

## RESEARCH ARTICLE

## Physicochemical and Sensory Properties of Protein Isolate from Anchovy (*Stolephorus insularis*)

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### Abstract

Anchovy is one of the primary fishery commodities in Indonesia; however its development as fishery products is currently suboptimal. Due to its high protein content, anchovy is potential to be developed as a source of value-added fish protein isolate. This study aims to produce anchovy protein isolate (API) and evaluate its physical, chemical, and sensory properties. The API was prepared from defatted anchovy flour. Isolation of anchovy protein was carried out using a pH-shifting method. The API was then analyzed for its physicochemical (bulk density, color, proximate) and sensory properties. The results showed that anchovy protein was more soluble at pH 11 and less at pH 5. Yield and protein recovery of API were 26.39 and 36.86% wb, respectively. The API had 92.20% protein, 3.64% moisture, 2.18% ash, 2.26% lipid, and 3.36% carbohydrate content on a dry basis. The results showed that the API exhibited good physical and sensory properties such as bulk density, color, the best score on sweetness, seaweed, bitterness, off-flavor, aroma, and rancid taste. There was no significant difference in sweet taste, off-flavor, aroma, and rancid taste between API and soy protein isolate (SPI) ( $p > 0.05$ ). Overall, API demonstrated satisfactory nutritional properties and potential use as food ingredients.

**Keywords:** anchovy, fish protein, protein isolate, physicochemical properties, sensory properties

## Introduction

Protein isolate is a product with a high protein of at least 90% db (Codex Standard, 2019). In the manufacture of various food products such as milk, baby food, and processed meats, protein isolate is often added as a protein source. There are several functions of protein isolate in food products, such as stabilizing emulsions, increasing color brightness, improving texture properties, increasing protein content/protein enrichment, and reducing cooking loss in processed meat products e.g., sausages (Canti et al., 2021). Protein isolates can also improve noodles' cooking quality, color, and sensory properties (Sofi et al., 2020). In addition, protein isolates can function to enhance the gel properties of surimi (Kudre et al., 2013). According to Statistics Indonesia (2020), Indonesia imported protein isolates of 15,684,076 kg. Therefore, an alternative source of raw materials is needed to produce the protein isolates.

Protein isolates are currently produced from various sources such as whey, beans, peas, cashews, canola,

and fish (Garba & Kaur, 2014). One of the methods used for protein isolation in the food processing industry is pH shifting. This method can effectively recover fish muscle proteins, including myofibrils, and water-soluble proteins, resulting in protein isolates with good color and functional properties and are safe for consumption (Tian et al., 2016).

Fish protein isolate (FPI) has been developed from rainbow trout, tilapia, haddock fish, carp, and freeze-dried saithe mince (Foh et al., 2012; Lone et al., 2015; Shaviklo et al., 2010a; Shaviklo et al., 2012; Tian et al., 2017). Freitas et al. (2011) have also made protein isolate from Argentine anchovies (*Engraulis anchoita*) residue such as viscera, head, fins, tail, and spikes. Their study reported that protein isolate from anchovy residue has adequate functional properties such as a higher ability to retain oil and emulsifying capacity. However, the determination of proximate, physical, and sensory characteristics of protein isolates from anchovy have not been carried out. In addition, there has never been a study on the preparation of protein isolates from *jengki* anchovies (*Stolephorus insularis*). In Indonesia,

studies on FPI have not been done extensively and are only limited to catfish and gourami (Haryati et al., 2020; Oktasari et al., 2015). The utilization of protein isolates in food products requires an assessment of their physical, chemical, and sensory properties. Bulk density and color of API need to be determined before formulating food products. The first plays a role in several food formulations, such as weaning food products and patient recovery, because it can increase the calorie density, nutrition, and digestibility of a product. Meanwhile, the color is an essential characteristic because it affects the overall acceptance of food products.

