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Effects of enzymatic hydrolysis using papain on the quality of fish protein hydrolysate from fringescale sardine (*Sardinella Fimbriata*)

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Fish protein hydrolysate is an extract of fish protein that can be used to supplement and fortify processed food products. One of the processing methods of fish protein hydrolysate is enzymatic hydrolysis of fish flesh by using papain. The aim of this study was to determine the optimum papain concentration (6, 8 and 10%) and hydrolysis period (4, 6 and 8 days) in the processing of fish protein hydrolysate using fringescale sardine as raw material. Parameters observed were pH, hydrolysis degree, yield, total soluble solid and sensory acceptability. Results showed that concentration of papain increased total soluble solid values, yield, and colour acceptability, but reduced hydrolysis degree. Longer hydrolysis period increased total soluble solid and hydrolysis degree as well as colour and clarity acceptabilities, but decreased pH values of the product. Recommended optimum conditions to process protein hydrolysate from fringescale sardine were by using 8% papain and 6 days hydrolysis period. Chemical composition of the hydrolysate was 73.1% moisture, 15.8% protein, 7.7% fat and 4.9% ash.

Keywords: Papain, fish protein hydrolysate, hydrolysis period.

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INTRODUCTION

Processing of fish protein hydrolysate (FPH) is one of alternatives that can overcome the problem of fish consumption. FPH is able to be used as food supplement and fortification materials for various food products. Addition of fish protein hydrolysate will improve protein content of food products.

FPH is a product which takes advantage the solubility of fish protein due to hydrolysis process. One of fish which can be utilized as protein source is fringescale sardine. Fringescale sardine is small pelagic fish, in which its total production from Indonesian territorial water. FPH can be defined as hydrolytic degradation of protein by catalyst, such as strong acid, strong base and proteolytic enzyme. According to Johnson and Peterson (1974), the most efficient protein hydrolysis method is by using protease as a catalyst. The Hydrolysis process produces peptides as simple compound and easily decomposed. One of proteases which can be used for hydrolysis process is papain. This

study was conducted to reveal the effects of papain addition level and hydrolysis period on the quality of fish protein hydrolysate. Quality of fish protein hydrolysate yielded was to be determined in terms of physical, chemical, and sensory acceptability.

MATERIALS AND METHODS

Fringescale sardine (*Sardinella fimbriata*) was used as raw material in this study. Proximate composition of fresh fringescale sardine was determined using AOAC method. Crude papain was obtained from a chemical shop in Jakarta.

Complete randomized factorial experimental design was applied with three replications. Factors studied were enzyme concentration (6, 8 and 10%) and hydrolysis period (4, 6 and 8 days). Analyses performed to reveal product quality were as follows: (1) physical analyses including yield and total soluble solid, (2) chemical analyses covering hydrolysis degree (N-α-Amino), total of nitrogen and pH, and (3) sensory acceptability in terms of colour, clarity, odour and taste.

Fish was dressed by removing viscera parts and then washed. The washed fish was eventually chopped into small pieces. Chopped fish was placed in covered jar, and added with enzyme and water as much as 25 % of fish weight. The jars were then covered tightly. The mixtures were incubated at 55°C - 60°C for a certain hydrolysis period. After hydrolysis processes completed, the enzyme was inactivated by heating at 85°C for 15 minutes. Dilution of hydrolysis product was filtered.

RESULTS AND DISCUSSION

As shown at Table 1, the increase of papain amount incorporated in the process, the higher pH value of the product. However, the higher hydrolysis period induced the decrease of pH value of fish protein hydrolysate obtained. The optimum pH and temperature levels for papain activity were in the range of 5 – 7 and 50 - 60°C respectively. To produce the best quality of fish protein hydrolysate, those optimum conditions for enzymatic hydrolysis have to be achieved (Liener, 1980).

