Scaling up process for fish peptone production

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Abstract. Fish peptone is one of protein hydrolysate products that can be used as nitrogen source for microbial growth. Peptone can be made from marine fish whereas protease enzyme was made from either papaya extract or commercial papain. Fish peptone is not only an eco-friendly approach but also can produce economic materials used in formulation of culture media. The objective of this research was to optimise the scaling up process of fish peptone production. An amount of 200 g of clean fish marine was blended with 1 L of water and added with 0.2 % of commercial papain at pH 8. Peptone production was carried out in a 1 L glass reactor with agitation (50, 100, 150, and 200 rpm) for 1-8 h. Fish slurry was then incubated at 50°C for 8 h. Fish protein hydrolysate was spray-dried with inlet and outlet temperature of 90°C and 80°C, respectively, at flow rate of 200 mL/h. Results revealed that the best condition for hydrolysis was shown by protein soluble content of 17.1 mg/L, α-amino content of 147 mM, and degree of hydrolysis of 8.3% which was achieved by agitation at 100 rpm for 8 h reaction. The yield of dried peptone product was 11.9 % (w/v). This peptone powder had higher soluble protein and α-amino content (442.3 mg/g, 124 mM) than the commercial peptone (258.5 mg/g, 65 mM). The microbial growth test of fish peptone products showed similar effectiveness compared with commercial bacterial peptone for the growth of *Escherichia coli* and *Streptococcus aureus*.

1. Introduction

Peptone is one of the ingredients used in the manufacture of microbial growth media. Peptone provides nitrogen for bacteria to grow. Peptone as an intermediate material between peptides and proteins is soluble in water, not coagulated by heat, and can be precipitated by ammonium sulphate and zinc sulphate [1]. Peptone can be extracted from animal proteins in the form of internal organs of meat, gelatine, milk, casein, plants, and khamir [2,3]. Due to its increasing demand and local peptone industry has not yet been developed, Indonesia imported 5.102 tonnes of peptone in 2013 at the value of US$ 20.76 million. The import increased compared to the year 2012, which was 3.296 tonnes at the value of US $ 12.15 million [4]. Fish peptone has an important economic value compared with other side products such as fish silage and fish meal [5].

Fish protein hydrolysates (peptone) were made by hydrolysing fish using proteolytic enzymes such as papain, ficin, trypsin, pancreatin, pronase, or enzymes isolated from proteolytic microorganisms [6]. Enzymatic processes do not require high temperature, it hydrolyses polypeptide specifically, and conserves all existing amino acids. This provides an advantage compared with acid hydrolysis that takes place at high temperatures, consequently damages either some or all amino acids. In addition, acid hydrolysis results in peptones with high salt content due to the formation of salts during the neutralization process [6].

Peptone production using enzyme from papaya latex has been carried out previously [7]. However, its practical application was difficult due to enzyme resource derived from dried papaya latex. Moreover, application of dried papaya latex based-enzyme in a large/industrial scale is ineffective since it is costly and impractical. Commercial enzymes are easier and cheaper compared to dried papaya latex with better quality and supply. The use of low valued fish for peptone production is promising because it is less expensive as well as provide better quality compared with commercial peptone.

This research aimed to produce peptone in a 1 L apparatus using commercial papain enzyme, commonly used in pilot scale.

2. Method

2.1. Enzyme preparation

A commercial enzyme (brand Xian Arisun ChemParm Co. Ltd, CAS no. 2323.627-2, Shaanxi, China, in the form of powder) was used in the experiment. A stock solution was made by dissolving the enzyme with aquadest at the concentration of 10% (w/v).

2.2. Enzymatic reaction

To investigate the influence of enzyme concentration, enzymatic reaction was performed with commercial papain enzyme at the concentration of 0.1, 0.2, 0.3, 0.4, and 0.5 %. The constant concentration of 25% substrate was used and the total volume was 40 mL.

