

Production of protein hydrolysates from fish by-product prepared by enzymatic hydrolysis

Murna Muzaifa, Novi Safriani, Fahrizal Zakaria

Department of Agricultural Product Technology, Agriculture Faculty, Syiah Kuala University, Banda Aceh, Indonesia.

Abstract. The objective of this research was to study the production of fish protein hydrolysate (FPH) from fish by-product prepared by enzymatic hydrolysis. Fish by-product were prepared using Alcalase and Flavourzyme enzyme and properties of FPH were analyzed. The results showed that FPH prepared using Alcalase enzyme had greater amount of protein (82.66%) than FPH prepared using Flavourzyme enzyme (73.51%). Solubility, emulsifying and foaming properties of FPH prepared using Alcalase were also better than those prepared using Flavourzyme enzyme. The FPH derived from fish by-product using enzyme may potentially serve as a good source of protein. It could be used as an emulsifier and as a foaming agent.

Key Words: fish by-product, protein hydrolysate, enzyme hydrolysis, alcalase, flavourzyme.

Copyright: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Corresponding Author: M. Muzaifa, murnathp@yahoo.com

Introduction

Large amount of fish by-product are currently disposed or used for low-value products. There is a large potential for reducing the amount of by-product and to utilize a larger amount of the by-product for value added product for human consumption (Diniz & Martin 1997; Haque & Mozaffar 1992; Sathivel *et al* 2003). Fish by-product contains the same valuable protein as the fish muscles. Recovery and alteration of protein present in the fish by-product is a feasible alternative. By using enzyme technology, it may be possible to produce a broad spectrum of food ingredients for wide range of applications (Kristinsson & Rasco 2000; Rustad *et al* 2011).

Enzymatic proteolysis and solubilization of proteins from various sources has been studied extensively and described by several different authors over the last 60 years (Aspmo *et al* 2005; Petreus *et al* 2011). Addition of proteolytic enzymes could make a hydrolytic process more controllable. Alcalase – an alkaline bacterial protease produced from *Bacillus licheniformis* - has been proven to be one of the best enzymes used in the preparation of fish protein hydrolysate (Hoyle & Merritt 1994; Shahidi *et al* 1995; Benjakul & Morrisey 1997; Kristinsson & Rasco 2000; Guerard *et al* 2001). Flavourzyme is a fungal protease/peptidase complex produced by submerged fermentation of a selected strain of *Aspergillus oryzae* which has not been genetically modified and is used for the hydrolysis of proteins under neutral or slightly acidic conditions. Flavourzyme has been used to produce a protein hydrolysate with acceptable functional properties (Kristinsson & Rasco 2000).

The characteristics of hydrolysate directly affect the functional properties and the uses as food ingredients (Kristinsson & Rasco 2000). Fish protein hydrolysates have been shown to have potential for nutritional or pharmaceutical applications (Wergedahl *et al* 2004). Functionality of food proteins has been defined as: any physicochemical property which affects the processing and behavior of protein in food systems as judged by the quality attributes of the final product (Kinsella 1976). Fish protein hydrolysates have been well studied and reported in terms of their production, biochemical, and functional properties (Kristinsson & Rasco 2000). Functional properties of protein can be improved by enzymatic hydrolysis under controlled conditions (Quaglia & Orban 1990). To improve the functional properties of proteins, enzymatic modification has been extensively employed. The objective of the present study was to evaluate the production and functional properties of protein hydrolysate from fish by-product prepared by enzyme hydrolysis using Alcalase and Flavourzyme.

Material and Methods

Material

Fresh fish was filleted and the leftover processing by-products, including the frame, dark muscle, cut offs, viscera, skin, scales, small bones and fins, were collected for protein hydrolysis. The fish waste was stored in a polyethylene bag at 40 °C until used for FPH production. The bacterial protease preparations Alcalase® 2.4L and Flavourzyme® 500L were obtained from Novozymes, Novo Alle, DK-2880 Bagsvaerd (Denmark).

