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Chemistry and Biochemistry

Effect of body weight on the chemical composition and collagen content of snakehead fish *Channa striata* skin

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Abstract

Snakehead fish (*Channa striata*) skin is a fishery by-product that has the potential for further processing because it contains a high amount of organic matter. This study investigates the effect of body weight on the chemical composition and collagen content of snakehead fish skin. This study used fresh snakehead fish of either gender. Their body weights were divided into three groups: small, medium, and large size. The characteristics of snakehead fish skin included proximate composition, amino acid content, collagen content, microstructure, and minerals. Snakehead fish skin from fish of different body weights indicated that the moisture content and ash tended to decrease, the protein content was relatively stable, and the lipid content tended to increase with increasing body weight. Glycine and proline comprised the highest percentages of amino acids at all levels of body weight, and the presence of hydroxyproline showed that snakehead fish skin was the source of collagen. There was no significant difference in body weight observed on collagen protein content of the skin. This study on the microstructure and mineral content of snakehead fish skin can be used as supporting information to promote the potential utility and economic value of the skin.

Keywords Amino acid · Hydroxyproline · Microstructure · Mineral content · Proximate composition

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Introduction

The snakehead (*Channa striata*) is a voracious, carnivorous freshwater fish species that consumes live animals (Mutmainnah 2013; Song et al. 2013). It is unique in its ability to survive under limited water conditions (Panchakshari et al. 2016) and adapt to muddy-swamp areas.

Snakehead fish have long been used as an alternative source of animal protein and are thought to have beneficial effects on health. Currently, snakehead fish have been employed to produce albumin-rich products useful for wound healing and health recovery (Haniffa et al. 2014). Various studies on the use of snakehead fish as a health food have been conducted. Some researchers investigated the chemical composition of snakehead fish albumin extract, which may contain amino acids, fatty acids, and minerals (Ab Wahab et al. 2015; Asfar et al. 2014; Berg et al. 2002; Gam et al. 2005; Mustafa et al. 2012; Santoso 2009; Zuraini et al. 2006). This presents an opportunity for a more intensive cultivation of these fish. However, high productivity increases the amount of waste produced by large-scale use of the fish. Therefore, characterizing the potential of skin

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as a valuable material is important to the development of a zero-waste process; moreover, it may increase the economic value of snakehead fish.

As the main by-product of the fishery industry (Shyni et al. 2014), the skin is high in organic matter with high contents of moisture and protein, followed by lipids and ash. Collagen, along with reticulin and elastin, are the main types of proteins arranged to form the connective tissue matrix and are the main constituents of the dermis layer in the skin of fish (Elliot 2011). Among these three types of fibrous protein, the most abundant is collagen, comprising one third of the total body protein (Li and Wu 2017). In general, collagen consists of several types, depending on the structure and cross-linking of the fiber, of which collagen contained in skin tissue is collagen type I (Muyonga et al. 2004), the content of which, according to Sotelo et al. (2016), can be estimated based on the value of hydroxyproline.

As a major protein component of the extracellular matrix, collagen is essential for maintaining normal structure, resistance, and strength in connective tissue (Blanco et al. 2017; Li and Wu 2017; Margaret 2008; Rangaraj et al. 2011; Sivakumar et al. 2000; Sotelo et al. 2016). However, these characteristics may differ between fish depending on species, age, and the method of collagen extraction.

Functionally, collagen has a broad spectrum of applications, such as food, packaging, biomedicine, pharmaceuticals, and beauty (Karim and Bhat 2009; Liu et al. 2015). In fact, it has been indicated as an active ingredient to help accelerate wound healing (Rangaraj et al. 2011). Therefore, information about the chemical composition of snakehead fish skin is considered important in efforts to explore its potential and utilization. This study aims to investigate the effect of weight on the chemical composition and collagen content of snakehead fish skin.