Anchovy is one of the primary commodities of marine capture fisheries in Indonesia. Based on Statistics Indonesia (2019), the volume of anchovy production reached 23,565.14 tons. One of the most common anchovies found in Indonesia is *jengki* anchovy. Currently, the utilization of anchovy is limited to fried and processed products such as flour, peanut brittle, *pepes*, *bebothok*, anchovy chips, and anchovy snacks. Anchovy flour has a protein content of 82.96% (Hendrayati et al., 2020). Based on their abundance and high protein content, the *jengki* anchovy is potential to be developed as a raw material for protein isolate production. This research aimed to produce the API and evaluate its physical, chemical, and sensory properties.

## Material and Methods

### Materials

The primary material used in this research was fresh *jengki* anchovy (*Stolephorus insularis*) obtained from the Intermoda Modern Market in Cisauk, Tangerang, Banten, Indonesia. Another material used was a soy protein isolate (MarkSoy90). The chemicals used were NaOH (Merck), 37% HCl (Merck), 97% H<sub>2</sub>SO<sub>4</sub> (Merck), BSA (Bovine Serum Albumin) (Sigma-Aldrich), ethanol (Merck), Coomassie Brilliant Blue G-250 (Merck), phosphoric acid (Merck), phosphate buffer solution (Sigma-Aldrich), protein catalyst tablet (3.5 g K<sub>2</sub>SO<sub>4</sub>; 3.5 g Selenium) (Behr Labor-Technik GmbH), H<sub>3</sub>BO<sub>3</sub> (Merck), Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (Merck), BCG-MR (Bromocresol Green-Methyl Red) (Merck), n-hexane (Merck), technical grade n-hexane (Bratachem), and distilled water.

### Preparation of Anchovy Flour

The preparation of anchovy flour was carried out based on the Yilmaz and Koca (2020) method with modifications to the temperature and sieve used. One kg of anchovy was soaked for 30 min in water. It was

washed thoroughly, then dried in a cabinet dryer (PT Agrowindo Sukses Abadi OVL-12, Indonesia) for 22 h at 55 °C. The sample was ground with a food processor (Philips HR-7627, China) and then sieved using an 80 mesh sieve. The anchovy flour then stored in a zipped polyethylene plastic bag containing packed silica gel and stored in a freezer (GEA AB-600TX, Indonesia) at -18 °C until used.

### Preparation of Defatted Anchovy Flour

The preparation of defatted anchovy flour was carried out according to Puteri et al. (2018) with modifications to the times and frequency of extraction. The anchovy flour was extracted for lipid using n-hexane for 30 min with a ratio of anchovy flour and n-hexane of 1:3 (w/v). The mixture was stirred using a magnetic stirrer and then was centrifuged at 2,822 x g for 20 min. Extraction was carried out in triplicate. The defatted flour was dried at ambient temperature overnight in a fume hood, transferred into a zipped polyethylene plastic bag prefilled with packed silica gel and stored in a freezer (GEA AB-600TX, Indonesia) at -18 °C until used.

### Protein Solubility at Various pH

The determination of protein solubility was performed according to Freitas et al. (2011) with modifications on the ratio, concentration of NaOH and HCl, times, and centrifugation conditions. One g of sample was put into a 50 mL beaker glass. Then a total of 20 mL of 0.1 N NaOH and 0.1 N HCl solution was added (1:20, w/v). The pH was adjusted using the addition of either 0.1 N NaOH or 0.1 N HCl (final pH 2; 3; 3.5; 4; 4.5; 5; 5.5; 6; 7; 8; 9; 10; 11; 12). The solution was stirred for 30 min using a magnetic stirrer at room temperature. The pH adjustment was performed every 15 min to maintain the pH of the solution. Centrifugation was executed for 30 min at 3,842 x g in a centrifugation tube. The dissolved protein in the supernatant was determined using the Bradford method by modifications to the blank, standard concentration, the number of samples, and reagents for analysis (Nouroozi et al., 2015). Distilled water was used as a blank. The standard protein used was a BSA solution of 1 mg/mL with a range of 0.1-1.0 mg/mL in phosphate buffer in a 0.5 mL tube. Bradford's reagent was prepared using 100 mg of Coomassie Brilliant Blue G-250 dissolved in 50 mL of 95% ethanol, then 100 mL of phosphoric acid was added. The solution was diluted with 1 L of distilled water. A total of 0.4 mL of the sample was pipetted into a test tube. A total of 8 mL of Bradford's reagent was added and vortexed. The absorbance was measured with a spectrophotometer (Thermo Scientific Genesis 10S

