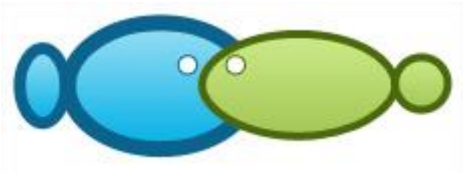

Physicochemical characteristics of Amazon sailfin catfish (*Pterygoplichthys pardalis*) skin gelatin

[1]Masrurah Ismail, [2]Kasmiati, [2]Metusalach

[1] Graduate Student in Fisheries Science, Faculty of Marine and Fishery Sciences, Hasanuddin University, Makassar, Indonesia; [2] Department of Fishery, Faculty of Marine and Fishery Sciences, Hasanuddin University, Makassar, Indonesia. Corresponding author: Metusalach, mminanga@hotmail.com

Abstract. Gelatin is a protein biopolymer obtained by partially denaturing collagen. Gelatin research on fish has been carried out, especially on economically important fish, but only a few studies have referred to non-economical fish. One of the non-economical fish with a high population and considered a pest by fishermen in Tempe Lake of South Sulawesi, Indonesia, is the Amazon sailfin catfish (*Pterygoplichthys pardalis*). This study aimed to characterize the gelatin extracted from the skin of the Amazon sailfin catfish. The experiment employed a completely randomized factorial design with two factors namely extraction temperature and duration of extraction. Each factor consisted of three levels with extraction temperatures of 70, 80 and 90°C, and duration of extraction of 2, 4 and 6 hours. The results showed that the extraction temperature and time affected the gelatin yield, and that the highest yield (3.99%) was obtained at 80°C and 6 hours extraction treatments. The gelatin contained 8.84% water, 81.08% protein, 9.07% ash, 0.73% fat, 0.2% crude fiber, 200.68 bloom gel strength, 4.33 cP viscosity, and pH of 4.25. FTIR test of the gelatin showed the wavenumber of 1635.64 cm^{-1} (Amide I), 1552.70 cm^{-1} (Amide II), 1242.16 cm^{-1} (Amide III), 3496.94 cm^{-1} (Amide A), and 2926.01 cm^{-1} (Amide B). The main amino acid constituents of the gelatin were proline 12.40% and glycine 21.48%. The heavy metal content of Amazon sailfin catfish skin gelatin was below the threshold value of the Indonesian National Standard (SNI), namely 0.0000 mg/kg (Cd); 0.0000 mg/kg (Hg); and 0.00122 mg/kg (Pb). The characteristics of the gelatin in this study were comparable with those of the commercial gelatin standards, and therefore the skin gelatin of the Amazon sailfin catfish is as a potential alternative source of fish gelatin.
Key Words: amide, amino acids, FTIR, gel strength, heavy metals, viscosity.

Introduction. Gelatin is a soluble polypeptide derived from collagen, which is the main constituent of animal skins, bones, and connective tissues. Gelatin is obtained by partial hydrolysis of collagen by acid or alkaline and thermal treatments. The fibrous structure of collagen is broken down irreversibly to produce gelatin (Zhou & Regenstein 2004). In Indonesia, gelatin is usually an imported product, mostly from Europe, America, and China. Gelatin has been widely used in the food industry as a stabilizer, thickener, emulsifier, jelly forming binder, and food wrapping (edible coating). In the pharmaceutical industry, gelatin is used as an ingredient for capsules, cosmetics, and films (Hashim et al 2010).

According to Abdullah et al (2016), in the last few years due to health problems and religious issues, increasing demands are made on the exploration of new alternative sources for gelatin raw materials. Health problems that arise, according to Kittiphattanabawon et al (2010), refer to Foot and Mouth Disease (FMD) and spiritual issues are related to Muslims who are not allowed to consume gelatin from skin and bones derived from pork.

On the other hand, processing of fish produces waste or byproduct that reaches 20-60% of the raw materials. Waste in the form of skin and fish bones accounts for 30% of the trash with high collagen content (Gomez-Guillen et al 2002). This waste is a potential raw material to produce fish-based industrial products, such as fish gelatin. One of the potential sources for gelatin production is the skin of the Amazon sailfin catfish (*Pterygoplichthys pardalis*). This fish is an invasive species in Tempe Lake of South

Sulawesi, Indonesia, and can survive in highly polluted environments. The Amazon sailfin catfish is an omnivorous fish that can eat about everything (Cook-Hildreth et al 2016). Because of the invasive nature and underutilized or unutilized status, its population density in the Tempe Lake is high. Unfortunately, neither scientific nor official data report on the population density of the Amazon sailfin catfish in Tempe Lake has existed. On the other hand, fishermen in the whole area of Tempe Lake claim that 70% of their daily catch is made up by the Amazon sailfin catfish.

Gelatin can be extracted using various types of extraction methods such as thermal, acid, alkaline, and enzymatic extraction. However, the most preferred method is the combination of acid and alkaline solvents. It can provide a better quality of gelatin than direct thermal treatment which produces low-quality gel (Herpandi et al 2011). Pre-treatment with a combination of acid and alkaline solvents is the best method for extracting gelatin from fish skin. Sinthusamran et al (2014) and Hashim et al (2017) reported that extracting gelatin using a combination of acid and alkaline not only removes non-collagen protein but also can improve the physicochemical quality of gelatin from the skin of barramundi (*Lates calcarifer*), cobia (*Rachycentron canadum*), kawakawa (*Euthynnus affinis*) and Japanese scad (*Decapterus maruadsi*). This paper describes the physicochemical characteristics of gelatin extracted from the skin of the Amazon sailfin catfish (*P. pardalis*) pretreated with acid and alkaline solvents prior to extraction with distilled water at various extraction temperatures and times. This gelatin could be a religiously accepted (halal) food ingredient.

Material and Method

Materials and chemicals. The Amazon sailfin catfish (*P. pardalis*) specimens were obtained from fishermen at Tempe Lake, South Sulawesi, Indonesia, from March to May 2020. As many as 200 specimens were studied, with the total length of the specimens ranging from 15 cm to 30 cm and with weights ranging from 158 g to 574 g. The fish skin was separated, cleaned from remaining meat, washed under running tap water, placed into a polyethylene plastic bag, and stored in a freezer at -20^{O}C until used. Other materials used were sodium hydroxide (NaOH), phosphoric acid (H_3PO_4), potassium bromide (KBr), hydrochloric acid (HCl), ammonium bicarbonate (NH_4HCO_3), sulfuric acid (H_2SO_4), sodium thiosulfate ($Na_2S_2O_3$), boric acid (H_3BO_3), trypsin, hexane, distilled water, Kjeldahl tablets, bromocresol green and methyl red indicators, filter paper, filter cloth, syringe filter, standard solution of cadmium (Cd), mercury (Hg) and lead (Pb), nitric acid (HNO_3), vanadium pentaoide (V_2O_5), hydrogen peroxide (H_2O_2), deionized distilled water, anti-bumping granules.

