Biochemical and physicochemical analysis of fish protein isolate recovered from red snapper (Lutjanus sp.) by-product using isoelectric solubilization/precipitation method

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Abstract. The effect of acid- and alkali-process on biochemical and physicochemical characteristics of fish protein isolate from red snapper (Lutjanus sp) by-product was evaluated. Protein recovered by alkali process (16.79%) was higher compared to acid process (13.75%). Reduction of lipid content and total volatile basic nitrogen (TVB-N) exhibited in both treatments indicated both process improved fish protein isolate recovered from red snapper by-product. In addition, the increasing of water holding capacity and oil binding capacity were observed. However, high peroxide value of fish protein isolate was showed in both treatment. This finding indicated that acid and alkali process can be used as a useful method to recover proteins from red snapper by-product. Alkali process gave a protein isolate with better overall quality compared to acid process.

1. Introduction

Consumer demand for functional food has been predicted to increase to a value of USD100 million in 2013 and has showed a positive trend from year to year [1]. Seafood-based functional food is developed from various sources such as krill, squid, and seaweed. Red snapper (Lutjanus sp.) is important for fillet production in Indonesia for its high quality and the demand for the fillet. During the filleting process, a large amount of low-value by-product is produced, and it is used as fish meal for animal feed. Head by-product from fish processing is about 20% of the fish weight [2], while the total by-product consisting of head, frame flesh and scale comprises 60% of the fish weight [3]. Due to the high value of fish head, many restaurants in Indonesia use red snapper head as traditional food. Meanwhile, the frame, skin, and scale are discharged or used as fish meal, plant fertilizer or human value-added foods.

Fish frame, scale, and skin contain valuable proteins such as myofibrillar proteins, stromata, sarcoplasmic proteins, and others that can be recovered for human consumption [4]. There have been some reports about the development of functional proteins and peptone using hydrolysis method from red snapper by-product for silage or fish feed [5, 6]. Currently, protein recovery with pH-shift method or isoelectric solubilization/precipitation has been studied. Protein recovery of bigeye snapper (Priacanthus spp.) with pH-shift method was evaluated to have a yield of 30% and acceptable characteristics [4].

Isoelectric solubilization/precipitation (ISP) processing is selective, pH-induced water solubility and removal of lipid, bone, scales and skin [7]. This method was proposed by Hultin and Kelleher [8]. The basic concept of ISP is about protein side-chains that have different electrostatic charges depending on
the pH condition. At certain pH levels, fish protein will solubilize (on), and at isoelectric point(pI), fish protein will precipitate (off) [9]. The pH for protein solubilization is low (acid) at around 2-3.5 or high (alkali) at around 11-13 [10].

Fish protein isolate produced with ISP can be applied as a raw material in the making of Frankfurter-type fish sausages [11], functional fish protein gel [12], or fish ball [13]. The yield of the fish protein isolate ranged at 50–87 % [4]. Therefore, ISP process is an effective way for recovering proteins from the by-product of fish processing industry. The aim of this study was to isolate and characterize the fish protein isolate recovered from red snapper (Lutjanus sp.) filleting industry by-product.

2. Methodology
2.1. Materials
The fish by-product was purchased from a filleting factory located in Sidoarjo, East Java, Indonesia. The sample was wrapped in polyethylene bag under frozen condition (-18°C) and transported to the laboratory. The sample was directly stored in the laboratory freezer (-18°C) prior to further process. The hydrochloric acid and sodium hydroxide were of p.a. quality.

2.2. Fish by-product preparation
The frozen fish by-product sample was thawed at 4°C overnight prior to processing. The skin, bone, flesh, and frame of the thawed sample were then separated from each other. The frame and flesh were homogenized with laboratory food processor (Bosch, Germany) three times for 3 minutes. The homogenized sample was stored in a polyethylene bag and frozen until further process.