UV Vis, USA) at 595 nm. The determination of protein solubility was carried out in duplicate. Once the standard curve was obtained, the protein concentration was determined using a standard curve equation,  $Y=ax+b$ ;  $x$ , concentration, and  $Y$ , absorbance.

### Preparation of Anchovy Protein Isolate (API)

The protein was isolated by the procedure described by Freitas et al. (2011), with some modifications i.e., the ratio of flour and solvent, duration, centrifugation, and lyophilization conditions. The defatted flour was extracted with a buffer solution of 0.1 N NaOH and HCl at pH of the highest protein solubility, the flour/solvent ratio used was 1:20, w/v for 30 min. Then protein solution was centrifuged (Eppendorf 5810R, USA) at 3,842 x g for 30 min. The soluble protein (supernatant) was precipitated at the pH of the isoelectric point. Then the separation was carried out using centrifugation at 3,842 x g for 30 min. The precipitated protein was called protein isolates. Lyophilization was carried out using a freeze dryer (Christ Alpha 2-4 LDPlus, Germany) at -35 °C for 96 h. The dry protein isolate was stored using polyethylene plastic at -18 °C.

### Yield and Protein Recovery of API from Fresh Fish

The yield and protein recovery of API from fresh fish was calculated using equations (1) and (2), respectively (Oliyaei et al., 2019):

$$\text{Yield (\% wb)} = \frac{W_i}{W_f} \quad (1)$$

$$\text{Protein recovery (\% wb)} = \frac{P_i \times W_i}{P_f \times W_f} \quad (2)$$

Where:  $W_i$  = weight of protein isolates (g)

$W_f$  = weight of fresh fish (g)

$P_i$  = protein content in protein isolates (% db)

$P_f$  = protein content in fresh fish (% db)

### Analysis of The Physical Properties of API

#### Bulk Density

Bulk density analysis was carried out according to Garba and Kaur (2014). A total of 10 g of protein isolate was put into a 100 mL measuring cup and tapped several times until solid formed. Bulk density was calculated using equation (3):

$$\text{Bulk density} = \frac{\text{sample weight (g)}}{\text{volume (mL)}} \quad (3)$$

#### Color

The color was measured using a colorimeter (NR200, China). Colors were analyzed using CIE  $L^* a^* b^*$  system. Measurements were performed by placing the sample in a cup that was already available and then measuring it on a scale of  $L^*$ ,  $a^*$ ,  $b^*$ . The  $L^*$  value was represented by the lightness parameter (0: black to 100: white). The value of  $a^*$  was defined as the red-green color ( $a^*+=0-100$  for red,  $a^*-=0-(-80)$  for green). The value of  $b^*$  was determined as a blue-yellow color ( $b^*+=0-70$  for yellow,  $b^*-=0-(-70)$  for blue).

### Proximate Analysis of Fresh Anchovy, Defatted Anchovy Flour and API

Proximate analysis of samples was performed according to the AOAC (2012) method. The proximate analysis determined moisture (Thermogravimetry method), ash (Gravimetry method), protein (Kjeldahl method), lipid (Soxhlet method), and carbohydrate (by difference method) contents.

