

## Effects of Extraction Temperature on the Total Protein and Vitamin C Levels of *Channa striata* Water Extract

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### Abstract

*Channa striata* are a freshwater fish, popularly known as snakehead fish in Indonesia, belonging to the family Channidae. The fish are usually consumed for their protein and their medicinal properties in treating wounds and pain. Previous study showed that the protein in *C. striata* can be extracted using water at 50°C for 60 minutes, and using acetone at 4°C for 24 hours. Considering all, this work aims to investigate the effects of temperature on the protein and vitamin C content of the water extract from *C. striata* fish collected in Rancaekek, West Java. The meat was extracted with water at 55-60 °C for 10 and 30 minutes, compared to the cold extraction at room temperature for 60 minutes. The resulting extracts were subjected to the qualitative Biuret color test and quantitative protein analysis using the Kjeldahl Method. The study revealed that cold extraction of *C. striata* meat for 60 minutes resulted in the highest protein content (0.29% in the residue and 0.35% in the supernatant). Moreover, the vitamin C levels, determined using the iodometric method, in the homogenate, supernatant, and residue were 60.64 mg/100 g, 60.82 mg/100 g, and 43.38 mg/100 g respectively.

**Keywords:** albumin, *Channa fish*, *Channidae*, snakehead fish, nutritional composition

### Efek Temperatur Ekstraksi terhadap Kadar Protein Total dan Vitamin C Ekstrak Air Ikan Gabus (*Channa striata*)

#### Abstrak

*Channa striata* merupakan ikan air tawar yang termasuk ke dalam famili Channidae, di Indonesia dikenal sebagai ikan gabus. Ikan gabus umumnya dikonsumsi karena kandungan proteinnya serta dimanfaatkan untuk pengobatan luka dan nyeri. Penelitian sebelumnya menunjukkan bahwa protein ikan gabus dapat diekstraksi menggunakan pelarut air pada suhu 50 °C selama 60 menit, serta menggunakan aseton pada suhu 4 °C selama 24 jam. Oleh karena itu, penelitian ini bertujuan untuk menelaah efek temperatur terhadap kadar protein dan vitamin C di dalam ekstrak air ikan gabus yang diperoleh dari Rancaekek, Jawa Barat. Daging ikan dikukus dengan air pada 55–60 °C selama 10 dan 30 menit, dibandingkan dengan ekstraksi pada temperatur ruang selama 60 menit. Ekstrak yang diperoleh dianalisis secara kualitatif menggunakan pereaksi warna Biuret dan secara kuantitatif melalui analisis protein menggunakan metode *Kjeldahl*. Hasil analisis menunjukkan bahwa metode ekstraksi dingin ikan gabus selama 60 menit menghasilkan kadar protein tertinggi (0,29% di dalam residu dan 0,35% di dalam supernatan). Selain itu, kadar vitamin C yang dianalisis menggunakan metode iodometri di dalam homogenat, supernatan, dan residu berturut-turut adalah 60,64 mg/100 g, 60,82 mg/100 g, dan 43,38 mg/100 g.

**Kata Kunci:** albumin, *Channa*, *Channidae*, ikan gabus, komposisi nutrisi

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## 1. Introduction

*Channa striata* fish (Figure 1) are a freshwater fish belonging to the Channidae family, popularly known as snakehead fish, with the local name of ikan gabus. They belong to the group of ferocious and predatory fish.<sup>1-6</sup> The fish are usually consumed by the population in Indonesia and Malaysia as a source of protein and also for their medicinal properties, particularly in treating wounds and pain.

