Effect of incubation time and pH on the protein characterization of the aqueous soluble phase of acidified mackerel by-product

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Abstract. This study aimed to evaluate the protein content from aqueous soluble phase (ASP) of mackerel by-product with a variation of time and pH. Mackerel by-product was hydrolyzed by different incubation time (0, 15, 30, and 45 h) and pH (3.2, 4.2, 5.2, and 6.2) to produce ASP. The study conducted on a completely randomized design (CRD) followed by the Tukey test. The different incubation times and pH showed a significant effect (P<0.05) on yield, crude protein, and soluble protein of ASP. The pH and yield values of ASP in the range of 4.1 to 6.3 and 11.16% to 30.69%, respectively. The crude protein and soluble protein content ranged from 1.28% to 3.27%, and 0.98 g/L to 2.31 g/L, respectively. The highest yield and crude protein of ASP was under pH 3.2 for 15 h. The amino acid analysis showed that several amino acids were detected in considerable amounts in the ASP. This study indicated that the ASP of mackerel by-product could be a potential material of fish peptone for bacterial growth media.

1. Introduction

The total volume of capture fisheries production in the world is increasing rapidly. FAO reported that the capture fisheries production reached 90.91 million tons in 2016. Mackerel is one of the economically important species in the world [1], accounting for 4.6% of total global fisheries production amounting to 4.17 million tons in 2016. Generally, mackerel processed in the form of a fillet and canned fish products. Among them, about 30% of the total mackerel processing industry are by-products, including head, viscera, fins, and scales. Mackerel by-products can be utilized as products that have economic values such as liquid fertilizer, fish meal, and animal feed. Besides, valorization fish by-products can prevent serious environmental problems.

Mackerel by-products can be recovered into products that are more acceptable and marketable. Fish can be acidified and separated into three phases using centrifugation, where one of them is an aqueous soluble phase (ASP). This phase is rich in protein content with high amino acid value. Khalil [2] reported that the ASP of bolti fish (Tilapia nilotica) viscera contained higher amino acid value when compared to the commercial peptone sources, such as Bactotryptone, Bactosoytone, and yeast extract.
As a high protein product, the ASP of fish can be produced as a peptone due to solubility in water. Several researchers have extensively studied fish peptones and their function as bacterial growth media, including tilapia and cobia [3], cod [4], boso [5], yellow-striped fish [6], and herring [7].

Peptones extracted from fish by-products have attracted researchers in recent years, mainly extracted by chemical compounds. Acid solutions are commonly used in the extraction of protein derived fish by-products. Córdova-Murueta et al. [8] revealed that the selection of extraction agents based on three factors: cost, availability, and bactericidal action. Shirahigue et al. [3] stated that the incubation process of fish under acidic conditions could promote autolysis. The peptide bonds of the fish protein are broken down by digestive enzymes into soluble products with low molecular weight. As a result, a complex mixture of peptides and amino acids that can be used as a nitrogen source in microbial culture media [9]. Nitrogen is one of the primary substrates for microbial growth, and peptones are the significant sources of this nutrient [10]. Furthermore, Shirahigue et al. [3] showed that peptones hydrolyzed using citric acid, formate, and propionate could be used as growth media for Escherichia coli and Staphylococcus aureus bacteria.

Mackerel by-product acidified by different incubation time and pH was less explored. Therefore, studies on incubation time (0, 15, 30, and 45) and pH (6.2, 5.2, 4.2, and 3.2) for the production of ASP of mackerel by-products are essential as a basic understanding of peptone production. The purpose of this study was to obtain the crude protein, soluble protein, and amino acid from ASP of mackerel by-product with different incubation time and pH.

2. Material and methods

2.1. Material
The mackerel by-product obtained from PT. Kelola Mina Laut (Gresik, East Java, Indonesia). The mackerel by-product was put in polyethylene plastic and conditioned at cold temperatures (4 °C) during transportation. After the sample arrived at the laboratory, the sample was washed with running water and grounded using a milling machine. Then, the sample was put into polyethylene plastic and stored in the freezer at a temperature of -20°C for up to 1 week. All chemicals used were of the analytical grade.

