Functional characterization of gelatin from freshwater fish scales using *Averrhoa bilimbi* acid pretreatment

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Abstract. This study aims to evaluate the characteristics of gelatin extracted from the scales of three selected freshwater fish species: Giant gourami, *Osphronemus goramy* (Lacépède, 1801); Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758); and Common carp, *Cyprinus carpio* (Linnaeus, 1758). The extraction process used an acid treatment with bilimbi (*Averrhoa bilimbi*) at a concentration of 3% (v/v) for 48 hours. The results showed that the protein content of fish scale gelatin ranged from 90.62 to 92.24 g (100 g)$^{-1}$. The average gelatin yield (dry weight basis) was 24.14 g (100 g)$^{-1}$ for Giant gourami, 20.21 g (100 g)$^{-1}$ for Nile tilapia, and 35.89 g (100 g)$^{-1}$ for Common carp. The extracted gelatin exhibited acidic properties, with a pH range of 6.65–6.68. Foaming ability (FA) was assessed based on foam volume, while foam stability (FS) was determined by volume loss. The FA and FS values were 4.85 and 0.65 for Giant gourami, 4.83 and 0.57 for Nile tilapia, and 5.06 and 0.29 for Common carp, respectively. Furthermore, the water-holding capacity (WHC) and fat-binding capacity (FBC) of the gelatin varied, with values of 720.00 and 249.67 g (100 g)$^{-1}$ for Giant gourami, 630.33 and 221.00 g (100 g)$^{-1}$ for Nile tilapia, and 312.00 and 44.33 g (100 g)$^{-1}$ for Common carp, respectively. Gel strength and viscosity of gelatin from Giant gourami (303.95 g and 7.85 cP) were higher than those from Nile tilapia (165.37 g and 7.78 cP) and Common carp (225.5 g and 6.83 cP). This study concludes that pretreating fish scales with *Averrhoa bilimbi* acid (3% v/v) for 48 hours is an effective method for gelatin extraction. Among the three species, Giant gourami scales show the greatest potential as a source of high-quality gelatin for industrial applications.
Key Words: fish scale gelatin, pretreatment, *Averrhoa bilimbi* acid, freshwater fish, functional properties.

Introduction. Fish is a source of high-quality protein due to its abundant availability, relatively affordable price, and safe nutritional content (Roberts et al 2023; Azrita et al 2024). Despite the widespread consumption, many people may be unaware of the large amount of scale waste generated by capture fisheries and aquaculture (Qin et al 2022; Thirukumaran et al 2022). The rapid expansion of the fish processing industry significantly supports global economic growth (Glaus et al 2024). Millions of tons of waste, including scales, are discarded in landfills worldwide each year (Manjudevi et al 2024). Additionally, the disposal of scales originating from mass fish mortality in floating net cage farming can contribute to aquatic environmental pollution (Syandri et al 2023).

Fish scales commonly considered waste have the potential to be developed into high-value products that provide environmental and economic benefits (Nurilmala et al 2024). These natural resources are widely applied as biomaterials (Segato et al 2024) and composed of hydroxyapatite, chitin, collagen, and gelatin structurally arranged in a hierarchical form identical to the hard tissue found in other animals (He et al 2022; López-Pedrouso et al 2023). Furthermore, the advantages are attributed to the mechanical properties, biodegradability, and biocompatibility.

Gelatin products from fish scales have been used over the past few years in the fields of food science and engineering, agriculture, nutraceuticals, adsorbents, pharmaceuticals, cosmetics, and tissue engineering (Venugopal 2022; Boronat et al 2023). The numerous applications prompt this study to show the need for an implementation of the circular economy concept in scale biomass conversion and future development prospects (Liu et al 2022; Cooney et al 2023).

Scale biomass waste can be processed into gelatin using conventional industrial chemicals such as glacial acetic acid during pretreatment or the initial acidification stage (Martins et al 2018; Zuraida & Pamungkas 2020; Xu et al 2021). In this process, alkaline solutions including sodium hydroxide (NaOH), are also applied as essential chemicals (He et al 2022; Venupriya et al 2022; Gaidau et al 2023).

The pretreatment stage carried out in several previous investigations has not applied the acid solution formula of the bilimbi plant (Averrhoa bilimbi, local name: Belimbing wuluh), which is locally sourced, environmentally friendly, and more economical. Therefore, this study aimed to analyze the proximate profile, amino acid composition, yield, and functional properties of gelatin extracted from scales of Giant gourami, *Osphronemus goramy* (Lacépède, 1801), Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), and Common carp, *Cyprinus carpio* (Linnaeus, 1758) using an initial acidification method with *Averrhoa bilimbi* acid solution.

Material and method

Animal materials. In August 2024, samples were collected from floating net cage fish farmers at Lake Maninjau, Agam Regency, West Sumatra Province, Indonesia. The samples contained 50 Giant gourami, with standard lengths ranging from 22.0 to 24.4 cm (mean: 23.26±0.73 cm) and weights of 451 to 549 g (mean: 500.84±28.13 g). Additionally, Nile tilapia had standard lengths ranging from 17.2 to 20.2 cm (mean: 18.77±0.83 cm) and weights of 200 to 290 g (mean: 245±29.01 g). The total length of the Common carp ranged from 22.0 to 24.4 cm (mean: 23.26±0.73 cm), and the body weights were 451.0 to 549.0 g (mean: 500.84±28.13 g). Before measuring length and weight, the fish samples were anesthetized in ice water at a temperature of 5°C. Euthanasia was performed by piercing the fish's brain using a 21G needle. Afterward, the fish were immediately placed in an ice box and transported fresh to the laboratory. The samples were then verified by the Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Bung Hatta University, Indonesia.

Preparation of formulation solution, fish scale, and gelatin extraction. Fresh bilimbi fruit (Averrhoa bilimbi) was washed with distilled water, then thinly sliced, and its seeds were removed. Next, 500 g of bilimbi pulp was blended using a Philips blender with a capacity of 1.25 L, manufactured in China. The blended pulp was then filtered using a tea strainer with a mesh size of 250 μm. The obtained solution, containing ascorbic, citric, and oxalic acids, was used as the formulation solution. Scales from three selected freshwater fish species were obtained using a stainless steel collector measuring 17.5 cm × 3.5 cm × 1.5 cm. These were washed with clean water, drained, and soaked in a 3% (v/v) *Averrhoa bilimbi* acid solution at a scale-to-solution ratio of 1:4, implying that 100 g of scales were immersed in a mixture of 12 mL of *Averrhoa bilimbi* acid and 388 mL of distilled water for 48 hours. Subsequently, scales were rinsed with distilled water until the solution's pH reached seven before extraction.