Materials and methods

Preparation of raw materials

This study investigates the effect of body weight on the chemical composition and collagen content of snakehead fish skin. Fresh snakehead fish of both genders were obtained from Bili Bili Dam, Gowa, South Sulawesi, Indonesia. Each fish was weighed and its length measured to determine the treatment group: small size (SS: 352.75 ± 25.10 g; 35.00 ± 1.03 cm), medium size (MS: 668.85 ± 33.08 g; 42.07 ± 0.75 cm), and large size (BS: 962.20 ± 23.10 g; 47.58 ± 1.69 cm). Fish body parts, such as scales, head, fins, viscera, meat, and fish bones, were removed. Fish skin was collected and cleaned to remove unwanted materials, washed, and stored in polyethylene plastic bags for further preparation. Fresh snakehead fish skin was analyzed for proximate composition. Skin samples for analysis of amino acids, minerals, and microstructure were reduced in size (approximately 2 × 2 cm) using a knife, then lyophilized (freeze dryer, ALPHA 1–2 LD plus, Martin Christ Gefriertrocknungsanlagen GmbH, Germany) until completely dry (about 72 h). The dried skin was milled to a finer size, packed under airtight conditions, and stored at < 4 °C for further analysis.

Proximate analysis

Proximate composition was determined using the AOAC (Association of Official Analytical Chemists) procedure (AOAC International 1995). Moisture content was determined using the gravimetric method. The Kjeldahl method was used for determination of crude protein content (conversion factor of 6.25 × N). Lipid content was determined by using the Soxhlet method. Ash content was determined by incineration for 16 h at 550 °C.

Amino acid analysis

The amino acid content was determined according to Nollet (1996). Amino acids were analyzed by using ultra-performance liquid chromatography (UPLC, ACQUITY UPLC-H Class, Waters, USA). The sample (0.1 g) was hydrolyzed in 5 ml of 6 N HCl and heated at 100 °C for 22 h. A solution containing 500 µl filtrate, 40 µl AABQ, and 460 µl distilled water was prepared. Following addition of AccQ-Fluor Borate and 20 µl reagent Flour-A, the solution (10 µl) was incubated for 10 min at 550 °C and then injected into a UPLC system.

Hydroxyproline analysis

The estimated content of collagen was determined based on the value of the amino acid hydroxyproline. The hydroxyproline content was measured using high-performance liquid chromatography (HPLC, 1200 Infinity Series by Agilent Technologies, US), according to the manufacturer's instructions. Prior to injection into the HPLC, the sample was hydrolyzed in 1 ml of 6 N HCl and heated at 110 °C for 24 h. A standard solution using L-hydroxyproline (Sigma, USA) was used. Estimated levels of collagen contained in snakehead fish skin were calculated on the basis of hydroxyproline values multiplied by a factor of 8.0 (Nagarajan et al. 2013). To determine the total content of collagen based on protein content, total collagen is divided by the amount of crude protein (wet base).

Scanning electron microscopy (SEM) analysis

The microstructure of snakehead fish skin was observed by scanning electron microscopy (SEM, Tescan Vega3SB, Česká Republika) at 500× magnification, according to the procedure of Ramadhan et al. (2014). When a sample was ready to be attached to the holder, it was coated with carbon tape. The sample was then coated by using palladium gold to prevent direct contact with electron samples. The coated sample was placed in a SEM chamber. The SEM chamber was placed under a vacuum by turning on an automatic pump in the VEGA software. The vacuum process must be performed perfectly. In the chamber, electrons are shot towards the sample, producing an image on a screen.

Energy dispersive spectroscopy (EDS) analysis

Energy dispersive spectroscopy (EDS, Tescan Vega3SB, Česká Republika) is performed with the same device as SEM. EDS was used to determine the mineral component of the sample (Yin et al. 2016). The mineral surface composition contained in the sample used SEM emission equipment with a detector for energy dispersive X-ray spectroscopy. The measurement was made with an electron beam at an acceleration voltage of 20 kV. To determine the mineral component of the sample, the sample was coated with palladium gold to make it conductive.

