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Synthesis of protein hydrolysate from *Scomberomorus commerson* by enzymatic hydrolysis

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ABSTRACT

Huge quantity of fishery wastes and by-products generated every year either doesn't been utilized properly or simply dumped in the sea to lead environmental issues. The discarded wastes are rich source of protein that can be made use in various commercial and industrial applications. The protein hydrolysate recovered from various fishes and fish parts were shown to have antioxidant, antihypertensive, immunomodulatory, neuroactive, and antimicrobial properties. The chemical methods used for the protein recovery does not produce the yield with required standards. Thus enzymatic recovery of protein hydrolysate is preferred choice and this technique is gaining momentum now. The present study was designed to produce a protein hydrolysate from the skin of *Scomberomorus commerson* by tryptic digestion. The results showed that the enzyme was capable to recover considerable quantity of protein and the protein hydrolysate prepared was undergone good degree of hydrolysis. Thus the protein hydrolysate of the skin of *Scomberomorus commerson* is a promising candidate for further characterization.

KEYWORDS

Scomberomorus commerson;
Trypsin;
Protein hydrolysate.

INTRODUCTION

The continuous increase in global fish resources results in 25% of wastage among total fish catch annually[1]. Every year, huge quantity of solid waste in the form of fish head, vicera, skin, bones, frames, and some muscle tissue[2] are discarded from seafood processing plants. Either these marine wastes are underutilized to produce low market value products such as fish meal or fertilizer, or dumped to lead the environmental issues. Complete utilization of fishery wastes for recovering high-end products would be fruitful strategy to overcome the issue and increase the economic gain. Fishery wastes and by-products are valuable sources of raw material for recovery of bioactive compounds. The fishery wastes converted by proteolytic hydrolysis into a more marketable and functional form is called as fish protein hydrolysate (FPH)[3].

Chemical and biological methods can be used for the production of hydrolysates. Protein substrates un-

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dergoing hydrolytic reaction with strong chemical and solvents yield the products of low nutritional value with poor functional ability and could not be utilized by food industry. The innovative method of enzymatic degradation of proteins with specific proteolytic enzymes to cleave particular peptide bond to produce fish protein hydrolysate with desired functional properties gaining momentum now[4]. Digestion parameters such as choice of enzyme, time of incubation, temperature and pH are tightly controlled to produce fish protein hydrolysates with desired functional and nutritional properties. Protein hydrolysates are produced for a wide variety of uses in the food industry, including milk replacers, animal food, media for growing microorganisms, protein supplements, stabilizers in beverages and flavor enhancers in confectionery products[5]. The development of fish protein hydrolysates as functional food ingredients have been relatively a recent technology gaining popularity due to the array of potential bioactive properties associated with them, including antioxidant, antihypertensive, immunomodulatory, neuroactive, antimicrobial, and mineral or hormone regulating abilities[6]. Production of protein hydrolysate from different fish species such as mackerel[7], herring[8], tuna cooking juice[9] by different enzyms have been reported earlier. The present study focus in the production and compositional analysis protein hydrolysate from *Scomberomorus commerson* (Narrow-barred Spanish mackerel), one of the important mackerel highly exploited in Indian seas[10].

EXPERIMENTAL

Fish sample

Enough quantity of the Marine fish, *Scomberomorus commerson* (Narrow-barred Spanish mackerel) was collected from coast of Chennai, Tamilnadu, India, and the skin portion was separated from the fishes, minced for uniformity and stored in plastic bags at -20^{0}C until used.

Chemicals and reagents

The enzyme trypsin for proteolytic digestion was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and all other chemicals and reagents (acids, bases, solvents and salts) used were of analytical grade obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India and Glaxo Laboratoreis, CDH division, Mumbai, India.

Preparation of fish protein hydrolysate

The proteolytic digestion of *Scomberomorus commerson* (Narrow-barred Spanish mackerel) was performed according to the method described by Je et al. (2007)[3] with minimal modification. To produce protein hyrolysate from fish skin, the enzymatic hydrolysis was carried out with the enzyme trypsin (phosphate buffer 0.1 M Na_2HPO_4- NaH_2PO_4; pH-8, temperature, 37 °C) at enzyme/substrate ratio (E/S) (1 / 100 w/ w). The 300 g minced fraction of *Scomberomorus commerson* was homogenized with blender and then thoroughly mixed with 3g of enzyme. The mixture was incubated for 6 h with continuous stirring and then heated in a boiling water bath at 100°C for 10 min to inactivate enzyme activity. The content was then centrifuged at 10000 rpm for 15 minutes and supernatant obtained was the fish protein hydrolysate. The hydrolysate was lyophilized to get a powdered sample and was stored at -20ºC.