#### Sensory Analysis of API

Sensory analysis was performed to evaluate aroma and taste attributes using the Quantitative Descriptive Analysis (QDA) method based on Meilgaard et al. (2016). Sensory evaluation of protein isolates was conducted by 12 trained student panelists (six males and six females) of the Department of Food Technology, Atma Jaya Catholic University of Indonesia. Panelists were selected and qualified based on Meilgaard et al. (2016). The panel leader guides the panelists to determine the attributes to describe the protein isolate solution. The protein isolate was dissolved in water to a concentration of 1%, then stirred, and the solution was prepared 1 h before evaluation. Panelists evaluate the intensity of sensory attributes using an unstructured scale (0-100%). The intensity of sensory attributes measured included aroma (fish liver oil, rancid, dried fish, trimethylamine (TMA)) and taste (fish liver oil, rancid, dried fish, TMA, sweet, bitter, seaweed, off-flavor) (Shaviklo et al., 2010b). All samples were presented to panelists on trays in individual booths at random. Drinking water was provided to panelists to neutralize the palate. Panelists evaluated two sample solutions in two sessions.

#### Statistical Analysis

The study was conducted with two treatment replications; each parameters analysis was performed in duplicate. The data were presented as the average

value and standard deviation. Data on physical and sensory properties were analyzed statistically using an independent t-test, where only two groups were compared. The chemical properties data obtained were statistically tested using analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT). The significance of the difference was defined at  $p < 0.05$ . Data were analyzed using SPSS version 24 software.

## Results and Discussion

### Solubility Profile of Anchovy Protein

The protein of the anchovy had the highest solubility at pH 11 (1.30 mg/mL) and lowest at pH 5 (0.71 mg/mL) (Figure 1). Anchovy's protein solubility increased at pH 3 and 5.5-11 but decreased at pH 3.5-5. Anchovy protein was soluble in either acid or alkaline pH. The solubility of protein at alkaline pH is higher than that at acidic pH because the number of negatively charged ions at isoelectric pH is greater than the number of positively charged ions at isoelectric pH (Fennema et al., 2017). Protein precipitation was carried out at pH 5 when the solubility of the protein was lowest (pH of the isoelectric point). At isoelectric pH, the protein becomes neutrally charged, or the number of positively charged equals the number of negatively charged amino acids. The interaction between protein molecules is maximized at the isoelectric point.

On the other hand, at the isoelectric point, proteins interaction with water is minimal. There is an increased protein-protein interaction, resulting in aggregation and precipitation. The hydrophobic inner layer of protein molecules will come out, while the hydrophilic part will be folded inward so that the protein solubility decreases and eventually clumps and settles (Jiang et al., 2015; Zou & Pang, 2018). Protein solubility is influenced by surface characteristics of amino acids, molecular weight, and conformational change in protein structure (Timilsena et al., 2016).

The protein solubility pattern of anchovy was U-shaped which is similar to fish meat and its by-products of other fish such as rainbow trout, tilapia, haddock, carp, Argentine anchovy residue (viscera, head, fins, tail, and spikes), salmon, cod, herring by-products, red snapper by-products, bigeye snapper head by-products, and yellowfin tuna roe (Abdollahi & Undeland, 2018; Foh et al., 2012; Freitas et al., 2011; Lee et al., 2016; Lone et al., 2015; Panpipat & Chaijan, 2017; Pramono et al., 2018; Shaviklo et al., 2010a; Tian et al., 2017).

### Yield and Protein Recovery of API

The yield of anchovy protein isolates obtained was  $26.39 \pm 0.75\%$  wb from fresh fish (Table 1). The API

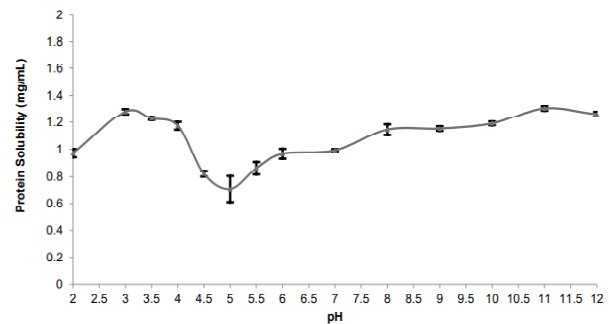


Figure 1. Protein solubility of anchovy at different pH.

