Kerong fish (*Terapon jarbua*) peptone production using papain enzyme as nitrogen source in bacterial media

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Abstract. *Terapon jarbua* fish is a widespread species distributed mainly in the Indo-West Pacific region and occurs in shallow coastal waters, mangroves and freshwater. In West-Java of Indonesia, the consumption of *T. jarbua* was not bound to arouse interest, but otherwise the fish contain the important protein, such as peptone. Peptone is used to support the growth of bacteria as nitrogen source. The present study was aimed to optimize the fish peptone of *T. jarbua* using papain enzyme concentration and to characterize the fish peptone product. The parameter of fish peptone development was enzyme concentration (0.1-0.5%), temperature (50-65^{o}C), hydrolysis time (4-7 h) and pH (5-8). The fish peptone product was characterized on the content of soluble protein, N-amino acid, AN/TN, and growth curve of *Escherichia coli* and *Staphylococcus aureus*. The optimum fish peptone production was obtained with 0.2% of papain enzyme pH 7 at 65^{o}C for 4 hr. The soluble protein content was of 3,63 mg/ml, N-amino content 0.29%, and AN/TN 0.54%. Fish peptone of *T. jarbua* was more selective for *E. coli* than *S. aureus*.

Introduction

Fish as one of marine resources occupies the certain and significant role in the aquatic contaminant studies and the monitoring water quality. Nowadays, fish production is still as one of producers of insignificant garbage due to waste mainly composed of fish body's parts [1]. Currently, fish and by products discarded by fisheries are becoming large in quantity and creating the serious pollution [2]. On the other hand, the by products contain protein-rich material and normally processes into low market such as animal feed, fish meal even laboratories support such as growth media for microbes. Fish waste can also be used for production of various value added product such as protein, oil, amino acids, mineral, enzymes, bioactive peptides, collagen and gelatine [3].

Terapon jarbua as one of fish in West Java Indonesia is over production, but the consumption of the fish is low due to the high salt level and commonly it is used as fishing bait [4]. In food biotechnology, material with the high protein content, good amino acid, and bioactive peptides have attracted much attention biotechnologist to explore the compounds for antioxidant, antihypertensive, immune-modulatory, and antimicrobial peptides application [1].

Peptone is a protein hydrolysate soluble in mixture of polypeptides and amino acids which is widely used in many biological and biotechnological applications such as microbial biomass [3]. Meanwhile, the literatures of fish peptone using papain enzyme was limited and it was needed to increase the economic value of the fish protein called peptone because it can be as nitrogen source in grow media of bacteria [5]. It has been showed that the trash fish peptone and marine fish peptone from ponyfish (*Eubleekeria splenders*), Yellow stripe travally (*Selaroides leptolipis*) and Mackarel

(*Decapterus maruadsi*) could be hydrolized using papain enzyme with various concentration [6][7]. The aim of the present study was to optimize of the production of fish peptone of *T. jarbua* using papain enzyme and to characterize the fish peptone product.

Experimental

2.1. Fish peptone preparation

T. jarbua fish was purchased from Indramayu, West-Java and transported to the laboratory in sealed foamed polystyrene boxes containing flaked ice. A total of 100 g of fish was mixed with water in a equal percentage of weight with water (ratio 1:4), and blended. The hydrolysis was carried out at various pH of 6-8 and at varied papain enzyme concentration (0.2-1.0% v/w). Papain enzyme was added into the mixture, heated at 50-65^{o}C for 4-8 h and inactivated at 85^{o}C for 15 min. The hydrolyzed mixtures were centrifuged (7000g, 20 min) at 4^{o}C. Finally, the supernatant was collected and dried to obtain soluble peptone.

2.2.Proximate composition

The proximate composition of fish samples was determined based on the Association of Official Analytical Chemist (AOAC) method. The moisture content was determined by drying samples overnight at 105^{o}C for 2 hr 30 min. The crude protein content was determined by the Kjeldahl method. The fat content was determined using soxhlet method and the ash content was obtained in furnace at 500^{o}C until fully becoming ash. The carbohydrate content was achieved by calculating the difference of total moisture, protein, fat, ash content.

