The production of Kerong fish (*Terapon jarbua*) peptone using enzymatic hydrolysis

Y Srikandace*, S Priatni, S Pudjiraharti, W Kosasih and E S Endah

Research Unit for Clean Technology, Indonesian Institute of Sciences (LIPI), Komplek LIPI Bandung, Jalan Sangkuriang, Gedung 50, Bandung, 40135, Indonesia

*Email: yoice_srikandace@yahoo.co.id

Abstract. The Kerong fish (*Terapon jarbua*) peptone was produced by using alcalase and papain enzymes. The parameters of fish peptone development were temperature (50-65°C), pH 7, and hydrolysis time (4-7 h). Papain and alcalase were used to digest the substrate with concentrations of 0.2% (w/w). The results showed that the highest yield of peptone was resulted by using alcalase and papain enzymes at 65°C for 6 h and 5 h, respectively. The maximum harvest of peptone using alcalase enzyme was shown by soluble protein content of 6.382 mg/ml with N total of 0.535. The highest peptone production using papain enzyme revealed soluble protein content of 5.781 mg/ml with N total of 0.252. It indicated that alcalase enzyme produced more fish peptone *T. jarbua* than papain enzyme. The resultant fish peptone supported the growth of *Pseudomonas aeruginosa*, *Salmonella thypii*, *Bacillus subtilis* and *Clostridium sporogenes*.

1. Introduction

Nowadays, large amounts of protein-rich raw materials from fish processing are discarded without recovery. The wastes generated from fish processing industry are approximately 100,000 tons per year [1], including fish that cannot be used in industrial process or as side dishes [2]. In West Java-Indonesia, Kerong fish (*Terapon jarbua*) is abundant in population and less desirable because the taste of fish is not tasty. This type of fish is usually sold as low-valued products even though they contain proteins and essential amino acids [3,4]. The *T. jarbua* fish contains water soluble protein hydrolysate in mixture of polypeptides and amino acids such as peptone [5]. Peptone is a cheap material for microbial growth medium and eco-friendly. Peptone is a protein generated from fish with the addition of proteolytic enzymes in order to accelerate the hydrolysis process [6]. Several proteolytic enzymes are used to hydrolyze fish peptone such as alcalase, papain, bromelain, ficin, pepsin, trypsin, and pancreatin [1,3]. Alcalase and papain are low cost per unit of enzyme activity compared to other enzymes; these enzymes are used to prevent enzyme waste and to increase the commercial viability of the peptone product, especially for medium culture [7,8].

Preliminary study [5] showed that the highest yield of peptone from *T. jarbua* was achieved by 0.2% w/w of non-commercial papain enzyme hydrolysis at pH 7. Therefore, this present study aims to optimize the production of *T. jarbua* fish peptone using commercial alcalase and papain enzyme at varied temperature of 50-65°C, hydrolysis time (4-7 h), as well as to characterize the fish peptone product.

2. Methods

2.1. Fish peptone preparation

The fresh *T. jarbua* fish was originated from Indramayu-West Java. The commercial enzymes (papain XF 1601001 and alcalase XF 3612001) were purchased from Arisumcompiharm-a local manufacturer. A total of 100 g of fish was mixed with water with a ratio 1:4, blended and separated from fish bone. The fish porridge was then hydrolyzed at pH 7 with 0.2% w/w with commercial alcalase and papain enzymes, respectively. The enzymes were added into the mixture, heated at 50-65°C for 4-8 h and inactivated at 85°C for 15 min. The hydrolyzed mixtures were centrifuged (7000*g*, 20 min) at 4°C. Finally, the supernatant was collected and dried to obtain soluble peptone.

2.2. Assay for total protein

The total protein of *T. jarbua* fish peptone was carried out using Lowry's method. Lowry A solution consisted of 4 mg/mL of NaOH and 20 mg/mL of Na_2CO_3 in water. Lowry B solution consisted of 10 mg/mL of potassium sodium tartrate and 5 mg/ mL of $CuSO_4$ in water. Lowry C solution was prepared by mixing 50 mL of Lowry A solution and 1 mL of Lowry B solution. While Lowry D solution was prepared by diluting Folin-Ciocalteu reagent with distilled water (dH_2O) at equal volumes. As much as 500 µl of fish peptone samples were added with 2.5 mL of Lowry C solution and dissolved for 10 min. Samples were then added with 0.5 mL solution D and dissolved for 30 min. Sample solution was measured at 500 nm.

