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CHARACTERISTICS OF GELATIN FROM SKIN AND BONE OF SNAKEHEAD (*CHANNA STRIATA*) EXTRACTED WITH DIFFERENT TEMPERATURE AND TIME

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ABSTRACT

This study aimed to determine the physicochemical properties of the skin and bone of snakehead fish as a potential source of gelatin through extraction at different temperatures and times compared to commercial gelatin. Extraction of skin and bones of wild snakehead fish (*Channa striata*) at different temperatures (50, 60, 70 ºC) and time (12,18, 24 hours). The pre-treatment process used a 0.1 M Ca (OH) 2 (1:6 w/v) immersion solution for 1 h and continued with 0.05 M citric acid (1:6 b/v) for 5 h. Before pre-treatment, the minerals of bones were degreased with 3% HCL solution for 24 hours. The results of the analysis showed that the differences in raw materials, temperature, and extraction time had a significant effect (p <0.05), as well as interactions among treatments (p <0.05) on the yield and gel strength. The yield of skin and bone tended to increase with extending extraction temperature and time, while the highest gelatin strength was found at 60 °C for 12 hours on the skin and 24 hours for the bone. The best gelatin was accomplished based on the highest performance of gel strength on skin and bones and compared to bovine commercial gelatin. The amino acids of the three types of gelatin showed higher levels of glycine and proline than other types of amino acids. Based on the total residues of each amino acid, skin gelatin and bone gelatin showed more dominant hydrophobic properties than hydrophilic properties, in contrast to bovine commercial gelatin. The three types of gelatin showed diverse chemical compositions, emulsion activity index, emulsion stability, water resistance, and fat binding capacity, which was reflected to be closely related to the source of the raw material and its amino acid content. The FTIR results showed that the extracted snakehead fish skin and bones have the potentiality to be used as gelatin equivalent to a commercial one.

Keywords: skin gelatin; bone gelatin; gel strength; snakehead gelatin; amino acid; FTIR

INTRODUCTION

Collagen is one of the important proteins that make up the connective tissue in animal bodies, which is found in skin, bones, teeth, scales, and tendons **(Lv et al., 2019; Schmidt et al., 2016)**. Each of the collagen characteristics is relatively different, depending on the cross-linking structure and the triple helix molecule. Collagen is the raw material for gelatin, through a partial hydrolysis process **(Montero and Gómez-Guillén 2000; Huda et al., 2013a)**. The very wide application of gelatin has prompted the search for sources of collagen for the raw material for gelatin, mainly from pigs and cows, and to a lesser extent from other animals such as chicken, duck, goat, and fish **(Ahmad and Benjakul, 2011; Nurkhoeriyati, Huda and Ahmad, 2011; Huda et al., 2013b; Kuan et al., 2017)**. Changes in social behavior, religious awareness, and understanding the importance of health become selective qualifications for the use of collagen raw materials for gelatin production.

Recently, the global market for halal gelatin has been steadily in demand. One source of raw materials that many researchers are currently exploring is the skin and bones of fish **(Karim and Bhat, 2009; Herpandi, Huda and Frederick, 2011)** because it contains high collagen so that it has the potential to be used as a source of gelatin **(Abd, Mirghani and Adam, 2013; Mirzapour, Moosavi-Nasab and Aminlari, 2018)**. Fish collagen is a potential byproduct of the fisheries industry and is allegedly able to accommodate the needs of gelatin for all ethnicities **(Gomez-Guillen et al., 2011)**. According to **Le Thi et al. (2020)**, the advantages of fish gelatin over mammalian gelatin are cheaper production costs, water solubility, and better thermal stability.

Fish skin and bones which are the remains of the fishing industry **(Cho et al., 2006; Herpandi, Huda and Frederick, 2011)** estimated at 30 to 41.5% of the total fish weight **(Gómez-Guillén et al., 2002; Stevens et al., 2018)**. The remaining products of the fish industry that have not been used optimally are the skin and bones of snakehead fish **(Rosmawati et al. 2018a)**. Snakehead fish are carnivores in freshwater and are an alternative source of animal protein in most parts of Indonesia which have a muddy area typology. Various studies have shown the potential of snakehead fish as a source of albumin which is proven to have a healing effect while restoring body health **(Haniffa et al., 2014)**. This is an opportunity for more intensive fish farming. However, high production has an impact on increasing waste products as a result of large-scale fish utilization. On the other hand, directing the potential of the skin and bones of snakehead fish as valuable materials is important so that they are not wasted. Thus, these efforts can increase the economic value of the skin and bone of snakehead fish.

Several types of solutions have been used in pretreatment for processing collagen extraction into gelatin. The alkaline solution usually helps to release non-collagen proteins, while the acid solution causes the process of breaking or weakens the bonds between the collagen molecule and other protein molecules, thereby facilitating the extraction process. Apart from solution, temperature and extraction time can affect the quality and quantity of gelatin produced **(Kittiphattanabawon et al., 2010; Kittiphattanabawon et al., 2012; Kołodziejska et al., 2008; Montero and Gómez-Guillén, 2000; Sanaei et al., 2013)**.

This study aimed to determine the physicochemical characteristics of the skin and bones of snakehead fish as potential sources of gelatin through extraction at different temperatures and times compared to commercial gelatin.

Scientific hypothesis

The study hypothesizes that different temperatures and times will affect the physicochemical characteristics of gelatine extracted from the skin and bones of snakehead fish.

MATERIAL AND METHODOLOGY

Samples

Wild Snakehead (*Channa striata*) fish of between 600 to 700 g with a length of approximately 42.07 ±0.75 cm were obtained from Bili Bili Dam, Gowa South Sulawesi, Indonesia (Figure 1).

Figure 1 Wild snakehead fish (Channa striata) used in the experiment.

Chemicals

The analytical grade chemicals $Ca(OH)_2$, Citric acid, HCl, KBr (Merck, Germany) were used in the experiments. The commercial gelatin used was bone bovine gelatin, 150 g gel strength (Green Valley, Global Capsule Ltd. Dhaka 1000-Bangladesh).

