

Physicochemical and sensory quality of mackerel fish ball formulated by dumbo catfish (*Clarias gariepinus*) protein isolate as a binder

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Abstract

Catfish is a freshwater fish with high protein content and productivity. However, its use is still limited. Food processing and formulation can be a solution to utilize catfish protein in fish-based products. This study investigated the physicochemical and sensory quality of mackerel fish balls (MFBs) formulated with catfish protein isolate (CPI) as a binder. The catfish protein was isolated using the pH-shifting method with extraction at pH 11 and precipitation at pH 5.5. The fish ball formulation contained mackerel crushed meat (MCM) and catfish protein isolate (CPI), with ratios of F1 (100:0), F2 (85:15), and F3 (70:30). The MFBs were also analysed for their physical (e.g., colour, water holding capacity (WHC), cooking loss, texture), proximate, and sensory properties. The results showed that the CPI had a high protein content (93.46% db), which followed the Codex Alimentarius standard. The addition of CPI had a significant effect on the colour, WHC, cooking loss, hardness, proximate composition, and sensory properties (colour, aroma, taste, texture, and overall attributes) of MFBs ($p < 0.05$). The CPI improved WHC, hardness and protein content while reducing the cooking loss of MFBs. The MFBs with the ratio of MCM: CPI of 85:15 (F2) were the best formulation based on the hardness value, protein content, and sensory properties. The protein content of F2 increased by 6.89% from the control. CPI can be an additive ingredient in highly nutritious fish-based products with good physical and sensory properties.

1. Introduction

Catfish, a fish that is widely cultivated in Indonesia, is commonly favoured by the public because of its affordable price and distinctive meat taste. As a popular commodity, catfish production in Indonesia in 2020 reached 993.76 thousand tons (Statistics Indonesia, 2022). In addition, the consumption level of catfish in 2021 was the highest compared to other types of fish, which counted up to 18.07% or 0.038 kg/capita a week (Statistics Indonesia, 2021a). One common species of catfish to be cultivated is the dumbo catfish (*Clarias gariepinus*), which is the result of a marriage between catfish from Africa (*Clarias mossambicus*) and catfish from Taiwan (*Clarias fuscus*). Dumbo catfish have advantages over local catfish, namely their larger size, faster growth, more resistance to diseases, and higher survival rates in various environmental conditions. Concerning its nutritional values, dumbo catfish normally contains 16.08% protein, 73.01% moisture, 2.03% lipid and 0.78% ash (Zuraida *et al.*, 2017). Currently, the utilization of catfish as a processed

product is nevertheless still relatively low, while most catfish are still consumed in fresh or direct form, such as fried catfish. Given that, a processing innovation is needed to maximize the potential of catfish production; one such innovation is to process the catfish in the form of protein isolate.

Protein isolate is a pure form of protein obtained through a purification process based on differences in solubility, which renders it free from lipids, moisture, carbohydrates, and fibre. Protein isolate is often considered in food production for its usefulness in increasing nutritional values and qualities. In products made from meat (e.g., sausages, patties), protein isolate is used to stabilize the water and oil composition in the products (emulsifier), improve the food structure due to its ability to form a gel and reduce cooking loss (Garba and Kaur, 2014; Shaviklo and Etemadian, 2019). Protein isolates can be isolated from plant and animal materials containing protein. Various plant protein ingredients that have been developed to produce protein isolates come from soybeans, mung bean, black bean, adzuki bean, rice

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bean, black kidney bean, speckled kidney bean, chickpea, cowpea, pea, amaranth, quinoa, chia, potato, Bambara groundnut seed, *Moringa oleifera* seed kernels and leaves (López et al., 2018; Chang et al., 2020; Ge et al., 2021; Arise et al., 2022; Bocarando-Guzmán et al., 2022; Illingworth et al., 2022). Animal protein has advantages over plant protein because it contains more essential amino acids and is easily digested (Derkach et al., 2022). The development of protein isolates from animal products is increasingly diverse, such as protein isolates from fish, molluscs, and insects. Varelziz and Undeland (2012) reported protein isolation from blue mussels using acid and base dissolution techniques. Monaya et al. (2022) also reported protein isolate production from slipper-cupped oysters using the alkaline pH shift method. Various types of insects have also been reported for the production of protein isolates, such as cricket, *Tenebrio molitor* larvae, and grasshoppers (Jiang et al., 2021; Razzak et al., 2022; Zhang et al., 2022).

In recent years research on the production of protein isolates from various fish species has been developed, such as Indian mackerel, salmon, cod, herring by-product, ponyfish, sardines, lanternfish, yellowfin tuna roe, tilapia, anchovy, Baltic herring (Abdollahi and Undeland, 2018; Kumarakuru et al., 2018; Oliyaei et al., 2019; Yoon et al., 2019; Zhou and Yang, 2019; Canti et al., 2022; Kakko et al., 2022a). That research studied the effect of protein isolation on yield, protein recovery, and physical, proximate, functional, and sensory properties of fish protein isolates. Research by Kumarakuru et al. (2018) and Haryati et al. (2020) reported the isolation and physicochemical, morphological, and functional characterization of protein isolates from catfish varieties *Batrachocephalus mino* and *Clarias* sp. The isolation of catfish protein from the *Clarias gariepinus* variety and its use in food products have not been studied.