Scales were extracted using distilled water at a scale-to-solution ratio of 1:4 in an electric oven set to 70°C for 4 hours. The extraction results were filtered using Whatman Grade 1 filter paper (110 mm), then the filtrate was poured into a 20 × 15 cm plastic tray with a thickness of 0.2 cm and left to dry at room temperature (28–30°C) for 48 hours until gelatin sheets formed. The dried sheets were cut into 3 × 2 cm pieces for water-holding capacity (WHC) and fat-binding capacity (FBC) analysis.

A portion of gelatin was ground using a Miller Powder Grinder and separated through a 150 μm sieve to obtain fine powder. The proximate composition and amino acid content of gelatin powder from each fish species were analyzed.

Nutritional evaluation of gelatin powder. Gelatin flour samples were analyzed for proximate composition (the moisture, crude protein (micro Kjedahl), and ash content of fish scales, and gelatin was determined using AOAC standard methods (AOAC 1990). Meanwhile, the Soxhlet method with ether was used to extract crude fat, and samples were burned for 16 hours at 550°C to determine the amount of ash present.
The procedures described by Cohen (2003) were followed for conducting amino acid analysis. The amino acid profile was assessed using high-performance liquid chromatography (HPLC), a Waters 1525 binary pump, a Waters 717 autosampler (Waters®), and a Waters 2475 multi-λ fluorescence detector with wavelengths set at 250 nm for excitation and 395 nm for emission. Furthermore, the materials were hydrolyzed in triplicate using 6 N hydrochloric acid for 24 hours at 110°C.

Amino acid analysis was conducted using the methods outlined by Cohen (2003). The amino acid composition was determined using an HPLC system, which included a Waters 1525 binary HPLC pump, a Waters 717 autosampler, and a Waters 2475 multi λ fluorescence detector with excitation and emission wavelengths set at 250 nm and 395 nm, respectively. The samples were hydrolyzed in triplicate using 6 N hydrochloric acid at 110°C for 24 hours.

Yield. The following formula was used to determine the results based on weight and protein content. Additionally, gelatin production was calculated using the following formula, which consists of weight and protein content, as described by Jamilah et al (2011):

(i) % Yield (wet weight basis)=(dry weight of gelatin)/(wet weight of scale) × 100

(ii) % Yield (dry weight basis)=(dry weight of gelatin)/(wet weight of scale - moisture content) × 100

(iii) % Yield (protein basis) =(dry weight of gelatin)/(protein content of scales) × 100

Acidity (pH). A 1.0 g gelatin sample was dissolved in 100 mL of distilled water, and the pH was determined at 25°C, as described by Cheow et al (2017).

Foam formation ability (FA) and foam stability (FS). Foam stability (FS) and foaming ability (FA) of gelatin were evaluated following the method described by Cho et al (2004). A gelatin solution of 1 g/100 mL was heated to 60°C to enable swelling and the initial sample volume was recorded as Vo. This was continuously homogenized using a magnetic stirrer for three minutes to generate foam, and the resulting volume was identified as V. After 30 minutes, the sample volume was measured as V1 before the calculation of FA and FS carried out using the following formulas:

Foam formation ability (FA)=(Volume of foam (mL) + Volume of liquid (mL))/(Initial volume of solotion (mL))

Foam stability (FS)=(Volume of foam + Volume of liquid (initial))/(Volume of foam + Volume of liquid (after 30 min.))

Water holding capacity (WHC) and fat binding capacity (FBC). The method described by Cho et al (2004) with slight modifications was used to assess the FBC and WHC of gelatin. A graduated cylinder containing 1.0 g of gelatin was filled with 10 mL of maize oil (for FBC) or distilled water (for WHC). The mixture was left to stand at room temperature for 30 minutes. After removing the excess water or maize oil through filtration, the moist sample was reweighed. WHC and FBC were determined using the following formula:

$$WHC/FBC\ (g/100\ g) = ((V1-V0)/V0 \times 100\%)$$

Where:
V1 = Weight of the sample after water/fat absorption (g);
V0 = Initial dry weight of the sample (g).

Gel strength. The gel strength of the gelatin was determined following the method outlined by Liao et al (2021), with slight modifications by Asiamah et al (2024). A total of 7.5 g of gelatin was dissolved in a bloom jar (150 mL capacity) containing 105 mL of distilled water to achieve a 6.67% solution. The mixture was left to stand at room temperature for approximately 3 hours to allow for full hydration. It was then heated at 40°C for 30 minutes using a magnetic stirrer and subsequently cooled for 15 minutes before being stored at 4°C (±1°C) for 17 hours (±1 h) before analysis. The gel strength was measured using a TAXT2 texture analyzer (Stable Micro System, UK) fitted with a standard 0.5-inch diameter cylindrical probe. The probe was lowered at a speed of 0.5 mm/s to a penetration depth of 4 mm, with the maximum force recorded during penetration representing the Bloom strength.

Viscosity. The viscosity of gelatin was determined using an Ostwald viscometer (Type No. 4) designed for high-viscosity liquids. To prepare a solution, 6.67 g of gelatin was added to 100 mL of distilled water and heated at 60°C until fully dissolved. Viscosity measurement was conducted at room temperature based on Shyni et al (2014).

Data analysis. The SPSS 16.0 software (SPSS, Chicago, IL) was used to analyze collected data, while the homogeneity of variances was assessed through Levene's test. For each species, the parameters of gelatin yield, pH, FA, FS, WHC, and FBC from three selected freshwater fish species were examined using a one-way Analysis of Variance (ANOVA). Subsequently, Duncan's multiple range test (Duncans 1955) was performed to carry out post hoc comparisons. The results for every parameter were presented as mean values ± standard errors.

