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Protein and albumin contents in several freshwater fish species of Makassar, South Sulawesi, Indonesia

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Abstract

Albumin of snakehead fish has been widely used in the health and nutritional applications for the past 10 - 15 years. An intensive exploitation to produce fish albumin has placed the natural stock of snakehead fish under a great pressure. Furthermore, its aquaculture production has not been significantly developed to balance the industrial demands. Therefore, the present work aimed to look for the potential sources of fish albumin of freshwater origin as an alternative to that of snakehead fish. The present work analysed seven freshwater fish species namely catfish (*Clarias gariepinus*), common carp (*Cyprinus carpio*), eel (*Monopterus albus*), Nile tilapia (*Oreochromis niloticus*), pangas catfish (*Pangasius pangasius*), snakehead fish (*Channa striata*), and three-spotted gourami (*Trichogaster trichopterus*). Albumin extraction was done by mixing 50 g of pre-homogenised fish meat with 200 mL of distilled water, homogenised in a laboratory homogeniser for 1 min, and heated in a water bath at 50°C for 60 min. The mixture was filtered using Whatman No. 40 filter paper under reduced pressure. The filtrate volume was recorded and stored at -20°C until further analysis. The parameters analysed were total protein, total soluble protein, and albumin level. Results indicated that the total protein content of meat was 17.93 - 21.87% (w/w), the lowest being in catfish and the highest in snakehead fish; total soluble protein was 2.43 - 5.43 g/100 g (w/w), the lowest being in eel and the highest in snakehead fish; and albumin content was 0.83 - 3.35 g/100 g (w/w), the lowest being in Nile tilapia and the highest in common carp. The higher albumin content in the common carp (3.35 g/100 g) and pangas catfish (3.22 g/100 g) as compared to that of snakehead fish (2.97 g/100 g) indicated that common carp and pangas catfish are highly potential to be used as alternative fish albumin source.

Introduction

Fish albumin is a water-soluble protein fraction derived from fish meat. In fish, albumin functions to help transport metabolites such as fatty acids, hormones, bilirubin, regulate blood colloid osmotic pressure system and osmoregulation processes, as well as filtering fluids in body tissues (De Smet *et al.,* 1998; Baker, 2002; Andreeva, 2011; Kovyrshina and Rudneva, 2012). Fish albumin is a bioactive protein useful for pharmacological applications, and has become a commercial nutritional product (Chasanah *et al.,* 2015a).

The content of albumin in fish is affected by intrinsic factors such as species, size, sex, maturity; and extrinsic factors such as environmental condition, habitat, food, and season. Hasnain *et al.* (2004) reported that the amount of fish albumin is also affected by genetic. Niwa *et al.* (2007) observed that for the cultured fish species, their albumin content is affected not only by fish species and size, but also by level of feeding, availability and quality of feed, and digestible energy content of the feed. Susilowati *et al.* (2015) analysed 17 species of cultured freshwater fish from Bogor and Cianjur, West Java, and found that the albumin content of these fish varied greatly between species. Six species of wild fish of Merauke (Papua) swamp water have also been reported to contain variable amount of albumin (Susilowati *et al.,* 2016). The albumin content of a fish species also varies according to geographical location. For example, albumin contents of *Channa striata, C. micropeltes*, and *C. pleurophthalma* from West were 107.23, 57.99, and 46.68 mg/g, respectively (Susilowati *et al.,* 2015), whereas those from central Kalimantan were 67.8, 89.3, and 89.6 mg/g, respectively (Firlianti *et al.,*

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2013). These may indicate the importance of analysing albumin content of fish not only according to their species, but also according to geographical location of their habitat.

Presently, the primary source of fish albumin is from snakehead fish (*Channa striata*). However, the availability of this fish in nature is experiencing depletion, thus affecting the stock for raw material in fish albumin production. Although efforts have been developed for fish cultivation, the success rate is not yet promising. The greatest difficulty faced by the snakehead fish aquaculture is feed. This species prefers natural as compared to artificial feed (Victor and Akpocha, 1992) due to its nature as a predatory fish species. Another obstacle for snakehead fish aquaculture is related to the breeding for mass production of fishlings. Bijaksana (2012) opined that snakehead fish reproduction process is greatly dependent on specific environmental factors such as fluctuations in water levels. As a consequence, the small production number of snakehead fish either in nature or in cultivation is unable to ensure sustainable provision of the snakehead fish albumin. Therefore, it is necessary to look for other potential sources of freshwater fish albumin as an alternative to that of snakehead fish. The present work investigated the albumin content of seven freshwater fish species, and classified them as potential source of fish albumin when their albumin content were found equal or higher than that of snakehead fish.