Statistical analysis

The experimental data were evaluated using analysis of variance (ANOVA) (Glenn et al. 2012) based on a completely randomized design. The significance of the variance was verified using Duncan's test. Data were analyzed using SPSS software (IBM Corporation, US). The other data were analyzed descriptively.

Results

The proximate composition of snakehead fish skin of different body weights is shown in Table 1. The body weights showed significant differences ($P < 0.05$) in the moisture content of snakehead fish skin. Fish of higher weight tended to have skin with lower moisture content (SS > MS > BS).

The essential and non-essential amino acid content of snakehead fish skin is shown in Fig. 1. The skin contains all essential amino acids, i.e., tryptophan, phenylalanine, leucine, isoleucine, valine, methionine, lysine, threonine, arginine, histidine, and some non-essential amino acids, i.e., tyrosine, cysteine, proline, alanine, glutamate, aspartate, glycine, and serine. Their presence was detected at all sizes of snakehead fish skin. The percentages of tryptophan, phenylalanine, methionine, tyrosine, proline, threonine, glycine, arginine, serine, and histidine were highest in MS, and the percentage of lysine was highest in BS. The percentage of leucine, isoleucine, valine, and cysteine tended to decrease with increasing body weight. However, the percentages of alanine, glutamate, and aspartate tended to increase with increasing body weight. The amino acid composition was a reflection of the various types of proteins contained in the skin.

Table 1 Proximate composition of snakehead fish skin based on different body weights (%)

Items	SS	MS	BS
Moisture	77.85 ± 0.52^{a}	76.06 ± 0.192^{ab}	74.33 ± 0.15^{b}
Protein	16.37 ± 0.60^{a}	17.49 ± 1.65^{a}	18.49 ± 0.59^{a}
Lipid	0.67 ± 0.17^{a}	3.32 ± 0.53^{b}	2.99 ± 0.42^{b}
Ash	0.60 ± 0.09^{a}	0.22 ± 0.05^{b}	0.20 ± 0.09^{b}

Values with different letters in the same row are significantly different ($P < 0.05$), ($n = 3$)

Table 2 shows the hydroxyproline content and estimated skin collagen content of snakehead fish. The results demonstrate that body weight was not significantly associated with either the hydroxyproline content, collagen content per weight of sample or collagen-based protein content ($P > 0.05$). For SS, MS, and BS, more than half of the ish skin was made up of collagen (51.55 ± 1.76 to 55.63 ± 2.73%: no signiicant diferencebetween sizes). In addition, different weights did not show any difference in the levels of collagen, which indicates that the utilization of skin tissue as a source of collagen (e.g., for gelatin production) can ignore the body weight in this range (300–1000 g).

A microstructural study of snakehead fish skin using scanning electron microscopy (SEM) is presented in (Fig. 2a, b, c). The snakehead fish skin profile resembles fibrils which are connective tissue containing collagen. The presence of white spots presumably reflected lipid deposits under the skin, which are likely to become the lipid contributors after the process of skin extraction into gelatin.

The ash content of the inorganic material is the accumulation of minerals contained in the raw material (Fig. 3). Analysis using scanning electron microscopy-energy dispersive spectroscopy (SEM–EDS) showed detectable minerals in snakehead skins. These minerals are Na, Mg, P, S, Cl, K, and Ca. The percentage of some minerals (Na, Mg, and P) was generally high in SS but low in MS and increased again in BS. The percentage of S was higher in MS than in SS and BS. The content of Cl, K, and Ca tended to decrease with increasing fish body weight.