Instruments

The instrument used in this study were oven (Memmert UM 400, Germany), dry blender (Miyako 152PF, PT. Kencana, Indonesia), weighted (Mettler Toledo, J81603-C/FACT, Germany), water-bath (Memmert W350, Germany), TAXT2 Texture Analyzer (Stable Micro System, UK), ultra-performance liquid chromatography (UPLC, ACQUITY UPLC-H Class, Waters, USA), vortex (IKA Labortechnik VF2, Germany), homogenizer (IKA T25 digital-ULTRA TURRAX, Germany), spectrophotometer (Thermo Fisher Scientific 4001/4, Genesys VF2, Germany), centrifuged (High speed refrigerated micro centrifuge, Tomy Mx-305) and Fourier Transform Infrared Spectrophotometry (FTIR) (IR Prestige-21, FTIR-8400, Shimadzu, Japan).

Description of Experiments

Extracting Gelatin

Process for Pretreatment

The pre-treatment process refers to **Saida et al. (2011)**. The skin or bone was soaked with 0.1 M $Ca(OH)_2$ (1:6 b/v) for 1 h in the early stages of the extraction process, washed vigorously with tap water three times, and then drained with a plastic filter. The next step was to soak the skin or bone with 0.05 M citric acid (1:6 b/v) for 5 h and then washed it with tap water three times again and rinsed it with a plastic filter.

Method of Extraction and Analysis

Using distilled water (1:6 b/v) at various temperatures and times, each 50, 60, and 70 °C for 12, 18, and 24 h in a water-bath on water-bath (Memmert W350, Germany), the snakehead skin or bone that had been processed by pretreatment was then extracted. Using four-folded cheesecloths, the extraction results were filtered. The liquid gelatin was dried in an oven (Memmert UM 400, Germany) for 48 h at 60 °C. To assess the yield of gelatin, the gelatin sheet was finely ground with a dry blender (Miyako 152PF, PT. Kencana, Indonesia) and weighted (Mettler Toledo, J81603-C/FACT, Germany), then stored in plastic Polyethylene at room temperature for further analysis.

The best method was used to obtain snakehead fish gelatin for the skin and bone at varying temperatures and extraction times. As the finest measure of gelatin consistency, the authors assign gel strength parameters. The selected skin and bone gelatin will be further characterized and compared to bovine commercial gelatin (BCG) gel strength. All analyses were conducted in triplicate.

Gelatin Yield

The gelatin yield (based on the wet weight base) was determined as the ratio between the dry weight of the gelatin and the total weight of the wet skin/bone base **(See et al. 2010)**.

Gel Strength of Gelatin

With some changes, the gel strength determination was based on **GMIA (2012)**. By mixing 7.5 g of gelatin and 105 mL of distilled water, a 6.67% (b/v) gelatin solution was prepared. To allow the gelatin to absorb water and swell, the mixture was left at room temperature for 30 minutes. The mixture was then heated in the water bath at 60 °C for 30 min to accomplish the gelatin solubility to obtain gelatin solution, which was then stored for 17 ±1 h at refrigerator temperature (4 ±1 °C). The strength of gel was determined using a TAXT2 Texture Analyzer (Stable Micro System, UK) with a 12.7 mm diameter plunger. The velocity of the plunger penetrates the gel at 10 $mm.min^{-1}$ at a maximum force of 0.5 $mm.sec^{-1}$ at a penetration depth of 4 mm. The result of the maximum force reading provided on the gel by the plunger was expressed in grams. Measurements were carried out three times.

Proximate Analysis

Using the methods of **AOAC (2005)**, the proximate composition of snakehead gelatin and bovine commercial gelatin was determined. Using the gravimetric method, moisture content was determined. For the determination of crude protein content (conversion factor of 5.55 × N), the Kjeldahl method was used. By using the Soxhlet method, lipid content was determined. The ash content of 16 h at 550 °C was determined by incineration.

Amino Acid Composition

According to **Nollet (2004)**, the amino acid composition was determined. Utilizing ultra-performance liquid chromatography (UPLC, ACQUITY UPLC-H Class, Waters, USA), amino acids were analyzed. The sample (0.1 g) was hydrolyzed with 5 mL of 6 N HCl and heated for 22 h at 100 °C. The prepared solution contained 500 µL filtrate, 40 µL AABQ, and 460 µL distilled water. The solution (10 µL) was incubated at 550 °C for 10 minutes after the addition of AccQ-Fluor Borate and 20 µL of Flour-A reagent and then injected into the UPLC testing method.

Emulsifying Properties Determination

A turbidimetric method based on **Pearce and Kinsella (1978)** with minor modification was used to determine the emulsion activity index (EAI) and emulsion stability index (ESI) of gelatin. A solution of 6 mL of gelatin with a concentration of 2% (w/v), an oil solution, and a gelatin solution combined with a vortex (IKA Labortechnik VF2, Germany) for 10 s was added with soy oil (2 mL). The mixture was then homogenized using a homogenizer (IKA T25 digital-ULTRA TURRAX, Germany) at 20,000 rpm for 1 min at room temperature (±28 °C) to prepare the emulsion. The emulsion, to acquire 1/100 dilution, was diluted with 1 $g.L^{-1}$ SDS. Using a spectrophotometer (Thermo Fisher Scientific 4001/4, Genesys VF2, Germany), absorption was measured against 1 $g.L^{-1}$ SDS at 500 nm. After emulsification, the absorbance was measured immediately at (A0) and 10 minutes (A10). The equations of **Ahmad and Benjakul (2011)** were used to measure EAI and ESI, as follows:

IAE ($m^2.g^{-1}$) = TDF / løC
ESI (min) = A0 / ΔA × Δt
Where, T = 2 × 2.303 × A500 × DF; DF = Dilution factor; l = long cuvette (m); ø = fat dispersion (0.25); C = gelatin concentration ($g.mL^{-1}$); A500 = wavelength; A0 = Wavelength at 0 min; ΔA = A0 - A10; Δt = 10 min.

Water Holding and Fat Binding Capacity

According to **Cho et al. (2006)**, water holding capacity (WHC) and fat binding capacity (FBC) are measured with partially modified input. Half a gram of gelatin was inserted and weighed (tube and gelatin) into a disposal centrifuge tube. A total of 10 mL of water or 10 mL of sunflower oil was applied to the tube, then left for 1 h at room temperature to swell and dissolve the gelatin in the water/oil. At 20,000 rpm for 20 min, the gelatin solution was then centrifuged (High speed refrigerated micro centrifuge, Tomy Mx-305). The upper step was removed and the centrifuge tube on the filter paper dried for 30 minutes. Based on **Shyni et al. (2014)**, the WHC and FBC gelatin values were measured, i.e. the difference between the initial volume of distilled water/sunflower oil applied to the gelatin sample and the supernatant volume was determined and the results recorded were absorbed as ml of water/oil per gram of gelatin sample.