2.3. Protein recovery with isoelectric solubilization/precipitation (ISP)
The thawed red snapper by-product (RSB) homogenate was processed to recover protein inacid and alkaline process. The solubilization was conducted at pH 2.5 and 11.5 for acid process and alkaline process, respectively. The recovery process followed Gehring et al. [3] method with a modification in the centrifugation speed. A total of 100 g of RSB was mixed with 900 mL of distilled water (4°C) and homogenised for 2x30 seconds using Food Processor (Bosch, Germany). The pH of the homogenate was then adjusted to either 2.5 or 11.5 using 1N HCl or 1N NaOH with constant stirring (400 rpm). The pH was monitored with calibrated pH meter (Labortechnik, Germany). The homogenate, the pH of which has been adjusted, was centrifuged at 6,000 rpm and a temperature of 4°C for 25 minutes. The supernatant was collected by filtering using three layers of cotton sheet. The pH of the filtrate was then adjusted to 5.5 using 1N NaOH or 1N HCl. Second centrifugation was performed to separate the pellet (recovered protein/fish protein isolate). The recovered protein was then collected in a polyethylene bag and weighed.

2.4. Proximate analysis
The proximate analysis of fish protein isolate was performed following AOAC (2002). The moisture, protein, fat, and ash content were evaluated. The total nitrogen of the fish protein isolate was determined with Kjeldahl method. The total crude protein was measured by multiplying the total nitrogen of the sample by 6.26.

2.5. Total volatile basic nitrogen (TVB-N)
The TVBN of the fish protein isolate was measured with Conway microdilution method. Briefly, 2 g of sample was added to 8 mL of 4 % (w/v) TCA and homogenized at 10,000 rpm for 2 minutes. The homogenate was centrifuged at 3,000 rpm for 15 minutes at room temperature. The supernatant as sample (1 mL) was placed in the outer ring of Conway apparatus. The inner ring consisted of 1 % boric acid solution. The reaction was initiated with an addition of 1 mL of K₂CO₃ to the sample at the outer ring. The Conway unit was closed and incubated at room temperature for 16 hours. The solution at the inner ring was then titrated with 0.02 N HCl until the green colour turned pink. The TVB-N value was expressed as mg of nitrogen released/100 gram of sample.
2.6. Peroxide value
The peroxide value was measured using Panpinat and Chaijan [4] method. A total of 2 g of sample was treated with 25 mL of organic solvent (with ratio of chloroform to acetic acid of 2:3). The mixture was shaken and added with 1 mL of saturated KI solution. The mixture was stored in the darkness for 5 minutes, then 75 mL of distilled water was added. A total of 0.5 mL of starch solution (1 % w/v) was added as an indicator. The PV was measured by titrating the iodine liberated from KI with standardized 0.01 N sodium thiosulfate solution. The PV was expressed as milliequivalents of free iodine per 100 g of lipid.

2.7. Water holding capacity
The water holding capacity was measured using method by Panpinat and Chaijan [4]. A total of 100 mg of fish protein isolate was homogenized with 10 mL of distilled water and centrifuged at 10,000 rpm for 30 minutes, then decanted. The difference between the initial weight and the final weight was measured as the water holding capacity of the fish protein isolate.

2.8. Oil binding capacity
The oil binding capacity was determined by adding 10 mL of coconut oil into 100 mg of fish protein isolate, and then the mixture was homogenized. The homogenate was then centrifuged at 2,500 rpm for 30 minutes. The free oil was decanted, and the weight difference was stated as oil binding capacity [14] with modification.

2.9. Amino acid profile
The amino acid profile was measured with a HPLC method [15].

2.10. Statistical analysis
All treatments except the amino acid profile were triplicated, and the differences between treatments were analyzed with analysis of variance (ANOVA), while the comparison of means was made by conducting Duncan’s multiple-range test for stating the significant difference.

3. Results and Discussion
3.1. Protein recovery of red snapper by-product
The protein recovery consisting of acid and alkaline process had different yields based on the protein content. The yield of fish protein made by alkali process was higher (77.30 %) than that of fish protein made by acid process (63.28 %). These results were in line with Chen and Jaczynski [10], who reported protein isolates recovered from trout processing by-product. High yields of alkaline process of salmon and pollock head of 50 % and 87 % were also reported by Bechtel et al. [16]. However, the results were contrary to Panpinat and Chaijan [4], who reported that the recovery protein from head by-product of big eye snapper had higher yield by acid process than by alkaline process. These results indicated that different raw materials had different suitability at different pH levels of the processing.