2.3. Assay for total N-amino

The total N-amino of *T. jarbua* fish peptone was estimated by formol titration method. A total of 10 mL sample was mixed with 20 mL of dH_2O, 40 mL of potassium oxalate and 3-4 drops of phenolpthalein indicator (PP). Sample was titrated using 0.1N NaOH, added with 2 mL of formalin and 3-4 drops of PP indicator. Sample solution was titrated using 0.1N NaOH and its protein content was measured.

2.4. Microbiology analysis

Bacteria *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella thypiimurium*, *Clostridium sprogenes*, *Enterobacter cloacae* and *Pseudomonas aeruginosa* were used for the growth test of fish peptone. Fish peptone with the highest yield was selected for this test. The sterilized broth test medium consisted of 1 g of fish peptone extract, 100 mL of dH_2O, 0.5% of yeast extract and 1% of NaCl at pH 7. The sterilized broth commercial medium contained 1 g of commercial peptone, 100 mL of dH_2O, 0.5% yeast extract and 1% NaCl at pH 7. Pure culture was regenerated into 100 mL of broth test media (conventional and commercial, respectively) and incubated at 37°C for 24 h and shaken at 150 rpm. Then, culture was incubated in Plate Count Agar (PCA). Total Plate Colony (TPC) method was applied to growth performance of fish peptone in PCA [6].

3. Result and discussion

3.1. Total protein and N-amino content

Analysis of Kerong fish (*T. jarbua*) was conducted to measure the protein and amino content of the raw material. The value of total protein and N-amino content from various temperatures and time incubations at pH 7 using commercial enzymes is described in Table 1.

Table 1 shows that the highest yield of fish peptone using papain enzyme and alcalase was obtained at incubation temperature 65°C. The highest yield of fish peptone that hydrolyzed with papain revealed total protein and N-amino were about 5.78 mg/mL and 0.35%, respectively. Hydrolysis with alcalase resulted in fish peptone with total protein and N-amino of 6.38 mg/mL and 0.54%, respectively. The research of fish peptone (protein content of 1.21%) from catfish (*Pangasius* sp) was obtained with the optimum temperature 60°C, 4% of concentration of papain for 48 h at pH 5 [7]. The fish peptone production using alcalase from ponyfish (*Eubleekeria splendens*), the yellow-stripped trevally

(*Selaroides leptolipis*) and mackerel (*Decapterus maruadsi*) were obtained at temperature 61.23°C, 6% alcalase concentration, and pH 8 in 27 min of hydrolysis [3]. The recent study showed that 0.2% papain and alcalase enzymes concentration at pH 7 produced high yield of fish peptone. The optimum yield was achieved might be due to different ability of the used enzyme to digest the fish substrate based on the temperature, enzyme concentration and hydrolysis time.

Table 1. Total protein and N-amino content from fish peptone at pH 7 using commercial enzyme.

Temperature	Incubation time (h)	Protein mg/mL		N-Amino (%)	
		Papain	Alcalase	Papain	Alcalase
50°C	4	1.69	2.21	0.08	0.43
	5	1.55	2.41	0.11	0.31
	6	1.66	2.54	0.24	0.38
	7	1.56	2.36	0.35	0.35
55°C	4	1.85	2.60	0.22	0.28
	5	2.08	2.70	0.21	0.25
	6	2.01	2.71	0.22	0.31
	7	2.01	2.28	0.18	0.38
60°C	4	4.30	2.42	0.17	0.25
	5	4.57	2.71	0.17	0.33
	6	4.43	3.13	0.17	0.34
	7	4.78	3.90	0.18	0.37
65°C*	4	5.45	5.99	0.25	0.50
	5	**5.78**	**6.41**	**0.35**	**0.58**
	6	4.80	6.38	0.30	0.54
	7	3.28	6.27	0.25	0.50

*Optimum hydrolysis condition for producing the highest yield of total protein and N-amino of fish peptone using commercial enzymes.

The chemical characteristic of fish peptone from *T. jarbua* showed the solubility and ratio of alpha-amino nitrogen (AN) to total nitrogen (TN) from fish peptone. Fish peptone resulted from hydrolysis with papain enzyme shows 92.17% of solubility and 6.06% of AN/TN. Fish peptone hydrolyzed by alcalase showed 95.11% of solubility and 8.48% of AN/TN. It indicates that fish peptone from *T. jarbua* can be used as culture media for bacteria. The study of silver carp filleting also showed fish peptone as a nitrogen source for growth medium of *S. aureus* [9]. The fish protein hydrolysate can be used as carbon and/or nitrogen source in microbial growth, which is not only an eco-friendly approach but can also produce cheaper raw materials for media culture formulation and other applications in food biotechnology [2,10].