had a higher yield than protein isolate from yellowfin tuna roe ( $11.6\text{--}14.1\%$ ); lantern fish ( $19.15 \pm 0.17\text{--}29.09 \pm 0.84\%$ ) (Lee et al., 2016; Oliyaei et al., 2019). However, the API yield was lower than that of isolate protein from catfish heads and frames, which was ( $36.6 \pm 1.3\text{--}55.8 \pm 1.5\%$ ) (Tan et al., 2019). The protein isolate yield was influenced by fish species, the method used to determine yield, centrifugation speed during protein isolation, and sarcoplasmic water concentration (Chen & Jaczynski, 2007). According to Tan et al. (2019), more than 20% of protein isolate yield is feasible for commercial production. Our findings thus indicate that *jengki* anchovy is a potential protein isolate source for commercial production.

Protein recovery is the amount of protein present in the precipitate (Kristinsson & Ingadottir, 2006). Protein recovery is an important parameter determining the economic feasibility of using a technology (Gehring et al., 2011). The protein isolates of anchovy had a protein recovery of  $36.86 \pm 1.04\%$  wb (Table 1). Protein recovery of API was higher than tilapia frame protein isolate  $15.32 \pm 0.34\text{--}19.19 \pm 0.47\%$  reported by Chomnawang and Yongsawatdigul (2013). According to Kristinsson & Liang (2006), the yield of protein recovery by isolation using an isoelectric point ranged from 42 to 90%. The more similar the mass of extracted protein to the original amount present in the

Table 1. Yield, protein recovery, physical properties of anchovy protein isolate (API) and soy protein isolate (SPI)

| Properties              | API                | SPI                |
|-------------------------|--------------------|--------------------|
| Yield (% wb)            | $26.39 \pm 0.75$   |                    |
| Protein recovery (% wb) | $36.86 \pm 1.04$   |                    |
| Bulk density (g/mL)     | $0.43 \pm 0.01^b$  | $0.39 \pm 0.01^a$  |
| <b>Color</b>            |                    |                    |
| L*                      | $74.36 \pm 0.87^a$ | $80.62 \pm 0.16^b$ |
| a*                      | $4.07 \pm 0.13^b$  | $0.87 \pm 0.01^a$  |
| b*                      | $18.04 \pm 0.43^b$ | $15.38 \pm 0.02^a$ |

Note: Different superscripts indicate significant different ( $p < 0.05$ ). Values are presented as mean  $\pm$  SD

starting material, the more efficient the protein recovery (Dunford, 2012). The difference in protein recovery can be due to several factors such as differences in fish species, centrifugation treatment, the concentration of water-soluble sarcoplasmic protein, time, and temperature of extraction (Foh et al., 2012; Surasani et al., 2017). Fish from freshwater and saltwater can give different protein recovery due to ionic bonds. The salt attached to marine fish can increase ionic bonds to increase protein recovery (Chen & Jaczynski, 2007). Increased centrifugation speed causes compaction to increase, resulting in efficient separation of solubilized proteins (Surasani et al., 2017).

## Physical Properties of API

### Bulk Density

Bulk density is used to determine sample mass, handling requirements, and the type of packaging suitable for storage and transportation. The API had a significantly higher bulk density than that of SPI ( $p < 0.05$ ) (Table 1). The API bulk density value was lower than that of isolate protein from rainbow trout ( $0.58 \pm 0.01$  g/mL) and catfish ( $0.64 \pm 0.01$  g/mL) (Lone et al., 2015; Haryati et al., 2020). However, it was higher than the protein isolate from Indian mackerel ( $0.31 \pm 5.12$  g/mL), pony fish ( $0.27 \pm 8.12$  g/mL), and sardine ( $0.36 \pm 5.32$  g/mL) (Kumarakuru et al., 2018). Flour generally has a bulk density ranging from 0.37–0.77 g/mL (Akinwale et al., 2017). Bulk density can be influenced by protein microstructure, particle size, the intensity of the attractive forces between particles, manufacturing method, drying procedure, and moisture content (Lone et al., 2015; Kumarakuru et al., 2018). According to Foh et al. (2012), the small bulk density is suitable for the formulation of weaning foods. Therefore a low density is required to increase calorie and nutrient density. Low bulk density can increase the digestibility of food products (Brou et al., 2018). However, protein isolates with higher bulk density are also needed to reduce the thickness of pasta products for children and in recovering patients (Ogunwolu et al., 2009). The API could be formulated into food products with high digestibility based on the bulk density value, such as foods for babies, children and patients in recovery. Bulk density is influenced by the structure of the starch polymers, and the loose structure of the starch polymers could result in low bulk density and increased digestibility (Malomo et al., 2012).