To investigate the influence of substrate concentration, enzymatic reaction was performed with substrate concentration of 5, 10, 15, 20, and 25 %. Commercial papain enzyme at constant concentration of 0.2% was used and the total volume was 40 mL.

2.3. Peptone powder production

An amount of 400 g of frozen fish was thawed, washed, and blended with 1.5 L of water. The fish slurry was then adjusted to pH 8 by adding 2 M NaOH. Aquadest was added to reach total volume of 1,960 mL. Fish porridge was put into a reactor and 20 mL of stock enzyme solution (10% concentration) was added, thus the final enzyme concentration was 0.2%. The mixture was heated at 50°C and stirred at 50, 100, 150, or 200 rpm for 8 h each. Enzymatic reaction was stopped by heating samples in boiling water for 10 min.

Protein hydrolysate was separated from its bone and fish scales by vacuum filtration using a Büchner flask coated with filter paper. The clear protein hydrolysate was spray-dried with inlet and outlet temperature of 90 and 80 °C, respectively and a flow rate of 200 mL/h.

2.4. Analysis of α-amino content

Analysis of α-amino content was performed using formol titration method [8]. Into a 100 mL beaker glass were added 5 mL of sample, 50 mL of aquadest, saturated K-oxalate solution in water (1: 3), and one drop of 0.01 % rosalin-chloride indicator. The mixture was adjusted to pH 8.2 by adding 0.1 M NaOH dropwise while stirred. A 10 mL of formalin (35%, v/v) was added. The titration was continued with the addition of 0.1 M NaOH to pH 9.2. The NaOH volume added was recorded as the amount of titre. Calculation of α-amino content is as follows:

$$\%\,\alpha - amino\ (\%N) = \left(\frac{\Delta V}{sample\ weight \times 10}\right) N.NaOH \times 14.008$$

Where:

%N = Amino concentration
ΔV = Added NaOH volume (titer)
N. NaOH = NaOH concentration (mol/L)

The degree of hydrolysis (% DH) was calculated as follows:

$$\%DH = \frac{\alpha - Amino}{Total\ N} \times 100\%$$

Total N was determined by the Kjeldahl method [9].

2.5. Analysis of soluble protein content

The analysis of the total protein of fish peptone was carried out using Lowry's method [10]. 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. Lowry D solution was prepared by diluting Folin-Ciocalteu reagent with aquadest 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 of solution D and dissolved for 30 min. Sample solution was measured with a spectrophotometer at 500 nm. A series concentration of bovine serum albumin (BSA) was used for standard curve of protein.

3. Results and discussion

The results of peptone production in a 1 L scale using commercial enzyme Xian Arisun ChemPharm are as follows:

3.1. Effect of enzyme and substrate concentration

The effects of varying enzyme and substrate concentration was observed as the formation of α-amino. The results are shown in Table 1 and 2.

Table 1. Effect of enzyme concentration on α-amino concentration.

Enzyme concentration (%)	α-amino concentration (%N)
0.1	0.516 ± 0.03
0.2	0.521 ± 0.02
0.3	0.544 ± 0.02
0.4	0.555 ± 0.02
0.5	0.527 ± 0.02

Table 2. Effect of substrate concentration on α-amino concentration.

Substrate concentration (%)	α-amino concentration (mM)
5	36.7 ± 3.05
10	51.3 ± 4.62
15	79.3 ± 20.13
20	102.7 ± 16.17
25	126.7 ± 32.33

Table 1 shows that varying enzyme concentration did not have significant effect on the resulting α-amino concentration. On the other hand, every 5% increase of substrate concentration resulted in significant increase of α-amino concentration (Table 2). The highest increase was observed at 20% substrate concentration.

Based on results shown in Table 1 and 2, 0.2% enzyme concentration and 20% substrate concentration were used in peptone production. pH 8 was selected as the optimum pH from a preliminary experiment and a previous research using the same substrate and papain enzyme from papaya latex [7].

3.2. Effect of time and agitation on fish hydrolysate production

The effect of time and agitation on fish hydrolysate production was observed as the formation of α-amino. The results are shown in Figure 1.