Proteins and fatty acids, found in abundance in the meat and skin of the fish, are believed to have contributed to its pharmacological properties.<sup>7</sup> One of the major advantages of *Channa striata* is its high albumin content, ranging from 4–6 g per 100 g of body weight. Albumin plays a crucial role in accelerating wound healing, maintaining fluid balance, and supporting immune function. In addition, albumin exhibits antioxidant properties and promotes tissue repair by enhancing fibroblast proliferation and angiogenesis. The albumin and essential amino acids present in *C. striata* protein extract directly contribute to increased cell proliferation and collagen synthesis, thereby facilitating the wound-healing process.<sup>8</sup>

*C. striata* was reported to contain bioactive proteins that are involved in the wound healing process via specific biological pathways.<sup>9</sup> It was reported that protein in *C. striata* fish can be extracted using water at 50°C for 60 minutes, as reported by Nurfaidah et al. (2021),<sup>10</sup> and using acetone at 4 °C for 24 hours, as reported by Prastari et al. (2023).<sup>11</sup> Moreover, another study by Dewita et al. (2022) on snakehead fish extraction reported water as the solvent at 100 °C for ten minutes of steaming and boiling. Then, the fresh fish meat, steamed fish meat for ten minutes, and boiled fish meat for ten minutes were analyzed and compared for their albumin content. The *C. striata* fish in this study were obtained from a traditional market in Pekanbaru.

It was delineated that the ten minutes of steaming resulted in the highest albumin levels ( $0.42 \pm 0.09$  g/dL), followed by the fresh meat without any heating ( $0.32 \pm 0.07$  g/dL), and the lowest albumin was found in the ten minutes of boiling ( $0.09 \pm 0.014$  g/dL).<sup>12</sup> Higher extraction temperatures increase oxidation and decrease the content of essential nutrients, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and fat-soluble vitamins; therefore, lower temperatures are recommended to improve oxidative stability and preserve nutritional quality.<sup>13</sup>

Taking everything into consideration, this work aims to study the effects of extraction temperature on the protein content of the water extract of *C. striata* obtained from Rancaekek, West Java.

## 2. Materials and Method

### 2.1. Tools

Instruments used were oven (Memmert UF30), Kjeldahl distillation apparatus (Iwaki), HPLC (Shimadzu), chemical glassware (Pyrex), digital analytical balance (Mettler Toledo AB204-S), furnace (Thermo Scientific Thermolyne F47925), desiccator (Kartell 2446), a modified steamer, blender, filter, digital scale, porcelain dish, measuring cup, and thermometer.

### 2.2. Materials

*Channa striata* fish (8 kg) with 20-30 cm in length and 300-400 g in weight were obtained from Rancaekek, West Java (Google coordinate at 6°58'26.2"S 107°45'12.1"E).

The fish sample was determined by the Zoology Museum, School of Life Sciences and Technology, Bandung Institute of Technology, Bandung, West Java, Indonesia (Ref. No. 2733/IT1.C11.2/TU/2025)

Chemicals used in this study were bovine serum albumin (BSA)(CAS No. 90604-29-8, USA), technical grade sulfuric acid (Brataco, Indonesia), a mixture of catalysts containing potassium sulfate and copper sulfate (Brataco, Indonesia), 30% sodium hydroxide (Brataco, Indonesia), hydrochloric acid 0.1 N (Brataco, Indonesia), n-hexane (Brataco, Indonesia), biuret reagent (1 g of copper sulfate pentahydrate and 4 g of sodium potassium tartrate tetrahydrate in 100 mL of distilled water, added with 4 g of sodium hydroxide pellets in 200 mL of distilled water), distilled water (Brataco, Indonesia), and filter paper (Whatman No.1).

### 2.3. Methods

#### 2.3.1. Preparation of the Extract

The fish were observed for their morphology and smell to confirm their freshness, and then washed under tap water to remove dirt and mud, and other pollutants. The fish flesh and meat were separated from the bones, scales, fins, head, and offal, then the meat and flesh were washed under tap water to remove blood and mucus, and then ground and weighed (resulting in 3400 g), and separated into three portions: CSE-I (500 g), CSE-II (500 g), and CSE-III (2000 g).

#### 2.3.2. Effects of Temperature on the Protein Content of CSE-I, CSE-II, CSE-III, CSE-IIIS, and CSE-IIIR

Two portions of each 500 g of the ground fish flesh and meat (CSE-I and CSE-II) were added with 500

mL of distilled water, and were heated at 55-60 °C using a boiler for 10 minutes for the first portion, and 30 minutes for the other portion. The resulting extracts were filtered and freeze-dried to remove the solvent. The freeze-dried extracts of CSE-I and CSE-II were subjected to the qualitative biuret color test, and the more positive biuret test results (indicated with a darker violet color) were further analyzed for their protein, fat, carbohydrate, and ash content using the Kjeldahl method, the result shown protein concentration from cold water extract 0.35% higher compare to hot water extract 0.06%, and other methods following the SNI 01-2891:1992.