### Color

Color is an important attribute because it can affect the acceptance of food products (Kumarakuru et al.,

2018). The lightness of the fish protein isolate depends on the connective tissue. Lipid retention and sample type can affect the level of yellowish color. The  $L^*$  and  $b^*$  values of API were significantly lower ( $p < 0.05$ ) than those of SPI (Table 1). However, the API had a significantly higher  $a^*$  value than SPI ( $p < 0.05$ ) (Table 1). The  $a^*$  and  $b^*$  values for both API and SPI were positive, indicating reddish to yellowish colors. The high pH changes during the pH-shift can affect the prooxidative properties of heme-pigments. In protein isolation with the pH-shift method, hydrophobic interactions may be more easily formed with misfolded proteins (both sarcoplasmic and myofibrillar) and thus co-precipitation improperly refolded and hydrophobic muscle proteins at pH 5.5 (Abdollahi et al., 2016). Co-precipitation of heme proteins in the recovered protein can affect the reddish color. Denaturation and oxidation of haemoglobin can cause a yellow-brown color in the product (Foh et al., 2012; Lone et al., 2015). Kamarakuru et al. (2018) reported the  $L^*$ ,  $a^*$ , and  $b^*$  values of Indian mackerel fish protein isolates were  $58.8 \pm 0.54$ ,  $1.59 \pm 0.15$ ,  $19.4 \pm 0.10$  and ponyfish were  $57.4 \pm 1.66$ ,  $1.68 \pm 0.05$ ,  $19.6 \pm 0.81$ , respectively. The color of the API ranges from reddish-purple to yellow. Differences in the color of various fish protein isolates were caused by fish species, fish freshness, pretreatment conditions, processing conditions, drying temperature, and lipid oxidation (Shaviklo et al., 2012). Since the color of the protein isolate could affect its application in food processing, the API could be used to formulate food products with neutral colors.

### Proximate Composition of API

Moisture content is an essential component because moisture content affects the length of storage, due to its ability to induce lipid oxidation which causes rancidity. The moisture content of defatted anchovy flour was not significantly different from that of API and SPI ( $p > 0.05$ ) (Table 2). The moisture content of the API was in the moisture content range of several fish protein isolates (1.41–10.64%) reported by Shaviklo (2015). High moisture content supports microbial metabolic activity, resulting in several volatile compounds and causing oxidative rancidity (Kulchan et al., 2016).

The API's ash content was significantly lower than those of the SPI and defatted anchovy flour ( $p < 0.05$ ) (Table 2). It was lower than those of protein isolate from rainbow trout which was 4% (Lone et al., 2015), and tilapia which was  $4.53 \pm 1.26\%$  (Foh et al., 2012). Tian et al. (2017) also reported that the ash content of protein isolate in carp was  $8.83 \pm 0.21$ – $8.88 \pm 0.36\%$  db. The ash content indicates the amount of impurities (minerals) that could be separated from the protein

(Foh et al., 2012). The ash content may be decreased during protein isolation, due to centrifugation process and protein solubility at alkaline pH (Taskaya et al., 2009). The type of fish and the process used to prepare the protein isolate influence the ash content of the resulting protein isolate (Pires et al., 2012).

