Characterisation of Snakehead Fish, *Channa striata* (Bloch, 1793), Byproduct and Its Gelatin Properties

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Abstract

This study aims to identify the characteristics of skin, bones, and scales as a biomaterial byproduct of snakehead fish, *Channa striata* (Bloch, 1793) before and after the extraction process into gelatin, based on 36 fresh snakehead fish samples. The biomaterials were identified, including their proximate composition (by weight of protein, fat, moisture, ash) collagen, amino acids. The physicochemical properties of gelatin in terms of yield, gel strength, viscosity pH, and functional groups were determined for scales, skin and bones. Combined, these biomaterials made up about 47.74 % of the total byproduct by weight of snakehead fish that would usually be discarded. Moisture and protein content of the skin was higher than that of scales and bone ($P < 0.05$), and fat content was higher in bone than in skin and scales ($P < 0.05$). The lowest ash content was in skin (<1 %), compared to scales and bone ($P < 0.05$). The collagen content of scales was higher than that of skin and bone ($P < 0.05$). Glycine and proline in the three biomaterials were nearly equal and highest in skin, scale, and bone, respectively. There were significant differences in viscosity and gel strength of the three types of gelatin ($P < 0.05$), the highest viscosity was in skin gelatin while the best gel strength was in scale gelatin, while pH was not significantly different ($P > 0.05$). The application of the same method and solvent can produce different properties and characteristics of gelatin, and the presence of functional groups helps determine the occurrence of changes in the secondary structure of gelatin from the three biomaterials.

Keywords: amino acid, biomaterial, functional groups, physicochemical gelatin

Introduction

The fish processing industry activities to supply quality food requires research to support the production of fishery products, which is a challenge for the fisheries processing industry and environmental management (Kim and Min, 2012; Marti-Quijal et al., 2020; Nawaz et al., 2020; Truong et al., 2021; Hassoun et al., 2023). Concerns about limited food sources, population growth, and improving socioeconomic conditions are why byproducts must be managed wisely. In addition, fish processing needs to reduce pollution's impact and increase the added value of byproducts (Choi and Regenstein, 2000; Shahidi et al., 2019; Nawaz et al., 2020). Collagen and gelatin are two important fishery byproduct derivatives that have been developed into industrial products with high economic value (Atef and Ojagh, 2017; Duan et al., 2018; Yang et al., 2022).

One of the important freshwater fish currently being studied is the snakehead fish. This fish includes wild fish that live in fresh water and contain high albumin (Gustiano et al., 2021; Irwan et al., 2021), which is effective as a wound healer (Haniffa et al., 2014; Ab Wahab et al., 2015; Ramadhanti et al., 2021). In the snakehead fish processing industry, the byproducts include parts that have not been utilized optimally, but still contain essential organic and inorganic components (Rosmawati et al., 2018a; Shahidi et al., 2019; Nawaz et al., 2020). Most of the biomaterial in snakehead fish byproducts is collagen (Rosmawati et al., 2018b; Md Zin et al., 2019) which, through heating

above the transition temperature, can cause protein fractionation to produce different peptides sizes (Lv et al., 2019).

Collagen protein is the dominant constituent of the extracellular matrix (Maschmeyer et al., 2020), consisting of triple helical strands that are interlocked to form a network of collagen fibrils with three distinct amino acids sequentially as the main encoding, namely glycine, proline, and hydroxyproline (Toppe et al., 2007; Alfaro et al., 2014; Blanco et al., 2017). Gelatin is a biopolymer derived from collagen protein (Kumar et al., 2017), through a partial hydrolysis process (Lv et al., 2019; Tan et al., 2020). Because of its functional properties, collagen has been widely used in various industries, such as food, cosmetic, biomedical, pharmaceutical, functional food, livestock, aquaculture, agriculture, and photographic application (Tanaka et al., 2018; Shahidi et al., 2019; Nurilmala et al., 2022; Oslan et al., 2022).

Utilisation of fishery byproducts is a measure to control the serious environmental impacts of the processing industry (Kim and Min, 2012; Truong et al., 2021), as well as increasing the economic value of the fish (Choi and Regenstein, 2000). Producing gelatin from fish also helps meet the need for fish gelatin which is still very small compared to pork and beef gelatin (Haug and Draget, 2011; Tavakolipour, 2011), even though demand for fish gelatin is increasing with increasing consumer awareness regarding religious boundaries and health quality (Karim and Bhat, 2009; Martins et al., 2018). The high demand for fish gelatin encourages the search for sources of productive raw materials. The skin, bones, and scales of snakehead fish, *Channa striata* (Bloch, 1793), are readily available biomaterial with potential economic value. These are byproducts of the processing industry and have not been appropriately handled so far. This study aims to characterise skin, bones, and scales as biomaterial byproducts of snakehead fish before and after the extraction process into gelatin. This study also analyses the potential of collagen in the skin, bone, and scales of snakehead fish with a different approach as a preventive effort to reduce the impact of pollution from byproduct processing on the surrounding environment.

Materials and Methods

Ethical approval

All animal research methods followed international, national, and institutional guidelines. The animal research was approved by the Research, Publication, and Community Service Institutions of Universitas Muhammadiyah Kendari, Indonesia (067/TGS/II.3AU/F-LPPM/2022).

Raw materials

Thirty-six wild snakehead fish were purchased from fishermen of the freshwater of Rawa Aopa National Park Watumohai, South Konawe, Southeast Sulawesi. Snakehead fish ranged in total length from 330 to 450 mm (mean 374.1 ± 31.2 mm) and their wet weight ranged from 291 to 792 g (mean 473.62 ± 125.97 g). Fresh snakehead fish were transported to the laboratory on ice in a cooling box. The chemicals used were of the analytical grade.

The fish's length and weight were measured, and the body parts were separated, including the scales, fins, head, viscera, skin, flesh, and bones. Each part was weighed to calculate the percentage based on the total weight of each. The frame (the bone that is still covered with meat) was cleaned by immersing it in water at a temperature of 60–70 °C for 30 min to remove any remaining meat. For amino acid and FT-IR assays, skin, bones, and scales were lyophilised. Proximate analysis, collagen content, and gelatin extraction were performed on fresh samples.

Proximate analysis and collagen content

Skin, bone, and scales were analysed for their proximate composition according to AOAC (2019) guidelines. The three samples were observed for protein content by the Kjeldahl method (AOAC 984.13), fat content by the Soxhlet method (AOAC 920.39), and ash and water content by incineration (AOAC 942.05), and gravimetrically (AOAC 930.15), respectively.

Collagen content analysis

The collagen content was determined by referring to the Total Collagen Assay used method (QuickZyme Bioscience, 2019) with microplate reader (WTW 21 Series, USA) at a wavelength of 540–580 nm.

In the initial stage, as much as 100 mg of the sample in a screw cap tube was hydrolysed with 12 M HCl and then 6 M HCl was added to obtain 100 mg.L^{-1}. The tubes were tightly closed and incubated for 20 h at 95 °C in an oven, then cooled to room temperature. The tubes were centrifuged for 10 min in a centrifuge (Eppendorf, Germany) at 13,000 ×g. The hydrolysed sample was diluted at a concentration of 2:1 (v/v), and 35 µL was used for further analysis.