One of the uses of protein isolate is as a binder in fish ball products. As revealed by Statistics Indonesia (2021a), as much as 88.13% of fish consumption in Indonesia comes from the fish and processed food (KIMJ) group, such as meatballs, nuggets, sausages, and others. However, until now, the protein isolates that are often applied to food products are soy protein isolates that are imported into Indonesia, not fish protein isolates (Tian et al., 2019; Samriddhi and Roshan, 2022). Haryati et al. (2020) reported that catfish protein isolate had a high protein content (90.46% db), water holding capacity (WHC) (1.52 g/mL), oil holding capacity (OHC) (4.08 g/mL), stable emulsions, and capable of forming a gel. Adding fish protein isolate as a binder improves the gel properties, texture and water-holding capacity of the resulting product. Kakko et al. (2022a) reported that

adding fish protein isolate from Baltic herring could improve fish balls' shape, water-holding ability, and texture. Ibrahim (2015) also reported that adding fish protein isolate from Nile boliti fish could increase protein content, cooking yield, and reduce fat content, energy, and cooking loss in fish balls and was accepted by the panellists. Moreover, adding fish protein isolate to fish balls can be a good source of minerals and amino acids for the body. The characteristics of the fish balls produced are preferred because of their excellent taste, high nutritional values, and good physical properties. The effect of the addition of catfish protein isolate on the characteristics of fish balls has never been known before.

Mackerel (*Scomberomorus commersoni*) was used as a raw material for fish ball formulation. Mackerel has a high protein content (22.89%), and productivity is abundant and stable in Indonesia (Statistics Indonesia, 2021b; Yilmaz, 2021). The high demand for white-fleshed fish such as big-eye snapper and threadfin bream fish has caused Indonesia's population decline (Statistics Indonesia, 2021b). However, mackerel has dark flesh, which has low gelling properties. Therefore, in the preparation of fish balls, washing fish meat was carried out to remove sarcoplasmic proteins and increase myofibrillar concentrations. Myofibrillar protein can increase the ability to form gels (Park, 2013). Considering the potential of fish protein isolate, this study aimed to evaluate the physical, proximate, and sensory properties of mackerel fish balls with the addition of catfish protein isolate as a binder.

2. Materials and methods

2.1 Materials

The materials in this study included dumbo catfish meat (*Clarias gariepinus*) obtained from the Metropolis Modern Market Tangerang, Indonesia; mackerel fish meat (*Scomberomorus commersoni*) obtained from the Intermoda Modern Market BSD, Tangerang, Indonesia; tapioca flour; garlic; salt; and pepper. The materials for the chemical analysis materials were ethanol (Bratachem Technical, Indonesia), NaOH (Merck, Germany), 37% HCl (Merck, Germany), 98% H₂SO₄ (Merck, Germany), catalyst tablets (CuSO₄ and K₂SO₄) (Behr Labor-Technik, Germany), Bromocresol green and Methyl red (BCG-MR) (Merck, Germany), H₃BO₃ (Merck, Germany), and n-hexane (Merck, Germany).

2.2 Preparation of catfish flour

The preparation of catfish flour, according to Yilmaz and Koca (2020), was mainly carried out by adjusting the temperature and drying time. Fresh catfish were first washed thoroughly to remove dirt, blood, scales, and mucus. The catfish meat was separated from the skin,

head, and other organs and then crushed in a food processor (Philips HR-7627, China). The crushed meat was dried in a cabinet dryer (Agroindo Sukses Abadi OVL-12, Indonesia) at 55°C for approximately 22 hrs. After that, the meat was crushed with a food processor until coarse flour was obtained. This coarse flour was sieved with an 80-mesh sieve (Megah Gumilang Chemikatama ATE-210, Indonesia) to produce fine catfish flour, which would be stored in a plastic zip bag with silica gels in the freezer (GEA AB-600TX, Indonesia) at -20°C.

2.3 Preparation of defatted catfish flour

The defatted catfish flour was prepared using the method enumerated by Puteri *et al.* (2018) with some modifications in the extraction time, length of time and speed of centrifugation. The catfish flour was extracted using food-grade ethanol solvent with a ratio of 1:3 (w/v) and subsequently stirred for 30 mins to remove the lipid content. After that, the mixture was centrifuged (Eppendorf 5418R, USA) at a speed of 1811×g for 20 mins. Pellets resulting from centrifugation were defatted catfish flour. The fat extraction process was repeated three times. Then, defatted catfish flour was dried overnight in a fume hood (Biobase FH1000, China) to remove residual solvent. The defatted catfish flour was stored in a plastic zip bag with silica gels in the freezer (GEA AB-600TX, Indonesia) at -20°C.