Table 1. Effect of papain level and hydrolysis period on chemical, yield, and sensory evaluation of fish protein hydrolysate

Treatment		pH	Total soluble solid	Hydrolysis degree	Yield (%)	Sensory evaluation*)			
						Colour	Clarity	Odour	Taste
Papain Level (%)	6	5.8	19.8 [a]	0.33 [a]	66.2 [a]	3.4 [a]	3.4 [a]	3.4	2.8
	8	5.9	21.8 [c]	0.32 [a]	74.0 [b]	3.6 [b]	3.5 [a]	3.8	2.8
	10	6.0	22.7 [b]	0.29 [b]	76.6 [b]	3.7 [b]	3.5 [b]	3.7	2.9
Hydrolysis Period (days)	4	6.0 [a]	20.3 [a]	0.31 [a]	71.5	3.4 [a]	3.2 [a]	3.6	2.8
	6	6.1 [a]	22.3 [b]	0.29 [b]	72.2	3.8 [b]	3.3 [a]	3.4	2.9
	8	5.6 [b]	21.8 [c]	0.33 [a]	73.2	3.5 [a]	4.0 [b]	3.9	2.7

*) Scale : 1 –4

Average TSS value tended to increase with the increasing papain amouit level and hydrolysis period. According to Hadiwiyoto (1995), collagen is predominant protein in fish to decompose into peptides having lower molecule weight. Those compounds induced cloudy performance of the product. Pigott and Tucker (1990) informed that formed protein and decomposed carbohydrate will generate Maillard reaction (non enzymatic browning) bringing about colour change of fish protein hydrolysate.

Hydrolysis process was expected to convert insoluble fish protein into soluble nitrogen compounds. Decomposition of peptide compounds resulted in simpler compounds, such as α-amino acids, dipeptides and other compounds contributing to typical taste and odour of the product. Gildberg (1992) noted that longer hydrolysis period in the processing of fish protein hydrolysate increased the amount of α-nitrogen amino. Hydrolysis degree levels tended to increase according to the hydrolysis period. On the other hand, the more papain added to the process reduced hydrolysis degree.

The results indicated that the more papain used in the process produced the higher yield. According to Harrow and Mazur (1971), larger enzyme concentration will increase enzymatic hydrolysis rate of protein. The yield tended to increase due to longer hydrolysis period. This tendency meets to the statement of Pigott and Tucker (1990) that the longer hydrolysis period will result in decomposing protein and fat into small compounds, such as peptides and fatty acids. However, analyses of variance indicated that hydrolysis periods insignificantly influenced the yield of fish protein hydrolysate.

Result of sensory evaluation showed that increasing papain addition level and hydrolysis period tended to improve colour, clarity, odour and taste acceptability.

1. Papain Addition Level

Sensory analysis of fish protein hydrolysate demonstrated that product with the most acceptable colour was the one processed using 8% papain. Although, papain addition level statistically did not affect the product acceptability in terms of odour and taste properties, but the highest clarity and odour acceptability scores were found in the product hydrolyzed using 8% papain for any tested hydrolysis period.

Colour and clarity were product appearance affected by processing. Physically, product appearance was determined by total soluble solid (TSS) level. By minimizing soluble solid affected by impurity level in papain, the appearance of fish protein hydrolysate was improved. The most acceptable TSS amount was obtained from fish protein hydrolysate processed using 6% papain, i.e. 19.8. The highest hydrolysis degree value was obtained from fish protein hydrolysate added with 6% papain.

The highest α - nitrogen amino content was found in the product produced with 6% and 8% papain addition level. The formation of amino acids affected the acidity level of the product reflected by pH value. This indication was supported by the fact that the lowest pH was obtained from the product hydrolyzed using 6% papain, i.e. 5.8. The more papain involved in the process resulted in increasing the filtrate volume. In terms of the yield, fish protein hydrolysate was recommended to process using 8% papain.

2. Hydrolysis period

The highest hydrolysis degree value was found in the product hydrolyzed for 8 days, i.e. 0.33. Amino acids formed influencing pH value, but pH value was not influenced by hydrolysis period. The lowest pH was measured in the product processed using 8 days hydrolysis. Meanwhile, the lowest TSS content was obtained in the product hydrolyzed for 4 days.

Sensory evaluation indicated that the highest colour acceptability score of fish protein hydrolysate was found in the product hydrolyzed for 6 days. Product showing the highest clarity acceptability score was the one produced using 8 days. Statistical analysis indicated that all tested treatments insignificantly affected odour and taste acceptability scores. In respect to overall results, the best fish protein hydrolysate can be processed using 8% papain and hydrolyzed for 6 days. Proximate composition of fish protein hydrolysate made from fringescale sardine was 73.1% moisture, 15.8% protein, 7.7% fat and 4.9% ash.

CONCLUSION

Use of papain at various addition levels pronouncedly affected the quality of fish protein hydrolysate in terms of total soluble solid, yield, hydrolysis degree and colour acceptability. Hydrolysis period significantly influenced total soluhle solid, clarity, pH, hydrolysis degree, and colour acceptability. Increasing hydrolysis period did not influence yield.

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