Another 2,000 g of the flesh and meat portion was soaked in 1,000 mL of distilled water for 60 minutes at room temperature (26°C), homogenized (this portion is addressed as CSE-III), and centrifuged at 2,500 rpm for 25 minutes. The supernatant (CSE-IIIS) was separated from the residue (CSE-IIIR) and freeze-dried at a temperature below -20°C, then placed in a vacuum chamber for 24 hours. Both the freeze-dried supernatant (CSE-IIIS) and residue (CSE-IIIR) were analyzed for protein, vitamin C, fat, carbohydrate, and ash contents.

### 2.3.3. Protein Solubility in Water

The protein solubility in water was calculated by using the formula as follows:

$$\text{Soluble protein} = \frac{\text{protein in supernatant}}{\text{protein in homogenate}} \times 100$$

### 2.3.4. Vitamin C Solubility in Water

Vitamin C (ascorbic acid) content was determined by the iodometric titration method. Ascorbic acid reacts quantitatively with iodine, reducing iodine to iodide while being oxidized to dehydroascorbic acid. The endpoint was identified by the appearance of a persistent blue-black color after the addition of starch indicator, indicating the presence of excess iodine.<sup>14,15</sup>

## 3. Result

Effects of temperature on the protein and vitamin C content of CSE-I, CSE-II, CSE-III, CSE-IIIS, and CSE-IIIR are presented in Table 1 and Figure 2.

Figure 2 shows the flow-chart diagram of the hot and cold extraction process of *C. striata* fish. Of the 10 minutes and 30 minutes of the hot extraction, the 10 minutes of heating resulted in a darker violet color of the Biuret test, thus was further analyzed. The proximate analysis of the 10-minute hot extraction resulted in protein content at 0.06%, ash 0.6%, fat 0.29%, and carbohydrate 0.91%.

Moreover, the cold extraction of the *C. striata* fish resulted in CSE-III (the homogenate), and after centrifugation, CSE-IIIS (the supernatant) and CSE-IIIR (the residue). A darker violet color of the Biuret and based on Proximate test result was shown by CSE-III (the homogenate), and further analysis resulted in the protein content at 0.61% and vitamin C at 60.64 mg/100 g, while in CSE-IIIS (the supernatant), we found less protein at 0.35%, with a slightly higher vitamin C at 60.82 mg/100 g, ash at 0.34%, fat at 0.66%, and carbohydrate at 0.88%. Interestingly, the residue (CSE-IIIR) contained lower protein at 0.29%, vitamin C at 43.38 mg/100 g, ash at 0.35%, fat at 0.68%, and higher carbohydrate at 7.78%.