2.4 Preparation of catfish protein isolate

The catfish protein isolate (CPI) was prepared following the method designed by Oo *et al.* (2017) with some adjustments in the concentration of flour and solvent for extraction, extraction pH and isoelectric point, extraction conditions, centrifugation, and lyophilization. The defatted catfish flour was extracted at pH 11 (the optimum pH), using 0.1 N NaOH for 30 mins with a ratio of 1:20 (w/v). After that, the solution was centrifuged at a speed of 2,465×g for 30 mins. The supernatant was separated from the pellet and precipitated at pH 5.5 (isoelectric point) with 0.1 N HCl. The precipitated protein was lyophilized with a freeze dryer (Christ Alpha 2-4 LDPlus, Germany) at -70°C for 72 hrs. The protein isolates were stored in polyethene plastic bags in a freezer (GEA AB-600TX, Indonesia) at -20°C.

2.4 Surimi and mackerel fish ball preparation

The preparation of surimi and mackerel fish balls (MFB) followed the stages elaborated by Tee and Siow (2017). Mackerels were killed by the bleeding method. The bleeding process of mackerel was by cutting behind the base of the pectoral fin recess of the fish with a knife. Afterwards, the head, entrails and gills were separated.

The mackerel meat was washed to remove impurities such as mucus and blood. Then the fish's body was filleted using a knife. The mackerel fillets were initially soaked in cold water with a ratio of meat to water at 1:3 (w/v) at 5°C for 30 mins. Soaking was to inhibit the activity of microorganisms and enzymes in the fish body and reduce the lipid content of fish meat. The fish fillets were separated from the lipids using the knife. Then the fillets were crushed and washed using cold water at 5°C, 3 times, ratio of cold water: crushed meat of 1:3. The frequency of washing fish meat was 10 mins. The purpose of washing the meat was to remove the sarcoplasmic protein and increase the myofibril protein concentration. The mackerel crushed meat was filtered with a calico cloth and pressed at the end of the washing.

In the preliminary research, MFBs were prepared using the formulation of the ratio of mackerel crushed meat (MCM) to catfish protein isolate (CPI) of 65:35. The result showed that MFBs with that formulation had more hard texture, lower elasticity, and an intensive fatty flavour. Therefore, the addition of CPI to MFBs was less than 35%. The MFBs were prepared in three formulations with different ratios of MCM and CPI, namely 100:0 (F1), 85:15 (F2), and 70:30 (F3). Next, the MCM was mixed with other ingredients, such as 2 g of salt, 1 g of pepper, 2 g of garlic, and 15 g of tapioca flour. The resulting dough was moulded into balls and soaked in cold water at 4°C with a ratio of MFBs to cold water at 1:0.5 (w/v) for 5 mins. The MFBs were boiled at 100°C for 15 mins. Then the MFBs were cooled in water at 5°C for 5 mins. The MFBs were stored in polyethene plastic bags in a freezer (GEA AB-600TX, Indonesia) at -20°C.

2.5 Proximate analysis of fresh catfish, catfish flour, defatted catfish flour, protein isolate and fish balls

The proximate parameters were moisture, ash, protein, lipid, and carbohydrate and were examined based on Association of Official Analytical Collaboration (AOAC) International (2012). The moisture content analysis was performed using the Gravimetry method. The empty dish was dried in the oven for 15 mins and then cooled in a desiccator for 30 mins. After that, the empty dish and 1 g of the sample were weighed. The dish with the sample was dried in an oven at 105°C for 6 hrs or until a constant weight was reached. The ash content was scrutinized using the dry ashing method, where the sample (1 g) was dried in a furnace at 600°C for 4 hrs. The protein content was determined using the Kjeldahl method. A total of 0.3 g of sample was put into a Kjeldahl flask, and then two catalyst tablets (0.4 g CuSO₄; 3.5 g K₂SO₄) and 12 mL of 98% H₂SO₄ were added. The sample was boiled until the liquid became clear and then cooled. Next, 80 mL of

distilled water was added to the sample. The sample was distilled with 50 mL of 40% NaOH. The distillate was collected in an Erlenmeyer flask containing 30 mL of 1% H₃BO₃. Next, the distillate was added with two drops of BCG-MR indicator and titrated with 0.1 M HCl. The nitrogen value was converted into protein content by multiplying by a factor of 6.25. The lipid content was examined with hexane solvent using the Soxhlet method. Approximately 2 g of the sample was weighed and transferred into the extraction thimble. After that, the sample was extracted with hexane solvent for 4 hrs. The lipid flask was dried in the oven for 5 hrs at 105°C, cooled in a desiccator for 30 mins and weighed. Finally, the carbohydrate content was estimated using a different method by subtracting the sum of moisture, ash, protein, and lipid content from 100%.

2.6 Analysis of the physical properties of fish balls

2.6.1 Colour

The colour of the fish balls was identified using a colourimeter. The parameters tested are the value of L* (lightness level), a* (red-green colour), and b* (blue-yellow colour).

