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Characterization of Halal Protein Hydrolysates Extracted from Bycatch Fish *Rastrelliger Kanagurta*

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Abstract: *Rastrelliger kanagurta*, one of species from by-catch fish has been exploited for extraction of fish protein hydrolysates to determine the proximate composition of extracted fish protein hydrolysate through acidic and alkaline hydrolysis. The results of this study indicate that the protein hydrolysate obtained from both hydrolysis showed highest protein concentration which 64.1±0.2 and 50.5±0.1 respectively. Meanwhile, the composition of lipid at pH 3 is 2.5±0.1 and pH 12 is 10.0±0.2, much lower in hydrolysed samples compared to unhydrolyzed samples. The extracted fish protein hydrolysates showed the capability to provide nitrogen source in the culture medium for the growth of lactic acid bacteria, *Lactobacillus* species as good as commercial peptone. The lag phase of *Lactobacillus* sp. was shorter in the commercial peptone broth and fish protein hydrolysate from acid hydrolysis compared to fish protein hydrolysate from alkaline hydrolysis. In conclusion, the proximate composition of fish protein hydrolysates from *R. kanagurta* were successfully determined and had showed the potential as medium for *Lactobacillus* species growth.

Keywords: Acid hydrolysis, Chemical hydrolysis, Culture medium, Optimization

1. Introduction

Fisheries industry occupy a major place in promoting the socioeconomic development of the country due to main source of nutritional compounds for the world's population. Moreover, about 38.5 million tons of species of the global marine fish captures are known to be by-catch and discarded due to low market value (Prabha et al. 2016). These large quantities of waste need appropriate management to avoid chemical and microbial deteriorations if improper handling. However, these fish wastes are rich in protein and have various applications in industry such as products flavor enhancer, feed supplement, pharmaceutical grade applications and food ingredients such as sweeteners or food additives by applying biotechnology methods (Muhammad et. al.,2015). One of the species which found as bycatch fish at fishing jetties in Sekinchan is *Rastrelliger kanagurta. R. kanagurta* well known as *ikan kembung* is the most popular marine fish in Malaysia due to its

abundance, year-round availability, low cost and high polyunsaturated fatty acid (PUFA) content. An interesting possibility is to hydrolyze the waste to obtain fish protein hydrolysates (FPH)-containing proteins or peptone with desirable functional properties with different approach. Due to protein hydrolysate (FPH) limitation in human industry because of taste defects, this fish peptone is very useful in microbial growth media in fermentation industry (Nurdiyana et al., 2015). Peptone are a water-soluble mixture of polypeptides and amino acids are widely used in microbial biomass. Most of the peptones are obtained from bovine or porcine origin, which restricts their usefulness when generating halal and kosher products (Fallah et. al.,2015). However, the origins of peptone have to be considered due to non halal source (porcine) and the risks of bovine spongiform encephalopathy (BSE) and foot-and-mouth disease (FMD) in recent years (Safari et.al., 2012). Most of earlier studies on alternative peptones focused on peptones performance towards different types of microorganisms and methods of hydrolysis to produce peptones. Therefore, the aims of this research are to determine the proximate composition of protein hydrolysate from *R. kanagurta* extracted through different methods of hydrolysis and to validate the ability of the fish protein hydrolysate to be exploit as a microbial media component.

2. Materials and Methods

2.1 Preparation of Raw Fish

About 5kg of *R. kanagurta* were bought from fishing jetties at Sekinchan, Selangor. Every 1kg of *R. kanagurta* were washed, mixed with 1 litre of distilled water and minced twice using blender until homogenize and the sample mixtures were frozen at −10°C.

2.2 Acid and Alkaline Hydrolysis

The frozen homogenates were hydrolysed by alkaline hydrolysis using NaOH which at pH 12 meanwhile for acidic hydrolysis using HCL which pH value at pH 3. Each set of pH were prepared in triplicate in conical flask and then placed in incubator shaker with 200 rpm, 70℃ for 90 minutes. The reactions were terminated by adjusting the pH into pH7 using HCl and NaOH. The mixtures were centrifuged 6000 rpm for 15 minutes and then filtered using Whatman filter paper number 1. The filtered supernatants were stored in falcon tube in deep freezer for -80℃. The frozen sample that was stored at -80℃ were put into freeze-dryer for 4 days.

2.3 Protein Content

Protein concentration was determined through Bradford method. Bradford assay was prepared using 100 mg Coomassie Brilliant Blue G-250 then dissolved in 50mL 95% ethanol (C_2H_5OH). During the stirring, 100mL of 85% phosphoric acid (H_3PO_4) was carefully added. After that, water was added to a total volume of 1L. The preparation was done in dark place. The solution was filtered, wrap with aluminium foil and kept at 4℃. Standard curve for Bolvine Serum Albumine (BSA) protein concentration was prepared using Bradford reagent. A series of BSA concentration mixed with 1ml of Bradford reagent and the absorbance were read at 595nm using UV spectrophotometer. The concentration used were 0, 0.2, 0.4, 0.8, 1.2, 1.6 and 2mg/ml. A graph was plotted using the absorbance against BSA concentration. The equation of y=mx+c was retrieved. The extracted protein hydrolysate at pH 3, pH 12 and

unhydrolyzed samples were used to measure the protein concentration. The protein concentrations were then calculated using the equation from standard curve.