Yield and degree of hydrolysis

The extent of hydrolysis was determined by adapting the procedure described by Chuan-He Tang et al. (2009)[11]. Briefly, the sample was mixed with trypsin enzyme with different enzyme/substrate ratio (E/S) (1/100, 2/100, 4/200 w/w) and the reaction was conducted at pH 8.0 and temperature, 37 °C (optimal conditions) for 0.5, 1, 2, 3, 4, 5 and 6 h. The pH of the mixture was maintained constant during hydrolysis using 2 M NaOH. After hydrolysis, the pH of the broths was brought to 7.0, and the solutions were then heated at 100°C for 10 min to inactivate the enzyme. The Hydrolysates were centrifuged at 10000g for 15 min, and the supernatants were lyophilized to get a powdered sample and were stored at -20ºC. The degree of hydrolysis (DH) is defined as the ratio between the number of broken peptide bonds (h) and the total number of peptide bonds per mass unit (h_{tot}).

$$DH\% = \frac{h}{h_{tot}} \times 100$$

The degree of hydrolysis (DH) of hydrolyzed protein was determined by measuring the amount of free α-

amino groups based on the reaction between Sanger's reagent of fluorodinitrobenzene (FDNB) and the amino groups in the amino acids which resulted a yellow complex of amino acids[12]. The absorbance was measured spectrophotometrically at 410 nm.

The nitrogen recovery (NR) in the soluble fraction was calculated using the method of Benjakul and Morrissey (1997)[13] by the following formula.

$$NR\% = \frac{\text{Total nitrogen in the soluble fraction}}{\text{Total nitrogen in the substrate}} X\ 100$$

Proximate composition

Moisture content was determined by placing approximately 2 g of sample into a pre-weighed aluminum dish. Samples were then dried in a forced-air convection oven at 105°C overnight or until a constant weight was reached[14]. The total crude protein (N X 6.25) content of samples was determined using the Kjeldahl method[14]. Total lipids in each sample were extracted with a mixture of chloroform and methanol as described by Bligh and Dyer[15]. The content of minerals (expressed as percent ash content) was determined by charring approximately 2 g of sample in a crucible over a Bunsen burner and then heating in a muffle furnace at 550°C until the ash had a white appearance[14].

Statistical analysis

The statistical analysis of data was performed by using SPSS 16 for windows. The results were expressed as mean of triplicates ± SD.

RESULTS AND DISCUSSION

Protein recovery

The degree of hydrolysis has been used as an indicator of the cleavage of peptide bond, whereas nitrogen recovery reflects the yield of proteins that can be recovered from the hydrolysis process. Enzymatic digestion of protein results in the release of peptides during hydrolysis. Figure 1 describes the recovery of protein in terms of yield % with respect to incubation time. It's common that yield of protein increase with increase in the time of hydrolysis. Maximum 61.9% of protein was recovered at 6 hours of incubation. The results suggest that trypsin would be a choice of enzyme for preparing protein hydrolysate from the skin of *Scomberomorus commerson*.

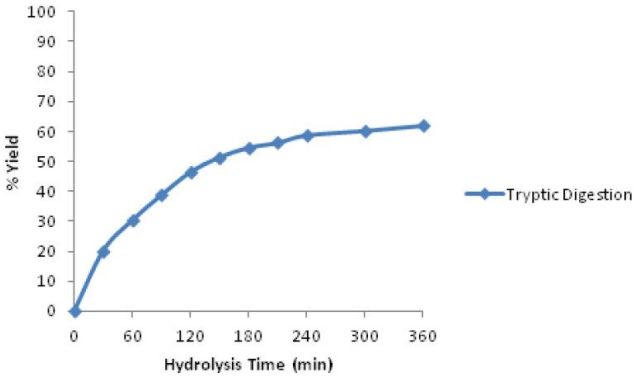

Figure 1 : Protein recovery from the skin of *Scomberomorus commerson* with respect to incubation time.

