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Characteristics of the fish protein isolate recovered from Sardine by-products using the Isoelectric Solubilization-Precipitation method

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Abstract. The purpose of this study is to restore and characterize fish-protein-isolates (FPI) from the by-products of the sardine canning industry through the application of the isoelectric method of precipitation-solubilization (pH shift) method. The study was conducted in the laboratory of the Department of Fisheries Microbiology at Airlangga University with two treatments of solubilization (pH 2.5 and 11.5). Fish protein isolates were then characterized by yield, water holding capacity, oil binding capacity, TVBN and microbiological analysis (total bacterial count, coliform detection, Salmonella and Vibrio). This study found that FPI which was processed by alkali had better results (39.50 ± 11.75 b / b) compared to acid-processed samples (36.00 ± 8.97 b / b). Water retention capacity from FPI produced by alkali treatment reached 0.96 ± 0.18 mg / L while FPI produced by acid treatment reached 0.52 ± 0.19 mg / L. The binding capacity of oil from FPI in processing alkali was also more high compared to acid treatment. However, the quality of microbiological acid is better, but both treatments do not meet microbiological standards. The research suggestion that is isoelectric precipitation-solubilization method can be applied to produce new values on the by-products of the fishing industry but by adding antibacterial substances as needed.

1. Introduction

A huge production of fishery by-products from the aquaculture and fish catch industries has been reported. The production of fish increased from 120 MT in 1996 to 128.8 MT in 2001, which implies the increased production of by-products. It was estimated that approximately 50% (w/w) of the by-products produced are from the raw material initial weight [1]. This is considered to be a loss during production. However, the by-products contain nutrients such as proteins, lipids and minerals. Another study reported that the by-product from the fisheries industry that consisted of frame, flesh, head, and scale was about 60% of the initial raw material weight [2]. It was often used for animal feed or fertilizer.

The high demand for fish protein has become a trigger to recover underused protein resources such as fish by-products. The frame, bone, skin and fish heads are a source of low cost protein [1]. Fish by-products contain sarcoplasmic, stromata, and myofibrillar protein, which are valuable to recover for human consumption [3]. Previous studies indicated that the production of fish protein - hydrolysate, peptone, and silage - for fish feed can be produced from fish by-products [4,5]. Myofibrillar protein can be recovered from the by-products by employing the pH shift method [6].



A recovery protein with a pH shift is a method of selective, pH-induced water solubility that encourages the removal of skin, scales, bone and lipids from the by-product [7] as proposed by Hultin and Kelleher [8]. The recovery of the protein with the pH shift or ISP method is due to the solubilization of protein at an alkali (around 11-13) or acidic pH (2-3.5) and with the precipitation of the myofibrillar protein at a pH of around 5.5 [9]. Myofibrillar protein in fish muscle homogenate commonly presents as an aggregate with a protein-protein hydrophobic interaction with different electrostatic charges, therefore the addition of a strong acid or alkali leads to the solubilization of myofibrillar protein [10].

Recently, the recovery of a protein with the pH shift or isoelectric solubilisation/precipitation (ISP) method has been developed to recover fish protein [3,4,6]. The recovery of the protein of the bigeye snapper (*Priacanthus* spp.) produced 30% (w/w) of the total fish protein isolate with acceptable characteristics [6], while a previous study reported that 16.79% (w/w) of the fish protein was recovered from the red snapper (*Lutjanus* sp.) by-products [3]. However, a study into the recovery protein of sardine by-products from the canning industry is limited. The aim of this study was to recover the fish protein from sardine by-products with the isoelectric solubilisation/precipitation method and to characterize the fish protein isolate recovered from the by-product.

2. Material and methods

2.1 *Sardine by-product Sample*

The sardine by-product was brought from a sardine canning industry factory located in Banyuwangi, East Java, Indonesia. The sample was wrapped in a polypropylene bag and packed to the laboratory of fish processing at Universitas Airlangga by train. After arrival, the sample was then stored in a laboratory freezer (-18°C) prior to further processing.

2.2 *Fish protein isolate preparation*

The preparation of the fish protein isolate was performed following a previous study [3]. Briefly, the sardine by-product sample was thawed at 4°C overnight before the preparation. The sample was then homogenized with a laboratory food processor (Bosch, Germany) three times for 3 min. The homogenate was then solubilized at pH 2.5 and 11.5 for the acidic and alkali treatments respectively. The pH was adjusted to either 2.5 or 11.5, employing a food processor with the addition of 1N HCl or 1N NaOH. After the pH was stabilized for 10 min, the homogenate was then centrifuged at 4,600 rpm at 4°C for 25 min. The supernatant was collected by filtering using three layers of cotton sheet. The pH of the filtrated supernatant was then adjusted to 5.5, employing 1N NaOH or 1N HCl. After the pH was stabilized for 10 min, the second centrifugation was performed at the same speed for 25 min to separate the protein and water. The fish protein isolate was collected in a polyethylene bag and weighed.