2.4 Proximate Analysis

2.4.1 Moisture and Lipid Content

The sample of 1g protein hydrolysate was spread on an aluminium dish foil that was pre-weighted. After that it will be put in an oven at 100˚C for 3 hours for drying process and then placed in desiccator until it reached constant weight. The moisture content was measured by the total weight loss during drying process. Meanwhile, the total lipid was determined by Soxhlet extraction method.

2.4.2 Ash Content

Before ash analysis was conducted, the crucibles were first prepared. All the crucibles were put into furnace with temperature 550˚C for 2 hours. After 2 hours, the furnace was turned off and the temperature was let to cooled down overnight. The next day, the crucibles were taken out from furnace and put into dessicator for 1 hour. The empty crucibles were weighted and recorded. After that, 1g of each sample was weighted and put into a furnace at 550˚C for 3 hours. After 3 hours, the furnace was turned off and let it cooled down overnight. The next day, the crucibles that contain ash were put into dessicator for 30 minutes or until it cooled down. The weight of the ash and crucibles were determined.

2.5 Broth Media Preparation

The broth media was prepared for extracted fish peptone and commercial peptone. The materials used for fish protein hydrolysate broth preparation were potassium dihydrogen phosphate (KH_2PO_4) 0.3 g, disodium phosphate (Na_2HPO_4) 0.3 g, dextrose 0.36 g, extracted fish peptone 0.6 g and nutrient broth 1.8 g. The same materials and amount were used to prepare commercial broth media but the buffered peptone water were used instead of extracted fish peptone. The broth media were prepared for 120 ml for each available pH and were done in triplicate.

The *Lactobacillus* culture were mixed into 40 ml of each broth media from extracted fish peptone and commercial peptone. The cultures were placed in incubator shaker with 150 rpm at 30℃. The absorbances were read at 600 nm for 0, 2, 4, 6, 8 and 10 hours for every culture. The growth curve of *Lactobacillus* in the broth media was plotted with absorbance against time.

2.6 Statistical Analysis

All experiments were carried out in triplicate and the results were presented as group means ± standard of deviation (SD) and statistically significant differences between mean values were determined by using SPSS 17.0 software.

3. Result and Discussion

The proximate composition of unhydrolyzed raw material and hydrolysate protein extracted in chemical hydrolysis process is shown in Table 1. Based on the results, it revealed that the *R. kanagurta* has high protein content with low lipid content from both hydrolysis, acid and alkaline. The protein content from acid hydrolysis was 64.1±0.2 and 50.5±0.1 for alkaline hydrolysis. Meanwhile low protein content discovered from unhdyrolysed sample or known as control. The high protein content for fish protein hydrolysates is because of solubilization of proteins during hydrolysis and removal of insoluble solid matter by centrifugation (Chalamaiah et al., 2012). Low lipid content in hydrolysed sample (2.57±0.1%) for acid hydrolysis when compared to unhydrolyzed raw sample (25.4±0.1%) mainly due to the interference of solvent in acid hydrolysis towards free lipids cross-linked to proteins and as well due to break-up protein particles. The result in line with previous studies where the protein content 83.62±1.05 and lipid 0.07±0.01 as reported by Saputra et. al., 2020.

Table 1: Proximate Composition (% ml) of control and Fish Protein Hydrolysate Sample[a] (Different pH)

Composition	Control	Fish Protein Hydrolysate
pH 3		
Protein	45.0±0.3	64.1±0.2
Lipid	25.4±0.1	2.5±0.1
Moisture	12.6±0.2	8.9±0.1
Ash	17.0±0.1	24.5%±0.1
pH 12		
Protein	43.0±0.1	50.5±0.1
Lipid	27.4±0.1	10.0±0.2
Moisture	15.6±0.2	15.6±0.02
Ash	15.0±0.1	23.9±0.1

a values represented mean±SE (n=3). FSH: Fish protein hydrolysate, SE: Standard error

The moisture content of protein hydrolysate from alkaline hydrolysis at pH 12 was highest due to the higher amount of alkali in order to increase the pH to 12. However, the moisture content from both hydrolysis and unhydrolyzed sample of *R. kanagurta* showed low moisture content. This result is contrast with Wisuthiphaet et al., (2015), reported the fish protein hydrolysate (FPH) of Ponyfish (*Eubleekeria splendens*), Yellow stripe travally (*Selaroides leptolipis*) and Mackerel (*Decapterus maruadsi*) give moisture content of 92.66%, 76.33% and 85.00% treated with 4, 6 and 8 M HCl respectively. The possibility of low moisture content in *R. kanagurta* sample due to ratio of water and sample added, which used 1:2 compared in Wisuthiphaet et al., (2015) used 1:1 ratio. The addition of water

hydrolysis during the preparation of fish protein hydrolysate play important role in determining the moisture content.