2.6.2 Water holding capacity

The water-holding capacity (WHC) was measured following the method described by Santana *et al.* (2015). A total of 5 g of the sample was put into a 50 mL centrifuge tube, into which 10 mL of distilled water was then added. The mixture was centrifuged at 2,000×g at 15°C for 10 mins. The supernatant was poured, and the final weight of the sample was determined. The WHC can be calculated using Equation (1):

$$\text{WHC (\%)} = \frac{\text{final sample weight (g)} - \text{initial sample weight (g)}}{\text{initial sample weight (g)}} \times 100\% \quad (1)$$

2.6.3 Cooking loss

The weight of the fish ball was measured before and after cooking to calculate the cooking loss using Equation (2) (Park, Lee, Lim *et al.*, 2021):

$$\text{Cooking loss (\%)} = \frac{\text{Before cooking weight (g)} - \text{After cooking weight (g)}}{\text{Before cooking weight (g)}} \times 100\% \quad (2)$$

2.6.4 Texture

Fish balls with a thickness of 2.5 cm were measured for their hardness using a texture analyser with a speed of 1 mm/s and a depth of 1.75 cm.

2.7 Sensory analysis of fish balls

The sensory analysis was performed using the acceptance test, which was developed by Meilgaard *et al.* (2016). The acceptance test involved thirty untrained student panellists (ten males and twenty females), aged

between 20-22 years, from the Biotechnology Faculty, Atma Jaya Catholic University of Indonesia. Fish ball samples were thawed at 25°C for 30 mins. Then the fish balls were heated using a microwave oven (Oxone OX-78TS, Indonesia) at 100°C for 3 mins. The sensory analysis used the hedonic scale of 1 to 7, with 1 - dislike very much, 2 - dislike moderately, 3 - dislike slightly, 4 - neither like nor dislike, 5 - like slightly, 6 - like moderately, and 7 - like very much. The parameters tested included colour, aroma, taste, texture, and overall attributes.

2.8 Statistical analysis

The statistical analysis was performed using SPSS. The data obtained were first tested for normality. If normal, the data were then analysed using the Analysis of Variances (ANOVA) method and Duncan's follow-up test. If not normal, the data were analysed using the Kruskal-Wallis method, followed by a stepwise step-down test. All data were statistically analysed at a significance level of $p < 0.05$. The data were obtained from three replicates.

3. Results and discussion

3.1 Proximate composition of fresh catfish, catfish flour, defatted catfish flour, and catfish protein isolate

The proximate composition of fresh catfish (FC), catfish flour (CF), defatted catfish flour (DCF), and catfish protein isolate (CPI) are shown in Table 1. In particular, the composition of CPI included moisture content (5.84±0.18% wb), ash (1.92±0.05% db), protein (93.46±0.76% db), lipid (4.09±0.43% db) and carbohydrates (0.53±1.09% db), all of which were lower than the proximate composition (moisture, ash, lipid, and carbohydrates) of FC, CF, and DCF. The moisture content of CPI was significantly different from that of FC, CF, and DCF ($p < 0.05$). The CPI had moisture content that met the Codex standard (Codex Alimentarius, 2019), which mentioned <10% as the acceptable level for soy protein isolate. The moisture content of CPI was higher than that of saithe fish (*Pollachius virens*) protein isolate (1.4±0.07-2.8±0.05%), tilapia (3.86±0.23-3.90±0.29%), rainbow trout (3.5±0.42%), Indian mackerel (5.71±0.07%), pony fish (5.65±0.04%), and anchovies (3.64±1.09%) (Foh *et al.*, 2012; Shaviklo *et al.*, 2012; Lone *et al.*, 2015; Kumarakuru *et al.*, 2018; Canti *et al.*, 2022). However, the moisture content of CPI was lower than that of protein isolate from myctophid fish (83.1%), tuna (69.74±0.69%), common carp (74.6%), catfish (5.86±0.09%), and sardine (6.05±0.05%) (Oliyaei *et al.*, 2015; Shabanpour and Eternadian, 2016; Shaviklo *et al.*, 2016; Kumarakuru *et al.*, 2018).

Table 1. Proximate composition of fresh catfish (FC), catfish flour (CF), defatted catfish flour (DCF), and catfish protein isolate (CPI).

Sample	Moisture (% wb)	Ash (% db)	Protein (% db)	Lipid (% db)	Carbohydrate (% db)
FC	74.39±0.11 ^c	5.35±0.04 ^d	72.62±0.46 ^a	18.92±0.26 ^c	3.11±0.58 ^b
CF	10.51±0.11 ^b	4.84±0.04 ^c	74.81±0.32 ^b	18.97±0.32 ^c	1.37±0.51 ^{ab}
DCF	10.69±0.07 ^b	4.33±0.01 ^b	85.64±0.12 ^c	7.13±0.07 ^b	2.91±0.19 ^b
CPI	5.84±0.18 ^a	1.92±0.05 ^a	93.46±0.76 ^d	4.09±0.43 ^a	0.53±1.09 ^a

Values are presented as mean±SEM of triplicate (n = 3). Values with different superscripts within the same column are statistically significantly different ($p < 0.05$).