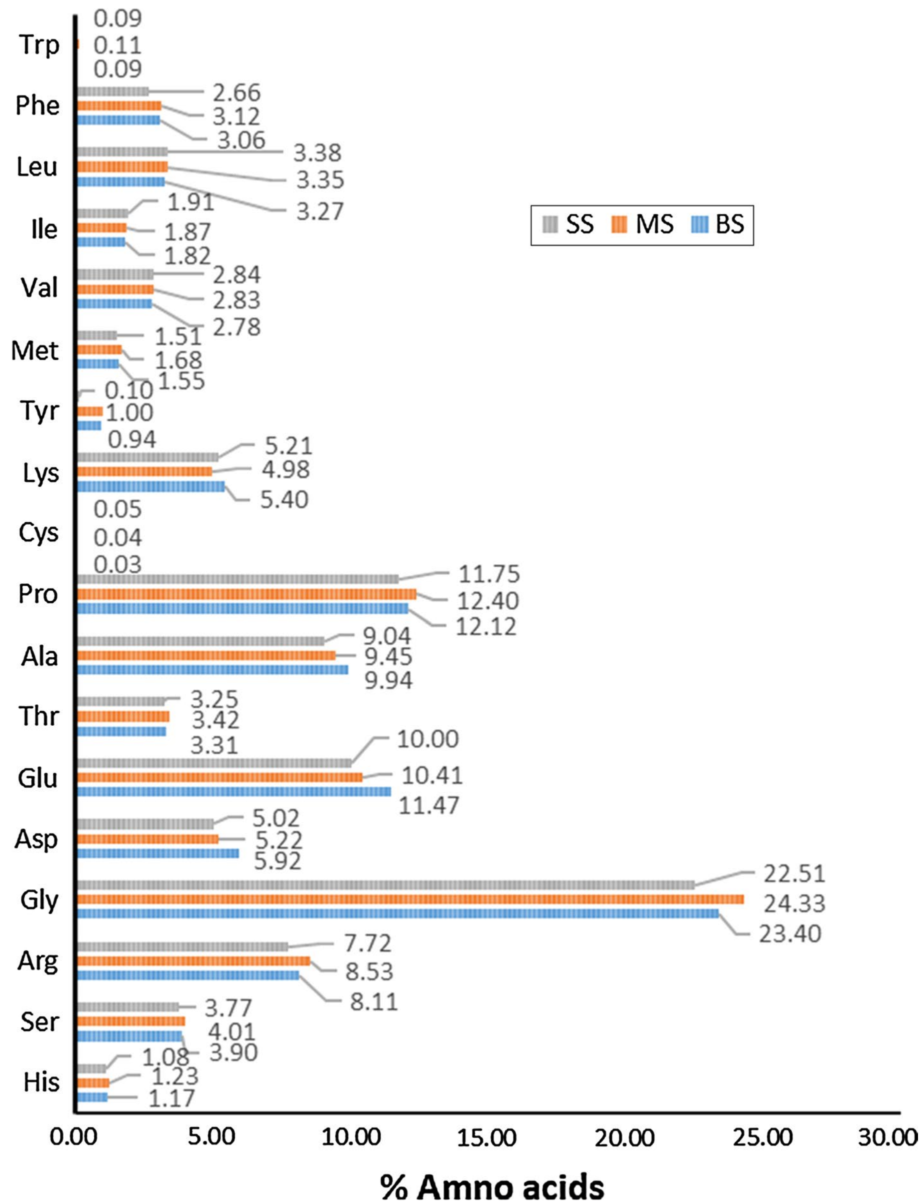

Fig. 1 The amino acid profile of snakehead fish skin based on different body weights

Table 2 The hydroxyproline content and collagen estimation of snakehead fish skin based on different body weights

Items	SS	MS	BS
Hydroxyproline, mg/100 g sample	11.37 ± 0.34	11.63 ± 0.60	11.91 ± 0.22
Collagen content, mg/100 g sample	90.99 ± 2.68	93.07 ± 4.83	95.25 ± 1.76
Collagen based protein content, (%)	55.63 ± 2.73	53.66 ± 7.32	51.55 ± 1.76

Values in the same row are not significantly different ($P > 0.05$), ($n = 3$)

Discussion

The current work is designed to investigate the chemical composition and collagen content of snakehead fish skin, which may contribute to the production of scientific information regarding its future use. Proximate composition is a meaningful characterization for the planning and commercialization of industrial processes (Boran and Karaçam 2011; Suseno et al. 2014) and may support further skin use through different technological applications (Yeannes and Almandos 2003). The composition of the raw materials could also affect the quality and final handling of the raw materials to be used in accordance with the application.

