Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 3| Issue 1 |JAN-MAR |2013|138-144

Research Article
Biological Sciences

PROXIMATE ANALYSIS AND PRODUCTION OF PROTEIN HYDROLYSATE FROM KING FISH OF ARABIAN GULF COAST - SAUDI ARABIA

*Sheriff Sheik Abdulazeez [1], Baranitharan Ramamoorthy[2] Ponmurugan Ponnusamy[3]

[1*&2]College of Applied Medical Sciences, Shaqra, Shaqra University, Kingdom of Saudi Arabia

[3]K. S. Rangasamy College of Technology, Thiruchengode, Tamil Nadu, India

*Corresponding Author Email: biosherif@yahoo.co.in

ABSTRACT

*Saudi Arabia documents continuous increase in the utilization of fish and fish based food products. However, the huge quantity of fishery wastes and by-products generated every year either doesn't been utilized properly or simply dumped. The discarded wastes are rich sources of protein that can be made use in various commercial and industrial applications. The protein hydrolysates 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 yields with required standards. Thus enzymatic recovery of protein hydrolysate is the preferred choice and this technique is gaining momentum now. The present study was designed to produce a protein hydrolysate from the skin of king fish (**Scomberomorus commerson)** by papain digestion. The results showed that the enzyme was capable of recovering significant quantity of protein from the skin of king fish. The protein hydrolysate produced was exhibiting good degree of hydrolysis to yield peptides in solution. Thus the protein hydrolysate is a interesting candidate to be explored for its bioactive properties.*

KEY WORDS

King fish, papain, protein hydrolysate, Saudi Arabia.

INTRODUCTION

The continuous increase in global fish resources results in 25% of wastage among total fish catch annually [1]. Fish and fish derived products are invariable diet of Saudi population. Fish consumption is an integral practice of Saudis and the rate of consumption is increasing continuously. 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 sea food processing plants. Either these marine wastes are underutilized to produce low market value products such as fish meal or fertilizer, or simply dumped. Complete utilization of fishery wastes for recovering high-end products would be an ideal strategy for an 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].

Protein hydrolysates can be prepared by conventional chemical methods with strong chemicals and solvents. The usage of such chemicals makes the products unsuitable to be used in food industry. The protein hydrolysates produced were of low nutritional value with poor functional ability and could not be utilized for commercial applications. Enzyme mediated

protein recovery from fishery wastes have been a recent research arena. This method suits to produce fish protein hydrolysate with desired functional properties [4]. Digestion parameters such as choice of enzyme, time of incubation, temperature and pH are tightly controlled to produce fish protein hydrolysates with the desired functional and nutritional values. The Protein hydrolysates thus obtained are already gaining reputation and widely used in variety of food industries in various forms, such as milk replacers, stabilizers of beverages, flavor enhancers in confectionery products, protein supplements, animal food, and microbial media[5] etc. The development of fish protein hydrolysates as functional food ingredients have been gaining popularity due to an 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 was designed to recover protein from the skin of king fish commonly known as Kanad in Arabic. King fish is one of the important commercial fish caught in Arabian Gulf coast of Saudi Arabia [10]. The proximate composition of crude sample and freeze dried hydrolysate were evaluated. The enzyme papain was selected for the study as it is a cheap, easily available, commercial enzyme.

MATERIALS AND METHODS

Collection of fish sample

King fish, commonly known as kanad in Arabic, caught from the Arabian Gulf Coast of Saudi Arabia was purchased from Saudi Fisheries Company. The sample was immediately sealed in a plastic bag, placed in icebox and immediately transferred to the Shaqra University Research Laboratory. In the University Laboratory, the skin portion was separated from the fishes, minced for uniformity and stored in plastic bags at -20^{0}C until used. The chemicals and reagents used were of analytical grade

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 papain (phosphate buffer 0.1 M Na_2HPO_4- NaH_2PO_4; pH-6, temperature, 37 °C) at enzyme ⁄ substrate ratio (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 -20 °C.

Yield and Degree of Hydrolysis

The extent of hydrolysis was determined by adapting the procedure described by Tang et al. (2009) [11]. Briefly, the sample was mixed with papain enzyme with different enzyme/substrate ratio (1/100, 2/100, 4/200 v/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 Hydrolysate was centrifuged at 10000g for 15 min, and the supernatant was lyophilized to get a powdered sample and was

stored at -20°C. 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})

$$DH\% = \frac{h}{h_{tot}} X100$$

The degree of hydrolysis (DH) of hydrolyzed protein was determined by measuing 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 have 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 increased with increase in the time of hydrolysis. Maximum 63.3% of protein was recovered at 6 hours of incubation. The results suggest that papain digestion recovers a considerable amount of protein from the skin of ***Scomberomorus commerson***.