2.3 *Yield*

The yield was measured by the percentage of the final weight of fish protein isolate compared with the raw material of the sardine by-product.

2.4 *Water Holding Capacity*

This method was used by Panpinat and Chaijan [6] to determine the WHC of FPI. A total of 100 mg of FPI was homogenized with 10 mL of distilled water and centrifuged at 10,000 rpm for 30 minutes and decanted. The difference between the initial weight and the final weight was measured as the water holding capacity of the fish protein isolate.

2.5 *Oil Binding Capacity*

A total of 10 mL of coconut oil was added to 100 mg of FPI and homogenized. The homogenate was centrifuged at 2,500 rpm for 30 minutes and the free oil was decanted. The weight difference was stated as being the oil binding capacity [11] with modifications.

2.6 Total Volatile Basic-Nitrogen

The Conway microdilution method was used to measure the TVBN. A total of 2 g of fish protein isolates (FPI) 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 sample (1 mL) was placed in the outer ring of the Conway apparatus. The inner ring consisted of 1 % boric acid solution. The reaction was initiated with an addition of 1 mL of K_2CO_3 to the sample at the outer ring. The Conway unit was closed and incubated at room temperature for 16 hours. The solution in the inner ring was then titrated with 0.02 N HCl until the green color turned pink. The TVB-N value was expressed as the mg of nitrogen released/100 gram of sample.

2.7 Microbiological analysis

The total plate count was evaluated by plating and counting the total visible colony from the fish protein isolate on Tryptic Soya Agar (TSA, Oxoid, UK).

2.8 Statistical analysis

All of the treatments were performed in triplicate and the differences between how the treatments were analyzed were examined using a t-test.

3. Result and discussion

3.1 Yield

Based on the measurement of the yield of the fish protein isolate, the acid solubilization (pH 2.5) and alkali (pH 11.5) were not significantly different, with the yield of the alkali being higher compared to the acidic treatment (Table 1). This finding was in accordance with the previous study as reported by [3, 12]. However, the yield of this study was lower compared to the previous study. Another study showed that the yield of the fish protein isolate recovered by the alkali and the acidic method was between 50 - 80% depending on the raw material used [3]. The total amount of protein recovered from the sardine by-product in this study was between 35.96 - 39.46% (w/w). Another study applied the centrifugation process with a speed of 10,000 rpm [10] while this study conducted this test at a speed of 4,600 rpm, therefore the possibility of recovering protein may have been affected by the centrifugal speed during the recovery process. This finding suggests that maybe the total myofibrillar protein from the raw material affects the yield of the fish protein isolate recovered from the raw material.

Table 1. Characteristics of the fish protein isolate from the sardine by-product

Treatment of solubilization	Yield (% w/w)	Water Holding Capacity (mg/L)	Oil Binding Capacity (mg/L)	TVBN (mg N/100 g)
pH 2.5	35.96 ± 8.89 ^a	0.52 ± 0.18 ^b	0.97 ± 0.14 ^b	0.47 ± 0.53 ^a
pH 11.5	39.46 ± 11.76 ^a	0.96 ± 0.17 ^a	1.04 ± 0.17 ^a	0.23 ± 0.20 ^a

Note: the data presented was done in triplicate and different annotations show the significant differences ($P < 0.05$)

3.2 Water Holding Capacity

The Water holding capacity (WHC) is an important physicochemical character of fish protein isolate. A higher WHC means that the capacity of the protein is higher when it comes to binding the water during processing or formulation. The gel matrix formation during processing is positively affected by the value of the water holding capacity. This is as if the FPI contains a higher number of sarcoplasmic proteins, then this will reduce the water holding capacity and reduce the gel strength of the product [3]. The fish protein isolate produced with alkali processing showed as having a significantly higher ($P > 0.05$) water holding capacity in this study (0.96 mg/L) (Table 1) compared to the fish protein isolate produced by acidic processing. This finding was different from what was previously reported [3, 13], as the previously reported study showed that the acid processed FPI had a higher water holding capacity. This finding indicates that the alkali treatment may produce a better gel in the final product matrices for the FPI produced from the sardine by-product.