Some factors that affect the moisture content of fish protein isolate include differences in drying methods, fish species, and protein isolate extraction methods. The drying method affected the composition and functional properties of the protein isolate (Shaviklo, 2015). Various drying methods are used, such as freeze drying, spray drying, and oven drying. Optimum temperature and drying time conditions are required to obtain good quality protein isolate. Brishti *et al.* (2020) reported that the moisture content of protein isolates by spray drying was lower than freeze drying and oven drying. Fish protein isolates with freeze-drying had lower moisture content than those without drying (Shaviklo *et al.*, 2012). Differences in fish species affected the chemical composition of the raw material and the resulting fish protein isolate. The composition of fish raw material is influenced by water temperature, quantity and quality of fish feed, movement, and physiological activity of fish (Bezbaruah and Deka, 2021). Previous studies found that protein isolates from red snapper by product, hemp, flax, and canola seed using the acid extraction method had a higher moisture content than the alkaline extractions (Teh *et al.*, 2014; Pramono *et al.*, 2018). The low moisture content of fish protein isolate is essential to semi-finished products because it can inhibit microbial activity, thereby extending the products' shelf life (Ikasari and Wijaya, 2021).

Catfish protein isolate had lower ash content than tilapia (4.53±1.26%), rainbow trout (4%), boliti fish (13.82±0.05%), and carp (8.83±0.21–8.88±0.36% db) (Foh *et al.*, 2012; Ibrahim, 2015; Lone *et al.*, 2015; Tian *et al.*, 2017). This indicated that the CPI had slight impurities. The difference in ash composition can be influenced by several factors, such as fish species, solvent, drying method, and extraction method (Haryati *et al.*, 2020). Following the Codex standard (Codex Alimentarius, 2019), the ash content of the protein isolate should not exceed 8% db.

The CPI had higher protein content than rainbow trout (75.61%), Nile boliti fish (83.90±0.00%), carp (82.96–83.20%), tuna (23.61%), Indian mackerel (87.27%), sardines (84.75%), and lanternfish (43.17–

84.89%) (Ibrahim, 2015; Lone *et al.*, 2015; Tian *et al.*, 2016; Shaviklo *et al.*, 2017; Kumarakuru *et al.*, 2018; Oliyaei *et al.*, 2019). Differences in the protein content of protein isolate products can be influenced by the source of raw materials, processing methods, and dewatering processes (Shaviklo and Etemadian, 2019). The protein content of the CPI was in line with the soy protein isolate standard in Codex Alimentarius (2019), which stipulated that the protein content should not be less than 90% in dry weight. This indicates that catfish protein isolate has the potential to be a good source of protein.

The lipid content of CPI was lower than that of protein isolate from yellowfin tuna roe (5.60±0.10–7.40±0.10%), tuna fish (5.7±0.54%), and carp (4.88±0.21% db) (Lee *et al.*, 2016; Shaviklo *et al.*, 2017; Tian *et al.*, 2017). The CPI had low lipid content due to the defatting process in CF. High lipid content can affect the quality of protein isolate products because it can cause oxidation, making the product rancid (Haryati *et al.*, 2020). The carbohydrate contents of FC, CF, and DCF were not statistically significant ($p > 0.05$). The production of CPI had significantly lower carbohydrate content than FC by 2.58%.

3.2 Physical properties of mackerel fish balls

The effect of adding CPI to colour, WHC, cooking loss, and hardness is shown in Table 2. Based on the colour analysis, the L* value in all samples showed no significant difference ($p > 0.05$). However, a* and b* values exhibited a statistically significant difference between MFBs with the addition of CPI (F2 and F3) and those without the addition of CPI (F1) ($p < 0.05$). The colour of MFBs can be influenced by the constituent materials, such as fillers and binders (Herdiana *et al.*, 2022). The difference in the colours of F1, F2, and F3 (i.e., the values of a* and b*) was attributable to the colour of the CPI added. The protein isolates of catfish tend to have a slightly reddish colour due to the deposition of heme protein and a slightly yellowish colour due to the lipid content. Similar to the research by Björkner *et al.* (2019), salmon fish balls with the addition of salmon protein isolate had a lower lightness,

Table 2. Physical properties of fish balls.

Properties	F1	F2	F3
Colour			
L*	70.82±0.76 ^a	69.70±0.18 ^a	69.59±0.16 ^a
a*	1.36±0.13 ^a	2.61±0.19 ^b	2.26±0.04 ^b
b*	6.81±0.36 ^a	11.16±0.31 ^b	11.59±0.26 ^b
WHC (%)	70.99±0.13 ^a	78.86±0.25 ^b	89.77±0.12 ^c
Cooking loss (%)	19.25±0.16 ^a	9.98±0.03 ^b	4.46±0.11 ^c
Hardness (gf)	3872±34.93 ^b	5211.50±297.50 ^c	2501±147.84 ^a

Values are presented as mean±SEM of triplicate (n = 3). Values with different superscripts within the same column are statistically significantly different ($p < 0.05$).

darker and brownish colour. Mackerel is a pelagic fish with dark muscle tissue rich in lipids and pigments such as haemoglobin and myoglobin, resulting in a darker protein mass. Kakko *et al.* (2022a) reported that surimi with the addition of protein isolate from Baltic herring had a lower L* value than commercial surimi. Commercial surimi production usually uses white-fleshed fish such as Alaska pollock. Haemoglobin in fish meat causes a yellow or brown colour in the protein because it can reduce the whiteness value.

