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COMPARISON OF PROXIMATE COMPOSITION, AMINO ACID, VITAMIN, AND MINERAL CONTENTS OF WHOLE FISH POWDER AND FISH PROTEIN CONCENTRATE FROM LOCAL INDONESIAN SNAKEHEAD FISH (*Channa striatus*)

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ABSTRACT

The gabus (*Channa striatus*) is an Indonesian indigenous predatory freshwater fish that traditionally known to have medicinal benefits in wound healing and energy boosting for sick people. The aim of this study was to investigate the nutritional content of two processed products made from the gabus fish: (1) whole fish powder (WFP) and (2) fish protein concentrate (FPC). Proximate analysis revealed that protein content was higher in FPC (85.85%) than in WFP (67.93%). Lipid, crude ash, and water contents of WFP were 5.74%, 10.76%, and 3.60% respectively, while those of FPC were 4.48%, 4.74%, and 4.61% respectively. Both products contained all essential amino acids ranging from 8.14 g (histidine) to 2.19 g (methionine) per 100 g of WFP protein and from 7.08 g (leucine) to 2.81 g (methionine) per 100 g of FPC protein. Non-essential amino acids contents was from 7.20 g (arginine) to 0.23 g (aspartic acid) in WFP protein and from 10.45 g (glutamic acid) to 2.7 g (serine) in FPC protein. Both products contained vitamin A, B2, E, D3, and B12. Calcium and phosphor were higher in WFP (2401 and 18.51 mg per 100 g respectively) than in FPC (178 mg and 7.1 g per 100 g respectively). FPC and WFP of *Channa striatus* have relatively high protein contents and complete amino acids composition and they can be considered as food supplement, especially as protein and amino acids sources.

1. Introduction

Gabus (*Channa striatus*) is a snakehead fish found in abundance in Asian countries. It is an indigenous predatory fresh water fish that can be found in all bodies of water, from small ditches, rice fields, to large rivers in Asian tropical countries such as Indonesia, Malaysia, India, and Pakistan (Mohsin and Ambak, 1983). In these countries, this particular fish has long been regarded as valuable food for its medicinal benefits. People suffering from

severe diseases have been traditionally suggested to include this fish in their diet to boost their immunological states. Post-natal women and post-surgery patients are often recommended to consume this particular fish in order to promote faster wound healing (Shafri and Manan, 2012). Several researchers have reported that gabus fish (*Channa striatus*) is rich in nutrients such as amino and fatty acids that are essential for wound healing and promotion of immune system in the body

(Zuraini et al., 2006; Dahlan and Daud, 2010; Schlenker and Long, 2007). However, due to its physical characteristics, fresh snakehead fish impractical and inconvenient to be used directly as food supplement for sick people. In addition, the protein content of fresh fish (around 20%) is much lower than that of fish protein concentrate (ranges from 60-90%) (Zuraini et al., 2006; Windsor, 2007; Murueta et al., 2007). Therefore, product innovation which can increase the usability of snakehead fish as a raw material for production of supplemental foods that are rich in protein and essential amino acids is timely important. This study was conducted to determine proximate composition, amino acids, vitamin, and mineral contents of two products from snakehead fish; namely, Whole Fish Powder (WFP) and Fish Protein Concentrate (FPC).

2. Materials and methods

2.1. Material

Samples of *Channa striatus* (1.0-2.0 kg/fish) were obtained from Bili-bili watershed in South Sulawesi. Analytical grade chemicals and solvents used in this study were procured from Merck and Sigma Aldrich, US.

2.2. Sample preparations

2.2.1. Whole fish powder (WFP)

Ten kg of fresh *Channa striatus* samples were gutted, weeded, and washed thoroughly with running tap water. The cleaned fish were then cooked in a pressure cooker with 8 L of clean water (all pieces were covered with water), at low heat. The cooking was continued for approximately 30 minutes after the initial boiling occurred. After that, the head and bones were removed from the flesh then the flesh was homogenized using a high speed mixer (Philips, Netherland) and dried at 60°C for 6 hours using an air dryer. The dried sample was ground using an electric grinder and sieved using a 100 mesh sieve. The sample was stored in an air-tight container at refrigerated temperature until used for analysis.