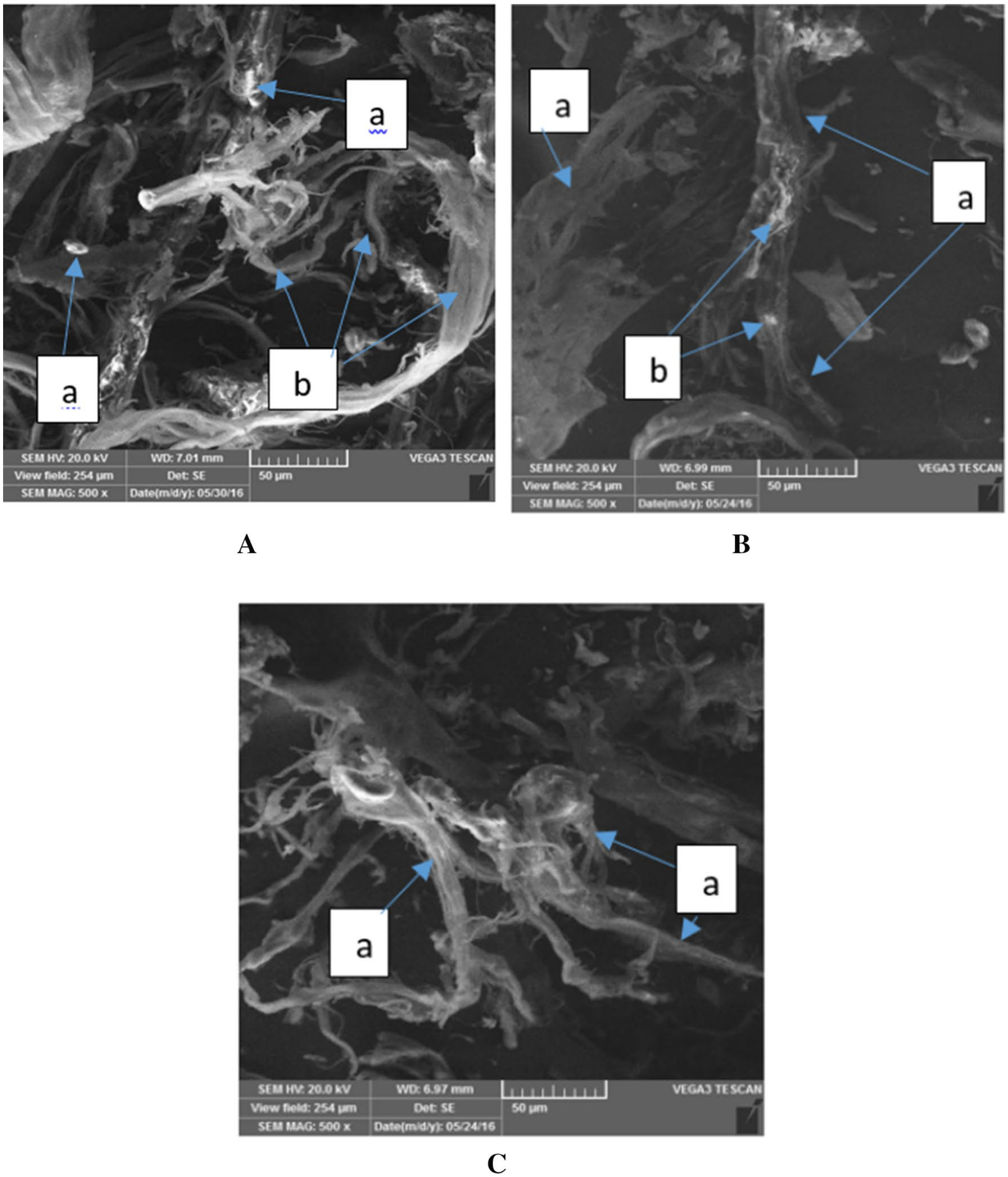

Fig. 2 Microstructure of snakehead fish skin sample using scanning electron microscopy (SEM) analysis with ×500 magnification. Lipid deposits **a**, connective tissue **b**. **A**: SS, **B**; MS, **C**: BS

Proximate composition of snakehead fish skin

Moisture was a major constituent of the fish body (Njinkoue et al.2016) and was bonded physically and chemically. As the main constituent, moisture is needed primarily in metabolic processes, transportation, and various vital activities in the body. The growth process leads to a change in the percentage of moisture in the fish body, especially the element of moisture bound by proteins. Breck (2014) suggested that increased body mass was responsible for decreased moisture and increased protein content, which was linked to biochemical activities of the body. Corroborating this idea, the moisture content of young fish was higher than that of older fish, which also changed the moisture content of their skin tissues. The moisture content of snakehead skin tends to decrease as fish body weight increases. Muyonga et al.

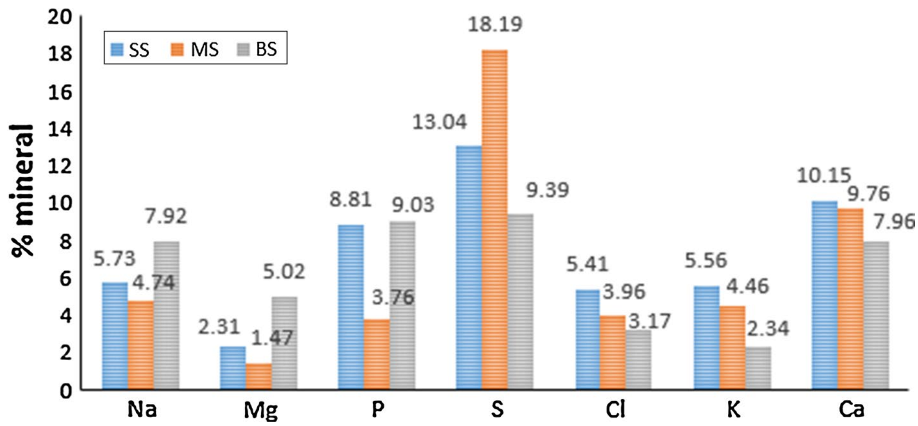

Fig. 3 Mineral content estimation of snakehead fish skin based on different body weights

(2004) reported a higher moisture content in the skin of young fish than that of older fish.

Protein was the second largest component of skin after moisture. Table 1 shows that the protein content of snakehead fish skin tended to increase with increasing body weight, as the protein content of SS was lower than that of MS and BS, but no significant effect ($P > 0.05$) on body weight was observed. The skin of snakehead fish at a weight of 300–1000 g showed a protein content ranging from 16.37 ± 0.60% to 18.49 ± 0.59%. The results were lower than those of previous studies, as reported by See et al. (2010) and Wulandari and Tarman (2015), namely 19.25 ± 3.40% and 20.36%, respectively. This difference might have been due to factors such as geographical aspects and fish habitats, as these factors contributed to the availability of feed. In addition, the availability of abundant feed showed a great impact on fish growth. In general, the skin consists of various types of proteins arranged to form skin tissue. Skin properties were more influenced by collagen than other types of proteins contained in the skin. Collagen or gelatin produced after the extraction process may affect the protein content of the skin (Sotelo et al. 2016). In addition, the protein has a high nutritional value as a source of essential amino acids, especially lysine and methionine (Shim et al. 2017).