Result and Discussion

Percentage of by-products from three selected fish species. The by-products of three selected fish species consist of parts frequently discarded during processing, such as the head, scales, fins, skin, bones, and viscera (Table 1). Composition analysis showed that the head represented the largest fraction of the by-products, accounting for 16.36% to 20.07% of body weight, followed by bones (11.26%–16.03%) and viscera (8.41%–21.04%). Scales contributed the least, ranging from 3.27% to 6.41%, while the skin accounted for approximately 5.37% to 7.09% of body weight. The results were consistent with the report by Thirukumaran et al (2022) stating that the head and skin contributed 21.5% and 3.3%, respectively, to the total fish by-products. This study corresponded with the report of Masood et al (2015) regarding fish scale waste comprising 3%–4.2% of body weight across various fish species. Similarly, Sari & Rohma (2024) found Striped snakehead and *Channa striata* (Bloch, 1793) scales occupied 5.12% of body weight, which correlated with the range observed in this study. The variation in by-product proportions among species is possibly influenced by factors such as body size, specific morphological characteristics, and processing methods. The higher proportions of the head and bones show the potential use as sources of collagen and gelatin. Scales present in smaller quantities remain valuable as raw materials in the biomaterial industry. Therefore, optimizing the application of fish by-products contributes to waste reduction and supports the principles of a circular economy in sustainable fisheries.

Table 1

Products from three selected freshwater fish species

Components	Fillet (%)	Head (%)	Scales (%)	Fins (%)	Skin (%)	Bones (%)	Viscera (%)
Giant gourami	37.42±0.97	20.07±0.50	6.41±0.33	3.72±0.27	7.09±0.95	16.03±1.45	8.41±0.84
Nile tilapia	33.61±3.91	21.35±3.74	3.27±0.31	3.81±0.55	5.57±0.89	14.63±2.24	8.88±1.40
Common carp	39.64±0.98	16.36±1.20	3.76±0.79	3.54±0.12	6.34±0.36	11.26±0.45	21.04±1.2

Sample number, n=25.

Biochemical composition of fish gelatin

Moisture. The moisture content of gelatin extracted from scales of Giant gourami, Nile tilapia, and Common carp was 8.24, 8.78, and 6.76 g (100 g)$^{-1}$, respectively (Table 2). Nile tilapia gelatin had the highest moisture content, followed by Giant gourami and Common carp. Approximately 9.20 g (100 g)$^{-1}$ of moisture and 1.18 g (100 g)$^{-1}$ of ash were similarly found in gelatin obtained from scales of Rohu labeo (*Labeo rohita*) (Jakhar et al., 2016). In comparison, 7.11 g(100 g)$^{-1}$ to 13.65 g (100 g)$^{-1}$ moisture content of gelatin was extracted from Bighead carp (*Hypophthalmichthys nobilis*) (Tu et al 2013). In animal tissues, moisture serves as an essential component present in physically and chemically bound forms (Njinkoue et al 2016). Factors such as water temperature and the acid or alkali type used during the pretreatment stage influence the moisture content of gelatin (Wahyuningtyas et al 2019). These play an important role in determining gelatin physicochemical properties, which affect the application in food and pharmaceutical industries.

Protein. In this study, the protein content of the extracted gelatin after undergoing pretreatment with a 3% (v/v) *Averrhoa bilimbi* acid formula (1:4 w/v) was 91.16 g (100 g)$^{-1}$, 90.62 g (100 g)$^{-1}$, and 92.24 g (100 g)$^{-1}$ for Giant gourami, Nile tilapia, and Common carp, respectively. For comparison, gelatin obtained from Nile tilapia scales using a 3% hydrochloric acid (HCl) pretreatment (1:6 w/v) had a protein content of 86.90 g (100 g)$^{-1}$ (Sockalingam & Abdullah 2014). Gelatin extracted from scales of Bighead carp using an ultrasound purifier at 200 W and 60°C for 1 hour was reported with a high protein content, ranging from 84.15 to 91.85 g (100 g)$^{-1}$ (Tu et al., 2013). In addition to the content, the physicochemical properties of gelatin are affected by protein composition (Wu et al 2023).

The food, cosmetic, and pharmaceutical industries popularly use gelatin as a hydrolyzed collagen product (Boronat et al 2023; Gaidau et al 2023). The valuable source of gelatin commonly processed are fish scales comprised of high collagen content. Protein content found in the extracted gelatin varies based on factors such as fish species (Jamilah et al 2011), farming systems and seasonal changes (Asiamah et al 2024), extraction methods (Arshad et al 2021), and pretreatment conditions (Zhang et al 2011). The quality and functional properties of gelatin are affected by the mentioned factors, thereby influencing the potential applications in different industries globally.

Fat. The fat content of three gelatin samples was each less than 0.15 g (100 g)$^{-1}$, signifying a minimal lipid presence in the extract. This low content is a desirable characteristic that enhances the purity and stability of gelatin, promoting its suitability for various industrial applications, such as the food, pharmaceutical, and cosmetic sectors. Fat content can vary depending on multiple factors, such as fish species and the extraction method used, including acid or alkaline pretreatment (He et al 2022). These pretreatment processes influence the efficiency of lipid removal, affecting gelatin's final composition and functional properties. Additionally, gelatin processed using different extraction methods shows variations in physical and chemical characteristics, including gel strength, viscosity, and thermal stability (Huang et al 2019). These variations are critical in determining the suitability of gelatin for specific applications, emphasizing the importance of optimizing extraction conditions to achieve the desired properties.

Ash. Ash content in gelatin derived from three freshwater fish species was relatively low, ranging from 0.48 to 1.19 g (100 g)$^{-1}$. This result suggests that the obtained gelatin has a minimal mineral residue, which is generally associated with higher purity and better functional properties. Previous studies reported similar results, with ash content of approximately 2 g (100 g)$^{-1}$ in gelatin extracted from small fish scales (He et al 2022; Hariyanti et al 2023; Nurilmala et al 2024).

The mineral composition in scales, which contributes to ash content, plays a crucial role in protecting fish from unfavorable aquatic conditions and enhancing mobility (Zhu et al 2012). The presence of inorganic components, such as calcium and phosphorus, affects the structural integrity of scales and may influence the composition of the resulting gelatin. However, a lower ash content is generally preferred, as it represents a higher degree of purity and improved quality. Chandra and Shamasundar (2015) reported that gelatin with reduced ash content had superior physicochemical properties, leading to enhanced suitability for applications in the food, pharmaceutical, and cosmetic industries. The relatively low ash content observed in this study reinforces the high quality of the extracted gelatin, showing its potential as an alternative source of fish-derived gelatin for various industrial applications.