Degree of hydrolysis

DH estimates the change of peptide content in a hydrolytic reaction. It is generally used as a proteolysis monitoring parameter[16] and an important factor highly related with the hydrolytic process yield[17]. The results of DH are presented in Figure 2. The typical shape of curve obtained in the present study was reported earlier in many investigations by different investigators[8,18,19]. The DH was observed to be 29.7% for enzyme substrate ratio 1:100, 32.2% for 2:100 and 34.2% for 4:100 which was well within the range of earlier observations made from the skin of different fish species[20]. The varied pattern of DH was closely reliant upon applied enzyme concentration, namely the E/S ratio. With E/S ratio increasing (from 1:100, 2:100 and 4:100 w/ w), the rate of DH increase during initial hydrolysis process. However, during final process (e.g., at a hydrolysis time >360 min), the rate of DH increase at different E/S ratios became similar.

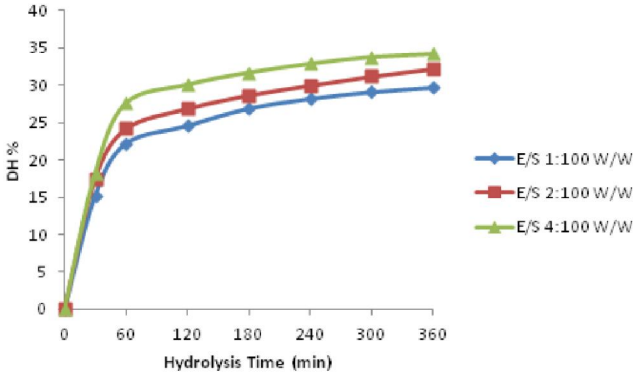

Figure 2 : Extent of hydrolysis influenced by E/S ratio with respect to incubation time.

Proximate analysis

The composition of protein hydrolysates generally depends on choice of enzyme, pH, incubation time and

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analytical methods used for estimation. In the present study the trypsin enzyme with appropriate pH and temperature optima was used to get the protein hydrolysate. The composition of protein hydrolysate in terms of protein content, lipid, moisture and ash contents of both the crude wet sample and freeze dried protein hydrolysate *Scomberomorus commerson* were measured. The results were expressed as a mean of triplicate ± SD and represented in Figure 3. The minced skin sample of *Scomberomorus commersoon* showed higher moisture content and least lipid content. The lyophilized protein hydrolysate showed protein content of around 86% which was in par with earlier findings[21].

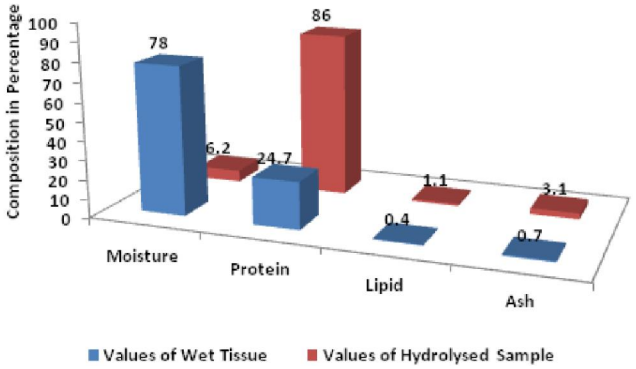

Figure 3 : Proximate analysis of crude sample and protein hydrolysate from *Scomberomorus commerson*.

CONCLUSION

The results of the present investigation clearly reveal that skin sample of *Scomberomorus commerson* could be utilized for the protein recovery by the proteolytic enzyme trypsin. The findings of the DH analysis clearly suggest that the sample is rapidly undergoing hydrolysis to produce small peptides during hydrolysis and not much influenced by higher E/S ratio. Proximate analysis revealed higher protein content in the sample and was justified by higher protein recovery during hydrolysis. The protein yield was high enough for further characterization. From this study it can be concluded that skin protein hydrolysate of *Scomberomorus commerson* is an ideal choice of protein substrate for further characterization to evaluate biomedical and commercial aspects.

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