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FULL PAPER

A comparison of physicochemical, functional, and sensory properties of catfish protein isolate (*Clarias gariepinus*) produced using different defatting solvent

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Abstract

The catfish has a high protein content and consumption level, but its utilization is not optimal. The defatting process can affect the characteristics of the protein isolate produced. This study produced protein isolates from catfish and investigated the effect of defatting solvents (n-hexane and ethanol) on physicochemical, functional, and sensory properties of catfish protein isolates (CPI). The CPI was prepared from defatted catfish flour using n-hexane (DCFh) and ethanol solvents (DCFe). The protein isolation of catfish used chemical methods to determine the solubility of protein at various pHs. The protein isolates were analysed for physicochemical, functional, and sensory properties. The results showed the highest solubility of DCFh and DCFe at pH 11 and the lowest at pH 5.5. The protein content of CPI from defatted flour with hexane (CPIh), and ethanol solvents (CPIe) were comparable, 92.02 and 93.47% db, respectively. Differences in defatting solvents affected the proximate composition (moisture; ash content), physical properties (bulk density; colour), functional (water holding capacity; foaming capacity), and sensory (dried fish taste) of CPI. Ethanol solvent could be used as an alternative for the defatting process. The CPI had good functional properties, i.e., water/oil holding capacity, emulsion capacity and stability, foaming stability, and gel strength, which could be used in food formulations and as an alternative to binders in processed meat products.

1. Introduction

Protein isolate is the purest form of protein because the protein content is at least 90% of the dry weight. Protein isolates can be used as a mixture in processed food products that can increase the nutritional value of these food products and increase their functional properties. There are several functions of protein isolates in food products: binding water and oil, emulsification in food product applications, and helping to maintain the structure of processed meat products (Garba and Kaur, 2014). In Indonesia, availability of protein isolates is still widely obtained from imports. According to Statistics Indonesia, imports of soy protein isolate up to 15,684,076 kg in 2020 (Statistics Indonesia, 2020). Therefore, an alternative is needed in the form of using local materials. During its development, protein isolates were produced from various sources such as whey, beans, peas, cashews, canola, and fish (Garba and Kaur, 2014).

The fish protein isolate (FPI) is generally not consumed directly and is used as raw material to produce value-added products. Fish protein isolates are protein concentrates prepared from fish muscle, without retaining the original shape of the fish muscle, frozen fish (surimi or minced fish), and dried fish (Foh *et al*., 2012; Shaviklo *et al*., 2012). The FPI has been developed from several raw materials such as tilapia muscles, rainbow trout, haddock fish, freeze-dried saithe mince, and carp (Shaviklo *et al*., 2010; Foh *et al*., 2012; Shaviklo *et al*., 2012; Lone *et al*., 2015; Tian *et al*., 2017).

Catfish (*Clarias gariepinus*) is one type of freshwater fish favoured by the people of Indonesia. Nationally, catfish production continues to increase every year. Catfish production in 2017 reached 1,125,526 tons (Statistics Indonesia, 2018). Based on Indonesia's Statistics report in 2020, the level of fish consumption reaches 0.32/kg/capita/weekly. The increase in production and consumption of catfish is because catfish has various advantages such as high

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adaptability to various environmental conditions, relatively fast growth, ease of cultivation, and high nutritional content. Catfish has a high protein content of 60.38% db (Olaniyi *et al*., 2017). However, catfish use has not been optimal because it is still limited to fish processing until the present, such as fried, roasted, boiled, steamed, and smoked. One of the utilizations of catfish as a food ingredient and protein source is fish protein isolate.

One of the important steps in protein isolation and application in food formulations is the defatted process. High lipid content can interfere with the protein extraction process. Catfish have a high enough lipid content of 24.65% db (Kurniawati *et al*., 2019). Therefore, it is necessary to take a lipid separation step to increase protein extraction. The defatted solvents can influence the produced protein isolate's physical, chemical, functional, and sensory characteristics. Currently, hexane is a solvent widely used to remove lipids from flour because efficient in extracting and easy to obtain. However, hexane has some disadvantages as a solvent to remove lipids, such as hexane residue, affecting food product safety, human health, and the environment. Ethanol could be used as an alternative defatting organic solvent because the ethanol residue has good sensory properties, i.e., taste, aroma, and colour, compared to hexane (Russin *et al*., 2011). The isolation and characterization of a protein from defatted catfish have not been studied before. In addition, the effect of organic solvents on catfish protein isolate (CPI) characteristics has never been known before. The purpose of this study was to produce protein isolates from catfish and investigate the effect of defatting solvents, including n-hexane and ethanol, on the physicochemical, functional, and sensory properties of CPI.