Materials and methods

The present work analysed seven freshwater fish species namely catfish (*Clarias gariepinus*), common carp (*Cyprinus carpio*), eel (*Monopterus albus*), Nile tilapia (*Oreochromis niloticus*), pangas catfish (*Pangasius pangasius*), snakehead fish (*Channa striata*), and three-spotted gourami (*Trichogaster trichopterus*). With the exception of snakehead and the three-spotted gourami, other fish species have long been cultivated in areas around Makassar, and are abundantly available at wet markets there. Snakehead and three-spotted gourami are also sold at certain wet markets by particular fishermen catching the fishes in swamp waters around Makassar. Snakehead fish was used as a reference species due to the popularity of its albumin. Other materials used were bovine serum albumin (BSA) as a standard, distilled water, H_2SO_4, NaOH, HCl, H_3BO_3, methyl red indicator, $NaHCO_3$, Na-K tartaric 1%, $CuSO_4$, phenol reagent, and filter paper.

Collection, preparation, and extraction of samples

All fish samples analysed in the present work were live fish obtained from wet markets around Makassar. The size of the fish collected was 340 - 824 g for carp, 263 - 361 g for tilapia, 140 - 172 g for eel, 116 - 162 g for three-spotted gourami, 271 - 374 g for catfish, 276 - 497 g for pangas catfish, and 243 - 640 g for snakehead fish. All fish were live-transported to the laboratory. Upon arrival, the fish were immediately killed by means of thermal shock at ≈10°C in a bucket filled with water, and crushed ice blocks. Each species weighed approximately 3 kg and divided into three groups of equal weights as replicates. The fish samples were then scaled, gutted, deboned, and filleted, before being thoroughly washed with clean running tap water, and allowed to drip. The meat was then minced, pre-homogenised in a commercial blender, placed in high density polyethylene (HDPE) zipped plastic bags, and stored at -20°C until further analyses.

After thawing the pre-homogenised fish meat at room temperature, 50 g of meat was accurately weighed in a 250 mL glass beaker, diluted with distilled water (1:4 ratio), and homogenised for 1 min with a laboratory homogenizer. The homogenised sample was then incubated at 50°C for 1 h in a continuously shaking water bath. After incubation, the sample was filtered under reduced pressure, and the volume of the filtrate was recorded. The filtrate was then transferred into a dark bottle glass with cap, and stored at -20°C until further analyses.

Total protein analysis

Protein analysis was performed following the micro Kjeldahl method. Briefly, 1 g of fish meat (2 mL of fish extract) was transferred into 100 mL Kjeldahl flask, and 10 mL of concentrated sulphuric acid was added. To accelerate the digestion process, a catalyst was added in the form of a selenium mixture. The Kjeldahl flask was heated at ≈400°C until the solution turned to a clear green shade. The flask was then allowed to cool at room temperature. Prior to distillation, the digested sample was diluted to 100 mL with distilled water. Then, 5 mL of the mixture were transferred into a distillation flask, and 10 mL of 10% sodium hydroxide were then added. The distillation flask was connected to the condenser, and the distillate was collected in an Erlenmeyer flask containing 3% of boric acid, to which 2 - 3 drops of methyl red indicator have been added. Titration was performed using 0.1 N HCl until pink colour appeared. The volume of HCl used was recorded, and the protein content (Mariotti *et al.,* 2008) was calculated using Eq. 1 and Eq. 2:

$$N\,(\%) = \frac{(a-b) \times N\,HCL \times 14.007}{Sample\ weigh\,t\,(g)} \times 100 \qquad \text{(Eq. 1)}$$

$$Protein\ Content\ (\%) = N\,(\%) \times 5.6 \qquad \text{(Eq. 2)}$$

where, N = nitrogen content; *a* = volume of HCl used in titration of sample; *b* = volume of HCL used in titration of blank; and N HCl = normality of HCl. For fish extract, the volume of extract (2 mL) was used as the sample weight.