Table 2

Composition of moisture, protein, fat, and ash in gelatin derived from scales of three selected freshwater fish species (dry weight basis)

Composition (%)	*Giant gourami*	*Nile tilapia*	*Common carp*
Moisture	7.52±0.02^{a}	8.58±0.02^{b}	6.76±0.01^{c}
Protein	91.16±0.05^{a}	90.62±0.01^{b}	92.24±0.02^{c}
Fat	0.14±0.01^{a}	0.15±0.01^{b}	0.12±0.01^{c}
Ash	1.19±0.02^{a}	0.65±0.02^{b}	0.48±0.01^{c}

Values are presented as the mean ± SD (%) based on triplicate measurements. Lowercase superscript letters a,b, and c denote significant variations among the three fish species ($p<0.05$); Sample number, n=25.

Amino acid profiles. Amino acid identified in the extracted gelatin showed significant differences, with total contents of 91.16 g(100 g)$^{-1}$, 86.82 g(100 g)$^{-1}$, and 89.64 g (100 g)$^{-1}$ in Giant gourami, Nile tilapia, and Common carp, respectively. A similar trend was observed in the compositional percentages (Table 2). Glycine, arginine, histidine, and lysine had higher percentages in Giant gourami scale gelatin (Table 3). Meanwhile, threonine, glutamic acid, and Aspartic acid had higher percentages in gelatin extracted from Common carp and Nile tilapia. Fish scale gelatin serves as a biomaterial rich in essential amino acids, including leucine, valine, phenylalanine, methionine, Arginine, histidine, lysine, proline, and threonine. This contains several non-essential amino acids, such as alanine, glycine, glutamic acid, and aspartic acid (Truc et al 2022; He et al 2022). Glycine is the most dominant in Giant gourami, accounting for 28.18 g (100 g)$^{-1}$ of total amino acid, which is significantly higher than the values of 21.37 g (100 g)$^{-1}$ obtained from Bighead carp scales (*Hypophthalmichthys nobilis*) (Tong and Ying 2013) and 23.83 g (100 g)$^{-1}$ found in carp (He et al., 2022). The amino acid composition of gelatin is influenced by the applied pretreatment method (Peng et al 2022).

The initial treatment of scales from three selected fish species using 3% (v/v) *Averrhoa bilimbi* acid (1:4) generated a total amino acid content of 91.15, 86.82, and 89.64 g (100 g)$^{-1}$ in Giant gourami, Nile tilapia, and Common carp, respectively. Meanwhile, the use of 4% (w/v) NaOH for the initial treatment of scales from carp, Bighead carp, and Peled (*Coregonus peled*) produced total amino acid contents of 97.70, 91.19, and 92.39 g (100 g)$^{-1}$, respectively (He et al 2022). This difference may be due to variations in gelatin sources, which can affect the total amino acid content.

The amino acid was classified based on the side chain properties related to water solubility, distinguishing between hydrophobic and hydrophilic (He et al 2022). Table 3 shows the hydrophobic and hydrophilic amino acid compositions of gelatin obtained from scales of three fish species, while Figure 1 presents the total amounts. The structure of amino acids in gelatin or protein plays a crucial role in determining WHC and FBC. The balance between hydrophilic and hydrophobic amino acids in protein structure is a key factor influencing the ability of gelatin to retain water and form a stable foam (Wu et al 2023). An optimal proportion of amino acid can enhance the capacity of protein to bind water and improve FS. Differences in amino acid composition may be influenced by various factors, including the source of gelatin, which subsequently affects WHC and FBC.

Table 3

Amino acid composition of three selected freshwater fish species

Amino acid (g (100 g)$^{-1}$ protein)	*Giant gourami scale gelatin*	*Nile tilapia scale gelatin*	*Common carp scale gelatin*
Leucine	2.04	2.16	2.65
Valine	1.3	1.2	2.02
Isoleucine	0.93	0.9	1.11
Tryptophan	0.09	0.08	0
Phenylalanin	1.02	1.86	2.3
Methionine	0.46	0.43	2.05
Proline	9.27	9.45	11.4
Alanine	8.9	8.56	10.36
Glycine	28.18	25.2	27.04
Arginine	14.75	12.56	3.06
Histidine	1.58	1.11	1.41
Lysine	3.99	3.86	3.7
Threonine	2.41	2.25	2.56
Tyrosine	0.28	0.33	0.18
Glutamic acid	7.14	7.86	10.1
Cystine	0	0.01	0.01
Aspartic acid	5.47	5.8	6.04
Serine	3.34	3.2	3.65
Total	91.15	86.82	89.64

Samples number, n=25.

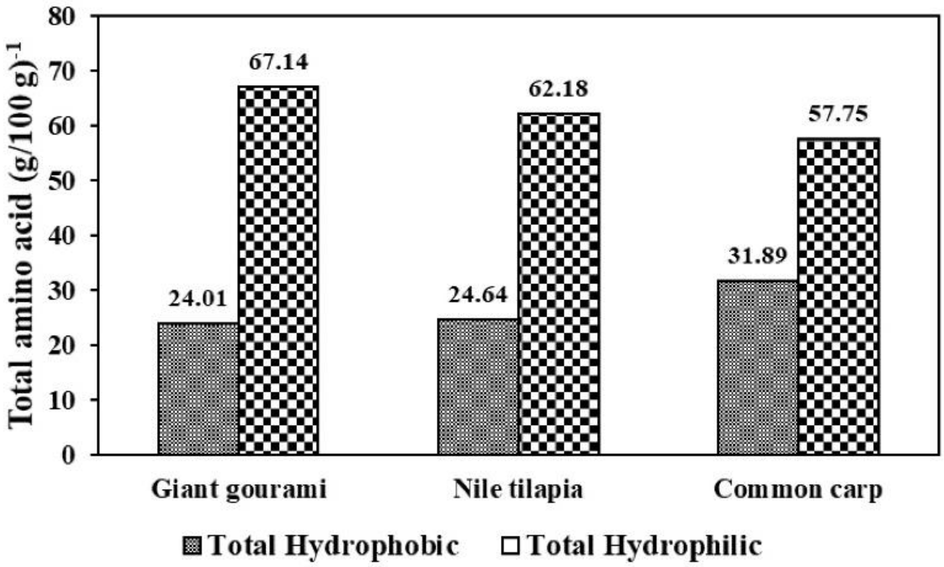

Figure 1. Comparison of hydrophobic and hydrophilic amino acids in gelatin extracted from fish scales of three species.