A collagen standard was prepared at a concentration of 1200 µg.mL^{-1} in 0.2 M acetic acid. A total of 125 µL of the standard solution was transferred to a screw cap tube and mixed with 12 M HCl (1:1 v/v). Then the tube was tightly closed and incubated for 20 h at 95 °C in an oven. The tubes were then cooled to room temperature, and centrifuged for 10 min in a centrifuge at 13,000 ×g. The resulting supernatant was analysed. Eight Eppendorf tubes were marked as S1–S8, with S1 to S7 containing hydrolysed dilution stock adjusted to a concentration of 4 M HCl, while S8 was a blank. The dilution process produced standards with the following concentrations: 300 µg.mL^{-1} (S1); 200 µg.mL^{-1} (S2); 100 µg.mL^{-1} (S3); 50 µg.mL^{-1} (S4); 25

μg.mL^{-1} (S5); 12.5 μg.mL^{-1} (S6); 6.25 μg.mL^{-1} (S7); 0 μg.mL^{-1} (S8). For analysis with the test solutions, 35 μL of each of the 8 solutions was run.

The final stage was to test samples and standards in duplicate. A total of 35 μL each of the standard and sample solutions was pipetted into the appropriate microplate testing wells. The sample solution was first diluted with 4 M HCl. A 75 μL buffer solution was added to each well and mixed well. The plate was covered with an enclosed adhesive plate seal and incubated at room temperature for 20 min while stirring. The detection reagent was added as much as 75 μL to each microplate well. The plate was closed with an enclosed adhesive plate seal and then incubated for 60 min at 60 °C. Finally, the plate was cooled with ice for 5 min until it reached room temperature and then the bottom of the plate was cleaned and read at 570 nm using a microplate reader.

Amino acid analysis

An ultra-performance liquid chromatograph (ACQUITY UPLC-H Class, Waters, USA) was used to determine the type of amino acids in the skin, scales, and bones, following Nollet (2004). The amino acid determination process was carried out by weighing 0.1–1.0 g of the test sample into a 20 mL vial and hydrolysed using 1 M HCl in a 50 mL volumetric flask. Distilled water was added and homogenised and the solution was filtered using a syringe filter. Before the derivation process, the filtrate was added to the internal standard. The filtrate solution was injected into the UPLC system in the final stage of the process to determine the amino acids present in the sample.

Gelatin extraction

The skin, bones, and scales were cleaned using running tap water. Before pretreatment, the bones and scales first underwent a demineralisation process using 3 % HCl for 24 h (Muyonga et al., 2004a; Rosmawati et al., 2021), cleaned using running water and rinsed in distilled water. Each skin, bone, and scale sample was immersed in a 0.5 M NaOH solution for 1 h (1:5 w/v) to remove tissue components such as pigments, including non-collagen proteins, mucopolysaccharides, and sulphur-containing compounds (Monsur et al., 2014). The skin, bones, and scales were cleaned with running water, rinsed with distilled water, and then filtered using four folded cheesecloths to avoid any effects of NaOH. The treatment was carried out by immersing each sample into each 0.05 M acetic acid solution for 6 h (1:5 w/v). Washing was carried out using running water carefully to avoid washing away the collagen and then rinsed with distilled water. The final stage was the extraction process using distilled water in a water bath for 12 h at 60 °C and then drying in an oven at 60 °C for 48 h.

Gelatin characteristics analysis

The yield, gel strength, viscosity, acidity and functional groups of the gelatin sample were analysed.

Yield

The gelatin yield was calculated based on the ratio of the dry weight of gelatin to the initial weight of the raw materials used (skin, scales, and bone) according to the equation:

$$Yield\ of\ gelatin = \frac{dry\ weight\ of\ gelatin}{initial\ weight\ of\ raw\ materials} \times 100\%$$

Gel strength

Gel strength was determined by making a gelatin solution from 7.5 g of gelatin in 105 mL of distilled water, according to GMIA (2012). The process of making a gelatin solution was preceded by allowing the water and gelatin solution to stand for about 30 min at room temperature so that the gelatin absorbs the water and expands. The mixture was then heated in a water bath for 30 min at 60 °C to reach gelatin solubility. Before measuring the gel strength using the TAXT2 texture analyser (Stable Micro System, UK), the solution was placed in a jar and stored for ~17 h (±1 h) in a refrigerator at ~4 ºC (±1). The gel strength was defined as the maximum force (in g) obtained when the plunger penetrated up to 4 mm into the gelatin gel.

Viscosity

The sample's viscosity was measured with a viscometer (DV-I Prime, Brookfield, USA) according to the method used by Shyni et al. (2014). The gelatin solution was prepared by adding 6.67 g of gelatin to a tube containing 100 mL of distilled water and heated at 60 °C until the gelatin particles were completely dissolved. Gelatin viscosity was measured at room temperature.

Acidity (pH)

The acidity value of gelatin was determined based on the standard test method (BSI, 1975) using a gelatin solution concentration of 1 % (w/v) in distilled water. The solution was measured at room temperature using a pH meter (Luthron pH 208, Taiwan).

FT-IR

Functional group analysis of the samples was determined using Fourier transform infrared spectroscopy (IR Prestige-21, FTIR-8400, Shimadzu, Japan) referring to Abedinia et al. (2017). A 2 g sample was mixed with 100 mg of potassium bromide (KBr) and placed in a crystal cell of an FTIR spectrophotometer. The spectrum was obtained at a resolution of 4 cm^{-1}, and the measurement range is in the wavenumber 4000 to 650 cm^{-1}. Spectrum results were read on a computer monitor.

Statistical analyses

Three replicate samples for each treatment were

analysed and the data were summarised as the mean ± standard deviation. Differences between types of biomaterials were tested by analysis of variance (ANOVA), using a completely randomised design and differences between means were evaluated by Duncan's multiple-range test (SPSS for windows: SPSS Inc., USA).

Results

Percentage of snakehead fish byproduct

The byproduct from snakehead fish consists of parts discarded in the processing industry, including fins, frames, heads, skins, scales, trimmings, and viscera (Table 1). The highest percentage of the parts considered as discards was the head (20.06 %), followed by frames (9.09 %) and scales (5.12 %) (Table 1). None of the other byproducts i.e. fins, trimmings, skins, and viscera, were more than 5 % of the fish. The total percentage of snakehead fish byproducts reached was 47.74 %.

Proximate composition and collagen content

The means for moisture and protein content of the skin (65.8 % and 31.7 %, respectively) were significantly higher than those of scales (50.9 %, 24.2 %) and bone (39.8 %, 16.1 %) ($P < 0.05$), but fat content was significantly higher in bone (8.5 %) than in skin and scales (both <1.2 %) ($P < 0.05$, Table 2). The ash content in the skin (<1 %) was significantly far lower than in scales and bone (both >20 %, $P < 0.05$, Table 2). The collagen content of scales (4.9 mg.g^{-1}) was slightly higher than that of skin (3.28 mg.g^{-1}) and bone (0.80 mg.g^{-1}, $P < 0.05$, Table 2).