FTIR

Fourier Transform Infrared Spectrophotometry (FTIR) (IR Prestige-21, FTIR-8400, Shimadzu, Japan) with a wavenumber of 4000 to 650 cm^{-1} is used in the analysis process. A pellet type preparation process, KBr (Potassium Bromide), was the tool used. Snippets (0.1 – 2% by weight) were pounded together with KBr, then pressed to achieve a pellet form at a pressure of 8-20 tons. Under an infrared lamp, the KBr was established in a dry state to prevent condensation of vapors from the atmosphere and then to discharge the water. Pellet samples were then inserted on an infrared spectrophotometer into the sample site, and the results of the spectrum would be read via a computer monitor.

Sample preparation: The processing of fish refers to the **See et al. (2010)** procedure. About 58 of Snakehead fish have been manually scaled and all body parts have been removed. The skin (6 kg) and bone (10 kg) of fish separately were both gathered and washed, then covered with a plastic bag and stored at -20 °C for a maximum of 2 months. The skin and bones of the thawed snakehead fish were prepared before the pre-treatment process in triplicate experiments. Small pieces of fish skin measuring approximately 2 to 3 cm in length and width were taken. With tap water, the skin was washed three times and then drained using a plastic filter. To promote the release of meat and fat still attached to the bone, the thawed snakehead bone was boiled (1:3 w/v) at 60 °C for 30 minutes. The bone was washed, and then the joints were separated using pliers. The process of bone mineralization was accomplished to release the potent inorganic elements bound with collagen in 3% (1:6 w/v) HCL for 24 h by soaking the bone fragments.

Number of samples analyzed: Number of samples analyzed were 54 samples which consist of 27 samples of skin gelatin, 27 samples of bone gelatin.

Number of repeated analyses: All analyses were conducted in triplicate.

Number of experiment replication: Sample of skin and bones were prepared in triplicate experiments.

Statistical analysis

The experimental data were tested using one-way analysis of variance (ANOVA) based on a completely randomized factorial design, namely yield, gel strength, proximate composition, amino acid composition, emulsifying properties, water holding and fat binding capacity of the skin and bone gelatin. The validity of diversity was checked using the Duncan test. The data was running using SPSS software (IBM Corporation, US).

RESULTS AND DISCUSSION

Snakehead gelatin yield

The yields were an important factor that was always considered in the conversion of collagen to gelatin because it was related to the effectiveness of the process applied and the economic benefits. The yield produced in the process of transforming collagen from the skin and bone of snakehead fish as raw material into gelatin at different extraction temperatures and times showed significant differences (p <0.05), as shown in Table 1.

The yield of snakehead gelatin was influenced by the type of raw material, temperature, and extraction time. According to **Karim and Bhat (2009)**; **Kołodziejska et al. (2008)**, that the conversion of collagen to gelatin can be influenced by processing parameters, such as the nature of the raw material and the method of extraction, including the pre-treatment conditions, temperature, and time. The higher the extraction temperature, the yield of SSG and SBG significantly increased. Likewise, the longer the extraction time, the yield of the two types of gelatin also significantly increased. Gelatin yield was influenced by differences in the type of raw material, temperature, and extraction time.

However, extraction at a temperature of 50 °C with different extraction times had not obtained an increase in the SBG yield (p >0.05). **Sims and Bailey (1992)** explained that skin collagen from immature animals contained an intermediate crosslink of dehydroxylysinonorleucine and when it reached adulthood it was converted into trivalent cross-linked of histidinohydroxylysinonorleucine which was more stable, while collagen in bones contained hydroxylysinocetonorleucine which was then converted to pyridinoline. According to **Bailey, Paul and Knott (1998)**, pyridinoline was more stable to heating. This possible phenomenon was one of the factors that cause the SBG yield to be lower than SSG due to the slower conversion process of collagen in bones compared to skin.

There were significant differences due to the interaction of raw materials and time; and the interaction of raw materials and temperature, as well as the interaction between temperature and time; and the interaction of raw materials, temperature, and time on gelatin yield (p <0.05). Table 1 showed that SSG was significantly higher than

SBG at the same temperature and extraction time. During the extraction process, temperature affects the solubility rate of collagen, where high temperatures caused the collagen bond structure to open and result in the breaking of the bonds from the collagen protein molecule. Extraction time was the most significant factor affecting gelatin yield **(Ahmad and Benjakul, 2011; Hanjabam et al., 2015)**. The higher temperatures used the gelatin yield tended to increase. During the extraction process in the hydrogen bonds, the heating process in a water bath which stabilized the triple helix of the parent collagen is damaged, causing a transition from helix to coil resulting in the conversion of collagen into gelatin solution **(Benjakul, Kittiphattanabawon and Regenstein, 2012)**.

In general, the yield of SBG was relatively lower than SSG, either due to a single factor of raw material, temperature or time, or the interaction between treatments. The skin was composed of organic material that can easily change its character due to chemical treatment rather than bone. This was because the bone structure consists of organic and inorganic composites **(Szpak, 2011)**. The demineralization process of bone to ossein at the beginning of the pre-treatment process causes the release of several minerals, as suggested by **Muyonga, Cole and Duodu (2004a)** and **Muyonga, Cole and Duodu (2004b)**. The demineralization process causes the release of mineral components that fill the spaces between collagen molecules in the bone matrix, allowing easier conversion of collagen to gelatin. The yields of SSG and SBG were 18.25 ±0.26% and 8.19 ±0.17%, respectively, higher than the results obtained by **Muyonga, Cole and Duodu (2004b)** on the skin and bones of *Lates niloticus* fish as much as 12.3 ±2.1% to 16.0 ±0.3% and 1.3 ±1.0 to 2.4 ±0.7%, respectively.

Snakehead Gelatin Gel Strength

The main parameter of gelatin was gel strength, which was used as a method for measuring the impact of production factors and optimizing the quality of the final product. According to **Karim and Bhat (2009)**, raw material characteristics, temperature, and extraction time play an important role in gelatin gel strength. There was a difference in gel strength between the skin and snakehead fish bones extracted at different temperatures and times (p <0.05). The effect of the gel strength gelatin of skin and bone snakehead fish on the temperature treatment and extraction time were presented in Table 1.