3.2. Proximate analysis
The protein, lipid, and moisture contents of fish protein isolate are exhibited in table 1.
Table 1. Proximate analysis of red snapper by-product (RBP) and fish protein isolate (FPI).

| Compounds | RBP     | Acid-made FPI | Alkali-made FPI |
|-----------|---------|---------------|-----------------|
| Protein   | 21.73±0.83<sup>b</sup> | 13.75±1.56<sup>a</sup> | 16.79±1.70<sup>ab</sup> |
| Lipid     | 3.74±0.32<sup>b</sup>  | 0.66±0.12<sup>a</sup>  | 1.18±0.07<sup>a</sup>  |
| Moisture  | 70.72±1.42<sup>a</sup> | 83.63±1.17<sup>b</sup> | 75.35±0.73<sup>ab</sup> |

Note: values are given as mean±standard deviation from triplicate measurement. Different letter in the same row indicate significant differences (P<0.05)

Based on the proximate analysis, some reduction of protein occurred in the acid and alkaline process. This result was contrary to Chomnawang and Yongsawatdigul [17], who reported that 85–90% of protein content in fish protein isolate was produced from tilapia by-product. The reduction of protein might be due to the denaturation of muscle proteins induced by pH shift, which caused the aggregation of protein and led to separation of bone, skin, and debris during centrifugation [4]. Another reason might be due to the use of centrifugation speed that was lower in this study than in the previous study.

Some significant reduction of lipid content showed in acid and alkaline process compared to the raw material. Lipids are susceptible to oxidation, which leads to rancidity or fishy odour [10]. Thus, the removal of lipid is an important characteristic. Both treatments (acid and alkaline) were effective in lipid removal. The effectiveness of alkaline process in lowering lipid content might be due to the saponification reaction of alkali and lipid, which produces soap that precipitated during centrifugation. This result was in accordance with [17], who reported that alkaline process was more effective than acid process in lipid removal.

Table 2. Total volatile basic nitrogen (TVB-N) and Peroxide value of red snapper by-product and fish protein isolates.

| Parameter       | RBP       | Acid-made FPI | Alkali-made FPI |
|-----------------|-----------|---------------|-----------------|
| TVB-N           | 28.06±6.08<sup>b</sup> | 10.52±0.01<sup>a</sup> | 10.51±0.01<sup>a</sup> |
| Peroxide value  | 0.01±0.04<sup>a</sup>  | 0.06±0.06<sup>b</sup>  | 0.04±0.01<sup>ab</sup> |

Note: values are given as mean±standard deviation from triplicate measurement. Different letter in the same row indicate significant differences (P<0.05). TVBN value (mg N/100 g) and PV (mEq/1000 g)

3.3. Total volatile basic nitrogen (TVB-N)

The total volatile basic nitrogen of fish protein isolate and raw material is exhibited in table 2. There was no significant difference between acid process and alkaline process (P<0.05) in terms of TVB-N value. Both processes improved the stability of cellular structure and prevented the spoilage of fish protein isolate produced. TVB-N value indicates the measurement of dimethylamine (DMA), trimethylamine (TMA), ammonia, and another compound, which indicates seafood spoilage due to the degradation of muscle proteins. The decrease in TVB-N might be due to the washing mechanism of the centrifugation and precipitation process when the structural protein at pH 5.5 precipitated, while other compounds (hydrophilic compounds such as DMA and TMA) leached out into the supernatant [4].

3.4. Peroxide value

Peroxide value depicts the formation of unstable compound (peroxide) due to lipid oxidation process. Fish muscle contains high amount of unsaturated fatty acids, particularly n-3 fatty acid that is susceptible to oxidation [4]. The peroxide value of this study (table 2) exhibited that the fish protein was susceptible to oxidation. Both acid process and alkaline process resulted in high peroxide values. Rapid lipid oxidation was reported in the fish protein isolate recovered by acid process [9]. However, this study showed that there was no significant difference in lipid oxidation between acid and alkaline process.
These findings were in accordance with Panpipat and Chaijan [4], who reported that both processes (acid and alkaline process) enhanced the oxidative instability of lipid in bigeye snapper head by-product.