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]. As expected the DH increases with increase in incubation time. The DH was observed to be 22.2% for enzyme substrate ratio 1:100, 23.6% for 2:100 and 24.7% 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 phase (e.g., first hour) of hydrolytic process. However,

at the end of 6 hours the rate of degradation does not much influenced by the E/S ratio and all the three E/S ratios showing similar percentage of digestion.

Proximate Analysis

The composition of protein hydrolysates generally depends on choice of enzyme, pH, incubation time and analytical methods used for estimation. In the present study the papain enzyme with appropriate pH and temperature optima was used to get the protein hydrolysate. The proximate analysis in terms of protein, lipid, moisture and ash contents of both the crude wet sample and freeze dried protein hydrolysate of king fish were measured. The results were expressed as a mean of triplicate ± SD and represented in **Figure 3** and **Figure 4** respectively. The minced skin sample of ***Scomberomorus commersoon*** showed higher moisture content (76.8%) and least lipid (0.43%) content. The lyophilized protein hydrolysate showed protein content of around 85.57% which was in par with earlier findings [21].

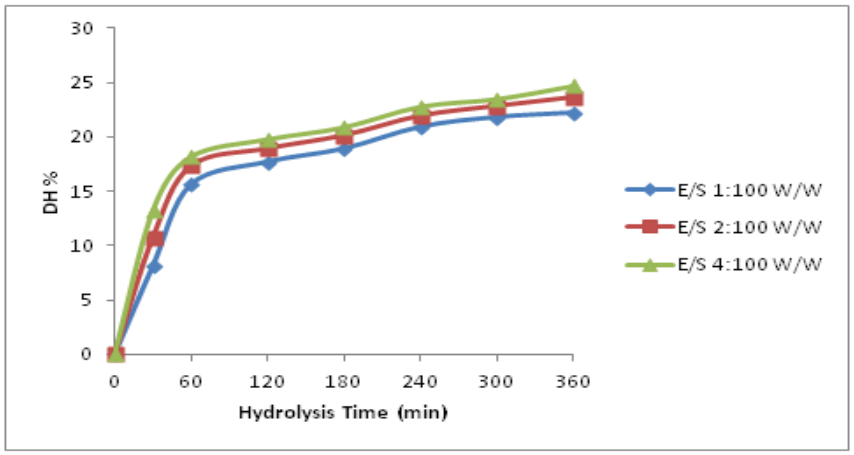

Figure 1: Percentage protein recovery of hydrolysis from the skin of kingfish by papain. Values are expressed as mean of triplicates ±SD

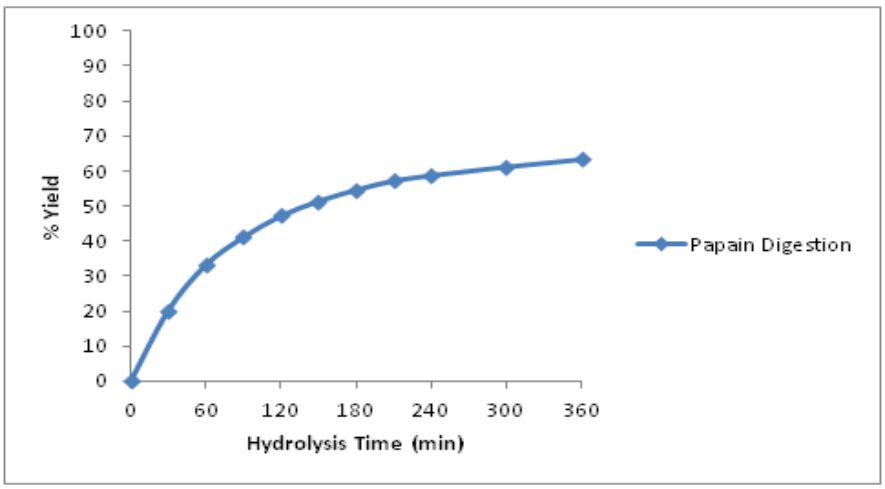

Figure 2: DH and influence of E/S ratio during hydrolysis of skin sample from kingfish by papain enzyme. Values are expressed as mean of triplicates ±SD