The interaction of treatment combinations between raw materials and temperature; raw materials and time; and between raw materials, temperature and time had different effects on the gel strength of snakehead fish gelatin (p <0.05). The existence of this interaction showed that there was a different gel strength on SSG and SBG. The longer the extraction time, the gel strength of SSG tends to decrease but vice versa in SBG was tending to increase.

Table 1 The characteristics of skin and bone of gelatin snakehead using different temperatures and extraction times.

Item	Yield (%)	Gel Strength (g)
SB1 (50 ºC, 12 h)	2.57 ±0.26^{a}	41.37 ±7.42^{a}
SB2 (50 ºC, 18 h)	2.63 ±0.37^{a}	55.37 ±5.12^{a}
SB3 (50 ºC, 24 h)	3.19 ±0.32^{a}	64.67 ±4.68^{ab}
SB4 (60 ºC, 12 h)	3.78 ±0.17^{ab}	48.00 ±5.32^{a}
SB5 (60 ºC, 18 h)	4.79 ±0.12^{b}	144.77 ±20.02^{cd}
SB6 (60 ºC, 24 h)	6.46 ±0.32^{c}	239.53 ±20.69^{gh}
SB7 (70 ºC, 12 h)	8.19 ±0.17^{d}	104.23 ±16.35^{bc}
SB8 (70 ºC, 18 h)	8.91 ±0.23^{d}	189.83 ±28.83^{ef}
SB9 (70 ºC, 24 h)	8.90 ±0.10^{d}	173.80 ±27.77^{de}
SS1 (50 ºC, 12 h)	14.22 ±0.18^{e}	225.33 ±7.67^{fg}
SS2 (50 ºC, 18 h)	16.06 ±1.09^{f}	126.40 ±13.22^{c}
SS3 (50 ºC, 24 h)	16.30 ±0.32^{f}	109.63 ±23.58^{c}
SS4 (60 ºC, 12 h)	14.33 ±0.07^{e}	278.37 ±22.28^{h}
SS5 (60 ºC, 18 h)	16.64 ±0.07^{f}	253.77 ±51.66^{gh}
SS6 (60 ºC, 24 h)	16.70 ±0.94^{f}	108.23 ±27.10^{c}
SS7 (70 ºC, 12 h)	16.83 ±2.16^{f}	246.13 ±47.22^{gh}
SS8 (70 ºC, 18 h)	18.25 ±0.26^{g}	173.23 ±16.16^{de}
SS9 (70 ºC, 24 h)	18.70 ±0.50^{g}	108.00 ±21.40^{c}

Note: Different superscripts in the same column indicate a significant difference (p <0.05). Values are presented as mean ±*SD* (n = 3). SB = snakehead bone; SS = snakehead skin.

Table 2 The chemical composition of snakehead skin gelatin (SSG) and snakehead bone gelatin (SBG) from the extraction process used the best temperature and extraction time compared to bovine commercial gelatin (BCG).

	Moisture	Protein	Fat	Ash
SSG	7.46 ±0.28^{b}	83.41 ±0.56^{b}	0.31 ±0.01^{b}	1.14 ±0.04^{a}
SBG	3.50 ±0.20^{a}	93.12 ±0.50^{c}	1.05 ±0.05^{c}	1.33 ±0.07^{b}
BCG	13.70 ±0.40^{c}	76.99 ±0.93^{a}	0.03 ±0.00^{a}	1.93 ±0.04^{c}

Note: Different superscripts in the same column indicate a significant difference (p <0.05). Values are presented as mean ±*SD* (n=3); SSG = Snakehead skin gelatin; SBG = snakehead bone gelatin; BCG = Bovine commercial gelatin.

The same properties were found in the gel strength of *Aluterus monoceros* skin gelatin **(Ahmad and Benjakul, 2011)** which tended to decrease with increasing extraction time. There was an increase in gel strength when the SSG was heated between 50 °C and 60 °C but decreased when the extraction temperature was increased by 10 degrees, while the gel strength of SBG tended to increase as the extraction temperature increased. It seems that the snakehead bone takes a relatively long extraction time to match the gel strength of snakehead skin. Compared to snakehead skin, the relatively long extraction process on the bones was thought to be related to the different chemical compositions of the two materials.

The skin consists of organic material and very little inorganic material, but bones contain organic material with a high proportion of inorganic material **(Olszta et al., 2007; Rosmawati et al., 2018a; Szpak, 2011)**. Demineralization of bone before pretreatment and extraction was an effective method for releasing inorganic materials **(Muyonga, Cole and Duodu, 2004a; Muyonga, Cole and Duodu, 2004b; Ranasinghe et al., 2020)**.

Based on Table 1 above, the shown that SSG and SBG have relatively the same gel strength when each extracted at different temperature and time combinations, SS4 (60 °C, 12 h) vs SB6 (60 °C, 24 h). Skin and bone have structural differences and cross-linkages **(Sims and Bailey, 1992)** so that different gel strengths can occur under the same extraction conditions. The extraction process allows the chain to break, the shorter the chain, the lower the gel strength. The temperature and extraction time contribute to the breaking of this chain. This is inseparable from the influence of the structure, sequence, and configuration of amino acids **(Kittiphattanabawon et al., 2012)**. The best gel strength was found in SSG extracted at 60 °C for 12 hours, while SBG at the same temperature with 24 hours extraction time was 278.37 ±22.23 g and 239.53 ±20.68 g, respectively. The gel strength of SSG and SBG was higher than BCG, namely 150 g (according to product label).

Chemical Composition

The chemical composition of SSG and SBG compared with commercial bovine gelatin (BCG) was shown in Table 2. Analysis of variance showed the type of gelatin had a significant effect (p <0.05) on moisture, protein, fat, and ash content.

The water content of the three types of gelatin showed significant differences (p <0.05), where the water content of SBG was lower than SSG and BCG. The difference in gelatin water content was influenced by differences in raw materials and extraction processes used. The maximum level of gelatin water content based on the **Indonesian National Standard (SNI, 1995)** was 16%, while the **British Standard Institution (BSI, 1975)** regulates maximum water content between 13 to 14%. Based on this, the snakehead gelatin has fulfilled the standard regulations imposed.