2.3.Assay for total protein

The total protein of *T. jarbua* fish peptone was carried out based on Lowry's method. A mount of 500 µl samples were added with 2.5 mL solution C (Lowry A 50 mL + 1 mL Lowry B) and dissolved for 10 min. Samples were then added with 0.5 mL solution D (Folin : dH_20 with ratio 1:1), dissolved for 30 min, and measured at 500 nm.

2.4.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 added with 20 mL dH_20, 40 mL potassium oxalate (1:3) and 3-4 drops of phenol phtalein indicator (PP). Sample was then titrated using 0.1N NaOH, added with 2 mL of 40% formalin and 3-4 drops of PP indicator. Sample was titrated using 0.1N NaOH and measured its protein content.

2.5.Bacterial growth

Bacteria of *Escherichia coli* and *Staphylococcus aureus* were used for the bacterial growth performance test. The sterilized test medium consisted 1 g of fish peptone extract, 100 mL dH_2O, 0.5% yeast extract, and 1% NaCl with pH 7. The sterilized commercial medium contained 1g of commercial peptone, 100 mL dH_2O, 0.5% yeast extract, 1% NaCl with pH 7. A mount of 1mL pure culture was added with 9 mL medium, incubated at 37^{o}C for 24 hr. The optical density of culture was measured at 600 nm every 2 hr for 24 hr.

3. Result and discussion

3.1. Chemical compounds

The proximate analysis showed that the *T. jarbua* fish contained total protein and fat were of 58.8 and 9.6% respectively. The fish *T. puta* contains 20-30% protein and 2-12% lipid [4]. The chemical compounds of fish can be varied between species. The proximate analysis showed the variation of results according to environment, age, sex species, migration, nutrient, and preservation method [8]. The value of total protein and N-amino content from various temperatures and time incubations for screening the optimal production of fish peptone was described in Table 1.

Table 1. Total protein and N-amino content from fish peptone on pH 7.

	Incubation time (h)	Protein(mg/mL)	N-amino (%)
50°C	4	2.8	0.07
	5	2.5	0.06
	6	2.6	0.07
	7	2.4	0.05
55°C	4	2.3	0.05
	5	2.5	0.06
	6	2.0	0.06
	7	2.4	0.06
60°C	4	2.8	0.07
	5	2.8	0.07
	6	2.9	0.07
	7	2.9	0.08
65°C*	4	3.6	0.07
	5	3.1	0.07
	6	2.9	0.07
	7	2.9	0.07

*Optimal condition for the highest yield of total protein and N-amino of fish peptone

Table 1 shows that the optimal condition was achieved at pH 7, incubation at 65°C time for 4 hr with total protein and N-amino were of 3.6 mg/mL and 0.07% respectively. Various protein and N-amino obtained might be due to the variety of achievement from protein whereas N-amino from the activity of papain enzyme. The papain enzyme activity was affected by the concentration and purity of enzyme, temperature, and time of hydrolysis. The recent study showed that 0.2% enzyme papain produced high peptone. In addition, fish carp contained high peptone using 0.26% papain enzyme, at 60°C for 3 hr hydrolysis [1]. Preparation of fish peptone as growth media for bacteria with the determination of enzyme concentration is one factor degradation speed rate of proteolytic enzyme [8]. Soluble protein content and N-amino of fish peptone *T. jarbua* product was of 3,63 mg/ml, 0.29%, respectively, and AN/TN 0.54%. Almost complete protein solubilisation could be achieved within an hour at 40°C, 1 % enzyme/substrate ratio (w/w) with papain and bromelain [9].

3.2. Bacterial growth analysis

The growth curve of *E. coli* and *S. aureus* on two kinds of peptone, peptone commercial and fish peptone of *T. jarbua*. were shown in Figure 1 and Figure 2.