Pre-treatment. The fish skin was first separated from the sticky meat and then cleaned under running tap water. The skin was drained using a perforated plastic container until no water dripped. The skin was weighed to the nearest 100 g per sample and soaked in water with a temperature of 55-60^{O}C for 2-3 min. The skin was then drained over a filter cloth to remove grease and dirt. Further, the skin was soaked in 0.05M NaOH (1:3, w/v) for 12 hours and then washed with running tap water until the pH of the skin reached a neutral value. The skin then was soaked in 4% H_3PO_4 (1:3, w/v) for 16 hours. Finally, the skin was washed again with running tap water until the pH of the skin reached a neutral value, then it was stored in the refrigerator (Modena VM-1350) for 24 hours.

Extraction of gelatin. The gelatin was extracted using distilled water with a ratio of skin to solvent of 1:4 (w/v). The extraction process was started by first setting the water bath (Shaking Water Bath YNC-SWB-50L) temperature according to the predetermined extraction temperature and time. The extraction temperature and time used were 70, 80 and 90°C, and 2, 4 and 6 h, respectively. For each predetermined treatment, three replicates of an approximately 100g each (total of 18 samples) of the skin samples were transferred into 500 ml beaker glasses and placed into the water bath with a continuous shake until the predetermined extraction time ended. After extraction, the gelatin was then subjected to a two-step filtration, first using a filter cloth and second with a Whatman No.1

filter paper. The separated gelatin was then dried in an oven (Memmert Oven Laboratory UN 55 53L) a temperature of 55^{O}C for 48 hours.

Yield of gelatin. The yield of gelatin was calculated from the ratio of the weight of dry gelatin obtained to the wet weight of the cleaned skin (Shyni et al 2014).

$$\text{Yield (\%)} = \frac{weight\ of\ dry\ gelatin\ (g)}{weight\ of\ cleaned\ wet\ skin\ (g)} \text{ x } 100$$

FTIR (Fourier Transform Infrared). FTIR analysis was used to determine the specific functional groups of the gelatin. Two gelatin samples (one was extracted at 80°C for 6h and the other at 90°C for 6h) were used for the FTIR analysis. A 6% (w/w) mixture of gelatin powder and phosphoric acid was prepared. Then 2 mg of the mixture were evenly mixed with 100 mg of potassium bromide (KBr) powder. This mixture was then used to prepare a KBr disc. Furthermore, the disc was scanned in an FTIR spectrometer in the range 500-4000 cm^{-1} wave length with a wave length resolution of 4 cm^{-1}. The resulting IR (infrared) absorption of the gelatin sample was then compared to that of the commercial gelatin.

Protein content (AOAC 2000). The protein content of two gelatin samples produced in experimental treatment of 80 and 90ºC for 6 h was performed in triplicates. Approximately 0.5 g of the gelatin was weighed and transferred into Kjeldahl flasks, then three Kjeldahl tablets and 10 mL of H_2SO_4 were added. The flask containing the sample was digested in a heating block at a temperature of 410ºC. Then, 10 mL of water were added, and the heating was continued until the solution became clear. The solution was cooled, then 50 mL of distilled water and 20 mL of 40% NaOH were added and filtered. The filtrate was transferred into a distillation flask and 50 mL of NaOH-$Na_2S_2O_3$ solution were added. Distillation was carried out and the distillate was collected in a 125 mL Erlenmeyer flask containing 25 mL of 2% boric acid (H_3BO_3) containing a mixed indicator of 0.1% bromocresol green and 0.1% methyl red with a ratio of 2: 1. Distillation was performed until the volume of the distillate in the Erlenmeyer flask reached 40 mL. At this point, the distillate in the Erlenmeyer flask displayed a bluish-green color. The resulting distillate was titrated with 0.1004N HCl until the color of the solution turned pink. The volume of the titrant was recorded. The protein content was then calculated as follows:

$$\text{\% N} = \frac{ml\ HCl \text{ used to titrate } sampel - ml\ HCl \text{ used to titrate } blank)}{Sampel\ weight\ (g) x\ 1000}\ x\ N\ HCl\ x\ 14{,}008\ x\ 100$$

% protein = % N x protein conversion factor (5.6) (Mariotti et al 2008)

Fat content (AOAC 2000). Six samples of ± 5 g each were crushed and then weighed (W1) and wrapped using filter paper and put into a thimble. The thimble was then fitted into a Soxhlet and hexane for 1½ cycles was added. Extraction was carried out for ± 6 h. The hexane was evaporated under vacuum using a rotary evaporator (50 rpm, temperature of 69°C). The hexane-free fat was then heated in an oven at 105°C for 1 h.

$$\text{Fat content (\%)} = \frac{W3-W2}{W1} \text{ x } 100$$

W1 = sample weight (g)
W2 = weight of empty crucible (g)
W3 = weight of crucible + extracted fat (g)

Ash content (AOAC 2000). Six samples of 3-5 g each of the gelatin powder were weighed into ashing crucibles and then heated in a muffle furnace at 500-600ºC until a constant weight was obtained. The remaining mineral were then weighed, and the ash content was calculated.

$$\text{Ash Content (\%)} = \frac{Weight\ of\ ash\ (g)}{Weight\ of\ sample\ (g)} \times 100$$

Ash weight = weight of crucible and sample after ashing - weight of empty crucible
Sample weight = weight of crucible and sample before ashing - weight of empty crucible

Moisture content (AOAC 2000). Six samples of 3-5 g each of gelatin powder were weighed and put into a dry cup of known weight, then the sample were dried in an oven at 105°C for about 6 h. The cup containing the sample was cooled and weighed, then dried again until a constant weight was obtained.

$$\text{Wet base moisture content (g 100 g}^{-1}\text{ wet material)} = \frac{W-(W1-W2)}{W} \times 100$$
$$\text{Dry base moisture content (g 100 g}^{-1}\text{ dry matter)} = \frac{W-(W1-W2)}{W1-w2} \times 100$$

W = weight of sample before drying (g)
W1 = sample weight and dry cup (g)
W2 = weight of empty cup (g)

Since the gelatin produced by the treatment of 80ºC for 6 h showed a better proximate composition, the remaining measurements (pH, viscosity, gel strength and amino acid composition) were done only for the gelatin from this treatment.