Yield. Table 4 presents the yield data determined based on wet weight, dry weight, and protein content. Considering all three parameters, the highest yield was observed in Giant gourami gelatin, followed sequentially by Nile tilapia and Common carp. Several studies reported different gelatin yields from various fish species and body parts. Jakhar et al. (2016) obtained gelatin yields of 6.52 g (100 g)$^{-1}$ and 7.21 g (100 g)$^{-1}$ from Catla and Rohu, respectively. Meanwhile, gelatin yields from fish scales have been reported to range between 2.12 and 15.12 g (100 g)$^{-1}$ (Wahyuningtyas et al 2019; Truc et al 2022; Nurilmala et al 2024; Asiamah et al 2024). The yield was affected by extraction pH, as well as the used alkali and acid concentrations (Ninan et al., 2011). In this study, the primary factor contributing to yield differences among species was protein content variation.

PH. pH values of gelatin derived from scales of Giant gourami, Nile tilapia, and Common carp were 6.68, 6.60, and 6.65, respectively (Table 4). Although there were slight variations in pH among species, a broader range of 4.02 to 6.24 has been reported for fish gelatin (Nurilmala et al 2024). The pH values of gelatin obtained from scales of Barramundi (*Lates calcalifer*) and Nile tilapia were recorded at 3.86 and 5.09, respectively (Hariyanti et al 2023). These differences are influenced by various factors, including different extraction pH (Weng et al 2014), liming process (Jamilah et al 2011), and extraction temperature (Liao et al 2021). The use of a 3% (v/v) *Averrhoa bilimbi* acid formula in the pretreatment process of scales from three selected fish species for gelatin production appears to be a novel method.

Table 4

Gelatin yield, and pH from three selected freshwater fish species

Characteristic	*Giant gourami scale gelatin*	*Nile tilapia scale gelatin*	*Common carp scale gelatin*
Yield (wet weight basis) (%)	11.22±0.02^{a}	8.32±0.03^{b}	7.53±0.08^{c}
Yield (dry weight basis) (%)	18.29±0.52^{a}	14.08±1.00^{b}	12.87±0.12^{c}
Yield (protein basis) (%)	24.14±0.96^{a}	20.21±0.74^{b}	17.39±0.46^{c}
Acidity (pH)	6.68±0.04^{a}	6.60±0.04^{b}	6.65±0.03^{c}

Values are presented as the mean ± SD (%)based on triplicate measurements. Lowercase superscript letters a, b, and c denote significant variations in three fish species ($p < 0.05$); Sample number, n=25.

FA and FS. The pre-acidification process (pretreatment) in this study using 3% (w/v) of locally sourced, environmentally friendly, and cost-effective *Averrhoa bilimbi* acid formula generated a higher FA ratio in gelatin derived from Common carp scales compared to Giant gourami and Nile tilapia scales. Meanwhile, the FS of gelatin from Giant gourami scales was higher, representing better FS. This suggests that the formed foam can last longer without collapsing compared to gelatin from Nile tilapia and Common carp scales (Table 5).

A study by He et al (2022) using 4% (w/v) NaOH for pretreatment reported FA ratios of gelatin from Peled fish, Common carp, and Bighead carp scales as 1.15, 1.45, and 1.62, while FS ratios were 0.40, 0.27, and 0.46, respectively. The higher FS observed in this study has potential applications in various industries, particularly in food production, including marshmallow and whipped topping manufacturing, as well as in cosmetics and pharmaceuticals, where FS is crucial in product formulations. Differences in FA and FS values can be influenced by several factors, such as protein composition, solubility, viscosity, gelatin molecular weight, and temperature, including used pretreatment with acetic acid or NaOH (Shakila et al 2012; Lin et al 2015; Derkach et al 2020; Gong et al 2025). Therefore, selecting the appropriate gelatin type depends on the specific application needs, which can be to emphasize foam FA or FS.

Table 5

Functional properties of gelatin from scales of three freshwater fish species

Properties	*Giant gourami*	*Nile tilapia*	*Common carp*
FA ratio	4.85±0.05^{a}	4.83±0.10^{b}	5.06±0.31^{c}
FS ratio	0.65±0.00^{a}	0.57±0.01^{b}	0.29±0.02^{c}
WHC (g (100 g)$^{-1}$)	720.00±6.55^{a}	630.33±8.96^{b}	312±15.71^{c}
FBC (g (100 g)$^{-1}$)	249.67±0.57^{a}	221±11.53^{b}	44.33±2.08^{c}

Values are presented as mean ± SD (%) based on triplicate measurements. Lowercase superscript letters a, b, and c denote significant variations in three fish species ($p<0.05$); Sample number, n=25.

WHC and FBC. According to Table 5, the WHC of gelatin extracted from Nile tilapia scales was lower than the values obtained from Giant gourami and Common carp scales. This lower WHC is primarily associated with a lower content of hydrophilic amino acid and a reduced hydroxyproline concentration, which play a crucial role in the ability of gelatin to retain water (Ninan et al 2011; Wu et al 2023). As reported in several studies, a higher hydroxyproline content enhances collagen network stability, contributing to greater water absorption capacity (Ninan et al 2011).

The results showed that the FBC of gelatin from Giant gourami scales was significantly high (249.67g 100g^{-1}), compared to the extremely lower values obtained in Common carp (44.33g 100g^{-1}). This difference is attributed to the exposure level of hydrophobic residues on the gelatin molecule surface and the amount of tyrosine, which influences foam formation and stability (Ninan et al 2011). Tyrosine promotes intermolecular interactions in protein solutions, affecting viscosity and foam structure stability (Wu et al 2023). Therefore, differences in amino acid composition and hydrophobic properties among these gelatin types contribute to variations in the functional characteristics.