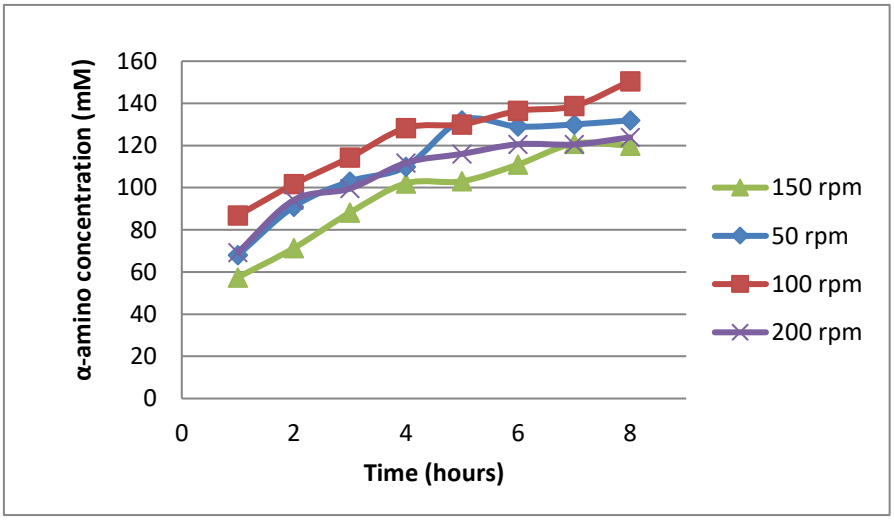

Figure 1. The effect of agitation and enzymatic reaction time on α-amino concentration of fish peptone.

During the 8 h reaction time, agitation of 100 rpm generally produced higher α-amino concentrations compared with other agitation speeds. The highest concentration of α-amino from 100 rpm agitation was obtained at 8 h with concentration of 150 mM. Agitation that produced the lowest α-amino was 150 rpm (123 mM at 8 h). Concentrations of α-amino from agitations of 50 rpm and 200 rpm were relatively similar up to 4 h, while the maximum α-amino concentrations at 8 h were 138 mM (50 rpm) and 124 mM (200 rpm).

3.3. Peptone powder production by spray dryer

Fish peptone resulted by the agitation of 100 rpm was spray-dried with inlet and outlet temperature of 90 and 80 °C, respectively, and flow rate of 200 mL/h. This condition was a modification from Lee [11]. The yield was 20.4 g and its powder performance is shown in Figure 2.

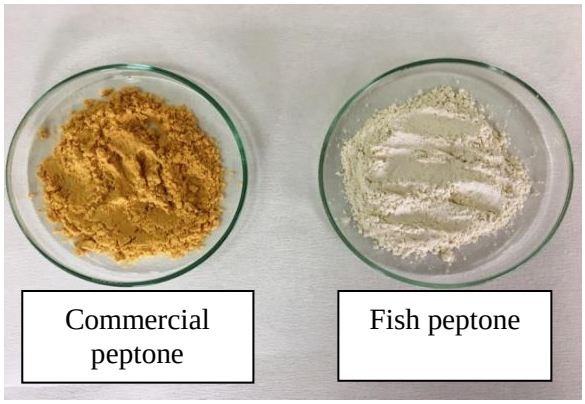

Figure 2. Fish peptone and commercial peptone.

To determine the protein content of fish and commercial peptones, as much as 1 g of each peptone was dissolved in 25 mL of aquadest (ratio1:25). Protein and α-amino contents of the peptone solutions are presented in Table 3.

Table 3. Protein and α-amino content.

Sample (1:25)	Protein content (mg/mL)	α-amino content (mM)
Fish peptone	17.69	124
Commercial peptone	10.34	65

The protein contents were calculated based on the data in Table 1. The protein content in fish peptone powder was 442.25 mg/g whereas the commercial peptone was 258.50 mg/g. Thus, protein and α-amino contents of fish peptone were higher than commercial peptone.