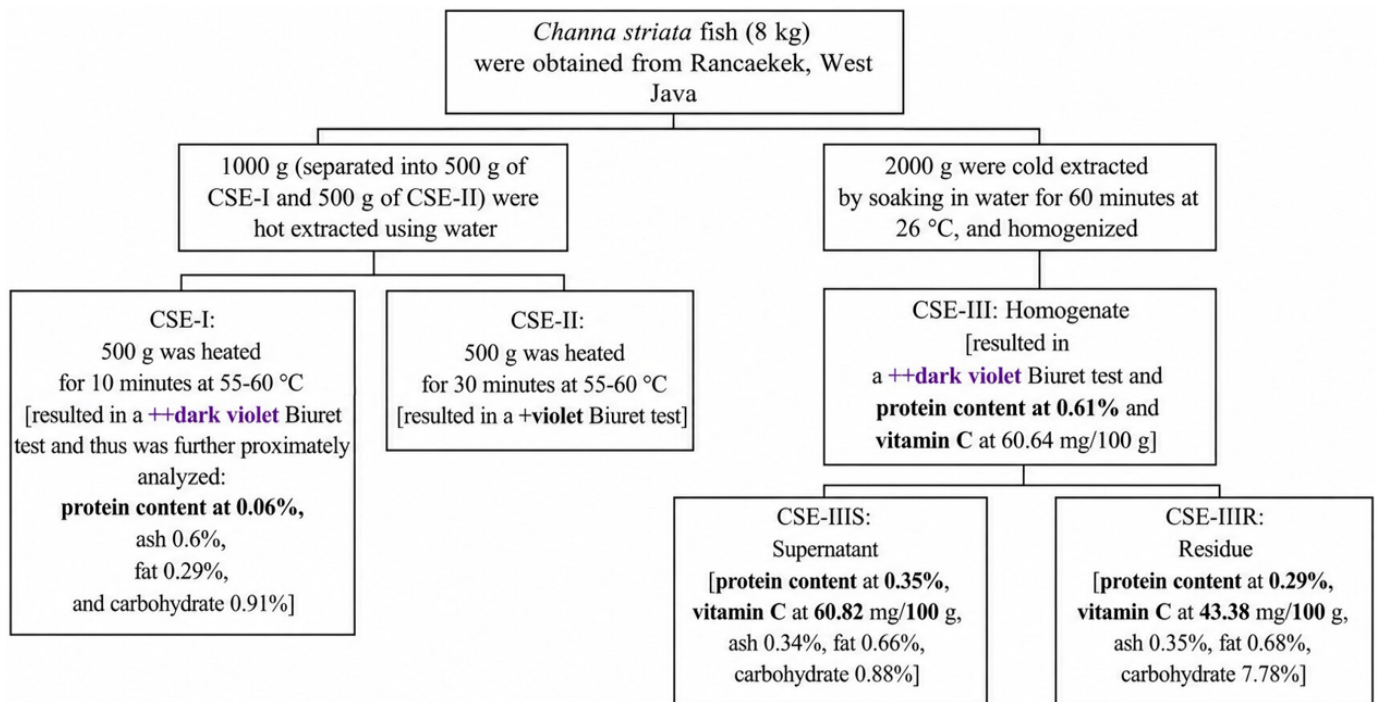
The total water-soluble protein in the *C. striata* meat is 57.38%.

## 4. Discussion

In this study, the biuret color test was employed for an initial qualitative analysis to detect the presence of protein in the extract. The assay is based on the principle that copper ions ( $\text{Cu}^{2+}$ ) in the form of copper sulfate in an alkaline condition (sodium hydroxide), react with peptide bonds in proteins, forming a violet color complex that absorbs light at a wavelength of 540 nm, which intensity is directly proportional to the



**Figure 1.** *Channa striata* fish, 30 cm in length, 400 g in weight, collected from Rancaekek, West Java.



**Figure 2.** The flow-chart diagram of the hot and cold extraction process and the results of protein and vitamin C analysis in the extracts

protein levels in the extract,<sup>16</sup> and compared to bovine serum albumin (BSA) as the standard. The use of BSA as a primary standard for serum protein assays was recommended by Doumas in 1975, after he compared it with human serum albumin (HSA). According to Doumas, BSA is more inexpensive, easily available, and exhibits better linearity in the standard curve of the biuret reaction.<sup>17,18</sup>

Our study revealed that extraction temperature affects the protein content. Heating the *C. striata* fish meat for 10 and 30 minutes at 55-60 °C (hot extraction method) resulted in lower protein, ash, and fat content compared to soaking the fish meat at room temperature (cold extraction method). These findings are in accordance with those reported by Rahmanifarah et al. (2014). In their study, the heating process in the extraction of *Clupeonella cultiventris caspia* fish

resulted in lower protein and ash content, which was thought to be caused by protein denaturation and a better separation process during protein extraction.<sup>19,20</sup> Extracting fish protein without heat exposure could maintain the natural structure of the protein, thus retaining its bioactivity.<sup>21</sup> The proteins contained in fish have shown special characteristics for water and lipid binding, albeit with their unpleasant odor.<sup>22</sup>

Interestingly, our study confirmed that vitamin C was also found in the cold-water extract with freeze-dry of *C. striata* meat fish as much as 1,520.27 mg/100 gr on 1st hour of water extraction, and it was found more (1,934.14 mg/100 gr) after 48 hours of extraction, although the protein levels decreased from 79.96% to 14.42%. Related to the vitamin C in the *C. striata* meat fish, very few studies reported the presence of this micronutrient in the fish meat. As a comparison,

**Table 1.** The protein, vitamin C, and other constituents resulted from the hot and cold extraction process of *C. striata*.