Determination of inorganic residue and impurities contain in the sample such as bone known as ash content. This content can determine the amount and type of minerals present in the sample and have influence towards the growth or retardation of microorganisms (Fatemah et al., 2015). The highest ash content of *R. kanagurta* protein hydrolysate was obtained through acid hydrolysis at pH 3 which is 24.5%±0.1. This indicated that there were present of the impurities and inorganic matter in the acid hydrolysis sample as well as detection of HCl during the hydrolysis process. These impurities can be obtained after ignition and oxidation of the organic matter (Simons, 2017). Based on data analysis using SPSS, all the criteria in proximate analysis such as protein, lipid, ash and moisture content showed no significance difference between hydrolysed samples from acid (pH 3) and alkaline (pH 12). However there are significance difference for unhydrolyzed sample with hydrolysed samples.

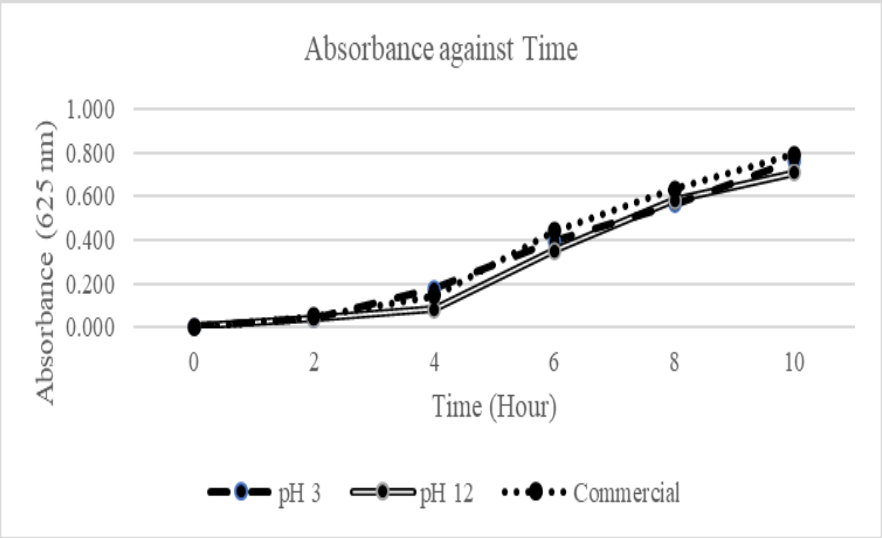

Fig. 1: Growth curve of *Lactobacillus* on broth medium containing the commercial peptone and fish protein hydrolysate

Lactobacillus sp. showed similar growth rate on the broth medium containing commercial peptone and fish protein hydrolysate. As shown in Figure 1, the lag phase of *Lactobacillus* sp. was shorter in the commercial peptone broth and fish protein hydrolysate pH 3 compared to fish protein hydrolysate pH 12. This indicated that the *Lactobacillus* sp. easily adapt to the new environment of the medium. Furthermore, the protein content of the peptone is in line with the nitrogen compound since nitrogen is an essential source in the microbial growth substrates (Abdul et. al., 2020). The exponential phase happened at 6 hrs (from 4 hrs to 10 hrs) for all the tested media broth as same as reported from previous study (Saputra et. al., 2020). From the Figure 1 above, the commercial peptone broth media have the highest growth of *Lactobacillus* sp. The fish protein hydrolysate extracted using pH 3 has the growth of *Lactobacillus* sp. that is almost the same with commercial peptone. It was followed by fish peptone from pH 12 that slightly below commercial peptone. The protein hydrolysate extracted using pH 3 have the highest *Lactobacillus* sp. growth due to the moisture content lower than protein hydrolysate extracted from pH 12.

The use of acid for hydrolysis also affect the growth because the type of amino acid it retain and destroyed. Both acid and alkaline hydrolysis destroy serine and threonine which are the inhibitory component of microorganism (Chasanah et. al., 2019). Besides, the amino

acid valine retained in acid hydrolysis increase the growth of bacteria compared to alkaline hydrolysis. There are several factors plays important role in characterize the extracted fish protein hydrolysate such as the types of fish, hydrolysis method, temperature and pH.

4. Conclusion

In conclusion, the bycatch fish *R. kanagurta* has the potential to be used as raw material to produce fish protein hydrolysate because it has a high protein content with low lipid content. This study revealed the optimum hydrolysis method of protein hydrolysate from *R. kanagurta* is acid hydrolysis at pH 3. The culture of *Lactobacillus* in fish protein hydrolysate broths showed the growth of bacteria is promising and can be used as medium components with further optimization and improvement.

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