Determination of pH. Triplicate measurements were carried out to determine the pH value of the gelatin. Gelatin powder (0.2 g) was dissolved in 20 mL of distilled water at a temperature of 80ºC. The resulting solution was homogenized with a magnetic stirrer. Then, the degree of acidity (pH) was measured at room temperature with a pH meter (pH mV Cond TDS PL-700PC).

Determination of viscosity. Measurement of the gelatin's viscosity was performed in three replicates. A 6.67% gelatin solution was prepared by dissolving 7 g of the gelatin in 105 mL of distilled water. Then, the solution's viscosity was measured using a Brookfield Synchro-Lectric Viscometer. The measurement was performed at 60ºC with a spindle shear rate of 60 rpm. The result was then multiplied by a conversion factor. The measurement was performed using the no. 1 spindle of the viscometer having a conversion factor of 1. The viscosity value was expressed in units of centipoise (cP).

Determination of gel strength. Triplicate determination was carried out to determine the gel strength of the gelatin. As much as 15 mL of the 6.67% gelatin solution was taken and placed in a glass vial of 20 mL volume. The sample was incubated at 10ºC for 17 h, then measured using a CT3 Texture Analyzer (Gomez-Guillen et al 2002). The result of the measurement was in the form of graphs, then the gel strength was calculated using the following formula:

$$\text{Gel Strength (dyne cm}^{-2}) = \frac{F}{g}\ x\ 980$$
$$\text{Gel Strength (bloom)} = 2.86 \times 10^{-3}\ G + 20$$

F = height of the graph before breaking
g = constant (0.07)
G = gel strength (dyne cm^{-2})

Amino acid analysis. The amino acid composition analysis was performed in triplicates. Digestion of gelatin was performed using trypsin, according to Cheng et al (2012). Approximately 100 mg of gelatin were dissolved in 50 ml of ammonium bicarbonate solution (1% NH_4HCO_3, pH 8.0). The solution was then filtered using a syringe filter of 0.22 µm pore size. After that, 100 µL of the gelatin solution was taken and 10 µL of trypsin were

added (1 mg mL^{-1} in 1% NH_4HCO_3, pH 8.0). The sample was incubated at 37°C for 12 hours and subsequently subjected to LC-MS/MS analysis.

Heavy metal analysis. The heavy metal content (Hg, Pb and Cd) of the gelatin was determined using the Thermo Scientific™ iCE™ 3000 Graphite Furnace Atomic Absorption Spectrometer (AAS).

Determination of Hg content (SNI 2354.6:2016). Three samples of gelatin powder (0.2 g each) were transferred into digestion flasks. Into each flask, 10 mg of V_2O_5, 10 ml of 65% HNO_3 and 95% H_2SO_4, and 3-5 anti-bumping stones were added and mixed thoroughly. The mixture was heated until a clear solution was obtained and, upon cooling at room temperature, 2 drops of 30% H_2O_2 and 15 ml of deionized water were added. The mixture was thoroughly mixed and transferred to a 50 ml volumetric flask. The volume was then adjusted using deionized water and then the solution was subjected to the AAS. A blank solution was also prepared following the same procedure as for the sample preparation. The absorbance of the solution was measured, and the content of the Hg was calculated. A calibration curve was prepared based on the absorbance of 1, 5, 10, 15 and 20 µg/L of standard Hg solution.

$$Y = a + bX$$

Y = absorbance
a = intersep
b = slope
X = concentration of Hg in the sample (µg/L)

$$\text{Hg (µg/g)} = \frac{(\text{D} - \text{E}) \text{ x Fd } \times \text{V(ml)} \; x \frac{1}{1000 \; ml}}{\text{W (g)}}$$

D = concentration of Hg in sample (µg/L) from AAS reading
E = concentration of Hg (µg/L) in blank solution from AAS reading
V = total volume of sample solution (mL)
W = sample weight (g)
Fd = dilution factor

Determination of Pb and Cd contents (SNI 2354.5:2011). Three samples (0.5 g each) of gelatin powder were transferred into porcelain crucibles and put into a furnace. The samples were then incinerated at 450°C for 18 h and then cooled down to room temperature. Upon cooling, 50 ml of 5% HNO_3 were added and mixed thoroughly. The volume of the mixture was reduced to about a half by heating over a hotplate. After cooling, the solution was transferred into a 50 ml volumetric flask and the volume was completed with deionized distilled water. The solution was further filtered into an Erlenmeyer flask over a Whatman number 45 filter paper and then transferred into a clean polystyrene tube prior to use for analysis. A blank solution of Pb and Cd (1 µg/ml) was prepared following the same procedure as for the sample preparation. The Pb and Cd contents were then determined at a wave length of 283.3 and 228.8 nm, respectively. A calibration curve for Pb and Cd was prepared based on absorbance of 1, 5, 10, 15 and 20 µg/L of standard solutions.

$$Y = a + bX$$

Y = absorbance
a = intercept
b = slope
X = concentration of Pb or Cd in the sample (µg/L)

$$\text{Pb or Cd (µg/g)} = \frac{(\text{D} - \text{E}) \text{ x Fd} \times \text{V(mL)}}{\text{W}}$$

D = concentration of Pb or Cd in sample (µg/L) from AAS reading
E = concentration of blank (µg/L) from AAS reading
V = total volume of sample solution prepared (mL)
Fd = dilution factor
W = sample weight (g)

Experimental design and data analysis. The experimental design used in temperature treatment and extraction time was a completely randomized factorial design. The Completely Randomized Design model is as follows:

$$Yijk = \mu + \tau i + \beta j + (\tau\beta)\, ij + \varepsilon ijk$$

Yijk = response to the effect of treatment in temperature and the duration to-j extraction to-k replication
µ = common mean
τi = the effect of temperature to-i
βj = effect of immersion time to-j
(τβ) ij = the effect of the interaction of the treatment temperature to-i with the extraction time to-j
εijk = error factor

The research data obtained was analyzed using the analysis of variance (ANOVA) at a 95% confidence level. When ANOVA indicated the presence of a significant difference, a multiple comparison analysis using the Duncan's Multiple Range Test (DMRT) at a 95% confidence level was employed to identify the difference among the treatments.

Results

The yield of gelatin. The yield of gelatin produced from the skin of the Amazon sailfin catfish in this study ranged from 0.89 to 4.17% (0.89-4.17 g of gelatin/100 g wet fish skin) (Table 1). The extraction temperature and time as well as their interaction were significantly ($p<0.05$) affecting the gelatin yield. The highest yield (4.17%) was produced by extracting the gelatin at 90^{O}C for 6 h, while the lowest yield (0.89%) was produced at 70^{O}C for 6 h. However, extraction of the gelatin at 90^{O}C for 6 h produced a similar yield ($p>0.05$) with that of the 80^{O}C for 6 h treatment.