The API had a protein content of  $92.20 \pm 1.98\%$  db that was significantly higher than that of SPI ( $79.59 \pm 1.06\%$  db) ( $p < 0.05$ ) (Table 2). The API protein content met the requirement of the International Food Standards Codex Alimentarius by  $>90\%$  db protein content (Codex Standard, 2019). The protein content of API was higher than those of yellowfin tuna roe ( $80.96 \pm 0.50$ – $92.13 \pm 0.70\%$ ) (Lee et al., 2016), rainbow trout ( $76.61 \pm 0.95\%$ ) (Lone et al., 2015), sardines ( $84.74 \pm 0.02\%$ ) (Kumarakuru et al., 2018), red snapper by-product ( $13.75 \pm 1.56$ – $16.79 \pm 1.70\%$ ) (Pramono et al., 2018), saithe fish ( $71.50 \pm 0.2$ – $73.60 \pm 0.35\%$ ) (Shaviklo et al., 2012), tuna ( $23.61 \pm 0.85\%$ ) (Shaviklo et al., 2017), and carp ( $82.96 \pm 1.80$ – $83.20 \pm 2.87\%$  db) (Tian et al., 2017). The protein extraction method, temperature and drying duration, centrifugation speed, and the relative concentration of fish protein sarcoplasmic in water-soluble affect the difference in protein content of fish protein isolate (Kumarakuru et al., 2018; Nolsoe & Undeland, 2009). Based on these results, the API could increase emulsion stability and protein content in formulation of processed meat products such as sausages, frankfurters, meatballs, burgers, and comminuted meat products.

The API lipid content ( $2.26 \pm 0.23\%$  db) was significantly higher than those of the SPI ( $0.59 \pm 0.11\%$  db) ( $p < 0.05$ ) (Table 2). The API had lower lipid content than those of yellowfin tuna roe ( $5.60 \pm 0.10$ – $7.40 \pm 0.10\%$ ) (Lee et al., 2016), sardines ( $2.65 \pm 0.15\%$ ) (Kumarakuru et al., 2018), tuna fish ( $5.7 \pm 0.54\%$ ) (Shaviklo et al., 2017), and carp ( $4.88 \pm 0.21\%$  db) (Tian et al., 2017). The lipid content of API was low due to the manufacture of API using defatted flour. The difference in lipid content in fish isolates was influenced by the raw materials used in the extraction

process (Pires et al., 2012). The carbohydrate content of API was significantly lower than those of the SPI ( $p < 0.05$ ) (Table 2). However, it was not significantly different with defatted anchovy flour ( $p > 0.05$ ). The results showed that the extraction process could increase protein content and reduce the carbohydrate content of defatted anchovy flour. Total carbohydrates were reduced (29.26%) after the API preparation process. Decreases in lipid and total carbohydrate contents were attributed to reagents used for the strong acid and alkali or alcohol during protein isolation (Syida et al., 2018).

### Sensory Properties of API

The sensory attributes of the aroma of fish liver oil, the smell of dried fish, and the smell of TMA on the API showed significantly higher scores than those of the SPI ( $p < 0.05$ ), except for the aroma attribute of rancid ( $p > 0.05$ ) (Figure 2). The taste scores of API (fish liver oil, dried fish, rancid, TMA, bitter, and seaweed) was significantly higher than those of the SPI ( $p < 0.05$ ). However, the sweet and off-flavor attributes scores were not significantly different compared to the others ( $p > 0.05$ ). Similar to a previous study by Abdollahi and Undeland (2018), protein isolates from cod, salmon, and herring had the aroma and taste of fish liver oil, dried fish, and TMA, with higher intensity than those of the SPI. In addition, Shaviklo et al. (2012) found that the intensity of aroma and taste of fish liver oil and dried fish was also high in protein isolates of saithe fish. The sensory attributes of smell, rancid taste, and off-flavor in API were higher than in SPI, though not significantly different. According to Secci and Parisi (2016), the taste and aroma such as rancid and off-flavor are caused by lipid oxidation during the processing. The high heme protein in fish can also cause lipid oxidation. Heme protein is an active pro-oxidant as long as changes in pH during protein isolation can cause lipid oxidation (Raghavan & Hultin, 2009). The addition of antioxidants is required during the protein isolation process to prevent lipid oxidation