Albumin analysis (Lowry method)

Reagent's preparation

The albumin analysis was performed following the method described by Apriyantono *et al.* (1989). Reagent A was prepared by dissolving 2 g of sodium carbonate in 1.1 mol/L of NaOH to a volume of 500 mL. Reagent B was prepared by dissolving 0.5 g of copper sulphur in 1% Na-K tartaric solution to 100 mL. Reagent C was prepared by mixing 50 mL of reagent A and 1 mL of reagent B. For reagent D, the phenol reagent was mixed with distilled water at a 1:1 ratio. Bovine serum albumin (BSA) of 0.25 mg/mL was used as a standard protein solution.

Preparation of albumin standard curve

The standard protein (BSA) solution between 0 - 0.8 mL (volume interval = 0.1 ml) was transferred into clean test tubes, and diluted with distilled water to a final volume of 4 mL. Then, 5 mL of reagent C was added to each tube, thoroughly mixed with vortex, and allowed to stand at room temperature for 10 - 15 min. Further, 0.5 mL of reagent D was added to each test tube, thoroughly mixed, and allowed to stand for 30 min to form a blue colour. Absorbance was then measured at 650 nm to create a standard curve.

Measurement of albumin in sample

Fish albumin extract (1.5 mL) was transferred into a clean test tube, and then treated following the method described by Apriyantono *et al.* (1989) for preparation of standard curve. Absorbance was then measured at 650 nm.

Statistical analysis

All data obtained were subjected to one-way analysis of variance (ANOVA) using SPSS 22 statistical software. Significant difference was determined at 95% level of probability ($\alpha = 0.05$) where ANOVA indicated the presence of a significant difference. Tukey's Test was employed to determine the difference between means of the analysed parameters.

Results and discussion

Total protein level in the fish meat

The freshwater fish samples analysed in the present work contained high amounts of total protein, ranging from 16.07 to 19.60% (w/w). Nurhayati (2007) stated that fish containing protein above 15% is classified as high-protein fish. These results were corroborated by Murray and Burt (2001) who stated that, in general, fish contained 17 - 22% of protein. Although the range was narrow, ANOVA showed that there was a significant ($\alpha < 0.05$) variation in protein content of the fish samples analysed. Tukey's Test showed that snakehead fish contained the highest protein, while the lowest protein content was shown

Table 1. The total protein, soluble protein, and albumin contents in the meat of seven freshwater fish species of Makassar.

Fish species	Meat protein* (%, w/w)	Soluble protein* (g/100 g meat, w/w)	Albumin (g/100 g meat, w/w)
Common carp	17.88 ± 0.19^{a}	4.06 ± 0.32^{ad}	3.00 ± 0.26^{a}
Tilapia	18.11 ± 0.23^{a}	2.98 ± 0.32^{b}	0.75 ± 0.14^{b}
Eel	18.08 ± 0.14^{a}	2.18 ± 0.30^{c}	1.63 ± 0.22^{c}
Three-spotted gourami	17.83 ± 0.35^{a}	3.05 ± 0.34^{b}	2.34 ± 0.28^{de}
Catfish	16.07 ± 0.20^{c}	3.57 ± 0.20^{de}	1.86 ± 0.23^{cd}
Pangas catfish	18.14 ± 0.20^{a}	3.20 ± 0.27^{be}	2.88 ± 0.24^{ae}
Snakehead fish	19.60 ± 0.33^{b}	4.85 ± 0.40^{a}	2.63 ± 0.15^{ae}

*True protein content calculated using nitrogen to protein conversion factor of 5.6 (Mariotti *et al.*, 2008). Values are mean ± standard deviation of triplicate determinations ($n = 3$). Means followed by different superscript letters within the same column are significantly different ($p < 0.05$).

by catfish. Table 1 further presents the total protein, soluble protein, and albumin contents of freshwater fish samples analysed in the present work.