Water holding capacity (WHC) refers to the ability of meat to retain water (Öztürk and Serdaroğlu, 2018). The WHC of MFBs added with protein isolate was 78.86±0.25-89.77±0.12% (Table 2), which suggests that the MFBs formulation added with protein isolates could significantly affect the WHC ($p < 0.05$). The fish protein isolate can indeed retain water during food processing. The higher the addition of protein isolate, the higher the WHC of the MFBs, which was due to the increased ability to retain water in the meat matrix. This tendency for increased WHC was also reported by Toldrà *et al.* (2021) when adding protein isolate to the Frankfurter-type sausages formulation. According to Haryati *et al.* (2020), CPI had a high water absorption of 3.38±0.03 g/ml, indicating high isolate porosity that traps the CPI in the space between particles. The WHC can be influenced by pH, the presence of polar amino acids, the concentration of the peptides, and the amount of protein content (Peighambardoust *et al.*, 2021).

The addition of CPI also significantly affected the percentage of MFBs cooking loss ($p < 0.05$), which pertains to the loss of water during cooking. The higher the addition of CPI, the lower the cooking loss of MFBs. Of the three formulations, the F3 had the lowest cooking loss, which amounted to 4.46±0.11%. In a previous study, Ibrahim (2015) reported cooking loss of Nile boliti fish balls with the addition of protein isolate was lower than a fish ball without protein isolate. Jenkelunas and Li-Chan (2018) also reported that adding protein hydrolysates could significantly reduce the cooking loss of Pacific hake fish balls. Meatballs tend to shrink during

the cooking process due to meat protein denaturation, loss of water, and fat (Park, Lee, Lim *et al.*, 2021). The more water the protein can hold, the less water will come out, thereby reducing the amount of cooking loss (Toldrà, 2017). According to Cheetangdee (2017), adding fish protein isolate to sausages can reduce cooking loss because of the cross-linking between isolate protein and muscle protein through hydrophobic interactions, which in turn will increase the strength of the protein matrix.

The hardness level of MFBs in all treatments was significantly different ($p < 0.05$). The F2 had the highest hardness level (5211.50±297.50 gf), while F3 had the lowest (2501±147.84 gf). The addition of protein isolate to a food product can affect the physical properties of the resulting product, including its hardness. Correspondingly, adding CPI can increase the hardness of MFBs, but excessive addition can instead reduce the hardness. The increase in the hardness level of MFBs was influenced by the ability of the protein isolate to form a gel, resulting in a stricter fish ball structure. On the other hand, the addition of too many protein isolates caused the gel formation to be inhibited, which is due to the presence of sarcoplasmic proteins that can inhibit the cross-linking between myofibril proteins (Zuraida *et al.*, 2017). Björkner *et al.* (2019) reported that salmon fish balls with the addition of 100% salmon protein isolate had lower breaking strength and hardness than fish balls with the addition of 50% salmon protein isolate. Kakko *et al.* (2022a) also reported that adding protein isolate from Baltic herring fish by 5-30% affected the hardness, springiness, and chewiness of fish balls. However, the hardness of Baltic herring fish balls with the addition of 50% fish protein isolate was significantly lower than commercial fish balls. The hardness of Baltic herring fish balls with the addition of protein isolates of 5, 15, 28 and 50% was not significantly different from the control. The addition of higher soy protein isolates reduced the hardness of the composite gel, even though the gel had a high WHC value (Wang *et al.*, 2022). That implies that protein isolates limit the hardness of the composite gel.

Similar to that reported by Park, Kim, Na *et al.* (2021), adding high concentrations of soy protein isolate can reduce the hardness of cookies. The hardness of the product is influenced by moisture, air space formation, and other ingredients.

3.3 Proximate composition of mackerel fish balls

As seen in Table 3, there were significant differences in the moisture, ash, protein, and lipid content of the MFBs added with CPI ($p < 0.05$). However, the carbohydrate content of F2 was not significantly different from F1 ($p > 0.05$). The addition of fish protein isolate caused a decrease in the moisture content of the MFBs. The moisture content of MFBs in all treatments met the Indonesian National Standard (SNI), which determines 70% as the maximum moisture content (Indonesian National Standard, 2017).

Ash content refers to the amount of mineral content (inorganic substances) in a product when the organic substances contained in the product have been burned. The decrease in MFB ash content may be due to changes in nutritional value during the frozen storage process. Alkuraieef *et al.* (2020) reported that the ash content of Indian mackerel fish balls decreased after frozen storage. The ash content of all MFB treatments met the SNI standard.