2.2.2. Fish protein concentrate (FPC)

Samples of snakehead fish were gutted, weeded, and washed thoroughly, and then deboned and cut into small pieces. One hundred grams of fish meat sample was mixed with 100 ml HCl (ratio 1:1 w/v) and homogenized using a high speed mixer (Philips, Netherland) at 50-60 °C. The mixture was then filtered. The liquid obtained (filtrate) was mixed with 200 ml of hexane solvent and centrifuged for 30 minutes to separate the fat from the filtrate. The oil and the hexane solvent were separated using a separation funnel. The liquid extract of protein concentrate obtained from the fat separation process was then dried at 60-70°C. The fish protein powder obtained from the drying process was stored in an air-tight container at refrigerated temperature until used for analysis.

2.3. Proximate analysis

Proximate analysis was performed in duplicate for samples of whole fish powder and fish protein concentrate. Moisture, ash, and fat contents were analyzed using the Association of the Official Analytical Chemists (AOAC, 1984) methods 14004 (1984), 14009 (1984) and 14006 (1984), respectively. Nitrogen was determined using the Kjeldahl method. The quantity of protein contained in each sample was calculated as $6.25 \times N$ (Method 7015, AOAC, 1984).

2.4. Amino acids analysis

Amino acids content of whole fish powder and fish protein concentrate were analyzed using Waters Acquity UPLC H-Class and H-Class Bio amino acid analysis method (Waters Company, USA, 2012) and amino acid analysis (Nollet, 2004). Briefly, 0.1 g of powdered sample was transferred into a tube containing 5 ml of 6 N HCl and then vortexed. The mixture was then hydrolyzed for 22 hours at 110°C. After the hydrolyzation process, the solution was cooled to room temperature and transferred into a 50 ml flask and added with aquabidest until the total volume reached 50 ml. The

diluted solution was filtered through a 0.45 μm filter. The filtrate was pipette 500 μl and mixed with 40 μl of AABA and 460 μl of aquabidest. Ten μl of the solution was mixed with 70 μl of AccQ-fluor borate and vortexed. After that, 20 μl of fluor A reagent was added and kept for 1 minute and then incubated for 10 minutes at 55 $^{\circ}\text{C}$. The solution was then injected into the UPLC system. The net height of every peak produced by the chart recorder of the analyzer (each representing an amino acid) was measured and calculated.

2.5. Vitamin & Mineral

2.5.1. Fat Soluble Vitamins

Fat soluble vitamin contents of snakehead whole fish powder and fish protein concentrate were estimated by using the Association of the Official Analytical Chemists method 2002.05 (AOAC, 2007). By this method, 2 grams of sample was placed into a 50 ml tube. Meanwhile, 50 mg each of vitamins A, D3, and E were placed into a 50 ml centrifuge tube. Both tubes were added with 5 ml of ethanol-ascorbic acid 0.1% and 4 ml of 50% KOH solution. The tubes were heated at 70 $^{\circ}\text{C}$ for 30 minutes and then vortexed for 10 minutes. After the heating, the tubes were cooled to room temperature and 5 ml of hexane was added into each tube and shaken for 5 minutes. The tubes were then put aside until separation occurred. The solution of n-hexane was separated and transferred into a flask and added with 1 ml of methanol-ascorbic acid 0.1% and also add with 2 x 10 $^{-6}$ of n-hexane (in a centrifuge tube). After that, the solution was evaporated in a dark room until dry and then added with HPLC grade methanol. The diluted solution was then put into a 50 ml flask, homogenized, and filtered with 0.45 μm filter. The filtrate was pipetted into autosampler vial and injected about 20 μl into the chromatography system. The Chromatography used was equipped with octadecyl silane (RP-18) column with a flow rate of 0.7 ml/minute. λ for vitamin A, D, and E were 325, 264, and 292 nm, respectively. The vitamin standards

were prepared in mobile phase. The concentrations of the vitamin in the WFP and FPC samples were calculated in relation to the peak of the standard vitamins.

2.5.2. Water Soluble Vitamins

Vitamin contents (water soluble vitamins) of WFP and FPC of snakehead fish were estimated using ultra performance liquid chromatography (UPLC) system (Waters Company, USA 2012). Two grams of sample was diluted with a solution of acetonitrile: formic acid 2% in methanol (75:25) in a 25 mL flask and homogenized. The diluted solution was then filtrated through a 0.2 μm polytetrafluoroethylene (PTFE) membrane. About 5 μl of the filtrate was injected into the UPLC system and measurement was conducted using the following chromatography conditions: detector (photodiode array (PDA) 265 nm, λ range 3D 190-400 nm; 2D 265 nm, resolution 1.2 nm), column (Amide 1.7 μm , 2.1 x 100 mm), temperature 30 $^{\circ}\text{C}$, and flow rate (0.3 mL/min). Vitamin standards were prepared in mobile phase. Vitamin concentrations was calculated in relation to the peak of standard vitamins.