Gel strength. Gel strength is a crucial parameter for evaluating the functional properties of gelatin (Liao et al 2021). It also serves as the primary criterion for categorizing gelatin quality into three levels: low bloom (<150 g), medium bloom (150–200 g), and high bloom (220–300 g). In this study, the gel strength of gelatin extracted from Giant gourami, Nile tilapia, and Common carp was 303.95 g, 165.37 g, and 225.5 g, respectively (Figure 2A). These values indicate significant differences ($p<0.05$) in the gel-forming ability of gelatin derived from the scales of these three freshwater fish species. The results suggest that the gelatin produced in this study meets acceptable quality standards. Generally, gelatin with low gel strength yields a softer and less elastic texture, making it unsuitable for applications requiring a firm structure (Wu et al 2023). He et al (2022) reported that the gel strength of gelatin from Common carp and Bighead carp was 334.77 g and 643.28 g, respectively, both of which were higher than the values observed in this study. However, the gel strength of Nile tilapia gelatin in this study (165.37 g) was greater than that of Nile tilapia gelatin obtained from aquaculture during the rainy and dry seasons, which ranged between 100–120 g (Asiamah et al 2024).

Viscosity. The viscosity of gelatin obtained from Giant gourami scales was recorded as higher (7.85±0.30 cP) compared to Nile tilapia (7.78±0.10 cP) and Common carp (6.83±0.17 cP) (Figure 2B). Generally, tropical fish such as Common carp and Nile tilapia tend to have a higher viscosity than fish from cold climates, which is attributed to differences in amino acid composition and molecular weight distribution (Joy et al 2024). Previous studies reported that the viscosity of gelatin from Nile tilapia scales cultured during the dry season reached 25.56 cP, while the value decreased to 17.03 cP in the rainy season (Asiamah et al 2024). Variations in viscosity among species and body parts have been observed, with gelatin from Common carp scales showing a viscosity of 26.3 cP (Dinçer et al 2016), Milkfish, *Chanos chanos* (Fabricius 1775) had 5.23 cP (Ismail et al 2019), and Greater lizardfish, *Saurida tumbil* (Bloch, 1795) had 7.5 cP (Wangtueai & Noomhorm 2009). These results confirm that fish species and environmental conditions play a crucial role in determining the viscosity characteristics of gelatin produced.

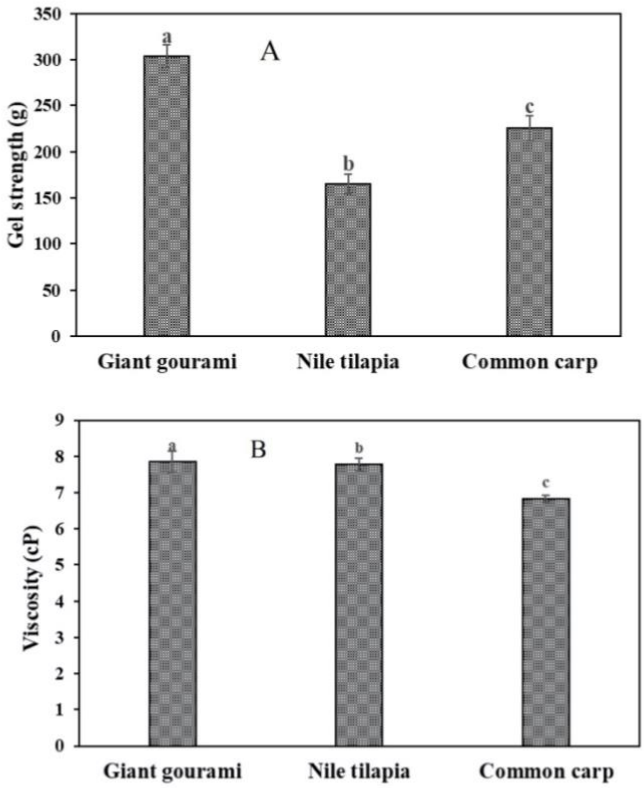

Figure 2. Gel strength (A) and viscosity (B) of gelatin extracted from three freshwater fish species.

Conclusion. In conclusion, the findings of this study demonstrate that the scales of Giant gourami, Nile tilapia, and Common carp, when pre-treated with a 3% (v/v) *Averrhoa bilimbi* acid solution, can be utilized to extract commercial gelatin with good functional properties and yield. However, gelatin extracted from Giant gourami exhibited superior functional properties and yield compared to the other species. This suggests that Giant gourami scales have greater potential as a source of high-quality gelatin for industrial applications. Further research is needed to explore its potential use as an ingredient in fish feed formulations and the treatment of aquatic animal diseases.

Conflicts of interest. The authors declare no conflicting interests.

Acknowledgment. The authors are grateful for the financial support provided by the Indonesia Ministry of Education, Culture, Research, and Technology, under grant number 112/E5/PG.02.00.PL/2024. The authors are also grateful to the students, fishermen, and fish farms that participated in this study as well as PT Internasional Translasi Edukasi, Jakarta, for the assistance rendered in editing the manuscript in English. Experiments were conducted according to the guidelines in the SOP (Standard Operating Procedures) of the Bung Hatta University Aquaculture Laboratory.

References

Arshad N. M., Ghaffar M. A., Mohtar N. F., 2021 Optimization of the extraction procedures and the characterization of fish gelatin from fringescale sardinella (Sardinella fimbriata) bones. AACL Bioflux 14(2):672-682.

Asiamah E., Atter A., Ofori H., Akanor P. T., Nkeita S., Koivula H., Lee Y., Agyakwah S., 2024 Effect of seasonal variation and farming systems on the properties of Nile tilapia gelatin extracted from scales. Heliyon 10(2):e24504.

