level. Each treatment contained three replications, whereas papain was control. The sample was kept in beaker and placed in water bath at 45⁰C for 2 hours for breakdown of protein. Mixing was done by a spatula and continuous stirring was carried out throughout the step.

2.5. Heating

When whole muscle part takes a form of slurry in beaker the temperature of the water bath was increased to 60⁰C for inactivation of protease enzyme.

2.6. Drying and grinding

After cooling, drying was carried out in hot air oven at 105⁰C for 12 hours. The dried content was collected from the crucibles and finely powdered by using a high speed grinder. The powder was collected in a sample jar, labeled properly and stored in a dry and cool place.

2.7. Proximate composition analysis

Protein, lipid, moisture and ash percentage of the fish peptide powder were analyzed in triplicates according to the method of AOAC (2000) and mean value was recorded. The protein and fat content were expressed on a dry weight basis.

2.8. Functional properties analysis

Determination of solubility were done according to Morr (1985). Emulsifying activity index (EAI) were determined according to method reported by Pearce and Kinsella (1978). Foaming ability and foam stability of fish peptide powder were analyzed according to the Shahidi et al. (1995).

2.9. Statistical analysis

Analysis of variance (ANOVA) was performed and means comparison were calculated by Duncan's multiple range tests. All experiment were carried out in triplicate. Analysis was performed using SPSS package (SPSS 12.0)

3. Results and Discussion

In the present study, fish peptide powder was successfully prepared in laboratory and it was gone through proximate composition and functional properties analysis.

3.1. Proximate composition analysis

There were no significant variation in moisture and fat content of fish peptide powder prepared using purified papain, papaya peel and ginger, while there were differences on protein and ash content (Table 1).

Fish peptide powder using purified papain enzyme had higher protein content (89.12%) compared to fish peptides produced from papaya peel (87.46%) and ginger (76.04%). Although protein concentration varied significantly but was still high and could be used as a potential source of protein. These findings are almost similar to the result of Kristinsson and Rasco (2000), where they found very high protein concentration (93.17%) through the enzymatic hydrolysis of lean fish. In the present study, the little bit variation in protein content was due to the scarcity of spray drying technique which is considered an ideal drying technique of fish peptide preparation. Nicharee *et al.* (2016) concluded that enzymatic hydrolysis with purified papain would be much more efficient than hydrolysis with other source. It would be the reason of getting high protein concentration in peptide hydrolyzed by pure papain compared to other sources. Although there is insufficient evidence of preparing fish peptides using papaya peel and ginger but these could be potential sources of low cost protease enzyme.

3.2. Functional properties analysis

In the present study, functional properties of fish peptide powder were analyzed by determining its solubility, emulsifying activity index (EAI) and foam expansion percentage.

3.2.1. Solubility

Solubility is one of the most significant aspect of functional properties of FPP. Good solubility of protein is required in many functional applications, especially for emulsions, foams and gel. Solubility of FPP using purified papain, papaya peel and ginger is presented in Figure 1. FPP from purified papain showed the highest solubility (84%) followed by 80% and 72% in FPP using papaya peel and ginger respectively.

3.2.2. Emulsification properties

The Emulsifying Activity Index (EAI) of FPP using purified papain, papaya peel and ginger are shown in Figure 2. The methods are generally used to measure the ability of FPP to form and stabilize emulsions (Kinsella, 1976). The present study found decreasing of emulsifying activity with increasing sample concentrations. Muzaifa *et al.* (2012) found similar decreasing trend in emulsifying activity with increasing concentrations. Gbogouri *et al.* (2004) reported higher emulsifying activity and emulsion stability in salmon by-product hydrolysate.

3.2.3. Foam expansion

Foaming properties are usually expressed as foam formation and stability. The stability of foam were increased when sample concentrations increased from 0.1% to 1% (Figure 3). Muzaifa *et al.* (2012) found similar increasing trend in foam expansion with increasing concentrations. In addition, this result is in agreement with Thiansilakul *et al.* (2007) who studied the foam ability protein hydrolysates from round scad (*Decapterus maruadsi*).

Table 1. Proximate composition of FPP using different enzymes.

Enzyme source	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Purified papain	5.33± 1.13 ^a	89.12±1.23 ^a	1.12±0.45 ^a	3.02±0.63 ^a
Papaya peel	5.54±0.78 ^a	87.46±2.34 ^a	1.47±0.32 ^a	5.11±0.23 ^b
Ginger	7.3±1.1 ^a	76.04±2.16 ^b	1.95±0.78 ^b	11.53±0.76 ^c

Values with the same superscript letters within the same column are not significantly ($p < 0.05$) varied.

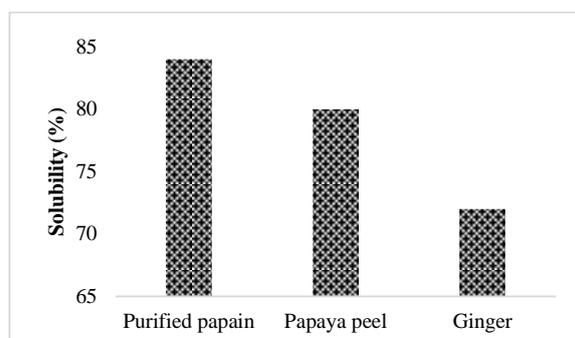


Figure 1. Influence of different enzymes on solubility (%) of fish peptide powder.

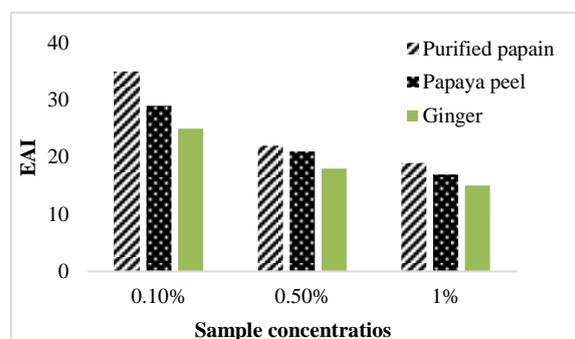


Figure 2. Influence of FPP concentration using different enzyme on emulsifying activity index (EAI).

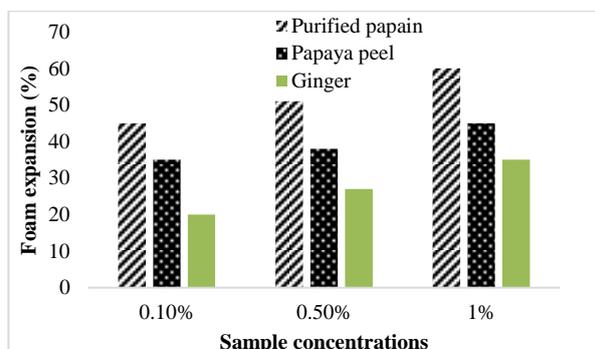


Figure 3. Effect of FPP concentration to foaming properties using different enzymes.

4. Conclusions

The fish peptide powder prepared from low-cost fish using purified papain, papaya peel and ginger may potentially serve as a good source of protein. FPP using purified papain and papaya peel have greater of protein content, solubility, emulsifying and foaming properties compared to FPP using ginger. It can be concluded that papaya peel would be a source of low-cost protease enzyme as an alternative of purified papain as the protein percentage was high in both cases.

Conflict of interest

None to declare.

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