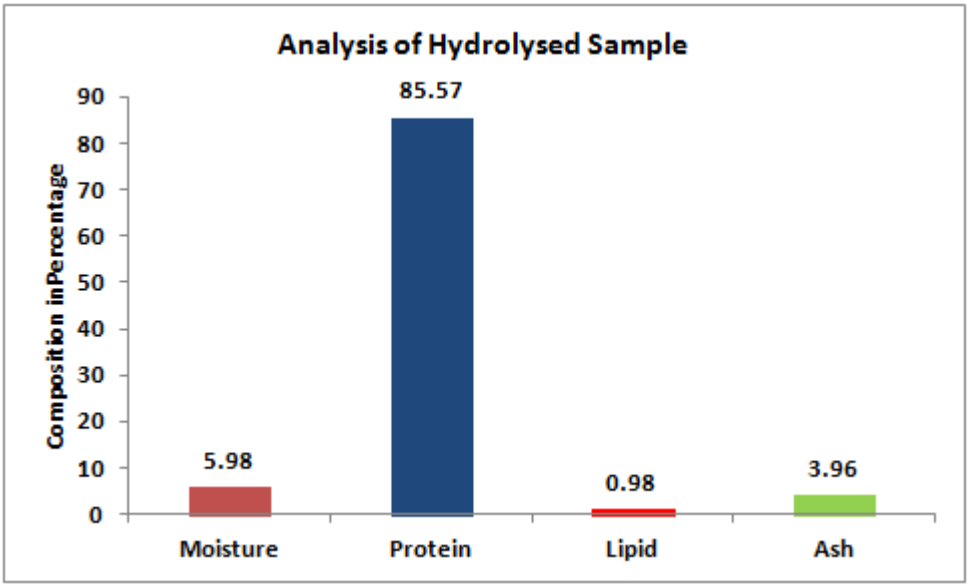

Figure 3: Proximate analysis of crude sample from the skin of king fish. Values are expressed as mean of triplicates ±SD

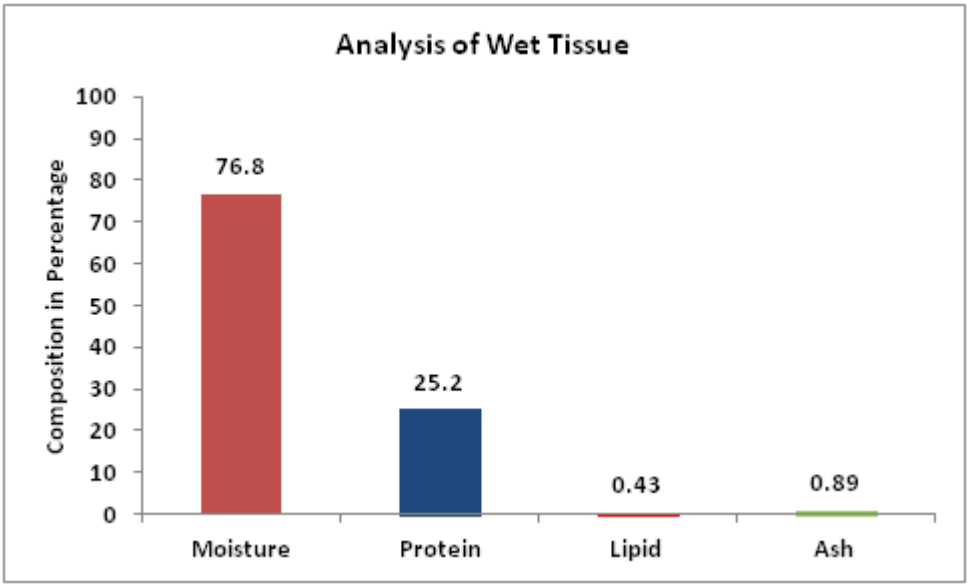

Figure 4: Proximate analysis of protein hydrolysate from the skin of king fish by papain enzyme. Values are expressed as mean of triplicates ±SD

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 papain. 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 biomedicinal and commercial aspects.