2. Materials and methods

2.1 Materials

The material used in this research was *Dumbo* catfish (*Clarias gariepinus*) meat obtained from the Metro Polis Modern Market Tangerang, Banten, Indonesia. The other materials were soy protein isolate (SPI) (MarkSoy 90), corn oil (Mazola), aquadest, n-hexane (Bratachem, technical), ethanol (Bratachem, technical). The other chemicals were an analytical grade purchased from Merck Millipore and Sigma-Aldrich Corporation.

2.2 Preparation of catfish flour

The preparation of catfish flour was performed according to Zebib *et al*. (2020) by modification, i.e., temperature and time drying, and size sieve used. Making flour began was prepared a sample of catfish by removing parts of the skin, bones, and other organs. The catfish meat was crushed in a food processor and dried in a cabinet dryer for 22 hrs at 55^{o}C. The dried meat was mashed in a food processor until it became coarse and sieved using an 80-mesh sieve. Then the catfish flour were stored at -18^{o}C until used.

2.3 Preparation of defatted catfish flour

The defatted catfish flour was prepared based on Puteri *et al*. (2018) by modification, i.e., duration and the frequency of total extraction. The production of defatted catfish flour (DCF) was done by extraction using two solvent treatments (n-hexane or ethanol technical). The defatted flour was named defatted catfish flour with hexane (DCFh) and ethanol (DCFe). The ratio of flour and solvent was 1: 3 w/v, then stirred for 30 mins. Next, the flour was put into a centrifuge tube for centrifugation at a speed of 3000 rpm for 20 mins. The separated solvent was stored for further extraction. Extraction was repeated three times. Then pellets were dried in a fume hood to remove the remaining solvent. The defatted flours were stored at -18^{o}C until used.

2.4 Determination of catfish protein solubility at various pHs

Determination of catfish protein solubility at various pH levels was performed based on Freitas *et al*. (2011) by modifications, i.e., the ratio and concentration solvent for extraction, conditions of extraction, and centrifugation. A total of 1 g of DCFh or DCFe samples were dissolved in 20 mL of 0.1 N NaOH solution and or HCl with a ratio of 1:20 (w/v). The pH of the solution was adjusted between pH 2–12 (pH 2; 3; 3.5; 4; 4.5; 5; 5.5; 6; 7; 8; 9; 10; 11; 12). Then the solution was stirred using a stirrer at room temperature for 30 mins, and the pH was adjusted every 15 mins to maintain the pH of the solution. After that, the dissolved protein was centrifuged for 30 mins at 3500 rpm. The dissolved protein content (supernatant) was determined using the Bradford method (Nouroozi *et al*., 2015). Determination of solubility at each pH was carried out in two repetitions.

2.5 Preparation of catfish protein isolate

The preparation of protein isolates was performed based on Oo *et al*. (2017) by modifications, i.e., the ratio of flour and solvent for extraction, extraction condition, centrifugation, and lyophilization. The catfish protein isolate (CPI) production used two defatted flour treatments, namely DCFh and DCFe. The defatted catfish flour was extracted using a solution adjusted to the optimum pH solubility using 0.1 N NaOH and or HCl for 30 mins with a ratio of 1:20 (w/v). Furthermore,

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the solution was centrifuged at 3500 rpm for 30 mins. The supernatant was precipitated at the lowest solubility pH (isoelectric point) and centrifuged at 3500 rpm for 30 mins. The precipitated protein was lyophilized for 96 hrs using a freeze dryer at -35°C. Protein isolates were obtained and stored at -18°C. The protein isolate was produced, named the catfish protein isolate from defatted flour with hexane (CPIh) and ethanol solvents (CPIe).

2.6 Proximate analysis of catfish protein isolate

The proximate parameters, i.e., moisture, ash, protein, lipid, and carbohydrate, were performed based on Association of Official Analytical Chemists (2012). The moisture content was analysed using the Thermogravimetric method by drying the sample in an oven at 105°C for 6 hrs or until it obtained a constant weight. Then, the ash content was determined by drying the sample in a furnace at 600°C for 4 hrs. Protein content was determined using the Kjeldahl method by destruction, distillation, and titration. The conversion factor for total nitrogen to a protein of 6.25 was used. Lipid content analysis was determined using the Soxhlet method by hexane solvent. Finally, carbohydrate content was determined by a different method by subtracting the sum of moisture, ash, protein, and lipid content from 100%.