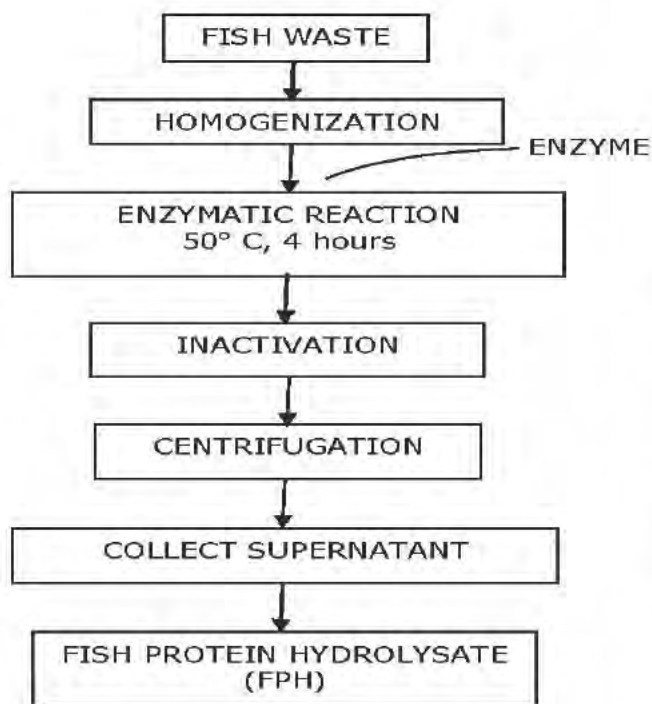


Figure 1. Flow sheet for production of fish protein hydrolysis.

Production of fish protein hydrolysates

The samples were partly thawed at room temperature for overnight and mixed with distillate water (1:1) and blended for 2-3 minutes. The homogenate samples were adjusted to pH 8.00 with buffer addition. The hydrolysis process was done in water bath (Memmert Schwabach, Germany) set up at 55°C. The enzymatic hydrolysis was started by adding of 2% enzyme (Alcalase and Flavourzyme). After 4 h of hydrolysis, the enzyme was inactivated by heating at 90°C for 15 min in a water bath (Model W350, Memmert, Schwabach, Germany). The mixture was then centrifuged at 3000 rpm at 4°C for 10 min using a Sorvall Model RC-5B Plus centrifuge (Newtown, CT, USA) and the supernatant was collected. Fish protein hydrolysate was freeze-dried using a Dura-Top™µp freeze-dryer (FTS systems Inc., Stone Ridge, NY, USA). The freeze-dried fish protein hydrolysate obtained was subjected to analyses. The common flow sheet of FPH making is presented in Figure 1.

Proximate analysis

Moisture content was determined by moisture analyzer (Denver Instrument IR-30). The protein hydrolysate powder was analyzed in triplicates and then mean value was recorded. Protein, ash and fat were determined according to the method of AOAC (2000). The protein and fat contents were expressed on a dry weight basis.

Functional properties analysis

Solubility and nitrogen solubility index (NSI) were calculated to determine the solubility of protein hydrolysate, following the procedure of Morr (1985). The emulsifying activity index (EAI) and the emulsion stability index (ESI) were determined according to the method proposed by Pearce & Kinsella (1978). Foaming ability and foam stability of protein hydrolysate were tested according to the Shahidi *et al* (1995).

Statistical analysis

Analysis of variance (ANOVA) was performed and means comparisons were run by Duncan's multiple range tests. All experiments were carried out in triplicates. Analysis was performed using a SPSS package (SPSS 15.0 for windows, SPSS Inc, Chicago, IL).

Results and Discussion

Proximate composition

There were no differences in moisture and fat content of FPH prepared using Alcalase and Flavourzyme enzyme, while there were differences on protein and ash contents (Table 1).

The FPH using Alcalase enzyme had higher protein content (82.66%) than FPH using Flavourzyme (73.51%). Protein content of this FPH was still high and could be an essential source of proteins. This finding is in agreement to Bhaskar *et al* (2008) who reported that the production of protein was as high as 14.25 % after hydrolysing Catla fish waste visceral with Alcalase enzyme (0.5-1.5%) for 55 to 165 minutes. In addition, Thiansikul *et al* (2007) claimed that 69% of protein was obtained from the hydrolysed round scad fish using flavourizyme enzyme.

Solubility

Solubility is one of the most important of FPH functional properties. Good solubility of proteins is required in many functional applications, especially for emulsions, foams and gels. Solubility of FPH using Alcalase and Flavourzyme are presented in Figure 2.