The protein content of SSG was lower than SBG and BCG (p <0.05). This difference may be related to the water content which tends to be low on SBG. Protein levels according to **Pranoto, Marseno and Rahmawati (2011)** can be used as a benchmark for evaluating the purity of gelatin. Protein levels in gelatin do not have a standard, but the levels can vary depending on the source of the raw material used. Different methods of extraction and pretreatment will affect the protein content obtained. The protein content of SBG was higher than SSG, in contrast to the results of research by **(Muyonga, Cole and Duodu, 2004a)** on the skin and bone (*Lates niloticus*) which produced a higher protein range in the skin (87.4 ±5.6 to 88.7 ±2.4) than bone (82.0 ±2.1 to 82.9 ±4.3) as was the case with **Wulandari, Suptijah and Tarman (2015)** and **Ratnasari et al. (2013)** in snakehead skin, were 96.21% and 87.27 ±0.78, respectively. Although **See et al. (2010)** were also only able to produce snakehead skin gelatin with lower protein levels, namely 75.63 ±1.05. Different contents of protein can be influenced by factors such as raw materials, pretreatment processes, and extraction methods **(Gomez-Guillen et al., 2011; Jamilah and Harvinder, 2002; Nagarajan et al., 2012)**.

The other important chemical compositions of gelatin were fat and ash. The highest SBG fat content was 1.05 ±0.05%. The relatively high-fat content in SBG compared to SSG can be attributed to the fat content of raw materials, where the fat of the snakehead bone was higher than the skin **(Rosmawati et al., 2018a)**. There was no definite standard for the minimum level of gelatin fat, but may be high levels of fat that can affect the quality of gelatin, including the shelf life. The fat content of commercial bovine skin gelatin was 0.23% **(Pranoto, Marseno and Rahmawati, 2011)**. This value was lower than the fat content of SSG but relatively higher than the fat content of BCG.

The gelatin ash content of the research results showed a fairly low value than **Indonesian National Standard (1995)**, which was less than 3.25%, whereas according to **GMIA (2012)** standard for type A gelatin which is about 0.3 to 2%. Ash content of SSG was lower than SBG and BCG, respectively (p <0.05). The demineralization process before the extraction effectively has reduced the ash content of SBG.

The variation in chemical composition between gelatin occurs mainly due to differences in methods during the process of changing collagen to gelatin **(Gomez-Guillen et al., 2011)**, protein content in raw materials, including collagen levels, amount of dissolved components in the skin, initial treatment and washing process and extraction method used **(Abedinia et al., 2017; Jamilah and Harvinder, 2002)**. Meanwhile, according to **Irwandi et al. (2009)**, the chemical composition of gelatin produced can be related to the environment and type of fish feed.

Amino Acid Composition

The amino acid composition of skin gelatin (SSG) and snakehead bone (SBG) compared to commercial gelatin (BCG) was presented in Figure 2. Glycine and proline were two types of the main amino acid **(Jamilah and Harvinder, 2002)** and were the highest in all types of gelatin were observed.

The characteristic feature of collagen and its derivatives was that the amino acid content of glycine and proline was higher than other amino acids **(Nagarajan et al., 2012)**, and glycine is the main amino acid **(Irwandi et al., 2009)**. However, specifically, the presence of proline and hydroxyproline were the main determinants of gelatin quality related to the gel strength, unfortunately,

hydroxyproline gelatin was not analyzed, and so it was rather difficult to predict gel strength really due to the presence of proline alone. It can be seen in Figure 2 that the highest proline levels of BCG then SSG and SBG, whereas the strength of the BCG gel was the lowest compared to SSG and SBG. The content of amino acids between fish and mammals was different, but its presence determines the stability of the gelatin conformation during the gel formation process **(Haug, Draget and Smidsrød, 2004)**. As **Gautieri et al. (2009)** emphasized that glycine was the most important amino acid in stabilizing collagen protein tertiary molecular structure whereas **Pranoto, Marseno and Rahmawati (2011)** suggested that the percentage of glycine can affect the binding properties of gelatin by water and lead to high values of gel strength and viscosity. The higher the temperature and the longer the extraction time affects the gel strength, this is assumed because the amino acids undergo cleavage in the peptide chain, and at the same time degradation occurs during the acid hydrolysis process **(Darragh et al., 1996)**.

Glutamate acid and alanine show levels that were not very different, and it seems that the levels of these two amino acids were higher in succession in the SSG than SBG and BCG. Arginine was also found to be quite abundant, respectively SBG, SSG, and BCG. The high levels of three amino acids may be linked to the source of the raw material of gelatin which was rich in these amino acids **(Rosmawati et al. 2018b)**. The other amino acids such as serine, aspartate acid, phenylalanine, threonine, leucine, isoleucine, methionine, tyrosine, valine, and histidine show an average of fewer than 5 residues/100 residues. Cysteine and tryptophan were not found in gelatin **(Haug and Draget, 2009)**, apart from the fact that both amino acids were present in sufficiently small amounts in their raw materials, these amino acids were thought to be susceptible to damage during the hydrolysis process. Amino acids were organic materials, that were bipolar by the presence of amine groups ($-NH_2$) and carboxyl (-COOH-), and side-chain groups (-R) that were specific and determine the level of reactivity of amino acids. Differences in side-chains cause differences in the physicochemical properties of proteins **(Aftabuddin and Kundu, 2007)**. Amino acids were grouped according to the type of side chain based on their solubility in water, hydrophilic, and hydrophobic amino acid **(Barrett and Elmore, 1998)**. Amino acids of gelatin with side chains were hydrophobic are alanine, leucine, valine, isoleucine, methionine, and phenylalanine, and hydrophilic side chains namely glycine, glutamate acid, lysine, serine, threonine, tyrosine, histidine, proline, arginine, and aspartate. The total percentage of amino acid groups based on their solubility in water was presented in Figure 3.

The hydrophilic amino acid of BCG was slightly higher than in SSG and SBG, and vice versa in the hydrophobic amino acids SSG and SBG were relatively slightly higher than the hydrophilic amino acids.