Table 1

The yield of skin gelatin from Amazon sailfin catfish

Temperature (^{o}C)	*Time (h)*	*Yield (%)*
70	2	1.22^{ab}
70	4	1.56^{b}
70	6	0.89^{a}
80	2	1.29^{ab}
80	4	2.65^{c}
80	6	3.99^{e}
90	2	2.4^{c}
90	4	3.25^{d}
90	6	4.17^{e}

Note: Different superscripts indicate significant difference.

Proximate analysis. The proximate composition (Table 2) was performed only for the gelatin powder extracted at 80^{o}C and 90^{o}C for 6 h. The gelatin extracted at 80^{O}C contained higher protein and ash, but lower fat content as compared to that of the gelatin extracted

at 90^{O}C. However, the moisture content in the gelatin produced by the two extraction temperatures was similar. The GMIA (2012) specified the standard quality of a gelatin which should contain maximum of 15% moisture, higher than 90% protein, less than 5% fat, 0.3-2% ash, and no crude fiber and extract material without nitrogen (EMWN).

Table 2

Proximate composition (%) of the Amazon sailfin catfish skin gelatin extracted at 80^{O}C and 90^{O}C for 6 hours

	Temperature (^{o}C)	*Moisture*	*Protein*	*Fat*	*Crude fibre*	*Extract material without nitrogen*	*Ash*
Research	80	8.84	81.08	0.73	0.2	0.07	9.07
	90	8.53	73.57	4.21	0.29	5.24	8.15
GMIA 2012		Maks.15	>90	<5	-	-	0.3-2

Statistical analysis (ANOVA) showed that there were significant variations ($p<0.05$) in the proximate composition of the analyzed gelatin. Among the treatments analyzed, the temperature of 80^{O}C was found to have the highest composition if compared with the 90^{O}C treatment (Table 2). A higher composition indicates a better proximate content in gelatin when compared to a lower composition especially for moisture, protein, ash, and fat. Crude fiber and EMWN were present in both treatments but the treatment at 90^{O}C had higher percentages of both.

FTIR analysis. The results of the FTIR analysis showed that the typical absorption pattern of the gelatin from the two treatments was relatively similar even though differences in intensities existed. Five peaks of amides were identified, namely amide I, amide II, amide III, amide A, and amide B, as shown in Table 3.

Table 3

The FTIR absorption peak of the Amazon sailfin catfish skin gelatin extracted at 80^{O}C and 90^{O}C for 6 hours

	Absorption Area				
	Amide I	*Amide II*	*Amide III*	*Amide A*	*Amide B*
80^{O}C 6 hours	1635,64	1552,70	1242,16	3496,94	2926,01
90^{O}C 6 hours	1633,71	1546,91	1236,37	3473,80	2924,09
Commercial gelatin	1651,07	1543,05	1242,16	3448,72	2939,52

The FTIR characteristics of the Amazon sailfin catfish skin gelatin extracted at 80^{O}C and 90^{O}C for 6 hours were shown in Figure 1 and Figure 2. The gelatin extracted at 80^{O}C for 6 hours showed amide peaks at wavenumbers of 1635.64 cm^{-1} (Amide I) and 1552.70 cm^{-1} (Amide II), indicating the presence of a carbon-nitrogen (C=N) bond. The absorption at a wavenumber of 1242.16 cm^{-1} (Amide III) shows the presence of nitrogen-hydrogen (N-H), carbon-oxygen (C=O), and carbon-hydrogen (CH_2) bonds. Further, the absorption at 3496.94 cm^{-1} (Amide A) indicates the presence of oxygen-hydrogen (O-H) and nitrogen-hydrogen (N-H) bonds, while the absorption at 2926.01 cm^{-1} (Amide B) shows a carbon-hydrogen (C-H) bond. These bonds indicated that the extract consisted of the gelatin.

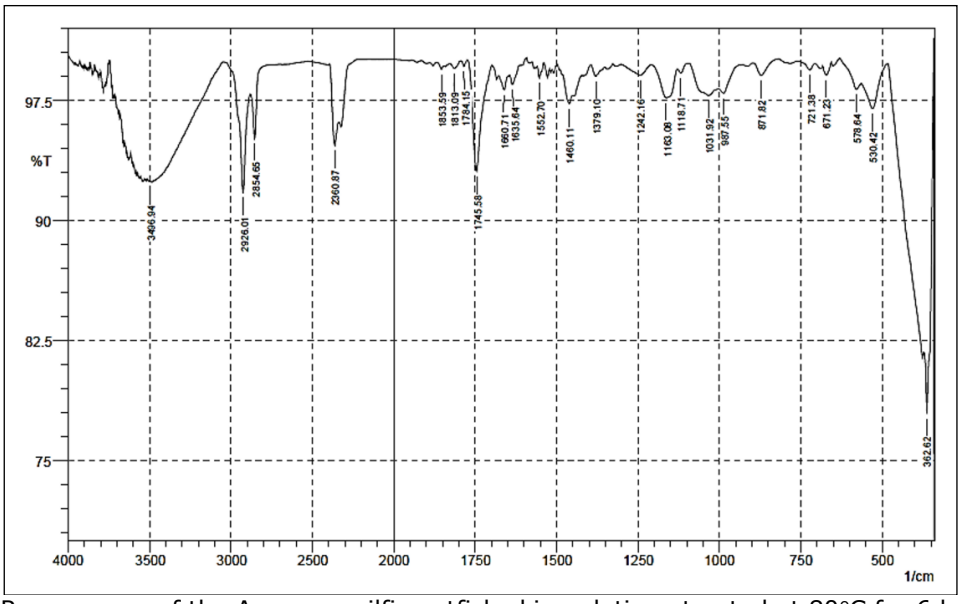

Figure 1. FTIR resonance of the Amazon sailfin catfish skin gelatin extracted at 80°C for 6 hours.

Treatment at 90°C for 6 hours attained a wavenumber of 1633.71 cm^{-1} (Amide I), and 1552.70 cm^{-1} (Amide II) indicating the presence of a carbon-nitrogen (C=N) bond. The absorption area with a wavenumber of 1242.16 cm^{-1} (Amide III) is an indicative of the carbon-oxygen (C=O), nitrogen-hydrogen (N-H), and carbon-hydrogen (CH_2) peptide bonds. The absorption area with a 3496.94 cm^{-1} (Amide A) wavenumber shows the O-H and N-H bonds, while the absorption area with a wavenumber of 2926.01 cm^{-1} (Amide B) indicates the presence of C-H bond. These bonds are characteristic of gelatins, and their presence in the molecular structures of the extract yielded from extraction at 90°C confirms that the extract consisted of gelatin.