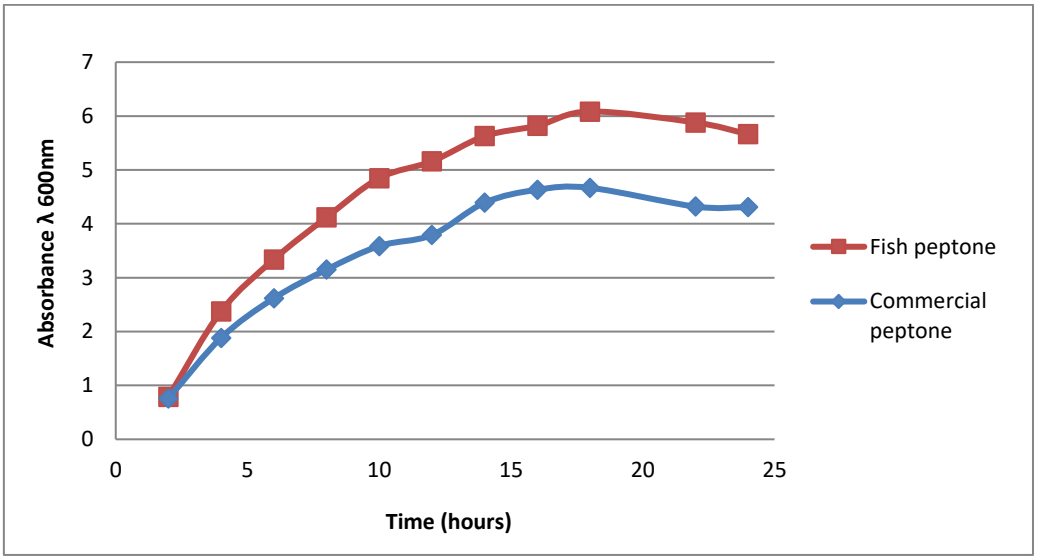

Figure 3. The growth curves of *E. coli* in microbial growth media.

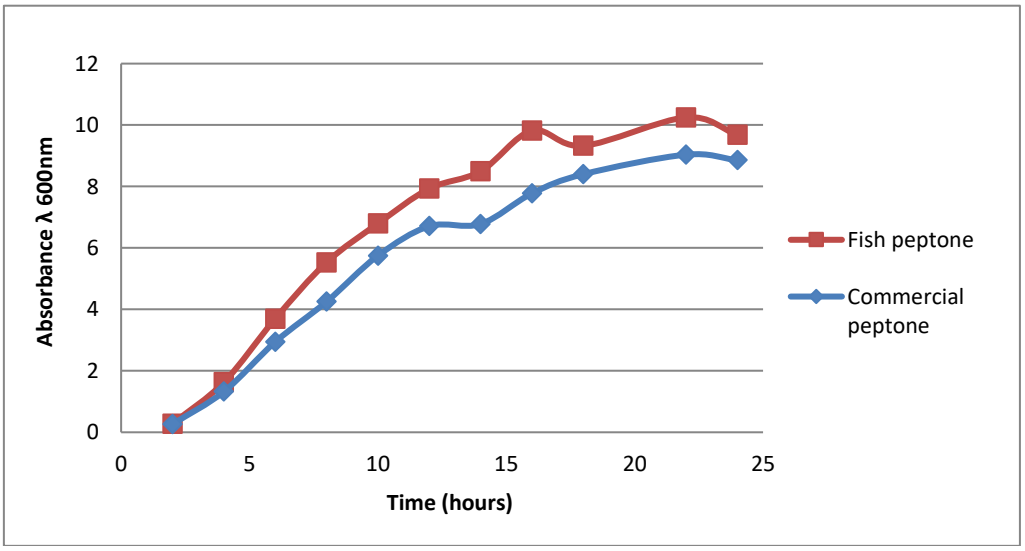

Figure 4. The growth curves of *S. aureus* in microbial growth media.

