Chemical Composition, Amino Acid and Collagen Content of Snakehead (*Channa striata*) Fish Skin and Bone

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Abstract- Utilization of snakehead fish as a health food has an impact on increasing by-product of skin and bone. This study aims to evaluate the chemical composition, amino acids and collagen content of the skin and bone of snakehead fish, as a consideration in its use further. This study used the skin and bone from snakehead fish in weight 900 to 1,000 g. Characteristics of skin and bone of snakehead consist of chemical composition and amino acid content. The percentage of water and protein is higher in the skin, and conversely, the fat and ash content is higher in bone. The highest percentage of amino acids in the skin and bone were glycine and proline, respectively, and the presence of hydroxyproline shown that the skin of snakehead fish was the source of collagen.

Index term- Skin of snakehead, bone of snakehead, chemical composition, amino acid, collagen content

I. INTRODUCTION

Snakehead fish (Channa striata) is a potential freshwater fish species and has become an important commodity in health food industry due to its abundant albumin content, and several researchers have verified it [1],[2]. Utilization snakehead fish for the raw material of albumin is an opportunity to develop this fish on a larger scale in the future.However, it's processing as a health food product has an impact on increasing its by-product.

The processing of by-products after filleting can reach 60-70% [3] or about 75% of total fish weight [4]. About 30% of byproducts are skin, bone, and scales [5], [6], [7]. Skin and bone are a major by-product fishery industry containing high organic and inorganic ingredients [8].

There is a relationship between fish production and by-products; Increased production causes increased costs to reduce impacts on environmental pollution [9]. In contrast, utilization of fishery byproducts can solve the problem of waste disposal and also create value-added products [10]. Therefore appropriate handling is required to prevent adverse environmental impacts. Valorization of skin and fish bones for production is one of the efforts to increase the added value of fishery by-products [11]. Utilization of skin and fish bones can reduce the impact of environmental pollution, in addition to increasing the value of use and economic value of the by-product. This study aims to evaluate the chemical composition and amino acid skin and bone fish snakehead.

II. MATERIALS AND METHODS

Regardless of their gender, the fresh snakehead fish obtained from the Bili Bili Dam, Gowa, South Sulawesi, Indonesia. Fish was weighed and measured in length. This study used the skin and bone of snakehead fish which the size were 900 to 1,000 g in the weight and length were 45.89 to 49.27 cm. Fish skin was collected and cleaned to remove unwanted material, washed and stored in polyethylene plastic bags for further preparation. For proximate analysis, fresh skin is used, while the bone first through degreasing process by boiling in water-bath temperature 60-70°C for 30 minutes. Skin and bone for the amino acids analysis used lyophilized skin processes. The skin was cut using a knife (approximately 2 × 2 cm), then lyophilized (frozen dryer, ALPHA 1-2 LD plus) until completely dry (about 72 hours). Dry skin is milled to reduce the size, packed airtight and stored at < 4°C for further analysis.

Proximate composition was determined using AOAC procedure [12]. Moisture content was determined using gravimetric method. Kjeldahl method was used for determination of crude protein content (conversion factor of 6.25 × N). Lipid content was determined by using Soxhlet

method. Ash content was determined by incineration for 16 h at 550°C.

The amino acid content was determined according to [13] Nollet (1996). The amino acids were analyzed by using an Ultra Performance Liquid Chromatography (UPLC, ACQUITY UPLC-H Class). Sample (0.1 g) was hydrolyzed in 5 mL of 6N HCl and heated at 100°C for 22 h. A solution containing 500 µL filtrate, 40 µm AABQ, and 460 µL distilled water was prepared. The solution (10 µL) added with AccQ-Fluor Borate and 20 µL reagent Flour-A was incubated for 10 min at 550°C, then injected in UPLC system.

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), according to Henderson Jr. and Brooks (2010) method. Prior to injection of HPLC, the sample was hydrolyzed in 1 mL of 6N HCl and heated at 110 °C for 24 h. 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).

The experimental data were evaluated using descriptive analysis.

III. RESULTS

Chemical Composition of Skin and Bone of Snakehead Fish

The proximate analysis results of skin and bone of snakehead fish (moisture content, protein, lipid, and ash), are presented in Table 1. Proximate analysis of snakehead fish skin and bone were conducted as a basis for the physicochemical characteristics of fish skin and bone. The analysis showed that there were a different skin and bone in the percentage; the content of moisture and protein were higher in the skin, whereas the content of lipid and ash in bone were higher.

Table 1. Chemical composition of skin and bone of snakehead fish (%)

Item	Skin	Bone
Moisture	74.33 ± 0.15	43.19 ± 0.16
Protein	18.49 ± 0.59	15.49 ± 0.59
Lipid	2.99 ± 0.42	4.19 ± 0.31
Ash	0.20 ± 0.09	32.05 ± 0.05

All values are mean ± standard deviation

Amino Acid Content

Table 2 shows the amino acid content of snakehead fish skin and bone. Amino acids contained in the skin was relatively higher than in bone. The Amino acid composition is a reflection of the mixture of proteinaceous material contained in skin or bone samples.

