# PHYSICOCHEMICAL PROPERTIES OF FISH PROTEIN HYDROLYSATES PREPARED FROM FISH BY-PRODUCT USING ALCALASE AND FLAVOURZYME ENZYME

## (Sifat Fisiokimia Hidrolisat Protein Ikan yang Diperoleh dari Limbah Ikan dengan Menggunakan Enzim *Alcalase* dan *Flavourzyme*

**Murna Muzaifa[1], Fahrizal[2], Novi Safriani[3]**
[1,2,3]Department of Agricultural Product Technology, Agriculture Faculty Syiah Kuala University,
Jl Tgk.Krueng Kalee Darussalam Banda Aceh, Indonesia.
E-mail: murnathp@yahoo.com

**Abstrak**

Limbah ikan banyak yang terbuang dengan percuma, padahal limbah ikan masih mengandung sejumlah protein. Protein tersebut dapat dimanfaatkan kembali dengan cara menghidrolisis limbah ikan menjadi hidrolisat protein ikan. Penelitian ini bertujuan untuk mempelajari sifat fisikokimia hidrolisat protein ikan yang diperoleh dengan hidrolisis menggunakan enzim. protease *alcalase* dan *flavourzyme.* Hasil penelitian menunjukkan bahwa hidrolisat protein ikan yang menggunakan enzim *alcalase* mempunyai kandungan protein yang lebih tinggi, kadar abu lebih rendah serta warna yang lebih gelap dibandingkan hidrolisat protein ikan yang menggunakan enzim *flavourzyme*.

**Key words:** *limbah ikan, hidrolisat protein ikan, hidrolisis, alcalase, flavourzyme.*

## 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 by-product and to utilize a larger amount of the by-product for value added product for human consumption. Fish by-product contains the same valuable protein as the fish muscles. Recovery and alteration of protein presents 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 2004). 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 enzyme 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 physicochemical properties of protein hydrolysate from fish by-product prepared by enzyme hydrolysis using alcalase and flavourzyme.

## METHOD

### *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 $^0$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).

***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 Scwabach, 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.

***Physicochemical analysis***.

Moisture content was determined by moisture analyzer (Denver Instrument IR-30), 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. The colour measurement were conducted using a colorimeter (Minolta CM-3500d) equipped with Spectra Magic Software version 2.11.

***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**

Physicochemical properties of FPH that consist of moisture content, protein content, fat content, ash content and color were analyzed. The result showed on the Table 1-5

.

Table 1. Moisture content of FPH prepared using Alcalase and Flavourzyme enzyme

| Enzym | Moisture content (%) |
|---|---|
| Alcalase | $8.14 \pm 0.07^{a}$ |
| Flavourzyme | $8.31 \pm 0.17^{a}$ |

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

Table 2. Protein content of FPH prepared using Alcalase and Flavourzyme enzyme

| Enzym | Protein (%) |
|---|---|
| Alcalase | $82.66 \pm 1.36^{a}$ |
| Flavourzyme | $73.51 \pm 3.53^{b}$ |

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

Table 3. Fat content of FPH prepared using Alcalase and Flavourzyme enzyme

| Enzym | Fat (%) | Protein (%) |
|---|---|---|
| Alcalase | $0.87 \pm 0.18^{a}$ | $82.66 \pm 1.36^{a}$ |
| Flavourzyme | $0.44 \pm 0.51^{a}$ | $73.51 \pm 3.53^{b}$ |