The F2 and F3 had different protein content that was significantly different from that of F1 ($p < 0.05$). Compared to F1, the F2 and F3 formulations saw an increase in their protein content, which amounted to 6.89% and 14.80%, respectively. The increase in protein content resulted from the addition of CPI, which had a protein content of 93.46% db. Compared with tilapia fish balls from Ibrahim's study (2015), tilapia fish balls with the addition of 50% fish protein isolate had a protein content of 60.46% db. This protein content of fish balls was even still lower than that of F3, which amounted to 72.60% db. This could be because the protein isolates of catfish had higher protein content (93.46% db) than tilapia (83.90% db). The protein content of all formulations of MFBs in this study met the SNI, which was more than 7% (Indonesian National Standard, 2017).

The lipid content of MFBs in all treatments was significantly different ($p < 0.05$). The MFBs with the

addition of CPI had lower lipid content (5.03-11.20%) than the control items, confirming that fish meat contained more lipid content than the CPI. The addition of CPI could reduce the lipid content of MFBs because of a decrease in the amount of crushed fish meat in the fish ball formulation. Lastly, the carbohydrate content of F2 and F3 was significantly different from that of the control formulation ($p < 0.05$). The F2 had a higher carbohydrate content (24.44% db) than F1 (22.53% db) and F3 (17.89% db). This is because CPI (0.53% db) and mackerel fish fillet (0.17%) typically have low carbohydrate content (Yilmaz, 2021).

3.4 Sensory evaluation of mackerel fish balls

Based on the sensory evaluation results (Figure 1), adding CPI to MFBs could significantly affect their aroma, taste, texture, and overall attributes. However, the analysis revealed no significant difference between F1, F2, and F3 on the colour attribute although all formulations exhibited a similar preference level, that is, preferable. The colour analysis of the MFBs shows that the addition of CPI can affect the a^* and b^* values in the fish balls. However, these differences did not influence the panellists' level of preference. Björkner *et al.* (2019) reported that salmon fish balls with the addition of 50% fish protein isolate were still favoured by panellists. The colour of the salmon fish balls was reddish. However, the panellists did not prefer salmon fish balls with the addition of 100% fish protein isolate because they had a darker and more brownish colour. Shaviklo *et al.* (2010) also reported that the colour appearance of haddock fish balls with the addition of fish protein isolate by 25% was not significantly different from the control. In another

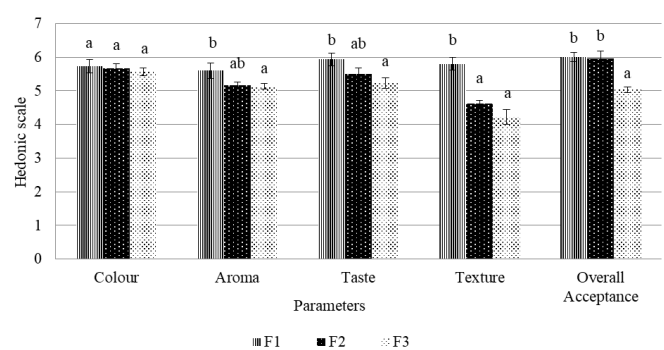


Figure 1. Sensory evaluation of fish balls. Bars with different notations within the parameter are statistically significantly different ($p < 0.05$).

Table 3. Proximate composition of fish balls.

Sample	Moisture (% wb)	Ash (% db)	Protein (% db)	Lipid (% db)	Carbohydrate (% db)
F1	68.63±0.32 ^c	7.08±0.13 ^c	47.21±0.28 ^a	23.18±0.67 ^c	22.53±1.21 ^b
F2	63.21±2.19 ^b	5.38±0.10 ^b	58.98±0.13 ^b	11.20±0.40 ^b	24.44±0.22 ^b
F3	59.22±0.40 ^a	4.48±0.11 ^a	72.60±0.50 ^c	5.03±0.06 ^a	17.89±0.26 ^a

Values are presented as mean±SEM of triplicate (n = 3). Values with different superscripts within the same column are statistically significantly different ($p < 0.05$).

study by Moosavi-Nasab *et al.* (2018), adding 4% lantern fish protein isolates to sausages produced a colour the panellists did not prefer. Oxidation and decomposition of proteins can cause undesirable effects such as colour, odour, and taste. Colour changes can occur due to the interaction of various components, such as hydrocarbons, ketones, aldehydes, and acids, with proteins.