2.2. Preparation of ASP of mackerel by-product
The hydrolysis of mackerel by-product was done by a modified method from Shirahigue et al. [3]. The first step was to prepare 900 g of mashed mackerel heads that had been mashed, and then 10% distilled water was added to dissolve the protein. The mackerel by-product obtained with the addition of HCl (6.2, 5.2, 4.2, and 3.2) in the sterilizing state. After the pH was measured, the samples were incubated at room temperature for 0, 15, 30, and 45 h. Followed by the inactivation of the hydrolysis process at 85°C for 20 minutes and centrifuged at 5000 rpm for 10 minutes to separate the solid phase, liquid phase, and phase oil.

2.3. pH value measurement
The pH values of raw material and ASP was measured using a pH meter (HI2002-01 edge®).

2.4. Chemical analysis
Proximate analysis of raw material consists of crude protein, fat, moisture, and ash were determined by the methods of AOAC [10]. For ASP of acidified mackerel by-product, only crude protein was measured by the AOAC method [10]. The soluble protein was analyzed using the method of Lowry et al.[11].

2.5. The yield of an aqueous soluble phase of mackerel by-product
The yield of ASP of mackerel by-product was calculated using the following equation:
Yield(%) = \frac{M}{M_o} \times 100 \quad (1)

Where M was the weight of ASP (g) obtained, and Mo was the weight of mackerel by-product.

2.6. Determination of amino acid
The amino acid was analyzed at the Indo Genetech Saraswanti laboratory, Bogor, West Java. The analysis followed the company method. Amino acids were separated using ultra-performance liquid chromatography (UPLC).

2.7. Statistical data analysis
Statistical analysis was done using analysis of variance (ANOVA) followed by Tukey’s posthoc test using SPSS 25.0 software. Statistical significance was considered at P<0.05.

3. Results and discussion

3.1. Proximate composition of mackerel by-product
The proximate composition of mackerel by-products (head portion) as a raw material is shown in Table 1. The by-products contained protein, fat, moisture, and ash, accounting for 17.37%, 3.56%, 74.23%, and 2.72%, respectively. Chemical composition of fish varies significantly among species and from an individual fish to another, depending on age, sex, environment, and season [12]. When compared with the chemical composition of sole fish by-products (protein 14%, fat 0.7%, ash 1.3%, and water 84.6%), mackerel by-products had higher protein, fat, and ash content than that of sole fish by-products [13]. The reason might be that the moisture content of mackerel by-products was lower than that of sole fish by-products. Theoretically, when the moisture in the foodstuff like fish, is high composition, consequently the protein content is low.

Table 1. Proximate composition of mackerel by-product.

| Component  | Value (%) |
|------------|-----------|
| Protein    | 17.37     |
| Fat        | 3.56      |
| Moisture   | 74.23     |
| Ash        | 2.72      |

Figure 1. The fraction of acidified mackerel by-product.
3.2. Yield of ASP

Centrifugation resulted in three fractions shown in Figure 1. The first phase was the pellet formed at the bottom side. Pellet contained insoluble materials, including bones, scales, and parts of phospholipids. The main phase in the middle tube was soluble protein. This fraction was selected as raw materials for peptone production. The last phase formed at the top, which contained many fats.

Yield is one of the essential parameters in the process of producing peptones. The higher ASP yields indicated the more efficient treatments. The yields of ASP obtained are tabulated in Table 2. There were significant differences between each treatment (P<0.05). It was indicated that the pH and incubation time affected the yield of ASP. The yields of ASP ranged from 11.6% to 30.69%. The highest yield was the treatment of pH 3.2 for 30 h, while the treatment of pH 6.2 with 45 h incubation expressed the lowest yield. In general, the low pH (3.2) showed the highest yield. These results were following by the statement of Nolsøe and Undeland [14], the more acidic pH used for protein hydrolysis would generate higher yields.