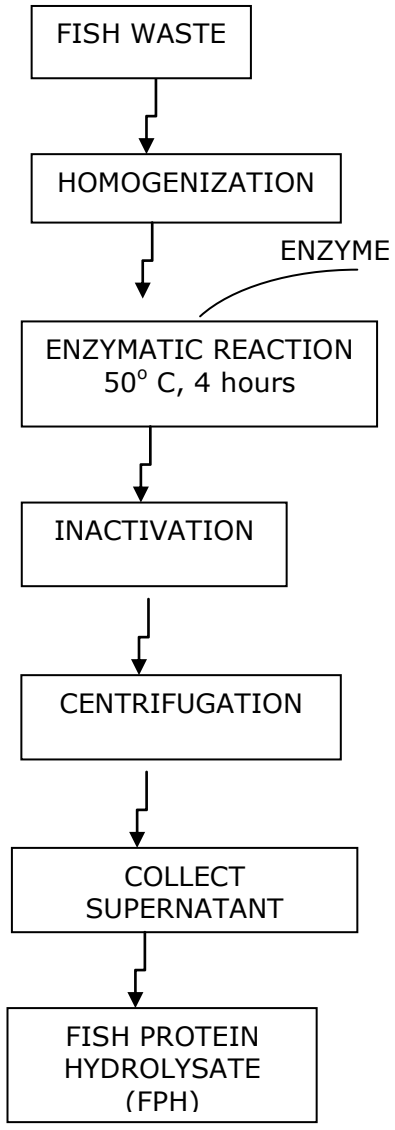


Figure 1. Flow sheet for production of fish protein hydrolysate

Table 4. Ash content of FPH prepared using Alcalase and Flavourzyme enzyme

| Enzym | Ash (%) |
|---|---|
| Alcalase | $9.61 \pm 0.78^{b}$ |
| Flavourzyme | $11.52 \pm 2.26^{a}$ |

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

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.

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.

As shown the table 5, the FPH using alcalase enzyme had brownish yellow in colour ($L^*$ = 78.00, $a^*$ = 3.04, $b^*$ = 36.37) and had darker colour than FPH using Flavourzyme enzyme ($L^*$ = 81.60, $a^*$ = 0.44, $b^*$ = 28.61). Alcalase and flavourzyme with a dark colour also contributed to the brownish colour of the resulting hydrolysate. From the study of Thiansilakul et al (2007), it was reported that the colour of protein hydrolysate from round scad, prepared using flavourzyme have similar colour with this product.

Whereas from the study of Sathivel et al. (2003), the colour of whole herring and herring byproduct hydrolysates, prepared using Alcalase. Herring gonad hydrolysate was the darkest ($L^*$ = 74.6) and most yellowish ($b^*$ = 18), whereas whole herring hydrolysate was the lightest ($L^*$ = 89.4) and least yellowish ($b^*$ = 8.0). Therefore, the varying colour of fish protein hydrolysates depend on the composition of the raw material and using different enzyme.

## CONCLUSION

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 had greater of protein and lower of ash content compared to fish protein hydrolysates using flavourzyme enzyme. While the color of fish protein hydrolysates using alcalase darker than fish protein hydrolysates using flavourzyme enzyme.

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

Table 5. Colour of FPH prepared using Alcalase and Flavourzyme enzyme

| Enzym | $L^*$ | $a^*$ | $b^*$ |
|---|---|---|---|
| Alcalase | $78.13 \pm 0.54^{b}$ | $3.04 \pm 0.52^{a}$ | $36.37 \pm 0.07^{a}$ |
| Flavourzyme | $81.6 \pm 0.51^{a}$ | $0.44 \pm 1.08^{b}$ | $28.61 \pm 1.11^{b}$ |

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

## REFERENCES

AOAC., 2000 Official methods of Analysis (16th ed.). Association of Official Analitycal Chemist. Washington, DC.

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.
Available online from November 07, 2008 at www.sciencedirect.com,doi:10.1016/j.biortech.2006.09.033.

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, Crit.Rev.Food Sci.Nutr. 8(4): 219.

Quaglia,G.B., Orban, E., 1990 Influence of enzymatic hydrolysis on structure and emulsifying properties of sardine (*Sardina pilchardus*) protein hydrolysate. Journal of FoodScience **55**:1571-1573.

Sathivel S., P.J. Bechtel, S., Crapo, K.D., Reppond., Prinnyawiwatkul, W., 2003 Biochemical and Fuctional Properties of Herring (*Clupae 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.