The aroma and taste attributes of F2 were not significantly different from those of F1. In contrast, F3 was significantly different from F1 though the former was still favoured by the panellists. Based on the proximate analysis, the CPI had a lipid content of 4.09% db. This high-lipid content, along with the content of haemoglobin and myoglobin during the extraction process and in the final product of protein isolate, can potentially trigger lipid oxidation. The lipid oxidation can eventually influence the product's aroma, taste or even colour, resulting in rancidity. Even if the intensity of the rancid aroma and taste in the protein isolates is relatively low, it can still produce a rancid taste and aroma in the final product (Shaviklo *et al.*, 2012; Canti *et al.*, 2022). The findings in this study are similar to those reported by Shaviklo *et al.* (2010); haddock fish balls with the addition of fish protein isolate by 25% and 50% had a rancid aroma and taste. Ibrahim (2015) reported that the panellists still favoured the taste and aroma of beef meatballs and Nile boliti fish balls with the addition of 25% and 50% protein isolate. Björkner *et al.* (2019) also reported that adding salmon protein isolate by 50% and 100% did not affect the aroma of salmon fish balls. However, panellists could still detect lower rancidity. Another study by Shaviklo *et al.* (2017) evaluated the sensory characteristics of the fish burger with the addition of protein isolate from tuna. Fish burger with the addition of 20% fish protein isolate, panellists detected a rancid aroma and taste. Kakko *et al.* (2022b) reported that protein isolates produced using an alkaline pH shift had a high peroxide value. Previous studies have reported that oxidation occurs in protein isolates produced using acidic pH shifts (Zhong *et al.*, 2016; Phetsang *et al.*, 2021). In addition, the freeze-drying process can also increase oxidation (Kakko *et al.*, 2022b). Halldorsdottir *et al.* (2014) also reported that freeze-drying could increase the value of Thiobarbituric acid-reactive substances (TBARs) from protein hydrolysis from cod bone mince, but not when antioxidants were added. Therefore, adding antioxidants is necessary during the pH shift and freeze-drying processes. According to Shaviklo and Etemadian (2019), the quality of dried or frozen fish protein isolate products can be improved by adding natural antioxidants and cryoprotectants such as tocopherol and sorbitol. Rancidity in mackerel fish balls can also be caused by the raw material used. Mackerel is a dark-fleshed fish

with a high concentration of myoglobin and heme compounds, so it functions as a pro-oxidant in muscle tissue. The storage process in the production of mackerel fish balls may increase lipid oxidation. Therefore, for MFBs with the addition of CPI, the preference level was lower than that of those without the addition of CPI, but the panellists could still accept the former.

The texture attributes of F2 and F3 were significantly different from those of F1. The F2 and F3 had a neutral level of preference among the panellists, which was related to the texture that could be either too hard or too soft. It was found that the hardness level of the F2 was the highest, which amounted to 5,211.5 gf, while the F3 had the lowest hardness, reaching 2,501 gf. Björkner *et al.* (2019) reported that salmon fish balls with 50% fish protein isolate had significantly higher texture (elasticity and firmness) than salmon fish balls with the addition of 100% fish protein isolate. Furthermore, as Ibrahim (2015) explained, adding fish protein isolate to Nile boliti fish balls might result in a grainy texture, which can consequently affect the level of the panellists' preference. Similar to the study reported by Shaviklo *et al.* (2010), haddock fish balls with the addition of fish protein isolate by 25 and 50% were detected to have graininess. In another study by Shaviklo *et al.* (2016), the grainy texture was also found in fish burgers with the addition of 20% tuna protein isolate. The grainy texture occurs due to the increased temperature, induction of pressure, and initial denaturation of protein during the pulverization of fish meat (Shaviklo *et al.*, 2010). Modifications in protein isolation and the preparation of fish balls, such as size reduction, can improve the texture of the protein isolate and fish balls produced (Shaviklo *et al.*, 2019).

Overall, there was no significant difference between F1 and F2, but F3 was significantly different from F1. Based on sensory evaluation, F1 and F2 were preferred by panellists over F3. Ibrahim (2015) reported based on the overall attributes of beef meatballs and Nile boliti fish balls with the addition of fish protein isolate of 25 and 50% s accepted by the panellists. Björkner *et al.* (2019) also reported that the panellist still accepted salmon fish balls with the addition of protein isolate of 50 and 100%. In another study by Moosavi-Nasab *et al.* (2018), the addition of 4% lantern fish protein isolate was still acceptable to panellists based on overall sensory attributes (aroma, taste, texture, and colour). The F2 was the best formulation considering the hardness level and the sensory evaluation for all attributes that the panellists could still accept.

4. Conclusion

The findings have demonstrated the successful

production of protein isolates from catfish and their use in producing mackerel fish balls. The CPI had protein content, which amounted to 93.46% db, in compliance with the CODEX standards (protein content at least 90% db). Adding CPI to MFBs could significantly increase colour (a^* and b^* value), WHC, and protein content. However, it significantly reduced the cooking loss value, moisture, ash, lipid, and carbohydrate content of MFBs. The MFBs for all treatments have met SNI 7266:2017. Adding CPI to F2 could increase the protein content, which was 6.89%, and F3, which accounted for 14.80%. The panellist still accepted the mackerel fish balls with additional CPI in overall attributes (colour, aroma, taste, and texture). The F2 (MCM: CPI, 85:15) was the best formulation based on its hardness, protein content and sensory evaluation.

Conflict of interest

The authors declare no conflict of interest.

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