Azrita A., Syandri H., Zakri H. A., Damanhuru H., Aryani N., 2024 Analysis of fatty acids and amino acids of three local freshwater Bagridae fish species in the Kampar Kanan

river, Indonesia, for food security. International Journal of Food Science 2024(1):6639837.
Boronat O., Sintes P., Celis F., Diez M., Ortiz J., Aguilo-Aguayo I., Martin-Gomez H., 2023 Development of added-value culinary ingredients from fish waste: Fish bones and fish scales. International Journal of Gastronomy and Food Science 31:100657.
Chandra M. V., Shamasundar B. A., 2015 Texture profile analysis and functional properties of gelatin from the skin of three species of fresh water fish. International Journal of Food Properties 18(3):572-584.
Cheow C. S., Norizah M. S., Kyau Z . Y., Howell N. K., 2017 Preparation and characterization of gelatins from the skins of sin croaker (Johnius dussumieri) and shortfin scad (Decapterus macrosoma). Food Chemistry 10(1):386-391.
Cho S. M., Kwak K. S., Park D . C, Gu J .S., Ji C . I., Jang D. H., Lee Y .B., Kim S. B., 2004 Processing optimization and functional properties of gelatin from shark (Isurus oxyrinchus) cartilage. Food Hydrocolloids, 18(4):573-579.
Cohen S. A., 2003 Amino Acid Analysis Using Pre-Column Derivatization with 6-AminoquinolylN-Hydroxysuccinimidyl Carbamate. Methods in Molecular Biology, 211:143-54.
Cooney R., de Sausa D. P., Fernandez-Rios A., Mellett S., Rowan N., Morse A P., Hayes M., Laso J., Regueiro L., Wan A HL., Clifford E., 2023 A circular economy framework for seafood waste volarisation to meet challenges and opportunities for intensive production and sustainability. Journal of Cleaner Production 392:136283.
Derkach S . R., Voron’ko N . G., Kuchina Y. A., Kolotova D . S., 2020 Modified fish gelatin as an alternative to mammalian gelatin in modern food technologies. Polymers 12(12):30-51.
Dinçer M. T., Erdem Ô. A., Kalkan H, Üçok M . C., 2016 Comparison of recovered carp scales (Cyprinus carpio) gelatin and commercial calf and pork skin gelatins. Ege Journal of Fisheries and Aquatic Sciences 33(4):335-341.
Duncan D. B., 1955 Multiple range and multiple F tests. Biometrics 11(1):1-42.
Gaidau C., Rapa M., Stanc M., Tanase M L., Olariu L., Constantinescu R R., Lazea-Stoyanova A., Alexxe C-A., 2023 Fish scale gelatin nanofibers with Helichrysum italicum and Lavandula latifolia essential oils for bioactive wound-healing dressngs. Pharmaceutics 15(12):2692.
Glaus K., Savau R., Brunnschweiler J.M., 2024 Characteristics of Fiji’s small-scale ray fishery and its relevance to food security. Marine Policy 163:106082.
Gong H., Chen L., Kan G., Zhang W., Qian Q., Wang X., Zhong J., 2025 Benzenedialdehyde-crosslinked gelatin nanoparticles for Pickering emulsion stabilization. Current Research in Food Science 10:100961.
Hariyanti., Nafiah K., Azizah N., Gantini S.N., 2023 [Effects of extraction temperature on the characteristics of gelatin from Lates calcarifer and Oreochromis niloticus scales]. JPB Kelautan dan Perikanan 18(2): 96-104. [in Indonesian]
He J., Zhang J., Ma Y. U. Y., Guo X., 2022 The structural and functional differences between three species of fish scale gelatin and pigskin gelatin. Foods 11(24):3960.
Huang T., Tu Z., Shangguan X., Sha X., Wang H., Zhang L., 2019 Fsih gelatin modifications: A comprehensive review. Trends in Food Science & Technology 86:260-269.
Ismail I., Hamzah N., Qurrataayyun S., Rahayu S., Tahir K. A., Djide M. N., 2019 Extraction and Characteristic of Gelatin from Milkfish (Chanos chanos) Scales and Bones with Variation in Acid and Base Concentrations, Extracting and Drying Method. Pp 210-215.
Jakhar J. K., Kumar A., Vardia H. K., 2016 Extraction of gelatin from skin and scale of Indian Major Carps. Environment & Ecology 34:1513-1518.
Jamilah B., Tan K. W., Hartina M. U., Azizah, A., 2011 Gelatins from cultured freshwater fish skins obtained by liming process. Food Hydrocolloids 25(5):1256-1260.
Joy J.M., Padmaprakashan A., Pradeep A., Paul P.T., mannuthy R.J., Mathew S., 2024 A review on fish skin-derived gelatin: Elucidating the gelatin peptides- preparation, bioactivity, mechanistic insights and strategies for stability improvement. Foods 13(17):2793.