REFERENCES

1. FAO, La situation mondiale des peˆches et de l'aquaculture 2006. De´partement des peˆches et de l'aquaculture, Rome (2007)
2. Awarenet (2004) Handbook for the prevention andminimization ofwaste and valorization of by-products in European agro-food industries. Agro-food waste minimization and reduction network (AWARENET). Grow Programme, European Commission, pp 1–7
3. Je J.Y., Qian Z., Byun H.G., Kim S.K., Purification and characterization of an antioxidant peptide obtained from tuna backbone protein by enzymatic hydrolysis. Process Biochem, 42:849-846, (2007)
4. Quaglia G.B., Orban E., Influence of enzymatic hydrolysis on structure and emulsifying properties of sardine (Sardina pilchardus) protein hydrolysate. Journal of Food Science 55: 1571–1573, (1990)
5. Tanuja S., Viji P., Zynudheen A.A., Joshy C.G., Composition, functional properties and antioxidative activity of hydrolysates prepared from the frame meat of Striped catfish (Pangasianodon hypophthalmus). Egyptian Journal of Biology, 14: 27-35, (2012)
6. Alasalvar, C., Shahidi, F., Quantick, P., Food and health applications of marine neutraceuticals. In: Alasalvar, C., Taylor, T., (Eds.), Seafoods- quality, technology and nutraceutical applications. Springer-Verlag, Berlin 2002, pp. 175-204,
7. Wu H.C., Chen H.M., Shiau C.Y., Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (Scomber austriasicus). Food Res Inter, 36: 949-957, (2003)
8. Sathivel S., Bechtel P. J., Babbitt J., Smiley S., Crapo C., Reppond K.D., Prinyawawitkul W., Biochemical and functional properties of herring (Clupea harengus) byproduct hydrolysates. J Food Sci, 68: 2196–2200, (2003)
9. Jao C.L., Ko W.C., 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging by protein hydrolysates from tuna cooking juice. Fisheries Science, 68, 430–435, (2002)
10. Younis E.M.,, Abdel-Warith A.A., Ali A., al-Asgah N.A., El-Shayia A. S., (2011) Chemical composition and mineral contents of six commercial fish species from the Arabian gulf coast of Saudi Arabia. Journal of Animal and vetenary advances, 10: 3053-3059, (2011)
11. Tang C.H., Peng J., Zhen D.W., Chen Z., Physicochemical and antioxidant properties of buckwheat (Fagopyrum esculentum Moench) protein hydrolysates. Food Chem, 115:672–678, (2009)
12. Goodwin J.F., The colorimetric estimation of plasma amino nitrogen with DNFB. Clin Chem, 14:1080-1090, (1968)
13. Benjakul S., Morrissey M., 1997. Protein hydrolysates from Pacific whiting solid wastes. J Agric Food Chem, 45:3423–3430, (1997)
14. AOAC, Official Methods of Analysis**.** Association of Official Analytical Chemists, Washington, DC, USA, (1995)
15. Bligh E. G., Dyer W. J., (1959). A rapid method of total lipid extraction and purification. Can J Biochem Physiol, 37: 91 l-17, (1959)
16. Guerard F., Guimas L., Binet A., Production of tuna waste hydrolysates by a commercial neutral protease preparation. J Mol Cat B; Enz, 19–20: 489–498 (2002).
17. Adler-Nissen, J., Enzymatic Hydrolysis of Food Proteins. Elsevier, New York 1986.
18. Hoyle N.T., Merritt J.H., Quality of fish protein hydrolysates from herring (Clupea harengus). J Food Sci, 59: 76–79, (1994).
19. Liceaga-Gesualdo A.M., Li-Chan, E.C.Y., 1999. Functional properties of hydrolysates from herring (Clupea harengus). J Food Sci, 64: 1000–1004, (1999)
20. Sathivel S., Huang J., Bechtel P.J., properties of pollock (Theragra chalcogramma) skin hydrolysates and effects on lipid oxidation of skinless pink salmon (Oncorhynchus gorbuscha) fillets during 4 months of frozen storage. J Food Biochem, 32: 247–263, (2008)
21. Yin H., Pu J., Yutingwan B., Xiang, Bechtel P.J., Sathivel S., Rheological and functional properties of catfish skin protein hydrolysates. J Food Sci, 75:11-17, (2010)

***Corresponding Author:**
Dr. Sheriff Sheik Abdulazeez * M.Sc., M.Phil., M.Tech., Ph.D.,
Assistant professor, Department of Biochemistry,
College of applied medical sciences, Shaqra University,
Post box.No.1383, Shaqra 11961. Kingdom of Saudi Arabia.
Phone: +966 532949275 Email: biosherif@yahoo.co.in