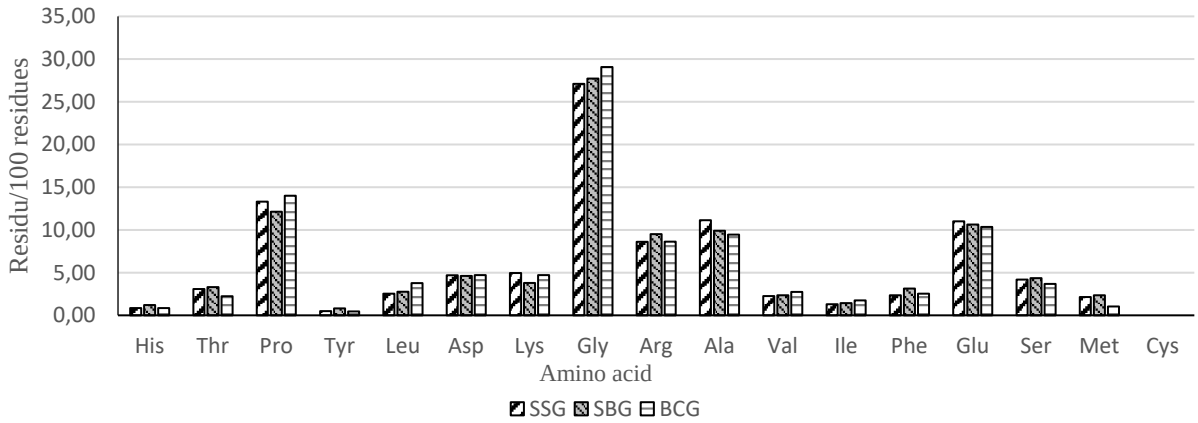

Figure 2 The amino acids composition of snakehead skin gelatin (SSG), snakehead bones gelatin (SGB), and bovine commercial gelatin (BCG).

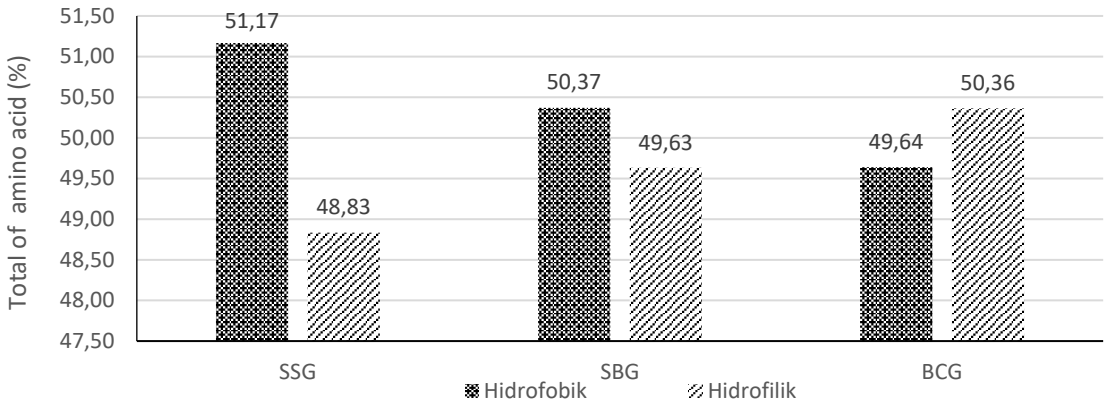

Figure 3 Comparison of total hydrophilic and hydrophobic amino acids (%) from snakehead skin gelatin (SSG), snakehead bone gelatin (SBG), and bovine commercial gelatin (BCG).

Table 3 Index of emulsion activity (IAE) and Index of stability emulsion (ISE) of snakehead skin gelatin (SSG) and snakehead bone gelatin (SBG) through the extraction process use the best extraction temperature and time and compared with bovine commercial gelatin (BCG).

	IAE ($m^2.g^{-1}$)	ISE (min)
SSG	32.49 ±0.28^b	26.25 ±0.19^c
SBG	28.47 ±0.36^a	19.16 ±0.18^a
BCG	28.92 ±0.09^a	25.42 ±0.16^b

Note: Different superscripts in the same column indicate a significant difference (p <0.05). Values are presented as mean ±*SD* (n = 3).

Tabel 4 Water holding capacity (WHC) and fat binding capacity (FBC) of snakehead skin gelatin (SSG) and snakehead bone gelatin (SBG) from the extraction process used the best temperature and extraction time and compared with bovine commercial gelatin (BCG).

	Water holding capacity (%)	Fat binding capacity (%)
SSG	192.53 ±4.76^b	223.97 ±4.80^a
SBG	166.48 ±2.87^a	227.67 ±3.28^a
BCG	298.85 ±4.30^c	219.10 ±3.14^a

Note: Different superscripts in the same column indicate a significant difference (p <0.05). Values are presented as mean ±*SD* (n = 3).

Aftabuddin and Kundu (2007) stated that the average level of the hydrophobic node tends to be greater than the hydrophilic node. Consequently, the ability of these amino acids to bind to fat greater than their ability to attract water. Different from BCG which has a hydrophilic amino acid that was relatively larger than its hydrophobic amino acids, it will have an impact on the ability to attract water that was greater than its ability to bind fat.

Between SSG and SBG tends to show differences that were not so striking in the amino acid levels. But this relatively small difference still influences the functional characteristics of the two types of gelatin, as well as those reported in the gelatin of the skin and bones of *Lates niloticus* **(Muyonga, Cole and Duodu, 2004a; Muyonga, Cole and Duodu, 2004b)**. Extraction methods and different types of raw materials can result in differences in amino acid composition between the three types of gelatin observed.

Index of Emulsion Activity (IAE) and Stability of Emulsion (ISE)

Gelatin is one type of hydrocolloid that has been widely used as an emulsifier in the industry, including food, pharmaceuticals, medicines, and other technical applications because it has surface-active properties **(Jellouli et al., 2011)**. This was a function of the ability of a surfactant to be hydrophilic and lipophilic at once, to reduce the surface tension caused by mixing oil and water. Gelatin was a type of natural surfactant, as a function of amino acids. The emulsion activity index and stability index of SSG, SBG, and BCG emulsion were presented in Table 3.

The index of emulsion activity of the three types of gelatin differed from one another (p <0.05).