Amino acid profiles

The amino acids identified in the skin, bones, and scales of snakehead fish were the same, but there were differences in their percentage compositions (Table 3). Glycine, alanine, proline, histidine, tryptophan, and cystine had higher mean percentages in the skin than in scales and bone (Table 3). In contrast glutamic acid, isoleucine, lysine, aspartic acid, tyrosine, and methionine, had higher mean percentages in the bone than in skin and scales. While in scales, serine, phenylalanine, valine, arginine, leucine, and threonine were higher than in skin and scales. Glycine and proline, which are the collagen molecule's main constituents were about 1 to 3 % higher in the skin than scales and bones.

Physicochemical properties of snakehead gelatin

The gel strength of the skin, scales, and bone of snakehead fish showed significant differences ($P < 0.05$), where the gel strength of the scales (351.6 g force) was significantly far greater than that of skin and bone (125 g force; Table 4). Bone gelatin viscosity was lowest in bone and highest in the skin ($P < 0.05$). The gelatin acidity (pH) did not differ significantly among the three tissues and ranged from 5.96 in skin to 6.59 in scales ($P > 0.05$; Table 4).

Using the NaOH solution combined with acetic acid far less gelatin was extracted from bone (0.80 %) than skin (22.7 %) and scales (6.02 %) (Table 4) i.e. the properties of collagen extracted from skin, scales and bone of snakehead fish differ.

Functional groups of biomaterials and gelatin from snakehead fish

The results from the Fourier transform infra-red (FT-IR) analysis identified the functional groups that make-up gelatin from the skin, scales, and bones of snakehead fish compared to their raw biomaterials (Supplementary Fig. 1).

Table 1. Mean ± SD percentage of snakehead fish *Channa striata* byproducts from different body parts.

Components	Fin	Frame	Head	Skin	Scales	Trim	Viscera
Mean ± SD (%)	1.67 ± 0.37	9.09 ± 3.18	20.06 ± 2.09	3.91 ± 0.93	5.12 ± 0.87	3.70 ± 2.32	4.19 ± 1.48

SD: standard deviation. n = 36.

Table 2. Mean proximate composition (percentage ± SE) for protein, moisture, fat, ash, and collagen content (mg.g^{-1}) of skin, scales, and bone of snakehead fish *Channa striata*.

Composition	Skin	Scales	Bone
Protein (%)	31.73 ± 0.02[c]	24.22 ± 0.09[b]	16.13 ± 0.08[a]
Moisture (%)	65.78 ± 0.00[c]	50.87 ± 0.00[b]	39.94 ± 0.00[a]
Fat (%)	1.16 ± 0.09[b]	0.45 ± 0.03[a]	8.46 ± 0.70[c]
Ash (%)	0.89 ± 0.02[a]	20.08 ± 0.27[b]	33.47 ± 0.17[c]
Collagen (mg.g^{-1})	3.28 ± 0.21[b]	4.88 ± 2.99[c]	0.80 ± 0.08[a]

SE: standard error. n = 3 for each mean. Different superscript letters in row indicate significant differences determined by ANOVA and Duncan's multiple range test.

Table 3. Amino acids and their percentage composition in the skin, bones, and scales of snakehead fish *Channa striata*.

Amino acid	Skin (%)	Scales (%)	Bone (%)
Serine	4.67	5.22	4.98
Glutamic acid	11.72	11.85	13.17
Phenylalanine	2.11	2.68	2.20
Isoleucine	1.47	1.11	1.85
Valine	2.58	3.05	2.87
Alanine	12.25	11.33	11.67
Arginine	6.89	7.57	6.64
Glycine	23.60	21.59	20.19
Lysine	5.02	5.81	5.86
Aspartic acid	5.89	6.75	7.37
Leucine	2.72	4.20	3.89
Tyrosine	0.72	0.97	1.01
Proline	13.06	11.93	11.64
Threonine	3.55	4.11	3.93
Histidine	1.18	1.11	1.10
Tryptophan	0.30	0.00	0.23
Cystine	1.37	0.14	0.45
Methionine	0.89	0.58	0.97

n = 1

Table 4. Yield, gel strength, viscosity and acidity of gelatin in the skin, scales, and bone of snakehead fish *Channa striata*.

Characteristic	Skin	Scales	Bone
Yields (%)	22.66 ± 0.27[c]	6.02 ± 0.31[b]	0.80 ± 0.27[a]
Gel strength (g force)	124.23 ± 30.63[b]	351.60 ± 21.90[c]	98.84 ± 4.23[a]
Viscosity (cP)	13.00 ± 1.00[c]	10.25 ± 2.99[b]	6.33 ± 1.53[a]
Acidity	5.96 ± 0.17[a]	6.27 ± 0.71[a]	6.59 ± 0.32[a]

SE: standard error. Mean ± SE; n = 3. Different superscript letters in row indicate significant differences ($P < 0.05$) determined by ANOVA and Duncan's multiple range test.

The FT-IR spectrum was useful for seeing changes in the secondary structure of a material. The changes that occurred after the extraction of skin, bone, and scales into gelatin were shown in the figure (Supplementary Figs. 1 a, b, c). Differences in the structure of a material can be characterised by differences in wavelength in each area of the amide. The data in Table 5 show that the functional group structure of skin, bone and scales changes slightly when derivatised into gelatin.

Discussion

Proximate composition of snakehead fish byproduct

Protein content

The skin contains high protein composed of layers of the epidermis, dermis, and hypodermis. The dermis was a source of the essential protein collagen (Tosh et al., 2003). Based on Rosmawati et al. (2018b) the skin is composed of various types of protein. However, its nature is more influenced by collagen than other types of protein. The protein content of snakehead skin was lower than that reported by Nurilmala et al. (2021) and Sai-Ut et al. (2012) in *Pangasius* sp. and *Pangasianodon gigas* which were 39.75 ± 0.12 %, and 43.00 ± 0.89 %, respectively. In contrast, the results were higher than those reported by Shyni et al. (2014) in *Scoliodon sorrakowah*, *Labeo rohita*, and *Katsuwonus pelamis*, which were 27.7 ± 0.36 %, 18.8 ± 0.06 % and 20.5 ± 0.26 %, respectively.

Scales of snakehead fish was a byproduct with a high protein content compared to bone, although it was lower than skin (31.7 %). Snakehead scales belong to the ctenoid type with functional adaptation for body protection from predators and environmental influences (Spinner et al., 2019). Scales are composed of calcium, hydroxyapatite (HAP) constituents, and Type I extracellular collagen matrix (Harikrishna et al., 2017; Rawat et al., 2021). The protein content of snakehead scales (24.2 %) was lower than that

Table 5. The functional group from Fourier transform infra-red analysis of a) raw biomaterial and b) gelatin of snakehead fish *Channa striata*.