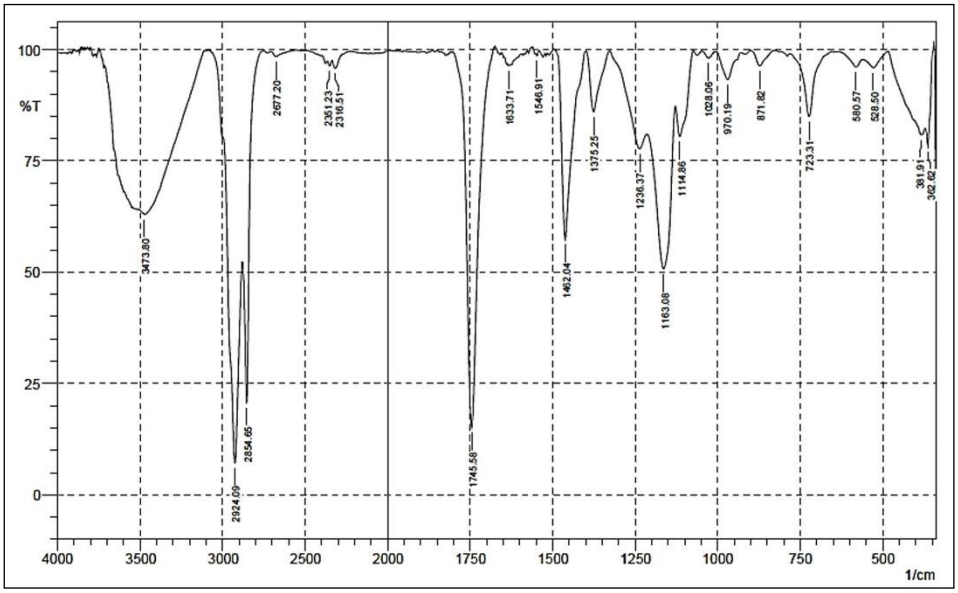

Figure 2. FTIR resonance of the Amazon sailfin catfish skin Gelatin extracted at 90°C for 6 hours.

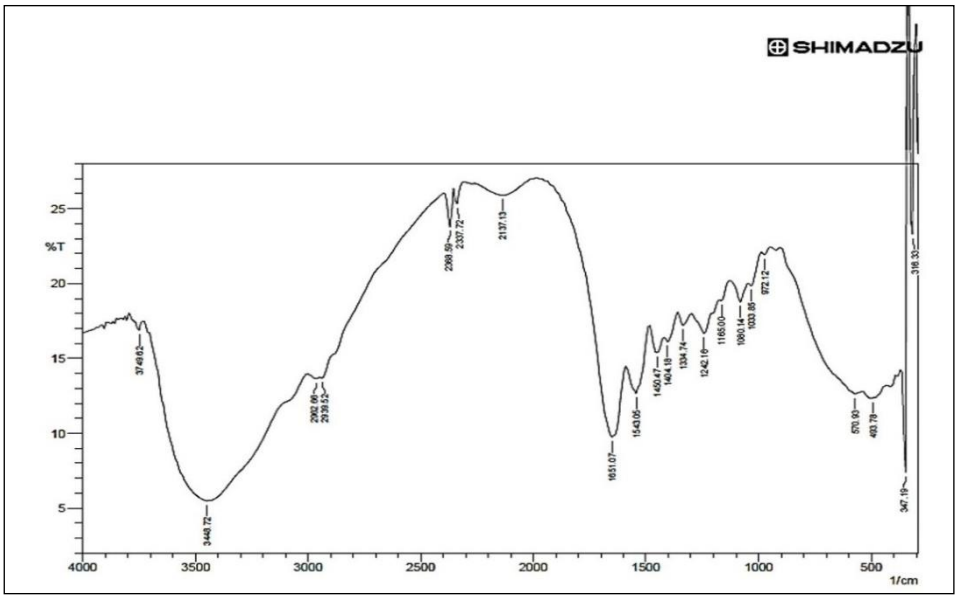

Figure 3. FTIR resonance of the commercial gelatin.

Commercial gelatin powder (bovine gelatin) was used as a reference for determining the Amazon sailfin catfish skin gelatin samples. The results of the FTIR (Figure 3) showed that the spectrum values of the commercial gelatin were almost the same with that of the Amazon sailfin catfish skin gelatin. Besides, the peak of Amazon sailfin catfish skin gelatin spectrum extracted at 80^{O}C was higher than that of the commercial gelatin in the absorption area of 3496.94 cm^{-1} (Amide A).

Degree of acidity (pH), viscosity, and gel strength. The pH, viscosity and gel strength of the Amazon sailfin catfish skin gelatin obtained in this research from the best gelatin treatment, which was at 80^{O}C for 6 h (Table 4).

Table 4

Physical and chemical properties of Amazon sailfin catfish skin gelatin

Parameter	*Value*	*The required standard (GMIA 2012)*
pH	4.25	3.8 - 5.5
Viscosity (cP)	4.33	15 - 75
Gel Strength (bloom)	200.68	50 - 300

Amino acid composition. The Amazon sailfin catfish skin gelatin contained 18 types of amino acids. Six amino acids were found to present in levels of more than 9%, including arginine, lysine, proline, glutamic acid, alanine, and glycine (Table 5). Glycine contributed to over 20% of the total amino acids present. Meanwhile, tyrosine, cysteine, and tryptophan, typically low in fish, were not detected.

Table 5

Types of amino acids in Amazon sailfin catfish skin gelatin

Types of amino acid	*Percentage (%)*
Arginine	14.75
Histidine	3.54
Lysine	9.22
Phenylalanine	0.37
Leucine	0.49

Isoleucine	0.58
Tyrosine	0
Methionine	1.32
Valine	1.84
Proline	12.40
Glutamic acid	12.66
Aspartic acid	5.00
Cysteine	0
Threonine	2.66
Serine	4.30
Alanine	9.38
Glycine	21.48
Tryptophan	0

Heavy metal contents. Based on the test results, the heavy metal content of cadmium (Cd), mercury (Hg), and lead (Pb) in the skin of the Amazon sailfin catfish (Table 6) was still below the threshold value for heavy metal content for fish and their processed products set by the SNI 7387 (2009), namely 0.1 mg/kg for Cd, 0.5 mg/kg for Hg, and 0.3 mg/kg for Pb. This research shows that the gelatin from the skin of Amazon sailfin catfish is safe to be used as a source of ingredients for food processing.