2.5.3. Minerals

Mineral contents were analyzed using the Association of the Official Analytical Chemists (AOAC, 2013) official methods 2011.14. Samples (0.5 g) were mixed with 5 mL nitric acid and heated in a closed-vessel microwave digestion system (MDS) from ambient to a temperature of 150 $^{\circ}\text{C}$ in 10 minutes and hold at that temperature for 10 minutes. After that, the ash was cooled to room temperature and added with H₂O to a total volume of 25 mL and homogenized. The mineral contents were measured using an inductively coupled plasma-optical emission spectrometry (ICP-OES) instrument.

2.6. Statistical analysis

Experiments were carried out in duplicate with mean values and standard deviation (SD) were calculated.

3. Results and discussions

3.1. Proximates

Proximate analysis results presented in Table 1 show that both of the products produced from snakehead fish contained a high amount of protein. The protein content was found to be higher in the FPC (85.85%) compared to that in the WFP (67.93%). The protein contents observed in this study were in the range of an ideal fish protein concentrate (Windsor, 2001). Moreover, the protein content of 85.85% for the FPC used in this study was higher than the average of 57-79% protein contents of several FPC produced from bycatch fish species reported previously (Muruet, 2007). In addition, the protein contents of the WFP and FPC used in this study were higher than the protein content of fresh snakehead fish ($\pm 20\%$) (Zuraini, 2006).

The crude ash component was much higher in whole fish powder (10.76%) than that in fish protein concentrate (4.74%). This may indicate that some of fine bones were present in the whole fish powder and contributed to the higher crude ash component found from proximate analysis. Total fat content was relatively low in both products, even though it was moderately higher in the whole fish powder (5.74%) than in the fish protein concentrate (4.48%). The lower fat content of the fish protein concentrate may significantly increase the stability of the product against lipid oxidation which may also enhance product quality (Windsor, 2001).

The moisture contents of WFP and FPC were 3.6% and 4.61% respectively. The two parameters (moisture and fat) are important for the storage quality of the products. High moisture content can increase the possibility of fat hydrolysis and the growth of microorganisms which will result in the

reduction of quality and safety of the product. The lower the amount of fat and moisture in the WFP and FPC, the better the quality and the longer the shelf life of the products.

Table 1. Proximate composition of whole fish powder and fish protein concentrate from snakehead fish (*Channa striatus*)*

	Whole fish powder	Fish protein concentrate
Protein (%)	67.93 \pm 0.44	85.85 \pm 0.22
Fat (%)	5.74 \pm 0.03	4.48 \pm 0.02
Moisture (%)	3.6 \pm 0.01	4.61 \pm 0.01
Crude ash (%)	10.76 \pm 0.01	4.74 \pm 0.01

*Value are means of duplicate samples

3.2. Amino acids

The amino acids composition of WFP and FPC of *Channa striatus* are shown in Table 2. It can be seen from the table that the two products contain 17 of the 22 amino acids found in nature. All essential amino acids were present in both WFP and FPC. The highest concentrations of essential amino acids per 100 g product were histidine (8.14 g) for WFP and leucine (7.08 g) for FPC and the lowest were Methionine (2.19 g and 2.81 g for WFP and FPC respectively). For non-essential amino acids, the highest concentration found was arginine (7.20 g/100 g) for WFP and glutamic acid (10.45 g/100 g) for FPC. It can also be seen from the values in the table that for every 100 grams of fish protein concentrate from snakehead fish, the amount of histidine, isoleucine, leucine, lysine, methionine, valine, and threonine contained were higher than the daily recommended intake (DRI) for children (WHO/FAO/UNU, 2007). Only phenylalanine and tryptophan was found to be lower than the recommended daily intake. The amount of histidine, isoleucine, lysine, and valine contained in 100 grams of whole fish powder were higher than the DRI for children while leucine, methionine, phenylalanine, and threonine contents were lower than the DRI for

children. However, the concentrations of all essential amino acids per 100 grams of WFP and FPC were higher than the WHO/FAO/UNU recommendation for amino acids intake in adult. Although several of the amino acids concentrations found in the WFP and FPC of snakehead fish were slightly lower than those found in protein hydrolysates from

Herring fish (*Clupeaharengus*) (Liceaga-Gesualdo & Li-Chan, 1999), the overall figures were generally similar. Since the amino acids found in the WFP and FPC were present in just the right balance for human nutrition, both products might be potentially used as functional foods for protein supplementation for human in need.