Functional group wave number (cm⁻¹)	Biomaterial snakehead fish		
	Skin	Scales	Bone
A) Raw biomaterial			
Amide A	3452.58	3477.66	3543.23
Amide B	2924.09	2929.87	2924.09
Amide I	1662.64	1664.57	1653.00
Amide II	1543.05	1548.84	1543.05
Amide III	1236.37	1242.16	1238.30
B) Gelatin			
Amide A	3454.51	3508.52	3462.22
Amide B	2924.09	2929.87	2924.09
Amide I	1658.78	1658.78	1658.78
Amide II	1527.62	1554.63	1560.41
Amide III	1240.23	1240.23	1242.16

n = 1

reported by Wangtueai and Noomhorm (2009) in *Saurida* spp., which was about 38.9 ± 0.27 %, similar to that of *Catla catla* (22.30 %) and slightly higher than *Cirrhinus mrigala* (20.36 %), respectively (Mahboob, 2015).

Snakehead bone had the lowest protein in these observations (16.1 %). However, the bone protein was considered a byproduct because the type of protein was similar to the protein found in skin and scales, so it was considered to have potential for utilisation as a byproduct. Snakehead fish bone protein content was relatively higher than that reported by in *Macrodon ancylodon* (14.80 %)(Da Trindade Alfaro et al., 2009)and *Priacanthus tayenus* (13.30 %) (Kittiphattanabawon et al., 2005).

Moisture content

Moisture is the main and most important animal constituent (Njinkoue et al., 2016), both physically and chemically bound. It functions for various metabolic activities, transportation, and other vital activities (Rosmawati et al., 2018a). In snakehead fish, the moisture content was higher in the skin, followed by the scales and the bones. This difference in moisture content has to do with the function and components of the constituent material. Snakehead skin moisture content (65.8%) was relatively higher than that reported for *Rachycentron canadum* (61.0 %), and lower than *Micropogonias furnieri* (66.3 %)(Silva et al., 2014).

The moisture content of snakehead fish scales was about 50 % which was markedly lower than the moisture content of scales for *Catla catla* and *Cirrhinus mrigala* being 72.3 and 73.4 %, respectively (Mahboob, 2015). However, it was higher than the moisture content of *Lates calcarifer* and *Mugil cephalus* scales (38.6%, 37.9 %)(Cao et al., 2017).

The moisture content of snakehead bones was low compared to skin and scales. The difference in the moisture content of these three types of materials has to do with the function and necessity of their biochemical processes, including the ash constituents which were high in bone. When compared with other fish bones, the moisture content of snakehead fish bones was higher than that of *Amblygaster sirm*, namely 10.47 ± 1.33 % (Hasan et al., 2020) and *Trachurus trachurus* which was 26.2 ± 0.2 % (Toppe et al., 2007).

Fat content

The fat content in snakehead fish bones was higher than that of the skin and scales. According to Toppe et al. (2007), this situation is related to particle absorption into the bone during the fractionation process. Fat is usually deposited in the subcutaneous tissue in the skin and because skin is thin, it is not possible to store fat in large quantities in the skin. Fat content may vary depending on species, sex, age, body weight, habitat, type of feed, and season (Anthony et al., 2000; Toppe et al., 2007; Breck, 2014; Guiry et al., 2016; Sousa et al., 2017).

The fat content of snakehead fish skin (1.2 %) was slightly higher than *Priacanthus tayenus* skin (0.98 %) but the fat was lower than snakehead bone fat, about 8.77 ± 0.46 % (Kittiphattanabawon et al., 2005). As for the scales, they have a very low fat content, as also found in the *Lates calcarifer* and *Mugil cephalus* scales of 0.20 ± 0.01 % each (Cao et al., 2017). In extracting collagen into gelatin, the fat content is considered because it can affect the functional properties of the resulting gelatin (Rosmawati et al., 2021).

Ash content

The ash content of snakehead fish skin was exceptionally low compared to scales and bone. The ash content of snakehead skin was less than 1 %, probably influenced by the biochemical composition and level of skin thickness (Muralidharan et al., 2013).

Bones and scales tend to contain high ash content regardless of their anatomical role. Bone functions in physical activity of the body to carry out active movements, so the elasticity and flexibility of bone were very dependent on the constituent components. Likewise, on scales, the ash content, which represented the minerals in its composition, was an important component that can protect the fish body from unfavourable aquatic environmental conditions and make the moving process more efficient (Zhu et al., 2012). Snakehead bone ash content was relatively lower than that of *Pseudotolithus elongatus* and *Pseudotolithus typus*, 39.30 ± 0.44 % and 45.54 ± 0.35 %, respectively (Njinkoue et al., 2016), while the ash content of scales was higher in *Cyprinus carpio*, i.e. 45.16 ± 0.97 % (Moosavi-Nasab et al., 2020).

Collagen content

The properties of the collagen extracted from snakehead fish varied between the three types of biomaterials. It was suspected to have something to do with the components that make up the network according to their respective functions. The high content of scale collagen may be related to its function as the outermost protector of the body, as well as to the skin to protect the muscles and cellular components around it, while the collagen in the bones maintains the flexibility of the bones so that they are not stiff and facilitate the movement of fish. Skin, scales, and bone are sources of bioactive biomaterials (Begum et al., 2021), especially as a source of collagen (Lv et al., 2019; Spinner et al., 2019; Nurilmala et al., 2022), and have the potential as a source of collagen and gelatin (Wang et al., 2014). Collagen was composed of three dominant amino acid markers for collagen biomaterial: glycine, proline, and hydroxyproline (Boran and Regenstein, 2010). Skin, scales, and bones belong to the category I collagen type (Wang et al., 2014; Jafari et al., 2020; Begum et al., 2021; Rawat et al., 2021; Nurilmala et al., 2022). Information on collagen levels will be a reference for utilising the biomaterials and the efficiency of the subsequent extraction process.

In this study, the levels of skin collagen, scales, and bones of snakehead fish were lower than collagen from *Saurida tumbil*, namely 659.94 ± 0.99 mg.g^{-1}, 653.33 ± 10.60 mg.g^{-1}, and 629.28 ± 3.61 mg.g^{-1} (Jaziri et al., 2022d). Differences in collagen content can be influenced, among other things, by habitat, type, and species of fish, including body tissue composition (Jaziri et al., 2022b, 2022c) and the method used to determine levels (Mahboob, 2015; Oslan et al., 2022).

Amino acids

Skin, bone, and scale biomaterials contained essential amino acids, such as histidine, arginine, threonine, lysine, methionine, valine, isoleucine, leucine, phenylalanine, and tryptophan). It also contained several non-essential amino acids, including serine, aspartic acid, glutamic acid, alanine, and tyrosine, and conditionally essential amino acids (glycine, proline, and cysteine)(Li et al., 2009; Li and Wu, 2017).

The primary marker amino acids to identify a potential biomaterial as a source of collagen are the presence of glycine, proline, and hydroxyproline. This study did not measure the levels of the amino acid hydroxyproline. However, the collagen content information was considered to indicate that the material can be used as a collagen source. Amino acid levels in the skin were slightly higher than in scales or bones. The results of previous observations by Rosmawati et al. (2018b) on the skin of different snakehead fish weights were around 23.81 to 24.83 % in glycine, which is similar to the level of glycine from the skin (23.60 %). However, the levels of proline determined previously for snakehead fish (11.45 to 12.12 %) were slightly lower than from the current study (13.06 %). The amino acid levels of glycine and proline from snakehead fish bones were 20.19 % and 11.64 %, respectively, relatively lower than those found in snakehead fish bones (weight 900 - 1000 g), 23.53 % and 12.18 %, respectively (Rosmawati et al., 2018a).