Table 6

The heavy metal content in Amazon sailfin catfish skin gelatin

Parameters	*Measurement unit*	*Test Results*	*Method Specification*	*Max. as per SNI 7387:2009*
Cadmium (Cd)	mg/kg	Not detected	Atomization	0.1 mg/kg
Mercury (Hg)	mg/kg	Not detected	Atomization	0.5 mg/kg
Lead (Pb)	mg/kg	0.00122	Atomization	0.3 mg/kg

Discussion. The yield of gelatin produced from the skin of the Amazon sailfin catfish in this study was low. The low yield was expected and related to characteristic of the Amazon sailfin catfish skin which is very thin and is covered by coarse scales. However, the yield of gelatin in this study was comparable with those of the skin of catfish *Gariepinus* spp. (0.44%) previously reported by Sivakumar et al (2000) and Muyonga et al (2004), Nile perch (*Lates niloticus*) young (1.3%) and adults (2.4%). The yield of gelatin in our study was much lower compared to the yield of gelatin from the skins of *Oreochromis niloticus* (7.81%) and *Oreochromis mossambicus* (5.39%) (Bakar & Harvinder 2002), *Johnius dussumieri* (14.3%) (Cheow et al 2007), *Pangasius pangasius* (21.93%), *Clarias batrachus* (20.57%), *Channa striata* (20.17%), and *Channa micropeltes* (20.76%) (Ratnasari & Firlianty 2016), fresh salmon (*Salmo salar*) (19%), smoked salmon (*Salmo salar*) (25%) (Ilona et al 2008). According to Koli et al (2012), the washing process on the skin will affect gelatin, making the resulting yield low. The conversion of collagen to gelatin depends on processing parameters, such as temperature, extraction time, pH, and raw material characteristics, and initial handling methods (Karim & Bhat 2009).

The gelatins obtained in this study were confirmed by the similarity of their spectral peak wavelengths with those of the commercial gelatin. The hydroxyl functional group (O-H) and amine group (N-H) spectra obtained in this study showed the same functional groups as those of the commercial gelatin. According to Brauner et al (2005), gelatins, like most proteins, have a structure consisting of carbon, hydrogen, a hydroxyl group (OH), a carbonyl group (C = O), and an amine group (NH), so it can be concluded that the compounds obtained in this study are gelatin.

The higher protein and lower fat contents indicate that the gelatin extracted at 80^{O}C has a better quality as compared to the gelatin extracted at 90^{O}C. However, only moisture (8%) and fat (0.73-4.21%) contents meet the GMIA (2012) standard (maximum of 15% and 5%, respectively). The protein content (73-81%) was lower than the GMIA standard of >90%, which might have been due to the high ash content (8-9%). The standard ash content for gelatin as set by the GMIA (2012) is 0.3-2%. The moisture content of the gelatin in this study was also much lower than the requirements set by FAO JECFA standard (2003) of 18%. The lower the water content the better the quality of the gelatin and vice versa. The gelatin's water content significantly affects its storage capacity because it is closely related to the metabolic activity that occurs while the gelatin is stored (Ninan et al 2012).

The protein content of the gelatin extracted at 80^{O}C in this study was higher when compared to the protein content of the commercial gelatin (bovine gelatin) (78.79%) as reported by Ratnasari and Firlianty (2016). Higher protein contents of fish skin gelatins were also reported by these authors for *Pangasius pangasius* (87.1%), *Clarias batrachus* (85.9%), and *Channa striata* (87.27%), but like that of *Channa micropeltes* (82.63%). The protein content of gelatin can be influenced by the quality of the ossein produced in the demineralization process, the type of raw materials and the freshness of the raw materials (Ward & Courts 1997).

The high content of ash in this study indicates that washing of fish skin with water is unable to reduce collagen-bound minerals and, therefore, demineralization must be performed prior to extraction of the gelatin. According to Liu et al (2009) and Duconseille et al (2015), the high ash content of gelatin is due to mineral components bound to collagen that have not been released during the washing process. Utama et al (2019) stated that ash is a component forming the extract material without nitrogen and affects the composition of organic materials. This is consistent with the data in this study which showed that gelatin was an extract containing materials without nitrogen. The crude fiber was also present in the gelatin. The higher temperatures used in the extraction process tended to increase the contents of the non-nitrogenous extract.

The fat content of fish skin gelatin is affected by whether the subcutaneous fat and grease are thoroughly removed. Therefore, the fat content in gelatin depends on the treatment during the process of gelatin preparation, both at the cleaning stage and the degreasing process until the filtering stage of the filtrate, where every fair treatment will reduce the fat content. Low-fat content in the gelatin is required to enable a longer duration of storage without causing rancidity (deMan 2007). Not only rancid gelatin may cause unpleasant odor and taste, but it may also cause toxic effects such as allergy, nausea, vomiting, and unease stomach. Oxidation of lipids produces aldehydes which in turn crosslink with amino acids causing insolubility of protein (Kussi et al 1975; Esterbauer et al 1991) and directly affect protein digestibility and utilization (Laohabanjong et al 2009).

The gel strength produced in this study was comparable with that of the commercial gelatin. The standard value of the gel strength according to Schrieber and Garies (2007) is 200-300 blooms. The gel strength of the gelatin is influenced by viscosity and pH, the high viscosity accompanied by a considerable gel strength indicates that the gelatin contains a substantial amount of pyrrolidine (proline and hydroxyproline) (Sarabia et al 2000).

The pH value of the fish skin gelatin in this study meets the standard pH criteria according to GMIA (2012), which ranges from 3.8 to 5.5. Gelatin pH is divided into low, neutral, and high. Neutral pH gelatin is applied for meat products, pharmaceuticals, chromatography, and paints. Low pH gelatin is used for the food industry, while high pH gelatin is applied for the pharmaceutical industry (Ninan et al 2012). In this study, the pH of the gelatin fell into a low category, and therefore is suitable to be used in the food industry. The resulting low pH is caused by the presence of acid residue in the gelatin. Ninan et al (2012) stated that when the immersion process is carried out, the skin collagen fibers will experience a swelling process which causes the structure of the amino acid bonds in the collagen molecule to open and the solvent is "trapped" between these bonds and does not dissolve during the neutralization process.

The viscosity value of the gelatin in this study confirms the standards set by GMIA (2012), which is between 1.5 - 7.5 cP. However, according to Mariod and Adam (2013), when the ability of a solution (NaOH) is suboptimal in hydrolyzing the collagen peptides the resulting gelatin will be unable to reach the highest standard point from GMIA. The gelatin peptide molecules' distribution strongly influences the solution's high and low viscosity values (Nishimoto et al 2005). High water content in gelatin can also reduce the viscosity value (Olivares et al 2006). The excessive drying process will break protein bonds in the gelatin, resulting in low viscosity, thus making the gelatin difficult to thicken.