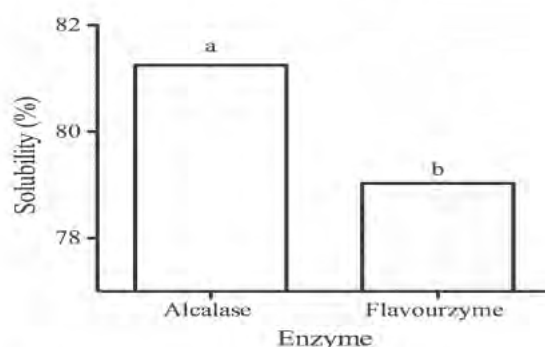


Figure 2. The influence of different enzyme on solubility of protein hydrolysate.

Table 1. Proximate composition of FPH prepared using Alcalase enzyme and Flavourzyme enzyme

Enzyme	Moisture content (%)	Protein (%)	Fat (%)	Ash (%)
Alcalase	8.14 ± 0.07 ^a	82.66 ± 1.36 ^a	0.87 ± 0.18 ^a	9.61 ± 0.78 ^b
Flavourzyme	8.31 ± 0.17 ^a	73.51 ± 3.53 ^b	0.44 ± 0.51 ^a	11.52 ± 2.26 ^a

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

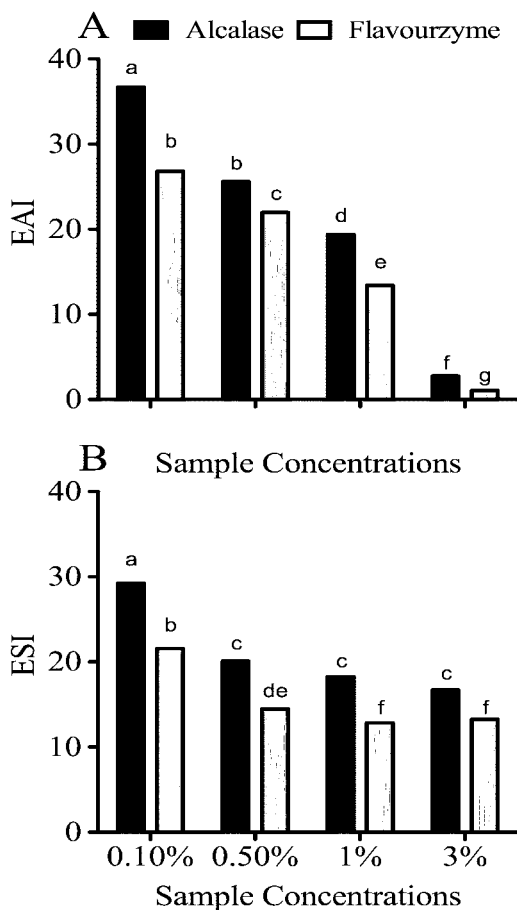


Figure 3. Influence of FPH powder concentration using different enzyme to; A. emulsifying activity index (EAI) and B. emulsifying stability index (ESI). Letters indicate significance.

Emulsifying properties

The emulsifying activity index (EAI) of FPH using Alcalase and Flavourzyme enzyme at various concentrations are shown in Figure 3 and 4. The methods are generally used to measure the ability of protein hydrolysates to form and stabilize emulsions (Kinsella 1976).

EAI of FPH produced using Alcalase and Flavourzyme enzyme were decreased with increasing concentrations of sample concentration. FPH obtained using Alcalase had higher EAI compared to FPH obtained using Flavourzyme at various concentrations. Emulsifying stability index (ESI) was also decreased with increasing hydrolysates at various concentrations. FPH produced using Alcalase had similar ESI with FPH produced using Flavourzyme enzyme. Protein hydrolysates produced using Alcalase have ESI higher than FPH produced using Flavourzyme at various concentrations.