Glycine is an amino acid that makes up all types of collagen and elastin and plays an important role in nutrition and metabolism (Li and Wu, 2017). Proline (along with glycine and hydroxyproline) is the main amino acid in collagen protein that contains three polypeptide chains (two 1 chains and one 2 chain). It is a major extracellular component in connective tissues such as skin and bone (Wu et al., 2011). Proline and hydroxyproline are essential for the biosynthesis, structure, and strength of collagen, as well as for strengthening the helical characteristics of the collagen molecule (Albaugh et al., 2017).

Characteristics of snakehead byproduct gelatin

Yields

The low yield of gelatin extracted from bone compared to skin and scales appears to be related to the chemical composition of bone, including amino acid composition (particularly glycine and proline) and collagen content (Jongjareonrak et al., 2010). The extraction method can also affect the yield produced (Koli et al., 2012). This extraction method was related to the use of the pretreatment solution (alkali/base) and treatment (mild acid), including the temperature and time of extraction. The results of previous studies using a solution of CaCO$_3$ and citric acid showed that the yield of snakehead fish bone and skin extracted at 60 °C for 12 h were 3.78 ± 0.17 % and 14.33 ± 0.07 %, respectively (Rosmawati et al., 2021).

The solubility of collagen largely determined the high/low yield during the extraction process (Muyonga et al., 2004a). During the extraction process, the hydrogen bonds that stabilize the parent collagen

triple helix are damaged, causing a transition from the helix to the coil, converting collagen into a gelatin solution.

As Table 4 shows, the skin gelatin yield was the highest. However, if it was associated with the appearance of gelatin, which tends to be yellow to blackish (data not shown), it was suspected that some protein impurities (pigments and other non-collagenous proteins) were not released optimally during the pretreatment process with NaOH solution. Thus, causing this protein to be included until the end of the extraction process. The gelatin yield can vary from 5 to 15 % (Tümerkan, 2021). Gelatin produced from *Pangasius* sp. skin and swim bladder ranged from 19 to 23 % (Nurilmala et al., 2022), and gelatin from *Oreochromis nilotica* and *Oreochromis mossambicus* were 7.81 % and 5.39 %, respectively (Jamilah and Harvinder, 2002).

The percentage of byproducts for each fish can differ depending on the species and the purpose for which it was used (Shahidi et al., 2019). Some studies reported that the total byproduct of fish can reach around 75 % (Songchotikunpan et al., 2008), 25 - 50 % (Venugopal, 2021), 40 - 70 % (Begum et al., 2021). In their research, Jaziri et al. (2022d) reported that the byproduct (consisting of skin, bones, and fins) of purple-spotted bigeye (*Priacanthus tayenus*) and barracuda (*Sphyraena barracuda*) only reached 19.69 % and 19.73 %, respectively which was about half of the byproduct of salmon (41.4 %). Meanwhile, the moisture content of snakehead skin was relatively higher than that of *Rachycentron canadum*, 61.0 ± 2.0 % (Silva et al., 2014) and *Saurida tumbil*, 67.05 ± 0.28 % (Jaziri et al., 2021). When compared to other fish bones, the moisture content of snakehead bones was higher than that of *Amblygaster sirm*, which was 10.47 ± 1.33 % (Hasan et al., 2020) and *Trachurus trachurus*, which was 26.2 ± 0.2 % (Toppe et al., 2007). However, its moisture content was lower than *Saurida tumbil*, which was 56.84 ± 0.13 % (Jaziri et al., 2021).

Gel strength

Gel strength is the main functional property of gelatin (Sinthusamran et al., 2014). This information will help guide the next use of gelatin. The extraction process with NaOH and acetic acid solutions with the same treatment on the three types of biomaterials showed very different gel strengths extracted from scales, skin and bone of snakehead fish. The gel strength of the scales was higher, while the gel strength of the bone was the lowest. This difference can be attributed to collagen content, raw material characteristics, method, concentration, and extraction time (Muyonga et al., 2004a; Karim and Bhat, 2009; Silva et al., 2014). In particular, the strength of the gel in this study was influenced by the characteristics of the biomaterial used. Even though they come from the same fish species, the information in Tables 2 and 3 shows that these three biomaterials have different compositions.

Thus, the gel strength of the three was strongly suspected to be related to the composition of the biomaterial.

Skin, bone, and scales have different chemical characteristics. It was one of the reasons why the same treatment can produce differences in the functional properties of the resulting gelatin, depending on the raw material, as Songchotikunpan et al. (2008), Taheri et al. (2009) suggested that the amino acid composition could cause the difference. Gel strength may vary between fish species and types of biomaterials, as reported by Cao et al. (2017) on *Lates calcarifer* scales and *Mugil cephalus* skin, which were 270.3 ± 1.3 g force and 249.1 ± 0.7 g force, respectively.

Viscosity

Viscosity is an important commercial physical property after gel strength. Producing gelatin using the same method on the skin, scales, and bones of snakehead fish produces different viscosity values from one another. It is thought to be the effect of differences in the types of biomaterials (Rera and Suprayitno, 2019), as Tables 2 and 3 shows that the three differ in chemical composition and amino acid content. Extraction methods give different responses to the viscosity value of gelatin due to changes in molecular weight (Muyonga et al., 2004a; Shyni et al., 2014) and triple-helical structure formation (Giménez et al., 2005).

The gelatin viscosity values of the snakehead byproducts varied from 6.33 to 13.00 cP for skin. Likewise, the different species showed different viscosity values, as previously reported in skin gelatin of *Scoliodon sorrakowah*. It was 5.60 ± 0.10 cP (Shyni et al., 2014), bone gelatin of *Anguilla bicolor* was 4.87 to 5.70 cP (Yudhistira et al., 2019), *Channa striata* from freshwater was 10.56 to 14.31 cP (Ayudiarti et al., 2020), skin gelatin of *Rachycentron canadum* and *Micropogonias furnieri* were 4.32 ± 0.11 cP, 3.54 ± 0.09 cP, respectively (Silva et al., 2014), bone gelatin of *Lutjanus campechanus* and *Epinephelus chlorostigma*, which were 15.30 ± 0.26 cP and 8.50 ± 0.30 cP, respectively (Shakila et al., 2012). Thus, the viscosity value variation was influenced not only by the raw material type but also by the species and habitat.