Significant differences in the lipid content of snakehead fish skin were observed ($P < 0.05$). The lipid content of the skin increased with increasing body weight. Table 1 shows that the lipid content of SS was lower than that of MS and BS, but the lipid content of MS was slightly higher than that of BS. This difference might be due to physiological factors such as gender and reproductive conditions, seasons, and environmental conditions under which the fish were caught (Boran and Karaçam 2011; Puwastien et al. 1999; Shim et al. 2017; Suseno et al. 2014). Saoud et al. (2008) suggested that the percentage of lipids in the tissue decreased during spawning, causing a varied chemical composition of fish (Boran and Karaçam 2011). Lipids found in the skin are usually in the form of lipid deposits. Generally, animals use lipids as a source of energy reserved in their bodies and stored not only in muscle but also in adipose tissue such as under the skin (Anusuya and Hemalatha 2014). Basically, the lipid content of fish is indispensable to biological processes in the body. However, during extraction of collagen or gelatin, the lipid is not required because its presence can affect the quality of gelatin. The presence of lipids facilitates faster oxidation, thus decreasing quality.

Ash content represents the mineral content of snakehead fish skin. Fish weight was positively and significantly ($P < 0.05$) correlated with ash content. This result was in agreement with Naeem et al. (2016), who stated that increasing body size is associated with decreasing moisture and ash content. The ash content of snakehead fish skin at a fish body weight of 300–1000 g ranged from 0.20 ± 0.09% to 0.60 ± 0.09%, which was slightly different from the previous results reported by See et al. (2010) and Wulandari and Tarman (2015), namely 0.55 ± 0.03% and 0.67%, respectively. The content of ash in snakehead skin appears to be lower than that in the entire body of snakehead fish. Boran and Karaçam (2011) reported that various levels of ash content were observed in some marine fish species such as gold mullet (1.90 ± 0.21%), shadows (1.98 ± 0.1%), garfish (1.73 ± 0.54%), and horse mackerel (1.80 ± 0.48%). However, Alfaro et al. (2013) found that the ash content in the skin of wamil tilapia *Oreochromis urolepis hornorum* fish at a weight of 700 ± 100 g was 4.2 ± 0.35%. This suggests that differences in ash content are associated with species differences, body size, tissue type, habitat, and feed availability.

Amino acid content

Amino acids are components of protein and have vital functions in biochemical processes (Berg et al. 2002). They are also important precursors for the synthesis of various molecules as well as the regulation of various metabolic functions, such as health, growth, development, reproduction, and homeostasis (Haniffa et al. 2014; Wu 2009; Zuraini et al.

2006). Snakehead fish skin contains an almost complete complement of amino acids, as does fish meat (Berg et al. 2002; Gam et al. 2005; Tan and Azhar 2014; Zuraini et al. 2006), even though the two types of networks have different mechanisms and functions.

In general, the most common amino acids found in snakehead fish skin were glycine and proline, of which the highest percentage was found in MS, followed by BS and SS. Glycine and proline were the most common types of amino acids found in collagen and gelatin (Giménez et al. 2005; Sotelo et al. 2016), which means that snakehead skins could be a good source of gelatin. Proline not only serves as a major component of collagen compounds but, nutritionally, proline functions in wound healing, antioxidant reactions, and immune responses (Wu et al. 2011). Glutamate, a major amino acid in snakehead fish meat, was also found at high levels in the skin, and its levels increased with increasing fish body weight (Tan and Azhar 2014; Zuraini et al. 2006). The percentage of alanine and aspartic acid tends to increase with increasing body weight; in contrast, alanine and aspartic acid in snakehead fish meat tend to decrease with increasing body size. Furthermore, arginine, another important amino acid, is an essential amino acid that plays a role in the production of hydroxyproline, which is used in the formation of connective tissue. Its presence was relatively higher in MS than in BS and SS. This suggests that arginine and other amino acids synergistically induce the formation of new tissues for wound healing. Some of these amino acids included valine, histidine, glycine, proline, and alanine (Gam et al. 2005). The aspartic acid levels in snakehead fish are usually found to be quite high, second to glutamate (Gam et al. 2005; Tan and Azhar 2014; Zuraini et al. 2006). However, aspartic acid in snakehead fish skin tends to increase with increasing body weight. Lysine, an amino acid, plays an essential role and, along with methionine, could be an indicator of protein quality. The highest content of lysine and methionine was attributed to BS and MS, respectively. In addition, cysteine and tryptophan were present in very small amounts; presumably they were damaged during hydrolysis by acid. The presence of relatively identical amino acids to those found in snakehead fish meat may be a consideration for utilizing fish skins on a broad spectrum.