2.7 Physical properties analysis of catfish protein isolate

2.7.1 Bulk density

A total of 10 g of protein isolate was put into a 100 mL measuring cup. The measuring cup was tapped on the table until there was no cavity in the sample. Bulk density was determined using equation (1) (Wani *et al*., 2013):

$$\text{Bulk density} = \frac{\text{sample weight (g)}}{\text{volume (mL)}} \quad (1)$$

2.7.2 Colour

Colour analysis was performed using a colourimeter which described the values of L* (indicated the level of lightness), a* (indicated the colour of red and green), and b* (indicated the colour of blue and yellow).

2.8 Functional properties analysis of catfish protein isolate

2.8.1 Water holding capacity

Water holding capacity (WHC) was measured based on Gao *et al*. (2018). A total of 1 g of protein isolate was put into a centrifugation tube, and then 10 mL of distilled water was added. The solution was vortexed for 2 mins and allowed to stand at room temperature for 1 hr, then centrifuged at a speed of 1,811×*g* for 25 mins. After that, the supernatant was poured into a 10 mL measuring cup, and then the volume was measured. The water holding capacity was determined using equation (2):

$$\text{WHC (mL/g)} = \frac{\text{volume filtrate (mL)}}{\text{sample weight (g)}} \quad (2)$$

2.8.2 Oil holding capacity

Oil holding capacity (OHC) was measured based on Gao *et al*. (2018). A total of 0.5 g of protein isolate was put into a centrifuge tube, and then 3 mL of corn oil was added. The solution was vortexed for 2 mins and allowed to stand at room temperature, then centrifuged at a speed of 1,811×*g* for 25 mins. The supernatant was poured into a 10 mL measuring cup, and the volume of oil was measured. The determination of oil holding capacity was calculated using equation (3):

$$\text{OHC (mL/g)} = \frac{\text{volume filtrate (mL)}}{\text{sample weight (g)}} \quad (3)$$

2.8.3 Emulsion capacity and emulsion stability

The emulsion capacity and stability were determined based on Zielińska *et al*. (2018). The protein isolate (1 g) was added with corn oil and distilled water of 25 mL each. The mixture was added with 2 N NaOH and or HCl to pH 8, then homogenized for 1 min. Then the mixture was put into a centrifugation tube and centrifuged for 10 mins at a speed of 3,220×*g*. The determination of the emulsion capacity was calculated using equation (4):

$$\text{Emulsion capacity (EC)} = \frac{\text{emulsion volume after centrifugation}}{\text{emulsion volume before centrifugation}} \times 100\% \quad (4)$$

Emulsion stability was evaluated by heating the emulsion in a water bath at 90°C for 30 mins. Next, the mixture was cooled for 10 mins and centrifuged for 10 mins at a speed of 3,220×*g*. The determination of the emulsion stability was calculated using equation (5):

$$\text{Emulsion stability (ES)} = \frac{\text{emulsion volume after centrifugation}}{\text{emulsion volume before heating}} \times 100\% \quad (5)$$

2.8.4 Foam capacity and foam stability

The foam capacity and stability were determined based on Guo *et al*. (2015). A total of 1.5 g of protein isolate was added with 50 mL of aquadest. The protein solution was adjusted to pH 8 using 2 N NaOH and or HCl. After that, the mixture was homogenized for 1 min and then poured into a measuring cup. The total sample volume was monitored at 0 min for foam capacity and 60 mins for foam stability. The foam capacity and stability were determined using equations (6) and (7):

$$\text{Foaming capacity (FC)} = \frac{\text{Final volume - initial volume}}{\text{initial volume}} \times 100\% \quad (6)$$

$$\text{Foaming stability (FS)} = \frac{\text{Foam volume after 60 mins}}{\text{initial foam volume}} \times 100\% \quad (7)$$

2.8.5 Gel strength

Gel strength analysis was measured based on Wang

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et al. (2018). The 1 g of protein isolate sample was added with 10 mL of aquadest, then adjusted to pH 8 using 2 N NaOH and or HCl. After that, it was heated at 95°C for 15 mins. Samples were refrigerated overnight at 4°C. Gel strength was measured using a texture analyzer.