Gbogouri *et al* (2004) reported that higher emulsifying activity and emulsion stability were found when salmon by-product hydrolysate using Alcalase enzyme. According to Wilding *et al* (1984), protein hydrolysates are a surface active and promote oil-in-water emulsions because they have hydrophilic and hydrophobic functional group. Emulsifying properties of FPH are

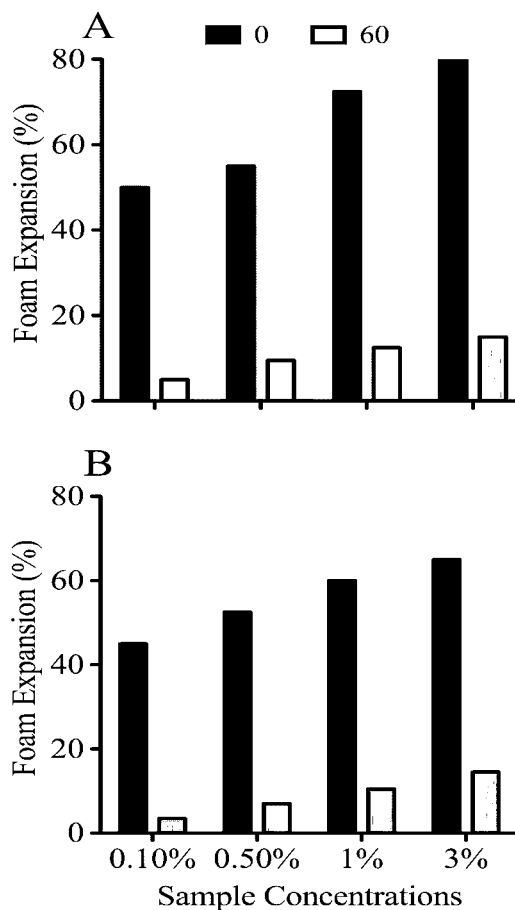


Figure 4. Effect of FPH concentration to foaming properties of FPH using; A. Alcalase and B. Flavourzyme enzyme.

directly connected to their surface properties, or how effectively the hydrolysate lower the interfacial tension between hydrophilic and hydrophobic components in food (Demetriades *et al* 1997).

Foaming properties

Foaming properties are usually expressed as foam formation and stability. Foam expansion at 0 min after whipping indicated the foam abilities of protein hydrolysates and foam expansion after whipping was monitored for 60 min to indicate the foam stability of protein hydrolysates. The ability and the stability foam of FPH were increased when hydrolysate concentrations increased from 0.1% to 3% (Figure 5 and 6). From the figure the study revealed that protein hydrolysates from fish waste have a good foam ability and stability. This result is in agreement with Thiansilakul *et al* (2007) who studied the foam ability protein hydrolysates from round scad (*Decapterus maruadsi*).

Conclusions

The protein hydrolysate derived from fish by-product using Alcalase and Flavourzyme enzyme may potentially serve as a good source of protein. Fish protein hydrolysates using Alcalase enzyme have greater of protein content, solubility, emulsifying and foaming properties compared to fish protein hydrolysates using Flavourzyme enzyme.

Acknowledgements

The author would acknowledge the support from Department of Agricultural Product Technology, Agriculture Faculty, Syiah Kuala University.