3.3 Oil Binding Capacity(OBC)

The oil binding capacity is an important characteristic of protein during formulation with lipid or oil, such as in salad dressing, butter, mayonnaise, sausage or cake [13]. This study found out that the alkali processing of the fish-protein-isolate had a significantly higher ($P>0.05$) oil binding capacity compared to the acid processed product (Table 1). This finding was different to that previously reported by Pramono et al [3], who reported that acidic process produced a fish protein isolate with a higher oil binding capacity. This finding indicated that the fish protein isolate produced from the different raw materials has different abilities when it comes to binding oil during fermentation.

3.4 Total Volatile Basic-Nitrogen

The total volatile basic nitrogen value indicates that the production of a volatile base due to the degradation of lipid and protein from the food material therefore, the higher TVBN value mean a higher level of deterioration in the product has occurred. In this study, both of the pH shift methods were not significantly different concerning the TVBN value, which was around 0.23 to 0.47 mg N/100 g sample. This finding was different than what was previously reported by Pramono et al [3], with the value of TVBN from the red snapper by-product being around 10 mg N/100 g. A lower TVBN value was reported by Panpipat and Chaijan [6], who reported that the TVBN of the fish protein isolate recovered from the big eye snapper by-product was around 2.0 mg N/100 g sample. The content of ammonia, dimethylamine, and trimethylamine in the product indicates the spoilage of the fishery product [6]. This finding indicated that the isoelectric solubilization-precipitation method reduces the total volatile compounds in the final product. The reduction of the TVBN may be due to the centrifugation process during the recovery of the protein [6].

3.5 Microbiological analysis

The microbiological analysis of the fish protein isolate recovered with the acid and alkali-processes has been illustrated in Table 2. The data was presented as Log CFU/g sample.

Table 2. Microbiological analysis of the fish protein isolate produced with the acid and alkali processes

pH processing	Log CFU/g			
	TPC	Coliform	<i>Salmonella</i>	<i>Vibrio</i>
2.5	6.09±0.13 ^a	3.13±0.11 ^a	Negative	Negative
11.5	7.34±0.23 ^b	3.57±0.07 ^b	Negative	Negative

Note: the value was averaged from the triplicate data, with the different annotations showing significant differences ($P<0.05$).

Based on the microbiological analysis, both fish protein isolates were not acceptable due to the high bacteria count (TPC higher than Log 5 CFU/g), and with a coliform of more than Log 3 CFU/g. The previous study mostly did not focus on the microbiological quality of the product due to the possibility of the reduction of the total bacteria during the acid or alkali treatment. However, our study figured out that the acid and alkali treatment were not effective enough at the reducing the microbes in the final product. Even though *Salmonella* and *Vibrio* sp. were negative in this study, it is important to determine the source of the biological contaminant during processing. The high TPC number may due to contamination post-processing. *Salmonella* and *Vibrio* are commonly found in fishery product material due to contamination. *Salmonella* may lead to typhoid fever due to the production of the toxin when infecting humans.

4. References

- [1] Gbogouri G A, Linder M, Fanni J, Parmentier M *J. Food Sci.* **69** 615-22
- [2] Gehring C K, Gigliotti J C, Moritz J S, Tou, J C, Jaczynski, J 2011 *Food Chem.* **124** 422-431
- [3] Pramono H, Pujiastuti D Y, Sahidu A M IOP Conference Series: Earth and Environmental Science **137** 012-061
- [4] Himaya S W, Kim S K 2014 Functional Proteins and Peptides from Fish Skin. In *Seafood Processing By-Products* (New York: Springer) p 197-205
- [5] Hernández A, García B G, Jordán, M J, Hernández M D 2014 *Anim. Feed Sci. Technol* **195** 112-119
- [6] Panpipat W, Chaijan M 2016 *Turk. J. Fish. Aquat. Sci.* **16** 041-050
- [7] Gehring C K, Gigliotti J C, Moritz J S, Tou J C, Jaczynski J 2011 *Food Chem.* **124** 422-431
- [8] Hultin H O, Kelleher S D *US Patent* **6** 451-975
- [9] Undeland I, Hultin H O, Richards M P 2003 *J. Agric. Food Chem.* **51** 3111-3119
- [10] Tahergorabi R, Matak K E, Jaczynski J *J. Funct. Foods.* **18** 746-56.
- [11] Jemil I, Jridi M, Nasri R, Ktari N, Salem R B S B, Mehiri M, Hajji M, Nasri M 2014 *Process Biochem.* **49** 963-972
- [12] Chen Y C, Jaczynski J 2007 *J. Agric. Food. Chem.* **55** 9079-9088
- [13] Foh M B K, Wenshui X, Amadou I, Jiang Q 2012 *Food Bioproc. Tech.* **5** 2192-2200