3.4. Microbial growth

The suitability of peptone fish as a source of nitrogen for microbial growth media was tested using *E. coli* and *S. aureus.* The microbial growth media consisted of 2% of fish or commercial peptone, 2% NaCl, and 1% yeast [12]. Measurement was carried out every two hours at 600 nm. Growth curves for *E. coli* and *S. aureus* can be seen in Figure 3 and Figure 4.

E. coli grown in media containing fish pepton showed a higher cell density compared with commercial peptone. The data shows that lag phase of *E. coli* in peptone was about 2 h. Log phase of *E.coli* in fish peptone and commercial peptone occurred for 18 h with maximum absorbances (A_{600}) of 6.084 and 4.668, respectively. After 18 h, the absorbance of *E. coli* decreased indicating the death phase had occurred. Vieira *et al* [13] reported the performance of peptones produced from different fisheries by-product for *E. coli* compared with commercial peptone. The better performance of fish peptone in comparison with commercial media was also reported by other researches [14].

As shown on Figure 4, the growth of *S. aureus* in fish peptone was higher than commercial peptone. The data shows that lag phase of *S. aureus* in peptone was about 4 h. Log phase of *S. aureus* in fish peptone and commercial peptone occurred for 22 h with maximum absorbances (A_{600}) of 10.248 and 9.036, respectively. The lag phase is defined as the transition to the exponential phase after the initial population has doubled. The lag phase is thought to be due to the physiological adaptation of the cell to the culture conditions. This may involve a time requirement for induction of specific messenger RNA (mRNA) and protein synthesis to meet new culture requirements. Meanwhile, during log phase or exponential growth the rate of increase of cells in the culture is proportional to the number of cells present at any particular time and the death phase is characterized by a net loss of culturable cells [15].

4. Conclusions

Low valued fish can be made into peptone using commercial papain enzymes. Peptone from fish has a high protein content for *E. coli* and *S. aureus* growth.

Acknowledgements

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References

[1] Neidhardt F C, Bloch P L and Smith D F 1974 *J. Bacteriol.* **119** 736–47
[2] Wisuthiphaet N, Kongruang S and Chamcheun C 2015 *J. Med. Bioeng.* **4** 466–70
[3] Al Bahri M B A G, Al Naimi S A and Ahammed S H 2009 *J. Al-Khwarizmi Eng.* **5** 1-19
[4] Badan Pusat Statistik 2014 *Tabel Impor Menurut Komoditi*
[5] Ariyani F, Heruwati E S, Murdinah, Wibowo T B S and Susetyo A R 2001 *J. Penelitian Perikanan Indonesia* **7** 75-84
[6] Dufossë L, De La Broise D and Guerard F 2001 *Curr. Microbiol.* **42** 32-8
[7) S Priatni, W Kosasih, T A Budiwati and D Ratnaningrum 2017 *IOP Conf. Ser.: Earth Environ. Sci.* **60** 012009
[8] Sudarmadji S 1997 *Prosedur Analisa Bahan Makanan dan Pertanian* (Yogyakarta: Liberty)
[9] AOAC 1995 Official Methods of Analysis of the Association of Analytical Chemists (Washington DC: AOAC)
[10] Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951 *J. Biol. Chem.* 193 265-75
[11] Lee G 2002 Rational Design of Stable Protein Formulations, ed Carpenter J F and Manning M C (New York: Springer) pp 135-58
[12] Becton, Dickinson and Company 2009 DifcoTM & BBLTM Manual of Microbiological Culture Media 2nd edition, ed Zimbro et al (Sparks: BD Diagnostics)
[13] Vieira, G H, Vieira R H, Macrae A and Sousa O V 2005 *J. Sci. Food Agric.* **85** 1235–7
[14] Fallah M, Bahram S and Javadian S R 2015 *Food Sci. Nutr.* **3** 153–7
[15] Maier R M 2009 *Environmental Microbiology* pp 38-54 (Academic Press. Inc.)