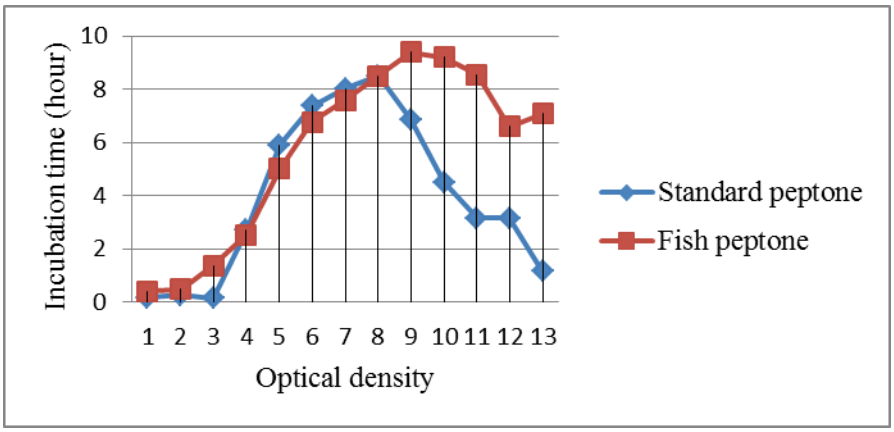

Figure 1. The growth curve of *E. coli* cultured in standard peptone and fish peptone.

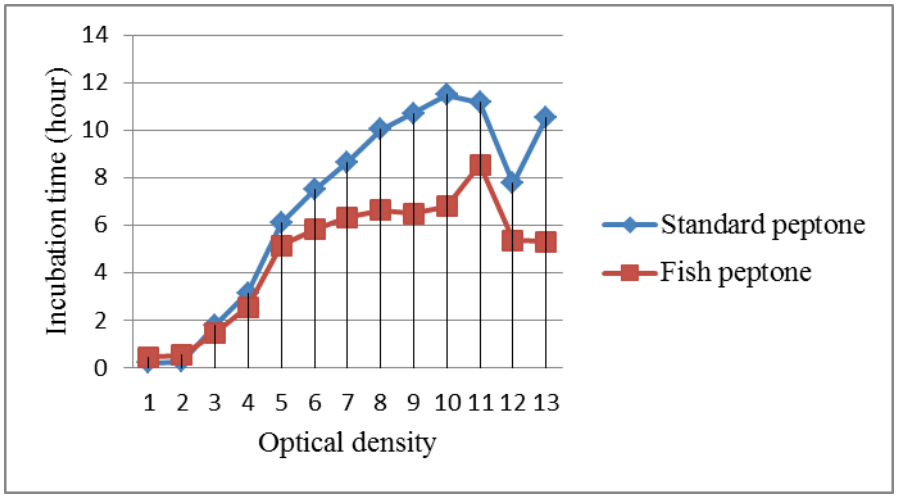

Figure 2. The growth curve of *S. aureus* cultured in standard peptone and fish peptone.

Figure shows that the growth of *E. coli* cells using fish peptone was higher than commercial peptone as well as a bit longer time to obtain maximum growth. On the other side, Figure 2 describes that the growth of *S. aureus* using fish peptone was lower as compared to commercial peptone. It indicated that fish peptone of *T. jarbua* was more selective for *E. coli* than *S. aureus*. Peptone from enzymatic hydrolysis by crude papain enzyme showed different performance in both bacteria growth profile**.** The hydrolysis process conducted the important roles in processing peptone which contributed to reveal the better performance of fish waste peptone in microbial growth [5]. It also might depend on the ability of bacteria to use the fish peptone to improve the cell growth. Fish peptone has a fairly high protein containing nitrogen easily for bacteria to absorp well [5][6]. The previous research showed that proteolytic enzyme papain for protein recovery from *Scomberomorus commerson* fish produced bioactive properties as well [10]. Bioactive compounds from fish protein hydrolysates also could be obtained by using base, acid, endogenous enzymes, bacteria, and digestive protease [11].

4. Conclusion

Peptone has been produced from from *T. jarbua* of which the optimum condition obtained with 0.2% papain enzyme, pH 7, at 65°C for 4 hr hydrolysis. The fish peptone was more selective for *E. coli* than *S. aureus*.

Acknowledgement

The authors greatly acknowledge the funding of the independently research project of LIPI 2016. The authors also thanked to the collaboration of staffs and supply the material.

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