Based on Table 1, five of seven freshwater fish species analysed in the present work showed a similar total protein content (≈18%, w/w). Variation in the protein contents of fish is influenced by many factors. Nianda (2008) stated that differences in protein content of fish are influenced by species, sex, age, habitat, season, and fish size. Meanwhile, Novia *et al.* (2014) reported that availability of food significantly contributed to the differences in protein content of fish. In the present work, the cultivated freshwater fish species (common carp, Nile tilapia, eel, pangas catfish) had similar protein content with that of three-spotted gourami (wild fish), and lower than that of snakehead fish (wild fish). This might indicate that the energy content of their feed could be comparable. Sexually mature fish tends to have higher water and lower protein in their meat since some of the meat proteins are transferred to developing gonads. Water is the main component of fish muscle, and any changes in water content will directly affect the proportion of other components in the fish meat.

The protein contents of freshwater fish samples analysed in the present work are similar to that of wild swamp water fish in Merauke, Papua reported by Susilowati *et al.* (2016); striped snakehead (*C. striata*) 18.31%, Nile tilapia (*O. niloticus*) 17.26%, grey mullet (*Liza tade*) 18.90%, catfish (*C. batrachus*) 18.43%, barramundi (*Lates calcarifer*) 17.11%, and climbing perch (*Anabas testudineus*) 18.92%. Sentosa and Satria (2015) analysed the feeding habits of the fish, and classified them as detritivorous (*L. tade*), herbivorous (*O. niloticus*), carnivorous (*A. testudineus, L. calcarifer*, and *C. striata*), and omnivorous (C. batrachus). Feeding habit, age, sex, environmental condition, and season affect the chemical compositions and nutritional values of fish (Fawole *et al.,* 2013) and crab (Ayas and Ozogul, 2011). According to Huss (1995), protein contents in fish ranged from 16 to 22% of the total muscle mass, and are the principal component of the solid matter.

Total soluble protein in fish meat extract

The average value of the total soluble protein in freshwater fish extract was between 2.43 - 5.43 g/100 g meat (w/w) (Table 1); lowest in eel and highest in snakehead fish and common carp. The ANOVA result showed that type of fish significantly ($p < 0.05$) affected the soluble protein content of the fish meat extracted, which might be related to the structure and composition of proteins in their meat. Different fish species have different structures and compositions of meat protein. Some fish species have higher proportion of sarcoplasmic protein, while others have higher proportion of myofibril protein in their meat. Fish having higher proportion of sarcoplasmic protein will subsequently produce higher amounts of soluble proteins. The protein contents of catfish, tilapia, and eel extracts differed significantly ($p < 0.05$) from that of snakehead fish. Eel contained the lowest ($p < 0.05$) soluble protein in its extract as compared to the other species. The protein in fish meat extract is water-soluble, therefore, its content in the extract is influenced by the amount of water released and recovered in the extraction process. Water, as a major constituent, accounts for 65 to 80% of the total muscle weight of fish, while the water soluble or sarcoplasmic proteins (myoalbumin, globulin, and enzymes) constitute 25 - 30% of the protein (Huss, 1995). McMan (1997) defined sarcoplasmic proteins as a fluid that exists between myofibrils. Sarcoplasmic protein, also known as myogin, includes albumin, myoalbumin, myoprotein, globulin-X, and myostromin. Albumin, myoprotein, and myoalbumin are highly water soluble. According to Sulastri (2004), different fish have different protein resistance, and are influenced by their myosin content.

Albumin content of fish

The average albumin level in the freshwater fish meat samples analysed in the present work ranged from 0.75 - 3.00 g/100 g (w/w) (Table 1). Nile tilapia was found to contain 0.75 g of albumin/100 g of meat, whereas common carp contained 3.00 g of albumin/100 g of meat. Eel and catfish contained fair amounts of albumin. Significant differences ($p < 0.05$) existed in the albumin contents of the freshwater fish analysed. The very low albumin content in Nile tilapia might have been due to albumin being transferred to the maturing gonads (maturing gonads were observed during filleting) or the high proportion of non-protein nitrogen in the extract. Both of this proposed reasons, however, require further analysis.