2.9 Sensory analysis

The sensory analysis used the Generic descriptive analysis (GDA) method based on Meilgaard *et al*. (2016) to evaluate the aroma and taste sensory attributes of the CPI and SPI samples. Sensory evaluation of protein isolates was carried out using ten trained panellists (male and female), Food Technology students at Atma Jaya Catholic University of Indonesia. The panellists determined the attributes to describe the protein isolate solution under the guidance of the panel leader. Protein isolate solutions (CPI and SPI) were prepared 1 hr before evaluation by a concentration of 1%. The scale was used unstructured of 0-100%. The sensory attributes were evaluated, i.e., aroma (fish liver oil, rancidity, dried fish, trimethylamine (TMA), taste (fish liver oil, rancidity, dried fish, TMA, sweet, bitter), and off-flavour (Shaviklo *et al*., 2012). Panellists evaluated 3 sample solutions over two sessions.

2.10 Statistical analysis

The study was conducted with two repetitions, and the results were presented as means ± standard deviation. Analysis of variance (ANOVA) was used to analyse the data and the differences in the means between samples were determined using Duncan's Multiple Range Test (DMRT). P-values lesser than 0.05 were considered significant. Statistical analysis was performed using SPSS Version 24.

3. Results and discussion

3.1 Protein solubility profile of catfish

The effect of lipid removal solvent and protein solubility profile of catfish (DCFh and DCFe) was shown in Figure 1. Based on protein solubility, DCFh (1.1 mg/mL) was the highest compared to DCFe (1.0 mg/mL) but did not show a significant difference (p<0.05). Therefore, using more polar lipid removal solvents such as ethanol, chloroform, and acetone, there was a higher protein denaturation than lipid removal solvents such as hexane and diethyl ether. In addition, the more protein maintains its original structure in the preparation process, the higher the protein solubility will be (Feyzi *et al*., 2017). Similar to L'hocine *et al*. (2006), the protein solubility of protein isolate did not show a significant difference from defatted flour with hexane, ethanol, and methanol solvents was influenced by the conditions used during protein isolate preparation, such as pH and extraction temperature. Ethanol solvent could be used as an alternative to removing the lipid of flour because the protein solubility of catfish did not show a significant difference between DCFh and DCFe. The highest protein solubility was DCFh and DCFe at pH 11, and the lowest was at pH 5.5. The solubility of protein at alkaline pH was higher than at acidic pH. The negative charge of amino acid residues in the alkaline pH range was greater than the total positive charge in the acidic pH range. Protein precipitation was carried out at a pH of 5.5 when the solubility of protein was lowest (pH of the isoelectric point). Protein solubility is influenced by the molecular weight, surface characteristics of amino acids, and conformation of the situation (Timilsena *et al*., 2016).

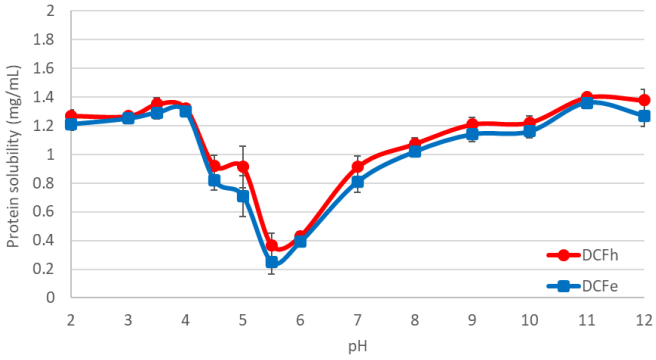

Figure 1. Protein solubility curve of catfish (DCFh and DCFe)

3.2 Proximate composition of catfish protein isolate

The proximate composition and yield of CPIh, CPIe, and SPI are shown in Table 1. The yield of CPIh (23.96±3.75%) prepared from DCF by the CPI process was significantly different and higher than CPIe (13.20±0.20%). Similarly, the protein yield of CPIh was higher than CPIe and significantly different. Lee *et al*. (2016) reported that protein isolates from yellowfin tuna roe yielded 11.6–14.1%. The yield of protein isolates was influenced by the fish species, the method used to determine the yield, centrifugation speed during protein isolation, and the sarcoplasmic concentration (Chen and Jaczynski, 2007).