Acidity of gelatin

The treatment process influenced the degree of acidity of gelatin during the conversion of collagen to gelatin (Da Trindade Alfaro et al., 2013). The soaking solution used during the pretreatment and treatment processes contributes to the acidity level of the final gelatin product. The pH of gelatins extracted from snakehead scales, skin and bones through immersion with NaOH and then with acetic acid ranged from 5.96 to 6.59. According to the GMIA (2012) standard, the acidity value was 5 to 7.5, including the gelatin of *Saurida* spp., which was 5.8 to 6.8 (Wardhani et al.,

2017). The acidity value of gelatin can be affected by the type and concentration of acid during the extraction procedure (Taheri et al., 2009). The washing process reduces or eliminates the effects of the previously used alkaline/acid solution. The near-neutral acidity of gelatin was a suitable pH value for food and medicine (Yudhistira et al., 2019). Therefore, gelatin with a neutral acidity will be more stable and have a wider range of applications (Wahyuningtyas et al., 2019).

Functional group of biomaterial and gelatin from snakehead fish

The Amide A area was found at wavenumbers between 3525–3356 cm^{-1}, where NH stretching occurs in pairs with hydrogen bonds. It was absorbed in wavenumbers 3440–3400 cm^{-1}. The process of changing raw biomaterials to gelatin was characterised by a minor shift in skin, larger shift in scale, and the greatest change in bone, from 3452.58 to 3452.51 cm^{-1}, 3477.66 to 3508.52 cm^{-1}, and 3543.23 to 3462.22 cm^{-1}, respectively. In the Amide B area, wavenumbers were absorbed between 2960–2874 cm^{-1}. Asymmetric stretching vibrations occur in -CH and NH_2, the three biomaterials remain in their original structure even though they have turned into gelatin. A unique thing happened in the Amide I area, where all biomaterials underwent structural changes from wave numbers of 1662.64 cm^{-1}, 1664.57 cm^{-1}, and 1653.00 cm^{-1}, respectively in the skin, scales, and bones to the same wavenumber for all biomaterials, namely 1658.78 cm^{-1}. According to Wahyuningtyas et al. (2019), Amide I was a characteristic of gelatin coil structure, where a combination of stretching vibrations-C=O of a hydrogen double bond with COO, strain-CN, deformation of CCN, and bending-NH. The Amide II area absorbed at wavenumber 1560–150 cm^{-1} occurred in the biomaterial change to gelatin. The structural change was indicated by wavenumbers from 1543.05 to 1527.62 cm^{-1} on the skin, 1548.84 to 1554.63 cm^{-1} on scales, and 1543.05 to 1560.41 cm^{-1} on bone. The difference in wavenumbers between the three types of gelatin in this area is related to the triple helical structure (Muyonga et al., 2004b). This Amide II area was associated with a combination of CN-stretching and NH-flexing of the peptide group. The area of Amide III gelatin observed underwent structural changes from wavenumbers 1236.37 to 1240.23 cm^{-1} on the skin, 1242.16 to 1240.23 cm^{-1} on scales, and 1238.30 to 1242.16 cm^{-1} on bone. This structural change was related to the random coil structure characterised by the presence of -CN stretching and -NH bending as wagging-CH_2 vibrations of the glycine backbone and proline side chain (Hu et al., 2017; Wahyuningtyas et al., 2019).

Conclusion

Skin, bones, and scales derived from snakehead fish have different proximate compositions (protein, fat, moisture, ash), collagen, and amino acid content. The highest protein content was found in the skin, collagen was highest in scales, while fat and ash were highest in bone. Glycine and proline were highest in the skin, then in the scales, and lowest in the bone. Chemical composition information can be used as a recommendation for the appropriate method for further handling of byproducts from snakehead fish to maximise their potential value.

There were differences in the physicochemical characteristics of the skin, scales, and bones of snakehead fish after being converted into gelatin, in terms of proximate, collagen, and amino acid levels. It indicates that the use of the same method and solvent can produce different gelatin properties and characteristics. Functional groups confirm that there are changes in the secondary structure of the biomaterial after the gelatin extraction process.