References

- AOAC, 2000. Official Methods of Analysis (16th ed.). Association of Official Analytical Chemist. Washington, DC.
- Aspmo, S. I., Horn, S. J., Eijsink, V. G. H., 2005. Enzymatic hydrolysis of Atlantic cod (*Gadus morhua* L.) viscera. *Process Biochemistry* 40:1957-1966.
- Benjakul, S., Morrisey, M. T., 1997. Protein hydrolysate from Pacific whiting solid waste. *Journal of Agricultural and Food Chemistry* 45:3423-3430.
- Bhaskar, N., Sudeepa, E. S., Rashmi, H. N., Tamilselvi, A., 2008. Partial purification and characterization of protease of *Bacillus proteolyticus* CFR3001 isolated from fish processing waste and its antibacterial activities. *Bioresource Tecnology* 98(14): 2758-2764. doi:10.1016/j.biortech.2006.09.033.
- Demetriades, K., Coupland, J. N., Mc Clements, D. J., 1997. Physical Properties of whey protein stabilized emulsions as related to pH and NaCl. *Journal of Food Science* 62(2):342.
- Diniz, F. M., Martin, A. M., 1997. Effects of the extent of enzymatic hydrolysis on the functional properties of shark protein hydrolysate. *LWT – Food Science and Technology* 30(3):266-272.
- Gbogouri, G. A., Linder, M., Fanni, J., Parmentier, M., 2004. Influence of hydrolysis degree on the functional properties of salmon byproduct hydrolysates. *Journal of Food Science* 69:615-622.
- Guerard, F., Duffose, L., de la Broise, D., Binet, A., 2001. Enzymatic hydrolysis of protein from yellowfin Tuna (*Thunnus albacares*) wastes using alcalase. *Journal of Molecular Catalysis B: Enzymatic* 11:1051-1059.
- Haque, Z. U., Mozaffar, Z., 1992. Casein hydrolysate II. Functional properties of peptides. *Journal of Food Hydrocolloids* 5:559-571.
- Hoyle, N., Merritt, J. H., 1994. Quality of fish protein hydrolysates from herring (*Clupea harengus*). *Journal of Food Science* 59:76-79.
- Kristinsson, H. G., Rasco, B. A., 2000. Fish protein hydrolysates: production, biochemical, and functional properties. *Critical Review in Food Science and Nutrition* 40:43-81.
- Kinsella, J. E., 1976. Functional properties of protein in foods: a survey. *Critical Reviews of Food Science and Nutrition* 8(4):219.
- Morr, C. V., 1985. Composition, physicochemical and functional properties of reference whey protein concentrates. *Journal of Food Science* 50:1406-1411.
- Quaglia, G. B., Orban, E., 1990. Influence of enzymatic hydrolysis on structure and emulsifying properties of sardine (*Sardina pilchardus*) protein hydrolysate. *Journal of Food Science* 55:1571-1573.
- Pearce, K. N., Kinsella, J. E., 1978. Emulsifying properties of proteins: evaluation of a turbidimetric technique. *Journal of Agricultural and Food Chemistry* 26:716-723.
- Petreus, T., Cotrutz, C. E., Sirbu, P. D., Neamtu, M., Filioreanu, A. M., Badescu, L., Neamtu, A., 2011. Bioinformatic lookup for metalloproteinases and their natural inhibitors. Structural relationships. *Annals of the Romanian Society for Cell Biology* 16(1):175-180.
- Rustad, T., Størro, I., Slizyte, R., 2011. Possibilities for the utilisation of marine by-product. *International Journal of Food Science and Technology* 46:2001-2014.
- Sathivel, S., Bechtel, P. J., Crapo, S., Reppond, K. D., Prinnyawiwatkul, W., 2003. Biochemical and functional properties of herring (*Clupea harengus*). *Journal of Food Science* 68:2196-2200.
- Shahidi, F. H., Xiao-Qing, Synowiecki, J., 1995. Production and characteristics of protein hydrolysates from capelin (*Mallotus villosus*). *Food Chemistry* 53:285-293.
- Thiansilakul, Y., Benjakul, S., Shahidi, F., 2007. Compositions, functional properties and antioxidative activity of protein hydrolysates prepared from round scad (*Decapterus maruadsi*). *Food Chemistry* 103:1385-1394.
- Wergedahl, H., Liaset, B., Gudbrandsen, O. A., Lied, E., Espe, M., Muna, Z., 2004. Fish protein hydrolysate reduces plasma total cholesterol, increases the proportion of HDL cholesterol and lowers acyl-CoA: Cholesterol acyltransferase activity in liver of Zucker rats. *Journal of Nutrition* 134:1320-1327.
- Wilding, P., Lilliford, P. J., Regenstein, J. M., 1984. Functional properties of protein in foods. *Journal of Chemical Technology and Biotechnology* 34B:182.

Authors

- Murna Muzaifa, Department of Agricultural Product Technology, Agriculture Faculty, Syiah Kuala University, Darussalam Banda Aceh, Indonesia, e-mail: murnathp@yahoo.com
- Novi Safriani, Department of Agricultural Product Technology, Agriculture Faculty, Syiah Kuala University, Darussalam Banda Aceh, Indonesia, e-mail: opi_riani@yahoo.com
- Fahrizal Zakaria, Department of Agricultural Product Technology, Agriculture Faculty, Syiah Kuala University, Darussalam Banda Aceh, Indonesia, e-mail: fahrizal.tse79@gmail.com

Citation Muzaifa, M., Safriani, N., Fahrizal, Z. Production of protein hydrolysates from fish by-product prepared by enzymatic hydrolysis. *Aquaculture, Aquarium, Conservation & Legislation* 5(1):36-39.

Editor Muchlisin Z. A.

Received 21 December 2011

Accepted 05 February 2012

Published Online 12 March 2012

Funding Department of Agricultural Product Technology, Agriculture Faculty, Syiah Kuala University.

Conflicts / Competing Interests No disclosures