Protein isolates are products with protein content reaching > 90% dry weight. The CPIh and CPIe had higher protein content (92.02±1.13 – 93.47±1.97% db) than SPI (76.07±0.20% db) and were significantly different (p<0.05). The CPIh and CPIe protein contents were not significantly different (p>0.05). Different defatting solvents in defatted catfish flour do not significantly affect the protein content of the catfish isolate produced. Gravel *et al*. (2021) reported that defatting steps could increase the protein content. Lipids can reduce the technical properties of proteins because the structure and size of large molecules can interfere with the interactions between molecules at the

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Table 1. Proximate composition and yield of catfish protein isolate from defatted flour with hexane (CPIh) and ethanol solvents (CPIe) and soy protein isolate (SPI)

Sample	Yield[a] (%)	Protein Yield[b] (%)	Moisture (% wb)	Ash (% db)	Protein (% db)	Lipid (% db)	Carbohydrate (% db)
CPIh	23.96±3.75[b]	22.04±3.45[b]	2.12±0.46[a]	2.47±0.39[b]	92.02±1.13[b]	4.18±0.41[b]	1.33±1.05[a]
CPIe	13.20±0.20[a]	12.33±0.18[a]	5.84±0.40[b]	1.92±1.12[a]	93.47±1.97[b]	4.09±0.97[b]	0.53±2.66[a]
SPI	-	-	6.75±0.12[c]	5.17±0.08[c]	76.07±0.20[a]	0.09±0.12[a]	18.68±0.04[b]

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significant different (p<0.05). -: not determined

[a]Yield (%) is the weight (g) of catfish protein isolate (CPI) obtained from weight defatted catfish flour (DCF) (g) × 100%

[b]Protein yield (%) = yield (g) × protein (%)

time of extraction (Rousseau, 2000). The protein content of CPI fulfilled the Codex Alimentarius International Food Standards (protein content for SPI products > 90% db) (Codex Alimentarius, 2019). The CPI protein content was higher than several fish protein isolates (Shaviklo *et al*., 2010; Shaviklo *et al*., 2012; Lone *et al*., 2015; Lee *et al*., 2016; Tian *et al*., 2017; Pramono *et al*., 2018). The protein content produced by each protein isolate is influenced by the method used for protein extraction, temperature and drying time, centrifugal separation, and the relative concentration of sarcoplasmic protein in water-soluble fish (Nolsoe and Undeland, 2009). In this study, the isolation process of catfish protein was successful at the highest protein solubility pH of 11 and isoelectric pH of 5.5. The CPI produced could be used as an alternative to the binding agent added to the emulsion of processed meat products.

The lipid content of CPIh and CPIe (4.09±0.97 – 4.18±0.41% db) were higher than SPI (0.09±0.12% db) and were significantly different (p<0.05). However, there was no significant difference in the lipid content of CPIh and CPIe. The CPI had lower lipid content than protein isolates from carp fish and yellowfin tuna roe (Lee *et al*., 2016; Tian *et al*., 2017). The high lipid and unsaturated fatty acids content can cause lipid oxidation (Liu *et al*., 2019). The solvent used during the preparation of defatted flour did not affect the lipid content of protein isolates. That showed ethanol could potentially be a good solvent alternative for defatting other than hexane.

3.3 Physical properties of catfish protein isolate

3.3.1 Bulk density

The CPIh and CPIe had a bulk density of 0.40±0.01; 0.45±0.01 g/mL respectively, significantly different from SPI (0.37±0.00 g/mL) (p<0.05) (Table 2). The bulk density of CPI was similar to some fish proteins (Foh *et al*., 2012; Shaviklo *et al*., 2012; Lone *et al*., 2015). The bulk density value depends on the protein's solubility and microstructure (Foh *et al*., 2012). Other factors that affect bulk density include particle size, the density of solids, the amount of interstitial air, the sphericity of the particles, the relative humidity, and the drying temperature (Ding *et al*., 2020). In addition, bulk density is also influenced by the drying method for protein isolates, the preparation of fish flour used, the number of addictive materials used in isolate production, and the *aw* value of the material (Shaviklo *et al*., 2012). Small bulk density is advantageous for weaning food formulations, where a low-density product formula is required (Foh *et al*., 2012).