The analysis of amino acids contained in the gelatin of the Amazon sailfin catfish skin showed that the main amino acids making up the gelatin were glycine, arginine, proline, and glutamic acid. However, glycine and proline were the two primary amino acids presented by Tong and Tiejin (2013), who stated that gelatin contains 9 out of 10 essential amino acids that the body needs; one essential amino acid that is barely contained in gelatin is tryptophan. The amino acid composition makes gelatin a multi-use ingredient in various industries.

The heavy metal analysis is important for gelatin, among others, to determine the safety of the product, especially if consumed in food products or medicines. Research on heavy metals contained in Amazon sailfin catfish skin gelatin has been investigated by Hermanto et al (2014), who obtained a metal content that is quite high when compared to this study. The heavy metal content reported by Hermanto et al (2014) included Hg 0.012 mg/kg, Pb 7.69 mg/kg, and Cd 2.49 mg/kg. It is suspected that the two gelatins produced came from different sampling locations. Heavy metal poisoning will damage organs and cause death (Parawita et al 2009).

Conclusion. Extraction of gelatin from Amazon sailfish catfish skin has been successfully conducted. The results showed that besides obtaining the best yield, the profiles of the gelatin produced at the optimum condition of 80^{O}C for 6 hours also met the characteristics of commercial gelatin. The physicochemical properties of the gelatin are indicated by the moisture content of 8.84%, protein content of 81.08%, ash content of 9.07%, fat content of 0.73%, crude fiber content of 0.2%, 0.07% extract without nitrogen, 200.68 bloom gel strength, viscosity of 4.33 cP and pH of 4.25. The functional groups of the gelatin consist of several types of amides detected at different wavelengths as follows Amide I (1635.64 cm^{-1}), Amide II (1552.70 cm^{-1}), Amide III (1242.16 cm^{-1}), Amide A (3496.94 cm^{-1}), and Amide B (2926.01 cm^{-1}). Glycine and proline are the main amino acids contained in the fish gelatin with a level of 21.48 and 12.40%, respectively. Heavy metal contamination of Hg and Cd was not detected but Pb was detected in a very small amount in the gelatin of the Amazon sailfin catfish skin, so it is safe to be used as a raw material. Thus, gelatin from the skin of the Amazon sailfin catfish has promising potential to be developed as an alternative source of religiously accepted gelatin (halal food).

Acknowledgements. The authors would like to thank the technicians of the Fishery Product Technology Laboratory, Faculty of Marine and Fisheries Sciences, Hasanuddin University, and Integrated Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Hasanuddin University, as well as to the Feed Chemistry Laboratory, Faculty of Veterinary Sciences, Hasanuddin University, for their excellent expertise in analyzing our samples.

References

Abdullah M. S. P., Noordin M. I., Ismail S. I. M., Nymathulla S., Jaamai M., Wai L. K., Mustapha N. M., Shamsuddin A. F., 2016 Physicochemical evaluation and spectroscopic characterization of gelatin from shank and toes of *Gallus gallus domesticus*. Sains Malaysia. 45(3):435-449.

Association of Official Analytical Chemists (AOAC), 2000 Official methods of analysis. 17th ed. Association of Official Analytical Chemists, Washington, DC:AOAC.

Bakar J., Harvinder K. G., 2002 Properties of gelatines from skins of fish—black tilapia (*Oreochromis mossambicus)* and red tilapia (*Oreochromis niloticus*). Food Chemistry 77(1):81-84.
Brauner J. W., Flach C. R., Mendelsohn R., 2005 A quantitative reconstruction of the amide I contour in the IR spectra of globular proteins: From structure to spectrum. Journal of the American Chemical Society 127:100–109.
Cheng X. L., Wei L., Xiaoa X. Y., Zhao Y. Y., Shi Y., Liu W., Zhang P., Maa S. C., Tian S. S., Lin R. C., 2012 Identification of five gelatins by ultra performance liquid chromatography/time-of-flight mass spectrometry (UPLC/Q-TOF-MS) using principal component analysis. Journal of Pharmaceutical and Biomedical Analysis 62:191–195.
Cheow C. S., Sarbon N. M., Kyaw Z. Y., Howell N. K., 2007 Preparation and characterization of gelatines from the skins of sin croaker (*Johnius dussumieri*) and shortfin scad (*Decapterus macrosoma*). Food Chemistry 101(1):386-391.
Cook-Hildreth S. L., Timothy H. B., David G. H., 2016 Female reproductive biology of an exotic suckermouth armored catfish (Loricariidae) in the San Marcos River, Hays Co, Texas, with observations on environmental triggers. BioInvasions Records 5(3):173-183.
deMan J. M., 2007 Principles of food chemistry. 3rd Edition. Aspen Publishers, Inc., United States of America.
Duconseille A., Astruc T., Quintana N., Meersman F., Santelhoutellier V. E., 2015 Gelatine structure and composition linked to hard capsule dissolution: A review. J. Food Hydrocoll 43:360-376.
Esterbauer H., Schaur R. J., Zollner H., 1991 Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic. Biol. Med. 11:81-128.
GMIA (Gelatin Manufacturers Institute of America), 2012 Gelatin Handbook. New York (US):GMIAInc.
Gomez-Guillen M. C., Turnay J., Fernandez-Diaz M. D., Ulmo N., Lizarbe M. A., Montero P., 2002 Structural and physical properties of gelatine extracted from different marine species: A comparative study. Food Hydrocolloids 16(1):25–34.
Hashim D. M., CheMan Y. B., Norakasha R., Suhaimi M., Salmah Y., Syahariza Z. A., 2010 Potential use of fourier transform infrared spectroscopy for differentiation of bovine and porcine gelatines. Food Chemistry 118(3):856–860.
Hashim E. F., Yan H. J., Abd Ghani I. F., 2017 Preliminary characterization on physical properties of selected marine fish skins as alternative sources of halal gelatine. Food Science and Nutrition Technology 2(2):1-5.
Hermanto S., Hudzaifah M. R, Anna M., 2014 The physicochemical characteristics of Amazon sailfin catfish (*Hyposarcus pardalis*) skin gelatin from acid extraction. Jurnal Kimia Valensi 4(2):109-120.
Herpandi N., Huda N., Adzitey F., 2011 Fish bone and scales as potential sources of halal gelatine. Journal of Fisheries and Aquatic Science 6(4):379-389.
Joint Expert Communittee on Food Additives (FAO JECFA), 2003 Edible Gelatin, In Compendium of Additive Specifications. Vol 1. Italy: Rome.
Karim A. A., Bhat R., 2009 Fish gelatine: properties, challenges, and prospects as an alternative to mammalian gelatines. Food Hydrocolloids 23:563-576.
Kittiphattanabawon P., Benjakul S., Visessanguan W., Shahidi F., 2010 Comparative study on characteristics of gelatin from the skins of brownbanded bamboo shark and blacktip shark as affected by extraction conditions. Food Hydrocolloids 24(2–3):164–171.
Koli J. M., Basu S., Nayak B. B., Pantage S. B., Pagarkar A. U., Gudipadi V., 2012 Functional characteristics of gelatine extracted from skin and bone of tiger-toothed croaker (*Otolithes ruber*) and pink perch (*Nemipterus japonicus*). Food and Bioproducts Processing 90(3):555–562.
Ilona K., Skierka E., Sadowska M., Kolodziejska W., Niecikowska C., 2008 Effect of extracting time and temperature on yield of gelatine from different fish offal. Food Chemistry 107:700-706.