Table 2. Amino acids composition (g/100 g protein) of whole fish powder and fish protein concentrate from snakehead fish (*Channa striatus*)*

Amino acid (g/100 g protein)	Whole fish powder	Fish protein concentrate	Herring (<i>Clupeaharengus</i>) protein hydrolysates***	Daily recommendation WHO/FAO/UNU***	
				Child (g)	Adult (g)
<i>Essential amino acids</i>					
Histidine	8.14±0.011	3.70±0.010	1.22	1.90	1.60
Isoleucine	3.33±0.013	3.94±0.003	3.15	2.80	1.30
Leucine	5.70±0.019	7.08±0.002	8.42	6.60	1.90
Lysin	6.56±0.040	6.63±0.004	8.46	5.80	1.60
Methionine	2.19±0.018	2.81±0.019	4.94	2.70	1.70
Phenylalanine	3.25±0.016	4.84±0.008	3.39	6.30	1.90
Valine	3.74±0.004	4.39±0.006	4.72	3.50	1.30
Threonin	3.39±0.004	4.56±0.006	4.74	3.40	0.90
Tryptophan	0.55±0.004	0.64±0.005	-		
<i>Non-essential amino acids</i>					
Tyrosine	2.21±0.012	3.27±0.005	2.64		
Aspartic acid	0.23±0.002	7.66±0.008	10.72		
Glutamic acid	4.37±0.005	10.45±0.014	15.87		
Serine	4.19±0.005	2.7±0.002	4.87		
Glycine	5.04±0.007	5.94±0.061	7.59		
Arginine	7.20±0.009	9.35±0.011	7.06		
Alanine	3.80±0.004	3.94±0.004	7.54		
Proline	3.29 ± 0.003	3.90±0.006	4.54		

* Values are means of duplicate samples

** Liceaga-Gesualdo & Li-Chan, 1999

***WHO/FAO/UNU, 2007

3.3. Vitamins and Mineral

The compositions of vitamins and minerals contained in WFP and FPC are presented in

Table 3 and Table 4. From Table 3, vitamin A, E, B2, and B12 were present in relatively small

amounts in both WFP and FPC compared to the DRI. On the other hand, vitamin D

concentrations were relatively high, which were 10.78 µg and 14.92 µg in WFP and FPC respectively. The adequate intake of vitamin D is 5 µg/day (Schlenker & Long, 2007).

Table 3. Vitamins content of whole fish powder and fish protein concentrate from snakehead fish (*Channa striatus*)*

Vitamin per 100g	Whole fish powder	Fish protein concentrate
Vitamin A (µg)	7.81±0.65	30.1±18.3
Vitamin D (µg)	10.78±1.82	14.92±4.03
Vitamin E (mg)	1.78±0.25	2.52±1.44
Vitamin B1	ND	ND
Vitamin B2 (mg)	0.68±0.01	0.69±0.00
Vitamin B6	ND	ND
Vitamin B12 (µg)	0.98±0.01	1.08±0.01

* Values are means of duplicate samples

ND: Not detected

Mineral contents of both WFP and FPC are shown in Table 4. The highest mineral content was Calcium in Whole fish powder (2401 mg/100 g), whereas in FPC calcium concentration was only 179 mg/100 g. The high concentration of calcium in WFP was the result of the inclusion of fine and small fish bones in the product, which on the other hand was absent in FPC. Other minerals such as Phosphor, Magnesium, Zinc, and Iron were also present but in relatively low concentrations.

Table 4. Mineral contents of whole fish powder and fish protein concentrate from snakehead fish (*Channa striatus*)*

Mineral (mg/100g)	Whole fish powder	Fish protein concentrate
Calcium	2401.20±10.52	178.91±9.33
Phosphor	18.51 ± 0.15	7.58±0.85
Magnesium	108.87±0.00	137.35±5.21
Zinc	0.032±0.000	0.026±0.00
Iron	0.086±0.001	0.154±0.003

*Values are means of duplicate samples

4. Conclusions

Whole fish powder (WFP) and fish protein concentrate (FPC) from snakehead fish (*Channa striatus*) are rich sources of protein and amino acids. Both products contain all essential amino acids and most of non essential amino acids. Furthermore, the amounts of essential amino acids present in both products are relatively high and meet or exceed the recommended daily intake per 100 grams of the products. In comparison to fresh snakehead fish, both WFP and FPC are more practical and convenient to use. In addition, the amounts that need to be consumed to achieve the daily requirements for protein and amino acids are relatively small. Therefore both products might be used for protein supplementation for sick people and others in need, particularly in tropical developing countries where *Channa striatus* are abundant.

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