Table 2. Amino Acids profiles of skin and bone of snakehead fish

Amino acid	Skin	Bone
Histidine	11.91	11.77
Serin	39.66	30.19
Arginine	82.52	81.53
Glisin	238.11	235.26
Aspartate	60.21	59.49
Glutamate	116.68	115.29
Threonin	33.67	33.27
Alanine	101.16	99.95
Proline	123.28	121.81
Sistein	0.33	0.33
Lisin	54.90	54.24
Tirosin	9.58	9.47
Metionin	15.77	15.58
Valin	28.32	27.98
Isoleusin	18.57	18.34
Leusin	33.22	32.83
Phenilalanin	31.13	30.76
Tryptophan	0.96	0.95

Collagen content

Collagen content can be estimated based on the amino acid content of hydroxyproline. The collagen content of snakehead skin relatively higher (95.25 ± 1.76) than in bone (89.01 ± 3.24).

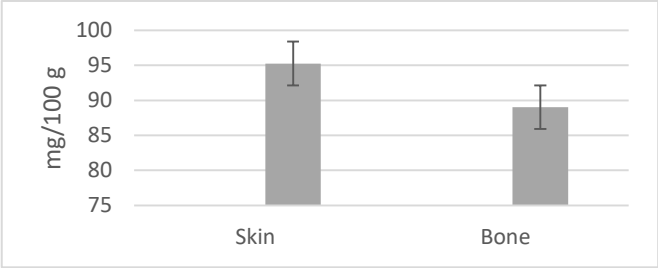

Figure 1. Collagen content estimation from skin and bone of snakehead fish. All values are mean ± standard deviation.

IV. DISCUSSION

The moisture content was the main body of fish [14] which was bonded physically and chemically. The moisture content is the main constituent it was needed primarily in the process of metabolism, transportation and various vital activities in the body. Proteins are the major constituents of skin and bone after water, which contain almost all of the amino acids [15], both essential and non-essential (Table 2). Both skin and bone contain lipid as one of its constituent components. The lipid content of fish bone was relatively higher than in skin and was same also found in the leather jacket [16]. In some processing processes, especially the process of bone extraction into gelatin, lipids need to be released because the lipid can decrease the quality of gelatin produced, and usually by degreasing process for that purpose. The snakehead fish bone contains higher levels of ash than the skin. As a representation of mineral content, the ash level high indicated a high mineral mass in bone, but skin leather jacket ash was higher than bone. This was according to [16] related to skin thickness and the characteristics of its biochemical composition.

Amino acids have vital functions in the biochemical processes of body [17], and are important precursors for the synthesis of various molecules as well as regulation of various metabolisms, such as health, growth, development, reproduction, and homeostasis [18], [19], [20]. In general, the

most common amino acids found in the skin and bones of snakehead were glycine and proline. Glycine and proline were the highest types of amino acids found in collagen and gelatin [21], [22]. The existence of this similarity becomes a reference that the snakehead fish skin and bone had the potential to be extracted into gelatin. Proline not only acts as a major component of collagen compounds, but nutritionally, proline works on wound healing, antioxidant reactions, and immune responses [22]. A number of other important amino acids, such as arginine, were essential amino acids that play a role in producing hydroxyproline which was used in the formation of connective tissue [23]. This reason reinforces that arginine along with other amino acids synergistically accelerates the process of forming a new tissue post-wound. Some of these amino acids were valine, histidine, glycine, proline, and alanine [23]. Alanine was one type of amino acid that plays a role in gelatin viscosity [21], and becomes a parameter of protein quality along with methionine. The percentage of amino acids cysteine and tryptophan were very small, the possibility of this amino acid was damaged during acid hydrolysis process. The presence of compounds of amino acids relatively identic to those found in snakehead fish meat [20], [17], [23], thus can being considered for utilizing the skin and fish bones on a broad spectrum.

Hydroxyproline is an amino acid found only in connective tissue containing collagen and elastin. The presence of hydroxyproline was a major feature of tissue matrix containing collagen, and its presence with proline in the amino acid chain can affect the quality of collagen or gelatin for triple helix stability [24]. Elliot [25] explains that collagen was one of the major proteins that form connective tissue along with other proteins, and is found in the dermis layer. This layer contributes to make the skin taut and flexible. Skin properties are more influenced by collagen than other types of proteins such as elastin and reticulin. In contrast, collagen in bone has a strong bond with minerals in the bone matrix. Protein content may represent the levels of collagen produced after the extraction process [20].

V. CONCLUSION

The chemical composition of snakehead fish skin and bone of originating from the same body size was relatively different. The content of moisture and protein were relatively higher in skin, conversely, the percentage of lipid and ash was higher in bone. The high presence of glycine and proline amino acids showed that both were collagen sources potential. The difference in the percentage of chemical composition between the skin and bone can be due to the physiological and biochemical properties and functions of each component.

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