Kussi T., Nikkila O. E., Sarolainin K., 1975 Formation of malonaldehyde in frozen Baltic herring and its influence on changes in protein. Zeitschrift fur Lebensmittel Untersuchung und Forschung. 159:285–289.
Laohabanjong R., Tantikitti C., Benjakul S., Supamattaya K., Boonyaratpalin M., 2009 Lipid oxidation in fish meal stored under different conditions on growth, feed efficiency and hepatopancreatic cells of black tiger shrimp (*Penaeus monodon*). Aquaculture 286(3-4):283-289.
Liu H. Y., Han J., Guo S. D., 2009 Characteristics of the gelatine extracted from channel catfish (*Ictalurus punctatus*) head bones. LWT-Food Sciens and Technology 42:540-544.
Mariod A. A., Adam H. F., 2013 Review: Gelatine, source, extraction, and industrial applications. Acta Sci. Pol., Technol. Aliment 12 (2):135-147.
Muyonga J. H., Cole C. G. B., Duodu K. G., 2004 Extraction and physico-chemical characterisation of Nile perch (*Lates niloticus*) skin and bone gelatin. Food Hydrocolloids, 18, 581–592.
Ninan G., Joseph J., Aliyamveettil Z. A., 2012 A comparative study on the physical, chemical, and functional properties of carp skin and mammalian gelatines. J. Food Sci. Technol 51:2085-2091.
Nishimoto M., Sakamoto R., Mizuta S., Yoshinaka R., 2005 Identification and characterization of molecular species of collagen in ordinary muscle and skin of the Japanese flounder (*Paralichthys olivaceus*). Journal Food Chemistry 90(1-2):151-156.
Olivares M. L., Peirotti M. B., Deiber J. A., 2006 Analysis of gelatine chain aggregation in dilute aqueous solutions through viscosity data. Food Hydrocolloids 20(7):1039-1049.
Parawita D., Insafitri, Wahyu A. N., 2009 Heavy metal concentration analysis of lead (Pb) in Porong River Delta. Jurnal Kelautan, 2(2):117-124.
Ratnasari I., Firlianty, 2016 Physico-chemical characterization and skin gelatine rheology of four freshwater fish as alternative gelatine source. AACL Bioflux (6):1196-1207
Sarabia A. I., Gomez-Guillen M. C., Montero P., 2000 The effect of added salts on the viscoelastic properties of fish skin gelatine. Journal of Food Chemistry 70:71-76.
Schrieber R., Garies H., 2007 Gelatine handbook. Wiley-VCH Verlag GmbH and Co. KGaA, Weinheim.
Shyni K., Hema G. S., Ninan G., Mathew S., Joshy C. G., Lakshmanan P. T., 2014 Isolation and characterization of gelatine from the skins of skipjack tuna (*Katsuwonus pelamis*), dog shark (*Scoliodon sorrakowah*), and rohu (*Labeo rohita*). Food Hydrocolloids 39:68–76.
Sinthusamran S., Benjakul S., Kishimura H., 2014 Characteristics and gel properties of gelatine from skin of seabass (*Lates calcarifer*) as influenced by extraction conditions. Food Chemistry 152:276-264.
Sivakumar P., Arichandran R., Suguna L., Mariappan M., Chandrakasan G., 2000 The composition and characteristics of skin and muscle collagens from a freshwater catfish grown in biologically treated tannery effluent water. Journal of Fish Biology 56:999-1012.
SNI 7387, 2009 [Maximum level of heavy metal contamination in food]. Indonesian National Standard 7387:2009 of the National Standardization Agency. 25 pp. [in Indonesian].
SNI 2354.5, 2011 [Chemical test method - Part 5: Determination of heavy metal lead (Pb) and cadmium (Cd) contents in fishery products]. Indonesian National Standard 2354.5:2011 of the National Standardization Agency. 6 pp. [in Indonesian].
SNI 2354.6, 2016 [Chemical test method - Part 6: Determination of heavy metal mercury (Hg) content in fishery products]. Indonesian National Standard 2354.6:2016 of the National Standardization Agency. 9 pp. [in Indonesian].
Tong Y., Tiejin Y., 2013 Gelling strength improvement and characterization of a gelatine from scales of bighead carp (*Aristichthys nobilis*). Journal of Food, Agriculture and Environment 2(1):146-150.
Utama C. S., Zuprizal, Chusnul H., Wihandoyo, 2019 Effect of heating time on the chemical quality of wheat pollard as a prebiotic potential agent. Journal of Food Technologists 8(3):113-122.

Ward A. G., Courts A., 1977 The Science and Technology of Gelatine. Academic Press, London.
Zhou P., Regenstein J. M., 2004 Optimization of extraction conditions for pollock skin gelatin. Journal of Food Science 69(5):393-397.

Received: 02 February 2021. Accepted: 24 February 2021. Published online: 08 August 2021.
Authors:
Masrurah Ismail, Graduate Student in Fishery Sciences, Faculty of Marine and Fishery Sciences, Hasanuddin University, Makassar, Indonesia, e-mail: masrurahismail@gmail.com
Kasmiati, Department of Fishery, Faculty of Marine and Fishery Sciences, Hasanuddin University, Makassar, Indonesia, e-mail: kasmiati74@yahoo.com
Metusalach, Department of Fishery, Faculty of Marine and Fishery Sciences, Hasanuddin University, Makassar, Indonesia, e-mail: mminanga@hotmail.com

How to cite this article:
Ismail M., Kasmiati, Metusalach, 2021 Physicochemical characteristics of Amazon sailfin catfish (*Pterygoplichthys pardalis*) skin gelatin. AACL Bioflux 14(4):2071-2085.