The albumin content in each freshwater fish observed was affected by extrinsic and intrinsic factors. The extrinsic factors that affect variations in fish albumin content, among others, are the availability and quality of feed, the energy content digested in the feed (Niwa *et al.,* 2007), and the condition of its ecosystem (Suprayitno, 2014). In the present work, five out of seven freshwater fish species used were cultured fish which were fed with different feeds. Although the feeds were not analysed, but in general, different feeds contain different ingredients, composition, quality, and energy content which will

eventually result in differences in chemical composition of the fish consuming them. In addition, stocking density and pond condition of these freshwater fish are different which will accordingly influence the physiological status of the cultured fish. High stocking density places fish under a lot of stress (a physiological parameter) due to the competition for space and food. The physiological status of the fish has a direct relationship with the biochemical parameters in the fish body. Kopp *et al.* (2010) documented that in fish from eutrohped habitats, their physiological status was differed, which was accompanied with the changes of basic biochemical indices including albumin concentration in plasma. While intrinsic factors influencing the chemical composition of fish include species, sex, age (Irianto and Susilo, 2007), and genetic (Hasnain *et al.,* 2004). Similarly, Kovyrshina and Rudneva (2012) reported that serum protein compositions and levels of their separate components depend on fish species, age, life cycle and sexual maturity, diet, health, and environmental factors. In the present work, the only intrinsic factor that could be related to the differences in the albumin contents of the freshwater fish analysed was difference in species. Other factors such as fish age, sex, life cycle and sexual maturity, diet, and health (despite the bulk reports on their effect on albumin in fish) were not investigated, and therefore, impossible to be confidently related to the variation in the albumin content of the freshwater fish.

The albumin levels in common carp, pangas catfish, and snakehead fish were similar ($p > 0.05$), but significantly higher ($p < 0.05$) than the albumin content of Nile tilapia, eel, and catfish. In the case of snakehead fish, its albumin content is comparable to that of large snakehead fish but was higher than that of medium and small size snakehead fish reported by Asikin and Kusumaningrum (2018). They found that water-extracted albumin content of snakehead fish was 2.89% in large fish (900 - 1200 g), 2.31% in medium fish (600 - 900 g), and 2.57% in small fish (300 - 600 g). Results of the present work indicated a high potential of several freshwater fish species to be an alternative to snakehead fish as a source of fish albumin.

The albumin contents of the freshwater fish analysed in the present work were higher than those reported by Susilowati *et al.* (2016); snakehead fish (1.39 g/100 g), and bawalmundi (0.57 g/100 g). Previously, it has been reported that the albumin content of snakehead fish from West Java was 1.07 g/100 g (Susilowati *et al.,* 2015), and from Central and East Java were 0.76 and 0.91 g/100 g, respectively (Chasanah *et al.,* 2015a). Variation in the albumin concentrations depend on fish species, size, diet consumption rate, dietary availability, and digestibility rate (Niwa *et al.,* 2011). Further, chemical compositions of fish depend on the species, age, sex, habitat, and environmental condition (Irianto and Susilo, 2007). Differences in the albumin content of fish reported by different studies might have been due to, in part, the differences in extraction and analytical methodologies. For example, in recovering the fish extract, our study and that of Asikin and Kusumaningrum (2018) used only filtration, while Susilowati *et al.* (2015; 2016) and Chasanah *et al.* (2015b) used centrifugation followed by filtration. For albumin analysis, our study used spectrophotometry, while the other studies used chromatography.

The data on albumin concentration in teleost fish vary greatly inter- and intra-species. The concentration of albumin (albumin-like proteins) in fish plasma of teleosts can vary from 10 to 50%, while in terrestrial vertebrate's albumin, more than 50% albumin of the total serum proteins have been reported (McDonald and Milligan, 1992). In few fish species, specific properties of albumin were characterised, and identified as albumin-like proteins (Hasnain *et al.,* 2004). Albumin-like protein was found in different bony fish and lamprey, while it was absent in some species of elasmobranchs (Metcalf and Gemmell, 2005). The present work indicates that common carp and pangas catfish have the potential as an alternative source of fish albumin due to their high albumin contents, easier to culture, as well as fishling and formulated feed availability as compared to snakehead fish. Nevertheless, further research is still needed to study the functionality of these proteins as compared to that of snakehead fish.

Conclusion

The freshwater fish species analysed in the present work were good sources of protein (> 16%, w/w). The majority of fish used contained similar protein content (≈19%, w/w). The soluble fraction of protein as well as the albumin in the meat of the freshwater fish showed some variations among species. It was surprising to note that *O. niloticus* contained extremely low level of albumin despite the total soluble protein being quite high. Common carp (*C. carpio*) and pangas catfish (*P. pangasius*) contained slightly, but not significant, higher albumin than that of snakehead fish (*C. striata*); nevertheless, both could be potential source of fish albumin.

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