The index of emulsion activity (IAE) of each type of gelatin was relatively different. The index of emulsion activity of SSG was higher than BCG and SBG. The value of IAE reported by **Jellouli et al. (2011)** in the gelatin of gray triggerfish (*Balistes capriscus*) skin was lower (21.44 ±0.09), and as was reported by **(Jridi et al., 2013)** in gelatin cuttlefish skin (*Sepia officinalis*) were 25.97 ±1.05, 25.42 ±0.95, 27.64 ±1.26 and 27.65 ±1.35 respectively. The difference of IAE of gelatin can be caused by differences in sources of raw material **(Jellouli et al., 2011; Jridi et al., 2013; Zhang et al., 2012)** and extraction processes applied **(Ahmad and Benjakul, 2011; Kaewruang et al., 2013)**, which has implications for the intrinsic character of gelatin **(Khiari et al., 2013)**. The important intrinsic factor that influences the activity of gelatin emulsion was the contribution of amino acids making up the gelatin protein. The amino acid of gelatin with an active side chain gives the gelatin the ability to be hydrophilic (polar charge) and hydrophobic (non-polar charge). The peptide chain of hydrophobic amino acids contributes to the emulsifying properties of gelatin, because of the presence of surface-active properties, where the greater the content of hydrophobic amino acids, the more emulsion activity tends to be higher **(Karim and Bhat, 2009)**. **Ahmad and Benjakul (2011)** further explained that surface activity in the interface of water and oil in gelatin gave the ability to facilitate the formation and stabilization of droplets during and after the emulsion process. The emulsion activity index was related to the area between surfaces, where the higher the emulsion activity index value, the smaller the size of the fat globule. The emulsion activity index shows the ability of proteins to stabilize oil and water interfaces.

In Table 3, there was a significant effect (p <0.05) of the gelatin type on the index of emulsion stability (ISE). The index of emulsion stability of SBG was smaller than BCG and SSG. Among the three types of gelatin, SSG appeared to show a better level of emulsion stability than BCG and SBG. However, the ISE of SBG was better than BCG, and in the same emulsion activity, SBG can achieve its stability in a shorter period. The same properties were reported by **Jellouli et al. (2011)** in gray triggerfish (*Balistes capriscus*) which had better ISE than bovine gelatin.

The emulsifying properties of gelatin were described as emulsion capacity or emulsion activity, which showed the ability of gelatin to aids the formation and stabilization of newly created emulsions and their ability to give emulsion

strength for the resistance of pressure. The index of emulsion activity was an interface measurement area. It was stabilized over a unit of protein weight ($m^2.g^{-1}$), and it was related to the ability of proteins to coat an interface **(Pearce and Kinsella, 1978)**. The emulsion activity index was influenced by the intrinsic factor of gelatin protein. Adding gelatin to an oil-water emulsion solution affects the oil-water interface. Emulsifying properties were produced due to the presence of hydrophobic regions in the peptide chain **(Karim and Bhat, 2009)**. The hydrophobic nature of the side chains of gelatin amino acid peptides reduces surface tension so that emulsion stability can be achieved. However, this depends on how large the ability of hydrophobicity of gelatin. The higher level of hydrophobic amino acid, the greater the IAE, and the shorter the ISE to achieve its stability. According to **Ahmad and Benjakul (2011)**; **Jellouli et al. (2011)**, the molecular weight can also influence the dispersion ability between oil and water, where the level of stability of emulsion from gelatin at high molecular weights results in better levels of stability than gelatin from lower molecular weights.

Water Holding and Fat Binding Capacity of Gelatin

Water holding and fat binding capacity were functional properties that were closely related to the texture of food products as interactions between components such as water, oil, and other components **(Ismail et al., 2010; Rawdkuen, Thitipramote and Benjakul, 2013)**. The results of the analysis in Table 4 showed that the type of gelatin has a significant effect (p <0.05) on the water holding capacity (WHC). Snakehead bone gelatin (SBG) has the lowest WHC and was highest in BCG and then SSG. The Differences in extraction methods, raw materials, and animal species were factors that influence the differences in the characteristics of gelatin. This difference also causes the WHC of the three types of gelatin to tend to be different.

Water holding capacity as in Table 4 show the percentage of BCG was relatively higher than the two types of gelatin from snakehead, but the percentage of both is still relatively higher than the WHC of cobia fish skin gelatin (97.03 ±1.53) **(Amiza et al., 2015)**. A study was carried out by **Balti et al. (2011)** in cuttlefish (*Sepia officinalis*) showed a relatively similar percentage with WHC on SSG and SBG, which was about 150 to 200%. The high WHC can be attributed to the hydrophilic amino acid content of gelatin **(Nurul and Sarbon, 2015)** and the main chemical component of gelatin, namely hydroxyproline **(Ninan, Jose and Abubacker, 2011; Zarai et al., 2012)**. Allegedly, if the hydroxyproline level was high then the ability to hold water of gelatin would also follow **(Jeya et al., 2012)**.

Water holding was one of the important functional properties in the processing process because of its function in improving the texture and compactness of food products. This trait according to **Zarai et al. (2012)** refers to the ability of proteins to absorb water and maintain it against the gravitational force in the protein matrix. Furthermore, **Zarai et al. (2012)** that its capacity to holding water makes it suitable and convenient to be applied to various frozen meat and fish products to reduce drip loss during the thawing or cooking process which impact protein denaturation resulting in partial loss of capacity to hold of water.

Table 5 The amide absorption area of snakehead skin gelatin (SSG), snakehead bone gelatin (SBG), and bovine commercial gelatin (BCG) were based on the FTIR spectrum.

Amide	Absorption peak area (cm^{-1})*	Wavenumber (cm^{-1})		
		SSG	SBG	BCG
A	3600 – 3200	3388.93	3383.14	3442.94
B	3100 – 2300	3080.32	3076.46	-
		2935.66	2929.87	2956.87
		2881.65	2879.72	2927.94
		2360.87	2360.87	2360.87
		2335.80	2335.80	2335.80
I	1656 – 1644	1653.00	1651.07	1653.00
II	1560 – 1335	1541.12	1521.84	1543.05
		-	-	1517.98
		1450.47	-	1454.33
		1413.82	1411.89	-
		-	-	1396.46
		-	-	1335.67
III	1240 – 670	1234.44	1234.44	1238.30
		1197.79	1197.79	1199.72
		-	1166.93	1163.08
		1076.28	1074.35	1078.21
		1028.06	1028.06	1031.92
		970.19	970.19	-
		923.90	921.97	923.90
		873.75	871.82	875.68
		-	-	673.16

Note: * **Muyonga, Cole and Duodu (2004c)**; **Uriarte-Montoya et al. (2011)**; **Kong and Yu (2007)**.