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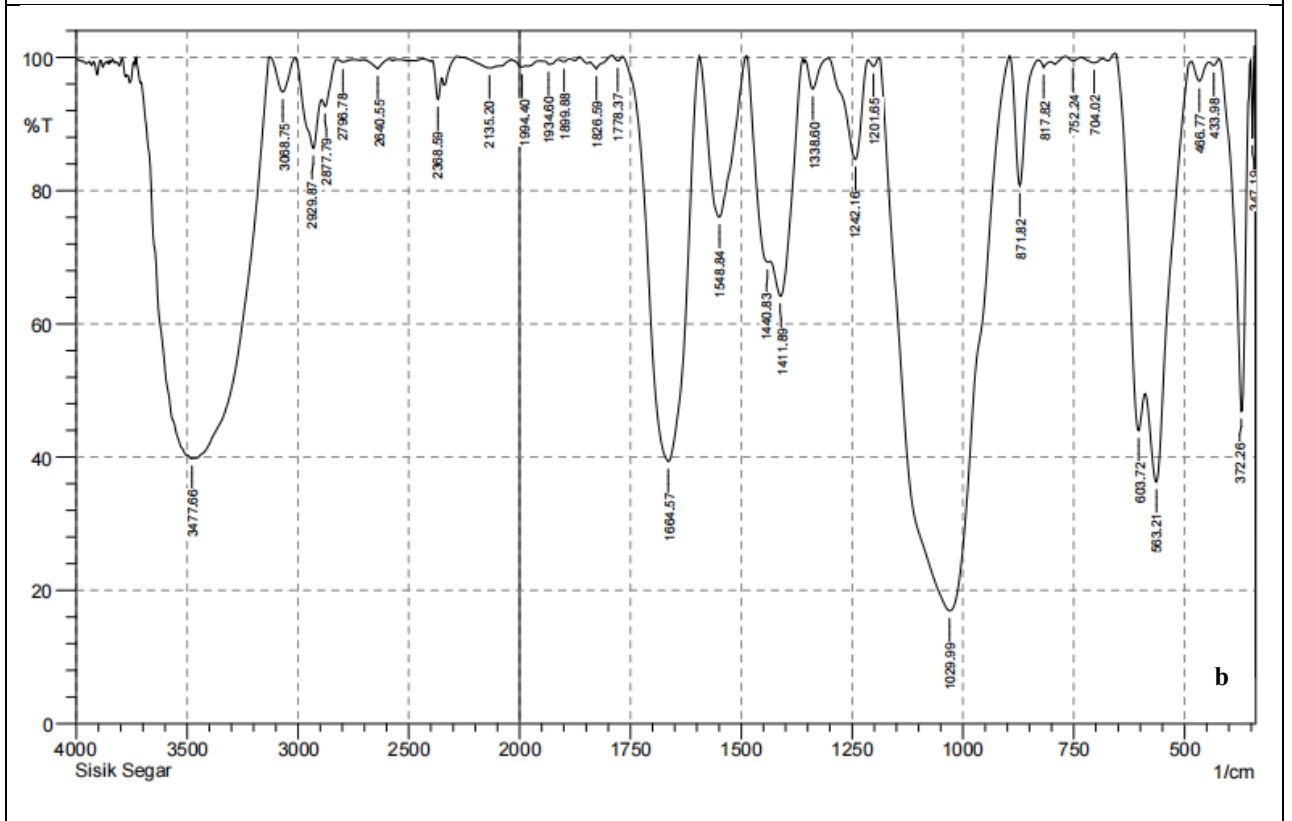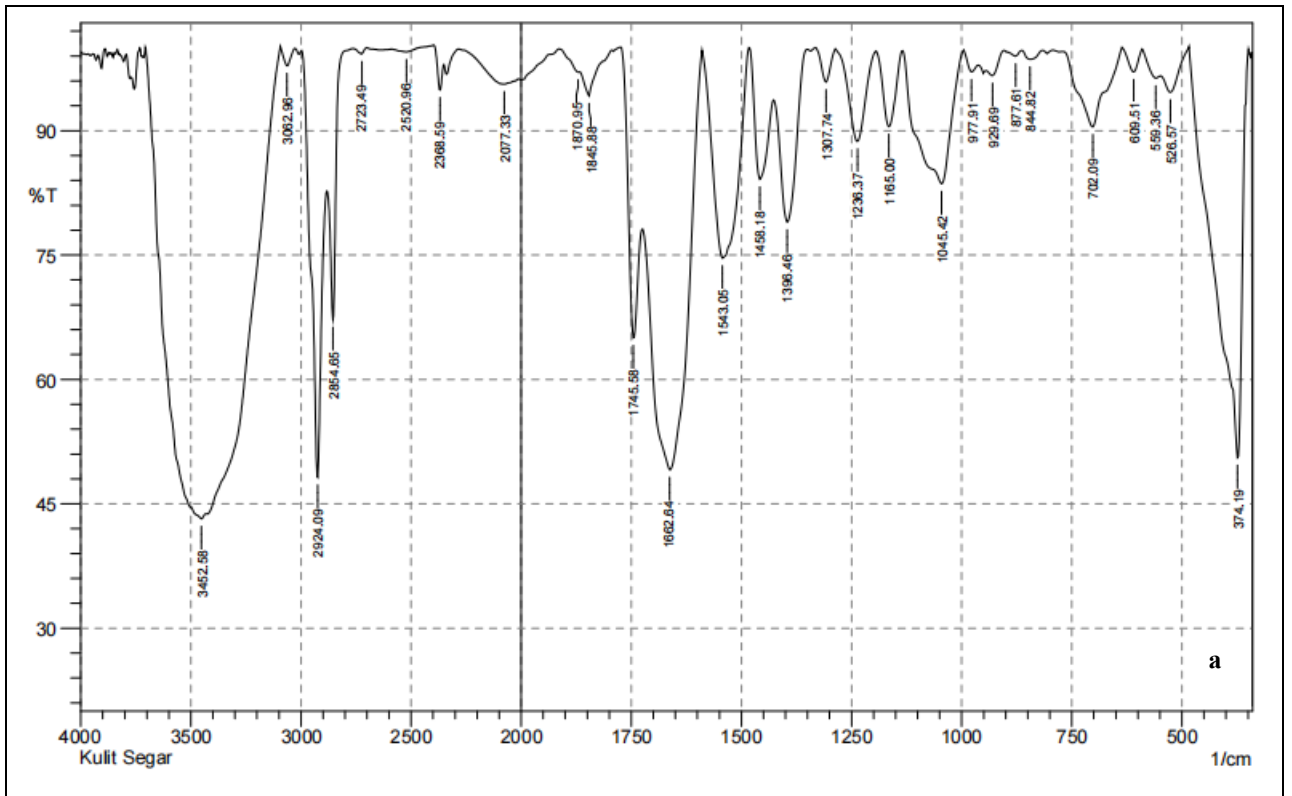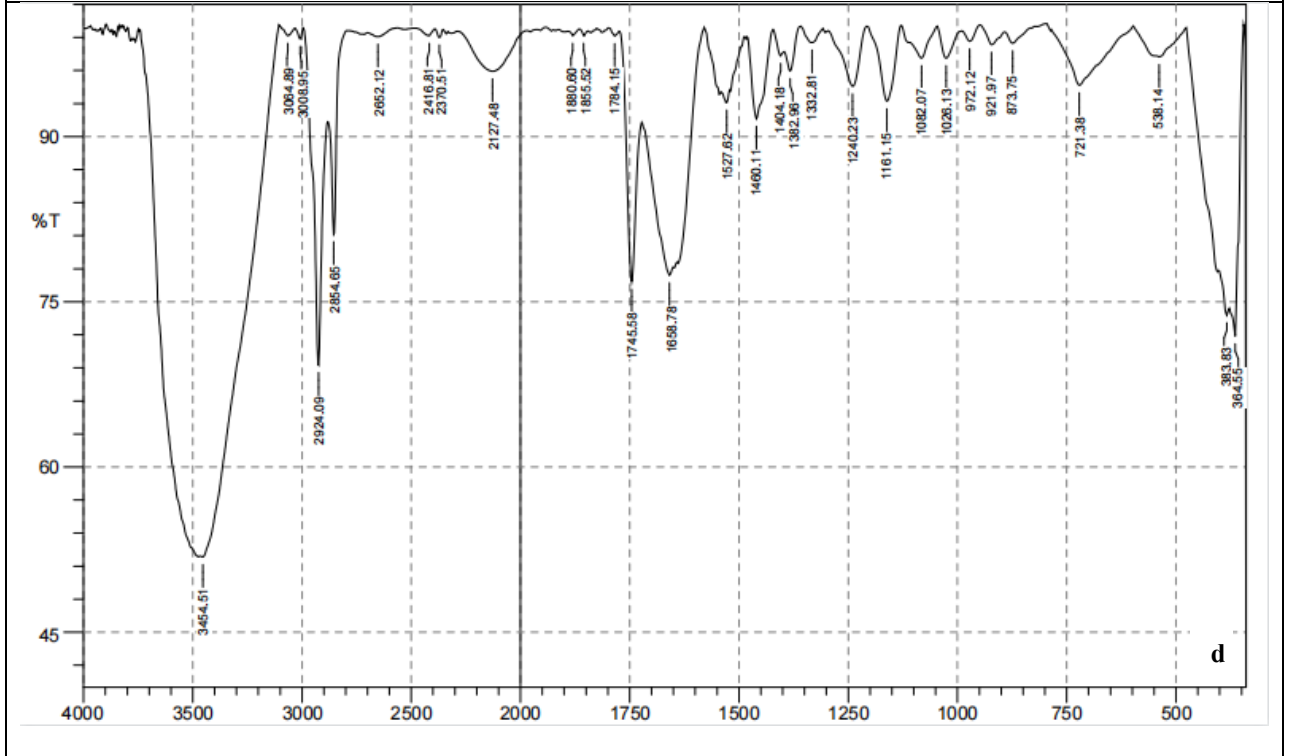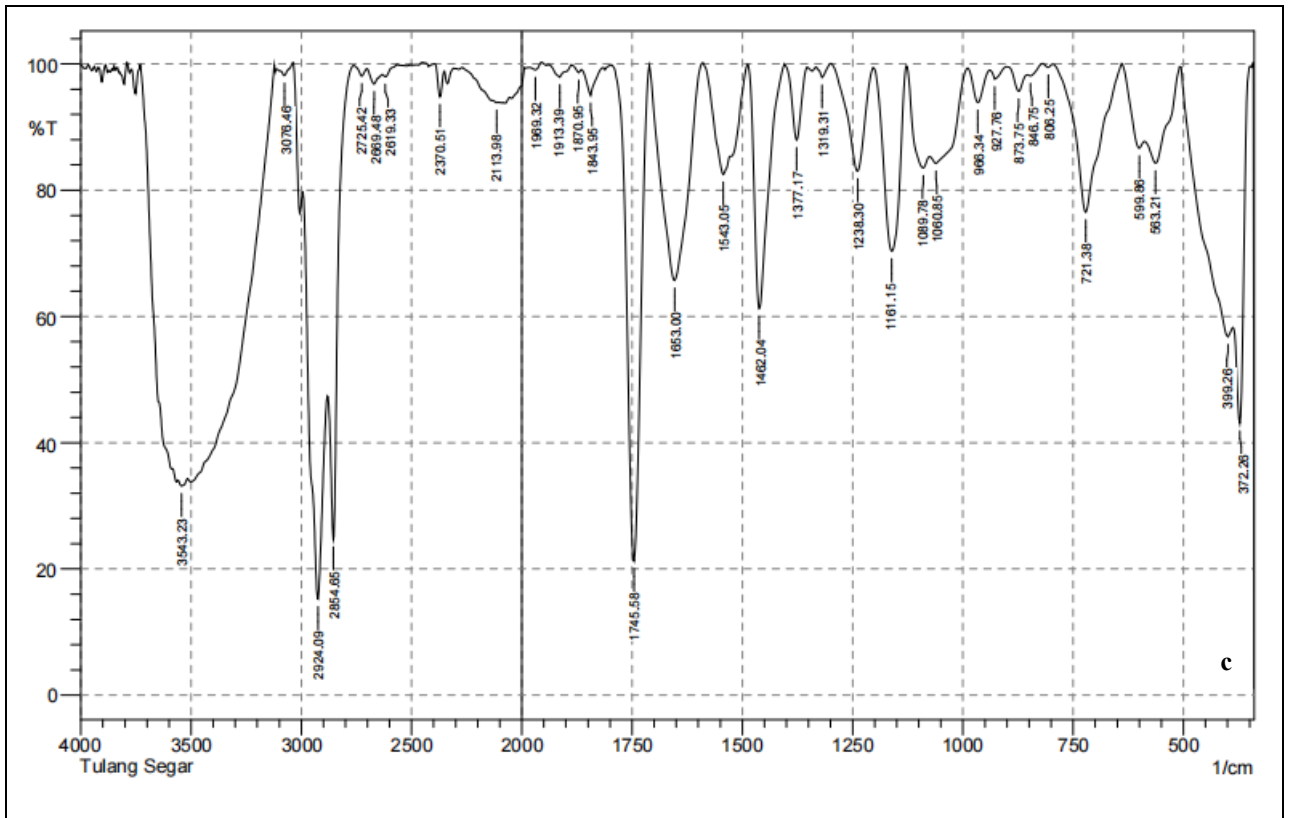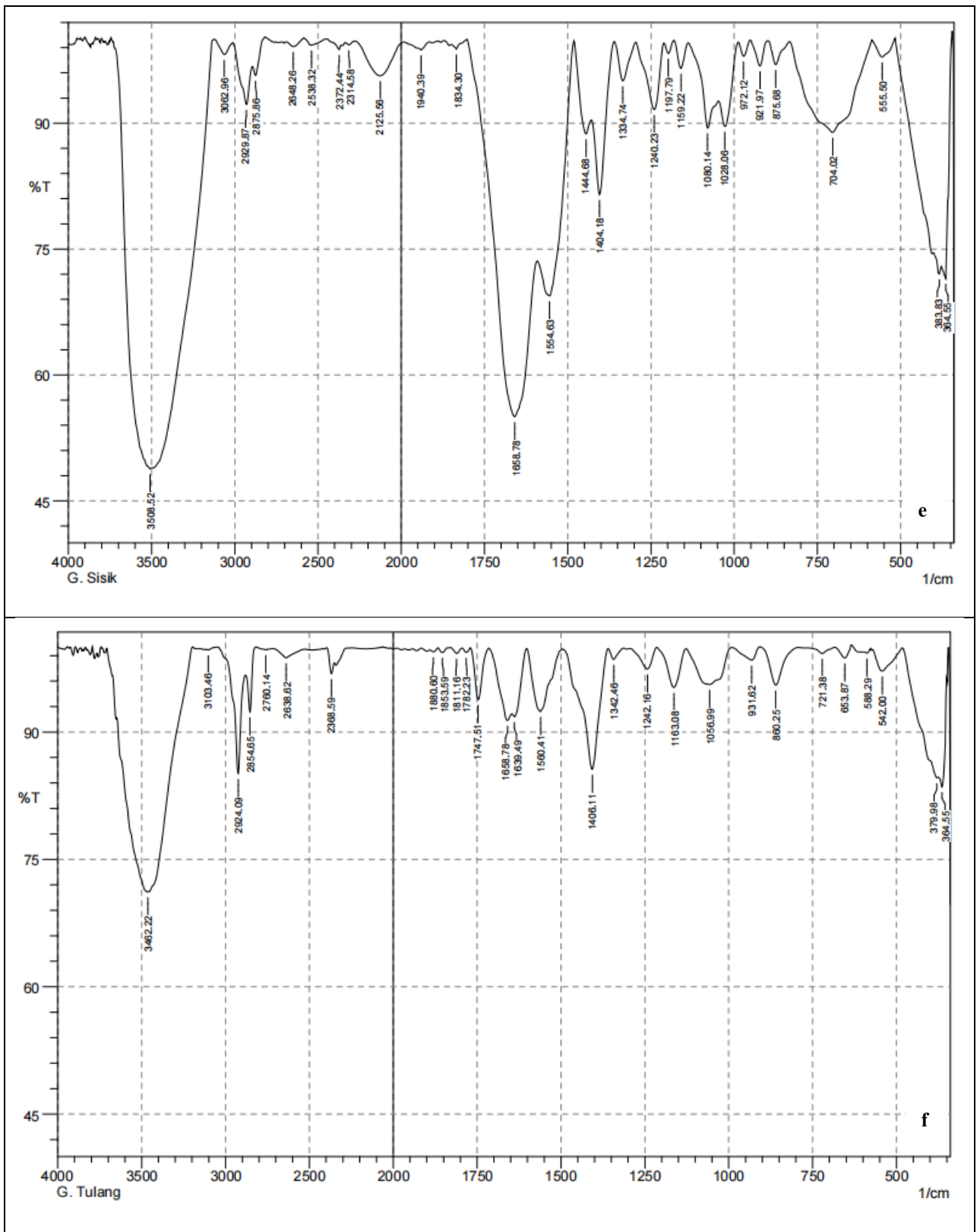
Supplementary Fig. 1. FT-IR spectra of raw material and gelatin of snakehead fish *Channa striata* (a = raw skin, b = raw scales, c = raw bone, d = skin gelatin, e = scales gelatin, and f = bone gelatin).