3.3.2 Colour

The values of L* (lightness), a* (red colour), and b*

Table 2. Physical and functional properties of CPIh, CPIe, and SPI

Properties	CPIh	CPIe	SPI
Bulk density (g/mL)	0.40±0.01[b]	0.45±0.01[c]	0.37±0.00[a]
Colour			
L*	77.03±0.61[b]	75.69±1.06[a]	80.65±0.01[c]
a*	3.61±0.18[b]	3.78±0.18[b]	0.85±0.01[a]
b*	16.54±0.31[b]	17.04±0.43[c]	15.33±0.01[a]
Water holding capacity (WHC) (mL/g)	3.69±0.47[b]	3.04±0.06[a]	3.05±0.06[a]
Oil holding capacity (OHC) (mL/g)	2.40±0.18[a]	2.51±0.34[a]	2.59±0.18[a]
Emulsion capacity (EC) (%)	52.33±2.04[b]	50.83±1.29[b]	55.00±0.00[a]
Emulsion stability (ES) (%)	53.83±2.32[b]	51.42±2.60[ab]	50.00±0.00[a]
Foaming capacity (FC) (%)	10.33±0.82[b]	3.00±1.26[a]	29.33±1.16[c]
Foaming stability (FS) (%)	95.83±8.33[a]	93.33±11.55[a]	91.67±3.61[a]
Gel strength (g/gf)	148.67±7.09[a]	136.17±15.90[a]	855.89±30.70[b]

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significant different (p<0.05).

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(yellow colour) CPIh, CPIe, and SPI are shown in Table 2. Each protein isolate had significantly different L* values (p<0.05). The L* CPI value (75.69±1.06–77.03±0.61) was lower than the SPI (80.65±0.01). The CPI lightness intensity was lower than SPI. The CPI had a higher a* value and was significantly different from SPI (p<0.05) but did not differ significantly between CPIh and CPIe (p>0.05). The CPI and SPI had a positive a* value, which means they point to redness. The value of b* for each protein isolate was significantly different (p<0.05). The CPI and SPI had a positive b* value, meaning they point to yellowness. The white colour of fish protein isolates depends on the connective tissue in fish meat. The reddish colour of CPI was due to the presence of heme protein in the final product. The yellowish colour in CPI was caused by lipid retention, denaturation, and oxidation of haemoglobin which caused the product to be brownish-yellow (Kristinsson *et al*., 2005). The difference in the solvent for defatted flour used for protein isolates affected the colour of the protein isolate produced (L* and b* values). This difference was caused by protein aggregation (Kim *et al*., 2021). Lone *et al*. (2015) reported the values of L*, a*, and b* of rainbow trout protein isolates, 44.08±1.95; 14.19±0.93; 42.10±1.17, respectively. The difference in the colour of various types of fish protein isolates can be caused by the processing conditions, the kind of fish, and the freshness of the fish. In addition, the treatment conditions before drying, drying temperature, and lipid oxidation can affect the colour of the resulting protein isolates (Shaviklo *et al*., 2012). The CPI could be applied to foodstuffs with neutral colours.

3.4 Functional properties of catfish protein isolate

3.4.1 Water holding capacity

The WHC value of CPIh (3.69±0.47 mL/g) was significantly different and higher than CPIe and SPI (p<0.05) (Table 2). The CPIe had a WHC value not significantly different from SPI (p>0.05). Similarly, L'hocine *et al*. (2006) reported that the WHC value of the SPI of defatted flour using hexane was higher than ethanol and significantly different (p<0.05). Modifications in the preparation of protein isolates also influence WHC values such as heat precipitated, acidic pre-treatment, and solubilization at various pHs. The CPI had a higher WHC value than several fish protein isolates (Foh *et al*., 2012; Shaviklo *et al*., 2012; Lone *et al*., 2015; Pramono *et al*., 2018). The WHC is influenced by the composition and conformation of protein molecules through hydrogen bonds. When producing the isolates, the difference in WHC values in various fish protein isolates can be influenced by pH and ionic strength, acids, and bases. The use of acids and bases can reduce the protein content of sarcoplasm and extract more myofibril protein, which plays a role in maintaining water content in gel formation (Foh *et al*., 2012; Lone *et al*., 2015). The CPI had a high WHC value to formulate processed meat products that could increase protein binding capacity. In addition, CPI could be applied to improve the characteristics of bakery products.