Hydroxyproline and estimated collagen content of snakehead fish skin

Some amino acids have been specifically examined because they can give certain characteristics to the raw material. The presence of glycine, proline, and hydroxyproline in the amino acid sequence is an indication that the raw material is a source of collagen (Toppe et al., 2007). Blanco et al. (2017) suggest that hydroxyproline content may represent collagen content in skin and bone. Hydroxyproline is present only in connective tissue containing collagen and elastin, and its presence with proline in the amino acid chain can affect the quality of collagen or gelatin. Total proline and hydroxyproline, also known as amino acids, contribute to triple helix stability (Schrieber and Gareis 2007). The content of hydroxyproline in the skin of snakehead fish increased with increasing body weight, which was higher than in *Rachycentron canadum* (12.1 ± 0.3 mg/kg) and *Micropogonias furnieri* (11.6 ± 0.2 mg/kg) as reported by Silva et al. (2014).

Although there was no significant difference ($P > 0.05$), between statistically different weights, there was a tendency for greater body weight to have less protein-based collagen. This condition was thought to be related to hydroxyproline levels as a key factor since the hydroxyproline levels at different weights were stable, but the bodies grew. Other factors may be attributed to the body's ability to synthesize, and the availability of food sources in its habitat. Supplementation of hydroxyproline in a cultivated snakehead fish diet could be a consideration for increasing skin tissue collagen, as Aksnes et al. (2008) reported with a salmon diet. Li et al. (2009) explained that hydroxyproline is one of the amino acids that must exist in the diet because its utilization rate is greater than the rate of synthesis by the fish.

Microstructure of snakehead fish skin

The potential of snakehead fish skin as a collagen source was augmented by the presence of fibrils in the snakehead fish skin observed in the micro structural analysis. Collagen is one of the major proteins of connective tissue, consisting of fibrils and arranged in a series of helices that form the structure of the skin. It was mostly found in the dermis layer and is the main constituent of the skin that gives it its strength. Microstructural images (Fig. 2a, b, c) depicted a dermis layer that resembled elongated fibril spindles. There were no remarkable differences observed between samples. However, Suárez et al. (2015) reported that fish age was also a notable factor affecting the strength of collagen; therefore, it is necessary to consider culture conditions.

Mineral content of snakehead fish skin

In general, SEM–EDS can indicate the presence of several types of minerals contained in the fish skin, although this tool was only able to detect the surface of the sample. However, it is not applicable to the quantitative determination of mineral content. Rivas et al. (2014) stated that minerals play important roles in metabolic processes; some minerals are responsible for controlling biological functions of the body. The levels of some minerals were not affected by fish weight but could be linked to such factors as season, age, reproductive conditions, feed source, and fish habitat (Atanasoff et al. 2013).

Conclusions

Increased weight of snakehead fish showed differences in chemical composition of the skin, including decreased moisture and ash content, and elevated lipid levels, but did not have a significant difference in protein content. Glycine and proline demonstrated the most abundant amino acids in all fish weight groups, while the presence of hydroxyproline showed that the skin of snakehead fish could be a promising source of collagen. The results also show that differences fish body weight observed did not indicate significant differences in protein collagen content. The study of microstructure and mineral content of snakehead skins can be an indicator for wider use of snakehead skin, thereby increasing its potential and economic value.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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