Liao W., Zhu Y., Lu Y., Wang Y., Dong X., Xia G., Shen X., 2021 Effect of extraction variables on the physical and functional properties of tilapia gelatin. LWT-Food Science and Technology 146:111514.
Lin C. C., Chiou T. K., Sung W. C., 2015 Characteristics of Gelatin from Giant Grouper (Epinephelus lanceolatus) Skin. International Journal of Food Properties 18(11):2339–2348.
Liu Q., Trevisan A.H., Yang M., Mascarenhas J., 2022 Aframework of digital technologies for the circular economy: Digital functions and mechanisms. Business Strategy and the Environment 31(5):2171-2192.
López-Pedrouso M., Lorenzo J. M., Bou R., Vasquez J.A., Valcarcel J., Toldrã M., Franco D., 2023 Valorisation of pork by-products to obtain antioxidant and antihypertensive peptides. Food Chemistry 423:136351.
Manjudevi M., Kamaraj M., Aravind J., Wong L.S., 2024 Application of the circular economy to fish scale waste. Sustainable Chemistry for the Environment 8:100170.
Martins M. E. O., Sausa J. R., Claudino R. L., Lino S. C. O., do Vale D. A., Silva A. L. C., 2018 Thermal and chemical properties of gelatin from Tilapia (Oreochromis niloticus) scale. Journal of Aquatic Food Product Technology 27(10):1120-1133.
Masood Z., Yasmeen R., Haider M.S., Tarar O.M., Hossain M.Y., 2015 Evaluations of crude protein and amino acid contents from the scales of four mullet species (Mugilidae) collected from Karachi fish harbour, Pakistan. Indian Journal of Geo-Marine Science 44(5).
Ninan G., Abubacker Z., Jose J., 2011 Physico-chemical and texture properties of gelatins and water gel desserts prepared from the skin of freshwater carps. Fish Technology, 48(1):67-74.
Njinkoue J. M., Gouado I., Tchoumbougnang F., Yanga Ngueguim J. H., Ndinteh D. T., Fomogne-Fodjo C. Y., Schweigert F. J., 2016 Proximate composition, mineral content and fatty acid profile of two marine fishes from Cameroonian coast: Pseudotolithus typus (Bleeker, 1863) and Pseudotolithus elongatus (Bowdich, 1825). NFS Journal 4:27-31.
Nurilmala M., Ramadhan W., Putri A. N., 2024 Characteristics of tilapia fish scale gelatin and its application in Surimi. Bio Web of Conferences 112:09001.
Peng J., Zi Y., Xu J., Zheng Y., Huang S., Hu Y., Liu B., Wang ., Zhong J., 2022 Effect of extraction methods on the properties of Tilapia scala gelatins. International Journal of Biological Macromolecules 221:1150-1160.
Qin D., Bi S., You X., Wang M., Cong X., Yuan C., Yu M., Cheng X., Chen X-G., 2022 Development and application of fish scale wastes as versatile natural biomaterials. Chemical Engineering Journal 428:131102.
Roberts N., Mengge B., Oaks B., Sari N., Irsan., Humphries A., 2022 Fish consumption pathways and food security in an Indonesian fishing community. Food Security 15(1):1-19.
Sari S. F., Rohma A.Y., 2024 Characteristics of Snakehead fish, Channa striata (Bloch, 1793) byproduct and its gelatin properties. Asian Fisheries Society 37(3):159-173.
Segato J., Calmanti R., Gnoato G., Cavarzerani E., Rizzolio F., Cretini C., Perosa A., Gigli M., Selva M., 2024 Fish waste for patches: Tailoring films assembled from fish waste gelatin, carbon dots and chitin nanocrystals. Advanced Sustainable Systems 8(12):2400413.
Shakila R. J., Jeevithan E., Varatharajakumar A., Jeyasekaran G., Sukumar D., 2012 Functional characterization of gelatin extracted from bones of red snapper and grouper in comparison with mammalian gelatin. LWT - Food Science and Technology 48(1):30–36.
Shyni K., Hema G. S., Ninan G., Mathew S., Joshy C. G., Lakshmanan P. T., 2014 Isolation and characterization of gelatin from the skins of skipjack tuna (Katsuwonus pelamis), dog shark (Scoliodon sorrakowah), and rohu (Labeo rohita). Food Hydrocolloids 39:68–76.
Sockalingam K., Abdullah H. Z. 2014 Extraction and characterization of gelatin biopolymer from black tilapia (Oreochromis niloticus) scales. In AIP conference proceedings 1669(1).

Syandri H., Azrita A., Mardiah A., Aryani N., Diharmi A., 2023 The proximate composition, amino acid profile, fatty acid content, and mineral content of scale flour from three fish species as potential feeds for fish fry. F1000 Research 12:1144.
Thirukumaran R., Priya V. K. A., Krishnamoorthy S., Ramakrishnan P., Moses J. A., Anandharamakrishnan C., 2022 Resource recovery from fish waste: Prospects and the usage of intensified extraction technologies. Chemosphere 299:134361.
Tong Y., Ying T., 2013 Gelling strength improverment and charactization of a gelatin from scales of Bighead carp (Aristichthys nobilis). Journal of Food, Agriculture and environment 11(1):146-150.
Truc T. T., Thom N. V., Ha N. T. N., Thuan N. H. D., Thuy L. T. M., 2022 Properties of gelatin extracted from snakehead fish (Channa striata) by-products at various temperatures and times. Food Science and Technology 42:e79922.
Tu Z., Huang T., Wang H., Sha X., Shi Y., Huang, Man Z., Li D. J., 2013 Physico-chemical properties of gelatin from Bighead carp (Hypophthalmichthys nobilis) scales by ultrasound-assisted extraction. Journal of Food Science and Technology 52:2166-2174.
Venugopal V., 2022 Green processing of seafood waste biomass towards blue economy. Current Research in Environmental Sustainability 4:100164.
Venupriya V., Krisnaveni V., Ramya M., 2023 Effect of acidic and alkaline pretreatment on functional, structural and thermal properties of gelatin from waste fish scales. Polymer Bulletin 80(9):10533-10567.
Wahyuningtyas M., Jaddid N., Burhan P., Atmaja L., 2019 Physical and chemical properties of gelatin from red snapper scales: temperature effects. Jurnal Teknik ITS 8(2):F95-F101.
Wangtueai S., Noomhorm A., 2009 Processing optimization and characterization of gelatin from lizardfish (Saurida spp.) scales. LWT-Food Science and Technology 42(4):825-834.
Weng H., Liang Y., Wang H., Zhang H., Wang M., Liu L., 2014 Physical-chemical properties of collagens from skin, scale, and bone of grass carp (Ctenopharyngodon idellus). Journal of Aquatic Food Product Technology 23(3):264-277.
Wu J., Xiao J., Zhu M., Yang H,. Liu J., Liu Y., 2023 Study of physicochemical and gelation properties of fish gelatin from different sources. Applied Sciences 13(9):5337.
Xu J., Zhanng T., Zhang Y., Yang L., Nie Y., Tao N., Wang X., Zhong J., 2021 Silver cap scale gelatins for the stabilization of fish oil-loaded emulsions. International Journal of Biological Macromolecules 186:145-154.
Zhang F., Xu S., Wang Z., 2011 Pre-treatment optimization and properties of gelatin from freshwater fish scales. Food and Bioproducts Processing 89(3):185-193.
Zhu D., Ortega C. F., Motamedi R., Szewciw L., Vernerey F., 2012 Structure and mechanical performance of a "modern" fish scale. Advanced Engineering Materials 14(4):B185-B194.
Zuraida I., Pamungkas B. F., 2020 Effects of acid pretreatment and extraction temperature on the properties of gelatin from striped snakehead (Channa striata) scales. AACL Bioflux 13(5):2937-2944.
***AOAC (Association of Official Analytical Chemists), 1990. Official Methods of Analysis. 15th Edn., Washington, DC., USA.

Received: 01 March 2025. Accepted: 09 April 2025. Published online: 09 April 2025.
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How to cite this article:
Syandri H., Azrita A., Elfrida E., Aryani N., 2025 Functional characterization of gelatin from freshwater fish scales using *Averrhoa bilimbi* acid pretreatment. AACL Bioflux 18(2):822-835.