| Treatment | Yield (%) | Crude Protein (%) |
|-----------|-----------|------------------|
| Hour      | pH        |                  |
| 0.0       | 3.2       | 28.86±0.29        |
|           | 4.2       | 27.48±0.36        |
|           | 5.2       | 21.39±0.15        |
|           | 6.2       | 18.19±0.18        |
| 15.0      | 3.2       | 29.37±0.46        |
|           | 4.2       | 24.26±0.23        |
|           | 5.2       | 13.36±0.18        |
|           | 6.2       | 23.98±0.39        |
| 30.0      | 3.2       | 30.69±0.34        |
|           | 4.2       | 23.67±0.39        |
|           | 5.2       | 13.52±0.27        |
|           | 6.2       | 19.38±0.16        |
|           | 3.2       | 26.16±0.26        |
| 45.0      | 4.2       | 21.10±0.23        |
|           | 5.2       | 19.73±0.24        |
|           | 6.2       | 11.16±0.20        |

3.3. Protein characterization of ASP

The crude protein of ASP was determined, and the results are tabulated in Table 2. The total protein of ASP was in the range of 1.28% to 3.27% based on a wet weight basis. The highest total protein was in the treatment of pH 3.2 for 45 h incubation, and the lowest total protein showed in the pH of 5.2 for 15 h. The results were in line with the nitrogen content. It was due to the total protein value was obtained from nitrogen content multiplied by a conversion factor of 6.25. Crude protein is an essential point in peptone production. The desired peptone is high in total nitrogen content.
The pH and incubation time of ASP had a significant effect on the soluble protein of ASP (P<0.05). The soluble protein ranged from 0.98 g/L to 2.1 g/L. The highest soluble protein of the ASP extracted from mackerel by-product was expressed in the treatment of pH 3.2 for 45 h, and the lowest of soluble protein was in the pH 5.2 for 30 h, around 0.98 g/L (liquid basis). Generally, the lower pH treatments would obtain the higher the soluble protein of samples. The longer incubation process will produce proteins and peptides with smaller molecular weights and form polar groups on the surface of the protein. This causes an increase in the ability of hydrogen bonds with water [18].

Table 3. Amino acid composition of ASP of mackerel by-product.

| Component     | Mackerel (%) | Bactopeptone (%) |
|---------------|--------------|------------------|
| L-Serine      | 5.54         | 1.5              |
| L-Glutamic acid| 12.74        | 8.1              |
| L-Phenylalanine| 3.05         | 2.8              |
| L-Isoleucine  | 3.21         | 2.1              |
| L-Valine      | 4.59         | 2.8              |
| L-Alanine     | 9.32         | 9.2              |
| L-Arginine    | 6.91         | 3.8              |
| L-Glycine     | 16.73        | 15.9             |
| L-Lysine      | 6.88         | 3.4              |
| L-Aspartic acid| 8.74         | 5.0              |
| L-Leucine     | 6.52         | 3.8              |
| L-Tyrosine    | 0.77         | 0.6              |
| L-Proline     | 8.73         | 8.8              |
| L-Threonine   | 4.10         | 1.1              |
| L-Histidine   | 2.19         | 0.8              |

The results of amino acid determination on ASP are shown in Table 3. The ASP had a slightly higher percentage of amino acids compared to the commercial peptone (Bactopeptone). The amino acid composition showed that several amino acids were present in considerable amounts in the hydrolysate,
among them glycine, glutamic acid, alanine, aspartic acid, and proline. The highest amino acid values in mackerel and commercial peptones were glycines with values of 16.73% and 15.9%. Amino acid test results had almost the same results as the study of Najim et al. [19] that peptone hydrolyzed from fish waste had a high content of glutamate acid.

Amino acids are an important aspect of the media used for bacterial culture in different microorganisms. This relates to the need for amino acids in each microorganism that can be used as a limiting factor in culture media. The results of various amino acids indicate that each amino acid has a different level of sensitivity to the pH value during the hydrolysis process [19]. However, ASP of mackerel by-product and commercial peptone showed amino acid values that were not much different.

4. Conclusion
In summary, the different incubation time and pH variations showed a significant effect on the yield, crude protein, and soluble protein on the ASP extracted from mackerel by-product. The yields of ASP in the range of 11.16% to 30.69% (liquid basis). The crude protein and soluble protein content ranged from 1.28% to 3.27%, and 0.98 g/L to 21 g/L, respectively. The highest content of yield, crude protein, and nitrogen content was under pH 3.2 for 15 h incubation time.

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