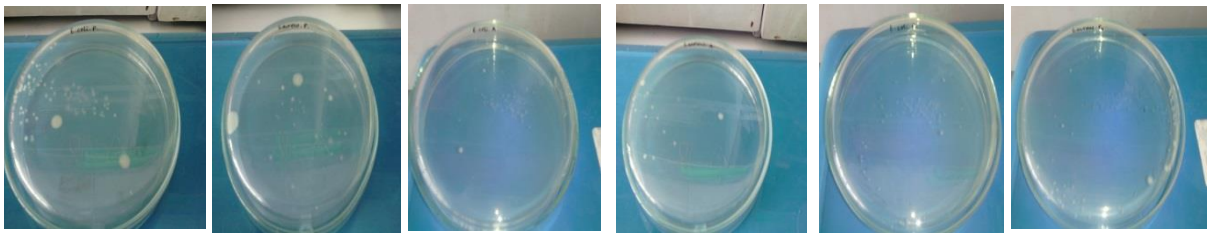
3.2. Microbiology analysis

The growth of bacteria on peptone from 10^{-5} dilution stage conducted in PCA medium is shown in Table 2.

Table 2 and Figure 1 describe that all commercial peptone and fish peptone supported the growth of bacteria. Bacteria *E. coli*, *S. aureus*, *B. subtilis* and *P. aeruginosa* could grow well in 1x24 h of incubation but others more times. The growth also depended on the nutrition element support in media and show the specific color of colonies in particular medium [11,12].

Table 2. Total bacteria growth (cfu/mL).

Bacteria	Commercial peptone	Fish peptone resulted from hydrolysis with papain	Fish peptone resulted from hydrolysis with alcalase
Escherichia coli	71 x 10^{-5}	**181 x 10^{-5}**	56 x 10^{-5}
Pseudomonas aeruginosa	>200 x 10^{-5}	>200 x 10^{-5}	45 x 10^{-5}
Enterobacter cloacae	132 x 10^{-5}	26 x 10^{-5}	46 x 10^{-5}
Salmonella tyhpii	>200 x 10^{-5}	>200 x 10^{-5}	10 x 10^{-5}
Bacillus subtilis	>200 x 10^{-5}	32 x 10^{-5}	>200 x 10^{-5}
Stapylococcus aureus	114 x 10^{-5}	30 x 10^{-5}	14 x 10^{-5}
Clostridium sporogenes	81 x 10^{-5}	6 x 10^{-5}	>200 x 10^{-5}



E. coli (P) *S. aureus* (P) *E. coli* (A) *S. aureus* (A) *E. coli* (Pn) *S. aureus* (Pn)

Figure 1. The colonies of bacteria cultured in commercial peptone and fish peptone.
P = Peptone hydrolyzed by papain, A = Peptone hydrolyzed by alcalase, Pn = Commercial peptone.

Figure 1 shows the growth of *E. coli* (negative gram bacteria) and *S. aureus* (positive gram bacteria) in PCA plate after all bacteria cultured in pepton. The result showed rapid growth of *E. coli*, *P. aeruginosa*, and *S. thypii* as negative gram bacteria using fish peptone hydrolyzed with papain enzyme. The total number of each bacteria were of 181x10^{-5}, >200x10^{-5} and >200x10^{-5}, respectively. On the other side, *B. subtilis* and *C. sporogenes* (positive gram bacteria) also showed rapid growth >200x10^{-5} cell on 10^{-5} dilution stage. In general, all bacteria grew well in commercial peptones. The interesting phenomena revealed from *E. cloacae* (negative gram bacteria) and *S. aureus* (positive gram bacteria). Bacteria *E. cloacae* and *S. aureus* were grown in peptones with papain and alcalase enzymes, respectively. Both bacteria did not grow well in the resultant peptone media, as shown by total colony of each bacteria of 26x10^{-5} and 14x10^{-5}, respectively. Previous study showed that the growth of *S. aureus* using fish peptone from *T. jarbua* was lower than that of commercial peptone [5]. It also indicated that fish peptone of *T. jarbua* was not suitable for the growth media of *E. cloacae* and *S. aureus*. Bioactive compounds from fish protein hydrolysates could contain base, amino acid, endogenous enzymes, bacteria, and digestive protease [13]. The present study also showed that peptone from *T. jarbua* could become specific growth medium for bacteria. It might replace expensive media for bacteria culture with the same quality.

4. Conclusion

Fish peptone as microbial growth media has been produced from *T. jarbua* through hydrolysis with commercial enzyme using an optimum condition of 0.2% papain enzyme concentration, pH 7 and 65°C for 5 h. Commercial alcalase enzyme produced more fish peptone *T. jarbua* than papain enzyme.

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