Table 3. Water holding capacity (WHC) and oil binding capacity (OBC) of red snapper by-product and fish protein isolate produced by acid- and alkali-process.

| Parameter | RBP        | Acid-made FPI | Alkali-made FPI |
|-----------|------------|---------------|-----------------|
| WHC (mL/g)| 0.13±0.02a | 0.50±0.26b    | 0.56±0.18b      |
| OBC (mL/g)| 0.06±0.04a | 0.17±0.06b    | 0.15±0.05b      |

Note: values are given as mean±standard deviation from triplicate measurement. Different letter in the same row indicate significant differences (P<0.05).

3.5. Water holding capacity
Water holding capacity indicates the ability of the food product to store water during processing. Sarcoplasmic protein has low water holding capacity, which does not allow water for a well-developed gel matrix formation [18]. The WHC of fish protein isolate produced by acid and alkaline process in this study was significantly higher than that of the raw material (P < 0.05), but there was no difference between acid and alkaline process. These results were in accordance with Foh et al. [19], who reported that the fish protein isolate produced from tilapia by acid process and alkaline process was around 2.63 and 2.51 mL/g, respectively. This finding indicated that both methods reduced the sarcoplasmic proteins and isolated the muscle/myofibrilar proteins. The isoelectric solubilisation/precipitation method (ISP) employing pH shift during processing was believed to affect the water holding capacity of the fish protein isolate produced [19].

3.6. Oil binding capacity
The oil binding capacity of fish protein isolate from red snapper by-product produced by acid and alkaline process was significantly higher than that of the raw material used (table 3). The acid process showed a higher score (0.17 mL/g), but the score was not significantly different from that of alkaline process (0.15 mL/g). This finding was in accordance with Foh et al. [19], who reported that the fish protein isolate of tilapia produced by acid and alkaline process had high oil binding capacity (3.38 mL/g). High oil absorption of fish protein is an important characteristic of formulation of food system such as cake, sausage, mayonnaise, butter, and salad dressing [19].

3.7. Amino acid profile
The amino acid profile of the fish protein recovered with ISP was affected by the pH treatment. Chen, Tou, and Jaczynski [20] reported that extreme pH during ISP process improved the essential amino acid profile of the FPI isolated from whole antarctic krill (Euphausiasuperba). Glutamic acid and lysine were higher than other amino acids. Meanwhile, threonine was the lowest amino acid in acid process, and histidine was the lowest in alkaline process. These findings were in accordance with [20], who reported that glutamic acid was the highest amino acid contained in krill protein isolate produced with isoelectric solubilisation/precipitation method.
Table 4. Amino acid profile of fish protein isolate produced with acid-and alkali-process.

| Amino acid   | Acid process | Alkali process |
|--------------|--------------|----------------|
| Aspartic acid| 8.05         | 8.46           |
| Glutamic acid| 14.11        | 12.91          |
| Serine       | 3.07         | 3.28           |
| Glycine      | 5.24         | 5.63           |
| Histidine    | 5.35         | 1.17           |
| Arginine     | 5.29         | 5.59           |
| Threonine    | 0.98         | 2.48           |
| Tyrosine     | 3.03         | 3.15           |
| Valine       | 5.08         | 4.37           |
| Methionin    | 2.85         | 3.62           |
| Isoleusine   | 4.57         | 4.84           |
| Phenilalanine| 3.94         | 4.06           |
| Lysine       | 12.12        | 9.51           |
| Leusine      | 7.16         | 6.87           |
| Alanine      | 5.20         | 5.72           |

4. Conclusion
Isoelectric solubilisation/precipitation with acid and alkali process can be an alternative process to recover proteins from red snapper by-product. This study demonstrates that alkali process gave a protein isolate with better overall quality compared to acid process.

5. References

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