3.4.2 Oil holding capacity

The OHC values for CPIh, CPIe, and SPI were not significantly different (p>0.05) (Table 2.). L'hocine *et al*. (2006) also reported that differences in solvent to remove lipids did not affect the OHC protein isolates produced. The CPIh and CPIe had higher OHC values than fish protein isolates in the previous study (Foh *et al*., 2012; Lone *et al*., 2015; Pramono *et al*., 2018). The absorption ability of oil depends on the protein structure. The lipophilic structure is due to the predominant content of the nonpolar protein branches, thus contributing to increased oil absorption. Hydrophobic proteins that are not water-soluble can absorb large amounts of oil. The ability to absorb oil in this protein isolate could be high because protein isolation was carried out at an alkaline pH (pH 11). Freitas *et al*. (2011) reported that the OHC value protein isolates with alkaline treatment were higher than acid treatment. The alkaline condition caused the denaturation of the protein and the unfolding protein structure. Thus, increasing hydrophobic interactions and thus increasing OHC (Oliyaei *et al*., 2019). In addition, high OHC in protein isolates was also influenced by the number of lipids present in the raw material to produce protein isolates (Freitas *et al*., 2011). The CPI had excellent potential for structural interactions in food, especially in improving palatability, flavour retention, and extension of shelf life of meat or bakery products where oil-holding capacity is desired (Chandra *et al*., 2015). In addition, the high OHC value in CPI is important in food formulations such as processed meat products, mayonnaise, batters, salad dressings, and making cakes and bread. The interaction of water and oil by protein is important in the formulation of food systems because of its effect on the aroma, taste, and texture (Foh *et al*., 2012).

3.4.3 Emulsion capacity and Emulsion stability

The EC on CPIh and CPIe differed significantly from SPI (p<0.05), but between CPIh and CPIe were not significantly different (p>0.05) (Table 2). The ES values on CPIh differed significantly from SPI (p<0.05) but were not significantly different from CPIe (p<0.05). Similar to Feyzi *et al*. (2017), hexane and ethanol solvents as a defatting of flour did not affect the EC. Kim *et al*. (2021) also reported that EC and ES were not significantly different from differences in the defatting solvents used, such as methanol, hexane, and ethanol

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(p<0.05). The EC and ES on CPIh were higher than CPIe because they were influenced by molecular weight and protein distribution. Proteins with low molecular weight have high stability and emulsion capacity (Mishyna *et al*., 2019). The EC and ES on CPI and SPI were lower than other fish muscle protein isolates (Foh *et al*., 2012; Shaviklo *et al*., 2012). The ability of the protein as an emulsifier will depend on its amphiphilic nature, protein solubility, level of surface denaturation, lipid-to-protein ratio, and emulsion viscosity (Lam and Nickerson, 2013). The EC of protein depends on the balance between hydrophilic and hydrophobic bonds between proteins and food ingredients. The nature of the emulsion can be affected by the number of hydrophobic peptide molecules. In addition, the nature of the emulsion can also be influenced by pH, where at an alkaline pH, the value of the emulsion capacity increases compared to acidic pH. The pH also affects the balance of the protein's hydrophobic and hydrophilic sides (Foh *et al*., 2012). The EC and ES in CPI could potentially be applied to food products containing oil emulsions, such as sausages, frankfurter, meatballs, comminuted meat products, burgers, mayonnaise, salad dressings, frozen desserts, cake batters, milk, and patties. Canti *et al*. (2021) reported that protein isolate successfully could improve the emulsion stability of chicken sausages.

3.4.4 Foam capacity and Foam stability

The FC of CPI and SPI ranged from 3–29.33% (Table 2.). The FC values for each protein isolate, CPIh, CPIe, and SPI, were significantly different (p<0.05), while the FS values were not significantly different (p>0.05). The CPIh had higher FC and FS than CPIe. That is because the protein solubility of catfish from defatted flour with hexane was higher than ethanol. According to Feyzi *et al*. (2017), proteins with high solubility mean that protein molecules migrate to the interface, causing air encapsulation in the foam system. The CPI had a lower FC than several fish protein isolates but higher FS (Foh *et al*., 2012; Shaviklo *et al*., 2012; Lone *et al*., 2015; Panpipat and Chaijan, 2017; Abdollahi and Undeland, 2018). Factors that influence the capacity and stability of the foam are protein concentration, surface tension, foaming time, and pH (Amankeldi *et al*., 2018). The foaming ability increases if the protein concentration increases because it will increase the thickness of the film layer at the interfacial. The FC depends on the strength of the protein film formed and gas permeability (Shaviklo *et al*., 2012). The foam capacity was also influenced by protein molecules. Globular proteins increase protein concentration, resulting in higher foam formation ability and stability (Indrawati *et al*., 2008). The nature of protein to form a stable foam is important in the production of some foods. The properties of FC and FS are suitable for bakery products, where the desired properties are to form a stable foam layer in the development of bakery dough, wafers, ice cream, whipped topping, meringue, mousse, marshmallows, and milkshakes.