Extraction Process		Biuret Test	Parameters				
			Protein (%)	Ash (%)	Fat (%)	Carbohydrate (%)	Vitamin C (mg/100 g)
Hot Extraction	CSE-I (10 minutes)	++	0.06	0.6	0.29	0.91	-
	CSE-II (30 minutes)	+	-	-	-	-	-
Cold Extraction	CSE-III	++	0.61	-	-	-	60.64
	CSE-IIIS	++	0.35	0.34	0.66	0.88	60.82
	CSE-IIIR	++	0.29	0.35	0.68	7.78	43.38

a study by Tawali et al. (2018) reported that vitamin A, vitamin E, vitamin B2, and vitamin B12, but not vitamin B6 and vitamin C, were present in relatively small amounts in the meat of *C. striata* fish collected in Bili-bili, South Sulawesi.<sup>23</sup> Calcium and iron were also reported in the meat of *C. striata* fish purchased from a traditional market in Ogan Ilir, South Sumatra, at a level of 33.44 mg/kg and 68.99 mg/kg, respectively.<sup>24</sup> A study by Putri and Febriansyah (2023) described the presence of essential fatty acids, such as omega-3 and omega-6, in *C. striata* fish obtained in Ogan Ilir, South Sumatra.<sup>25</sup> Another study by Mustafa et al. (2012) delineated the presence of protein at 3.36%, albumin at 2.17%, fat at 0.77%, glucose at 0.07%, zinc at 3.34 mg/100 mL, copper at 2.34 mg/100 mL, and iron at 0.20 mg/100 mL.<sup>26,27</sup> The 6-month-old snakehead fish (*C. striata*) obtained from the Griya Arka Kendal Farm in Central Java, Indonesia, weighing 800-900 g, were reported to contain 17.09-19.02% protein, 2.45-4.26% lipid, and 1.30-30.18% ash, in the head, bones, fins, and tail, while fatty acids EPA and DHA was 0.19% and 7.97% in the head, 0.1% and 9.41% in the bones, 0.1% and 9.83% in the fins and tail.<sup>28</sup>

Based on our study, the total water-soluble protein in the *C. striata* meat is 57.38%. This result is lower than that reported in a previous study, that snakehead fish originating from the Mahakam watershed of East Kalimantan has a water-soluble protein content of 63.59 %, as reported by Asikin and Kusumaningrum (2017),<sup>29</sup> while that of *C. striata* from Tarakan, North Borneo, was 64.18%, as reported by Hutapea et al. (2022).<sup>30</sup>

Water-soluble protein is contained in fish, such as albumin. Albumin is a globular protein that has good solubility in water and diluted saline and is denatured by heat.<sup>31,32</sup> Consuming the steamed water of *C. striata* fish is believed to accelerate the recovery in post-delivery women, as described in a review article.<sup>7</sup>

Albumin was reported to increase the expression of EGFR (epidermal growth factor receptor), ERKs (extracellular signal-regulated kinases), TGF- $\beta$  (transforming Growth Factor-beta), and collagen, and decrease that of MMP-8 (matrix metalloproteinase-8) in male Sprague-Dawley rats, thus explaining its wound-healing properties. Moreover, the administration of albumin could activate the signaling pathway of nuclear factor-kappaB (NF- $\kappa$ B).<sup>33</sup>

## 5. Conclusion

The extraction temperature affects the protein content of *Channa striata* fish. The cold extraction of *Channa striata* fish meat with water for 60 minutes resulted in the highest protein content, which were 0.61% in the

homogenate, 0.29% in the residue, and 0.35% in the supernatant, while the vitamin C levels were found at 60.64 mg/100 g in the homogenate, 60.82 mg/100 g in the supernatant, and 43.38 mg/100 g in the residue.

## Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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