Table 2. Proximate composition of API and SPI

| Parameters          | Fresh anchovy        | Defatted anchovy flour | API                | SPI                | Protein isolate standard values* |
|---------------------|----------------------|------------------------|--------------------|--------------------|----------------------------------|
| Moisture (% db)     | $319.16 \pm 11.67^b$ | $3.54 \pm 1.14^a$      | $3.64 \pm 1.09^a$  | $8.56 \pm 0.13^a$  | < 10                             |
| Ash (% db)          | $3.37 \pm 0.38^b$    | $10.12 \pm 0.60^d$     | $2.18 \pm 0.19^a$  | $4.59 \pm 0.17^c$  | < 8                              |
| Protein (% db)      | $66.15 \pm 1.85^a$   | $78.54 \pm 3.98^b$     | $92.20 \pm 1.98^c$ | $79.59 \pm 1.06^b$ | 90 or more                       |
| Lipid (% db)        | $17.53 \pm 0.58^d$   | $6.58 \pm 0.56^c$      | $2.26 \pm 0.23^b$  | $0.59 \pm 0.11^a$  | -                                |
| Carbohydrate (% db) | $12.94 \pm 1.39^b$   | $4.75 \pm 3.87^a$      | $3.36 \pm 1.91^a$  | $15.23 \pm 1.15^b$ | -                                |

Note: a, b, c, d superscripts indicate significant different ( $p < 0.05$ ). Values are presented as mean  $\pm$  SD.

\*Codex Standard (2019)



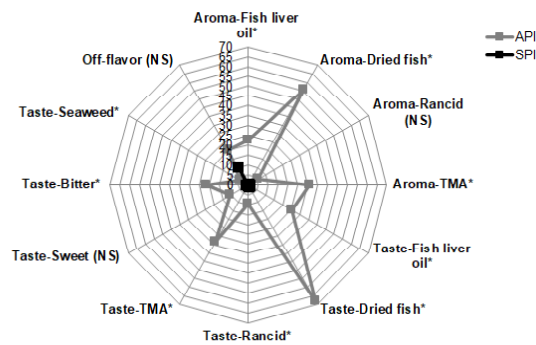


Figure 2. Sensory attributes of API and SPI values are means of two evaluations. \* = Significant difference ( $p < 0.05$ ). NS = Not significant.

(Shaviklo et al., 2012). API had a higher bitter taste than SPI. The bitter taste is influenced by lipid content, ash, and bile in whole fish and fish offal (Dauksas et al., 2004). The taste of sweet and seaweed in API was also higher than in SPI. According to Aspevik et al. (2021), the high content of free amino acids in the ingredients can contribute to sweet, bitter, and umami tastes. The amino acid content of glutamate and aspartate gives rise to a seaweed taste in API (Mouritsen et al., 2019). The sweet taste in API is due to the amino acids D-histidine, D-phenylalanine, D-tryptophan, glycine, L-alanine, L-glutamine, L-proline, L-serine, and L-threonine. Amino acids that cause a bitter taste include L-histidine, L-tryptophan, and L-valine (Bachmanov et al., 2016).

## Conclusion

Isolation of anchovy protein was successfully performed by the pH-shifting method. The API had satisfactory physical characteristics such as bulk density and color. Based on its chemical composition, the API met the standard of protein isolate. The sensory properties of API showed the best scores on the aroma and taste of rancid, off-flavor, bitter, sweet, and seaweed taste. However, the smell and taste values of fish liver oil, dried fish, and TMA of API were higher than those of SPI. Overall, API could be used as an excellent source of protein and can be applied in the formulation of foodstuffs.

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## Supplementary Material

Supplementary material is not available for this article.

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