3.4.5 Gel strength

Gel strength at CPIh and CPIe was 136.17–148.67 g/gf (Table 2). The CPIh and CPIe had lower gel strength than SPI and differed significantly (p<0.05). Different solvent treatments for defatting protein did not affect the strength of the resulting protein isolate gel. Shaviklo *et al*. (2012) showed that saithe fish protein isolate had a gel formation strength of 6.0±0.5 to >10%. The ability to form gel was influenced by the denaturation of myofibrils protein, the concentration of myofibrils protein content, stroma, sarcoplasm, and additives to protect proteins during the drying process. The gelation properties of CPI play a role in the texture formation of processed meat products such as sausages, frankfurter, meatballs, and comminuted meat products. Elasticity in the final product can be formed because the matrix in the gel structure retains water and lipids.

3.5 Sensory properties of catfish protein isolate

The sensory evaluations of CPIh, CPIe, and SPI are shown in Figure 2. All sensory attributes, aroma, and taste of the CPIh and CPIe samples differed significantly from SPI (p<0.05). The CPIh samples had all sensory attributes that differed significantly from CPIe (p>0.05), except for the dried fish taste. The CPI had a higher intensity than SPI in aroma and taste attributes. The sensory evaluation showed that the CPI sample had a higher intensity on the aroma and taste attributes of fish liver oil, dried fish, and off-flavour. In addition, CPI had also described an aroma and taste of rancidity but low in intensity. Shaviklo *et al*. (2012) reported that fish protein isolates from freeze-dried saithe had a high aroma and taste such as rancidity, fish liver oil, and dried fish. That is due to lipid oxidation which can cause off-flavour and damage to fish muscles. According to Undeland *et al*. (2005), the aroma and taste of lipid oxidation were produced during the isolation process of fish protein. Furthermore, the drying process caused lipid oxidation at high temperatures to accelerate peroxide breakdown into carbonyl components, peroxide value, generally in primary and secondary oxidation products (Secci and Parisi, 2016). According to Raghavan and Hultin (2009), the high heme protein in fish, an active pro-oxidant during pH changes during protein isolation, could cause lipid oxidation. Several enzymes in meat, such as peroxidase and dioxygenase, could initiate lipid oxidation processes (Dominguez *et al*., 2019).

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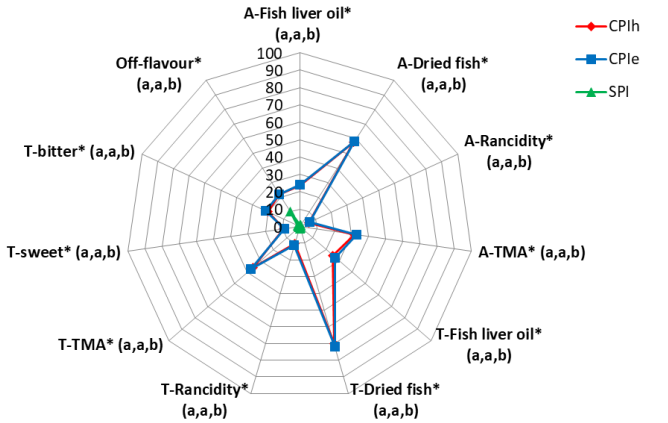

Figure 2. Sensory attributes of catfish protein isolates (CPIh, CPIe) and soy protein isolate (SPI) (scale: 0-100 evaluated by panellists). Values are means of two evaluations.
*Statistically significant different (p<0.05).

4. Conclusion

Catfish protein isolates from defatted flour have been successfully produced. The CPIh and CPIe had a high protein content which fulfilled Codex Alimentarius International Food Standards. Different lipid removal solvents affected the proximate composition (moisture; ash content), physical (bulk density; colour), functional (WHC; FC), and sensory (dried fish taste) properties of the resulting protein isolates. Ethanol could be suggested as a defatting solvent to produce CPI with good WHC, OHC, EC, ES, FS, and gel strength. Ethanol could potentially be a good solvent alternative for defatting other than hexane. The CPI had good functional properties such as WHC, OHC, EC, ES, FS, and gel strength, while FC was poor. In addition, CPI obtained the best sensory score on all attributes, except for the aroma and taste of dried fish. CPI could be used in food formulations to increase nutritional value based on its chemical, physical, functional, and sensory properties.

Conflict of interest

The authors declare no conflict of interest.

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