The three types of gelatin observed did not show any significant difference (p >0.05) on FBC. The fat binding capacity of SBG was relatively slightly higher than in SSG and BCG. Both showed lower percentages than FBC from red snapper bone gelatin (*Lutjanus campechanus*) and brown spotted grouper (*Epinephelus chlorostigma*) which were 493.90% ±4.20 and 429.57% ±4.73 respectively **(Jeya et al., 2012)**, but it is higher than the cobia skin gelatin (*Rachycentron canadum*) which was 164.0% **(Amiza et al., 2015)**. The difference in FBC from gelatin cannot be separated from the characteristics of the gelatin itself, which among others was influenced by raw materials and extraction methods so that it may result in differences in the composition of amino acids. The ability of gelatin to bind fat was mainly facilitated by hydrophobic amino acids **(Jellouli et al., 2011)**, the greater the ability to bind fat tends to be higher Hydrophobic amino acids include tyrosine, isoleucine, valine, leucine, proline, phenylalanine, methionine and alanine. **Ninan, Jose and Abubacker (2011)** state that high levels of tyrosine were thought to be responsible for the binding capacity of fat in gelatin.

Functional Group of Gelatin

Analysis using Fourier transform infrared (FTIR) spectroscopy was carried out to identify changes in the functional group and secondary structure of gelatin **(Muyonga, Cole and Duodu, 2004b)** based on the vibration spectra produced by the compounds contained therein at certain wavelengths. The absorption value of snakehead fish gelatin (SSG and SBG) compared to BCG was presented in Table 5.

Amide A peaks from SSG, SBG, and BCG in the absorption area of 3388.93 cm^{-1}, 3383.14 cm^{-1}, and 3442.94 cm^{-1}, respectively. The smaller value absorption that appears in the spectra, indicates a lower free amine group, which means less degradation. Absorption values of BCG which were in the range above 3400 cm^{-1} indicate that wavenumbers were in the vibration region of free NH stretch **(Ahmad and Benjakul, 2011; Kittiphattanabawon et al., 2015; Sinthusamran, Benjakul and Kishimura, 2014)**. The SSG and SBG absorption areas show a slightly lower wavenumber, that was in the absorption area of 3400 – 3200 cm^{-1}, which means there was a shift in wavenumbers to a lower frequency, this was because there were NH groups of peptides involved with hydrogen bonds **(Ahmad and Benjakul, 2011; Muyonga, Cole and Duodu, 2004c)**. A relatively high SSG wave number compared to SBG shows that the hydrolysis process of snakehead fish skin at a temperature of 60 °C for 12 hours causes relatively more hydrogen bonds to be released than hydrogen bonds in snakehead bone at the same temperature. This indicates that the type of raw material and the relative extraction process affect the secondary structure of the gelatin protein, besides this difference according to **Kittiphattanabawon et al. (2015)** may also be due to differences in amino acid composition and sequence.

The amide I band between SSG and BCG were at the same wavenumber, which was slightly higher than SBG, namely 1653.00 cm^{-1}, 1653.00 cm^{-1}, and 1651.07 cm^{-1}, respectively. The difference in the type of raw material and the extraction method used causes the wavenumbers of the two types of snakehead fish gelatin to be different. At the same temperature (60 °C) with different extraction times, the SBG wave number (24 hours of extraction) was slightly lower than SSG (12 hours of extraction) indicating that the level of C=O reactivity in the skin was able to open and act reactively more quickly on the chain- α compared to snakehead fish bones, so this situation is according to **Jridi et al. (2015)** has the potential to cause the loss of the triple helix due to excessive hydrolysis of the intermolecular cross-linking. Furthermore, this situation by **Yu et al. (2014)** is alleged to have caused the loss of amino acid reactivity, including lysine, hydroxylysine, and histidine, which function to maintain inter and intra-molecular cross-links in the telopeptide region.

In the amide band II, the peak wavenumbers between SSG, SBG, and BCG have shown the absorption values were 1541.12 cm^{-1}, 1521.84 $cm^{-1,}$ and 1543.05 cm^{-1}, respectively. The amide region II showed an abnormality in the molecular structure due to the transformation of α-helix into a random coil structure due to the partial hydrolysis process during extraction **(Muyonga. Cole and Duodu. 2004b)**, which was characterized by the occurrence of NH flexural vibrations coupled with CN stretching vibrations **(Yu et al., 2014)**. The temperature and length of extraction time can cause changes in the triple helix as a result of the denaturation of collagen to gelatin **(Muyonga, Cole and Duodu, 2004b)**. The smaller the wavenumber, the smaller the triple helix damage rate observed.

The SSG and SBG wavelengths in the peak region of the amide band III showed the same absorption value, namely 1234.44 cm^{-1}, while BCG was at 1238.30 cm^{-1}, indicating a greater disturbance in the molecule, and this according to **Sinthusamran, Benjakul and Kishimura (2014)** associated with the loss of the triple helical structure. Amide region III was a spectrum region of 1220 – 1320 cm^{-1} and is associated with CN stretching and NH deformation as well as absorption arising from vibrations of CH2 groups from the glycine backbone and proline side chains **(Ahmad and Benjakul, 2011)**.

CONCLUSIONS

The snakehead skin and bone gelatin had different characteristics, and this was greatly influenced by the temperature and time of extraction. The higher the temperature and extraction time, the yield of both gelatins tended to increase. The gel strength between both gelatins was more influenced by the extraction time, which was 60 °C 12 h for the snakehead skin, and 60 °C 24 h for the snakehead bones. The difference gel strength as a characteristic indicator of snakehead fish skin and bone gelatin parameters was compared with commercial bovine gelatin. The amino acids of the three types of gelatin showed higher levels of glycine and proline compared to other types of amino acids. Based on the total residues of each amino acid, both skin gelatin and bone gelatin showed more dominant hydrophobic properties than hydrophilic properties, however vice versa in commercial bovine gelatin. The three types of gelatin showed different differences in chemical composition, index of emulsion activity, the stability of the emulsion, water holding, and fat binding capacity, and this was thought to be closely

related to the source of the raw material and its amino acid content. The characteristics of the three types of gelatin were supported through FTIR analysis and showed snakehead fish skin and bones as potential gelatin equivalent to commercial gelatin.

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