

Article

Valorization of Rohu (*Labeo rohita*) Fish Scale Waste Through Gelatin Extraction: Physicochemical, Thermal, Rheological, and Functional Characterization

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Abstract

Fish scales are collagen-rich processing by-products with considerable potential as a sustainable source of non-mammalian gelatin. This study aimed to extract gelatin from Rohu (*Labeo rohita*) fish scales and comprehensively characterize its physicochemical, thermal, structural, rheological, textural and functional properties for potential food and industrial applications. Gelatin was extracted by acid demineralization followed by hot-water extraction and characterized using physicochemical analyses, Fourier transform infrared spectroscopy (FTIR), sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), texture profile analysis (TPA) and functional property measurements. The extracted gelatin exhibited a yield of 21.86%, a protein content of 88.37%, an ash content of 1.5%, a gel strength of 182.82 g, a viscosity of 6.56 cP, a solubility of 87.49%, a melting temperature of 23.38 °C, a setting temperature of 20.4 °C and a setting time of 660 s. FTIR and SDS–PAGE confirmed the characteristic structural features of type I collagen-derived gelatin with well-preserved α_1 , α_2 and β chains. The gelatin also demonstrated favorable water-holding capacity (169.70%), fat-binding capacity (188.50%), foaming capacity (193.33%) and foaming stability (77.65%), while emulsion capacity and stability increased with gelatin concentration, reaching 44.11% and 42.15%, respectively, at a gelatin concentration of 2.0%. These findings demonstrate the potential of Rohu fish scale gelatin as a sustainable, value-added non-mammalian biomaterial for food, packaging, pharmaceutical and related industrial applications while promoting the valorization of fish-processing waste.



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Keywords: fish scale gelatin; fish-processing by-products; waste valorization; functional properties

1. Introduction

Gelatin is a natural, biodegradable biopolymer obtained through the partial hydrolysis of collagen and is widely used in the food, pharmaceutical, biomedical, photographic, cosmetic, and packaging industries due to its excellent gelling, thickening, emulsifying,

foaming, and film-forming properties. Owing to its high biocompatibility and biodegradability, gelatin remains one of the most versatile biopolymers employed across diverse industrial sectors [1]. The global gelatin market was valued at approximately USD 3.7 billion in 2023 and is projected to grow at a compound annual growth rate (CAGR) of 6.6% through 2028, driven by increasing demand from the food, pharmaceutical, nutraceutical, cosmetic, and biomedical sectors [2]. This sustained market growth highlights the increasing demand for sustainable and high-quality gelatin from alternative sources. Traditionally, gelatin has been produced from bovine and porcine skins and bones. However, these conventional sources are associated with several limitations, including religious and cultural restrictions, concerns regarding zoonotic disease transmission, and environmental impacts associated with livestock production [3]. Consequently, increasing attention has been directed toward fish-processing by-products as sustainable alternative sources of gelatin. The valorization of these by-products supports circular bioeconomy principles by reducing environmental impacts while converting processing waste into value-added biomaterials for food, pharmaceutical, biomedical, and packaging applications [4].

Global fisheries and aquaculture production reached 235 million tonnes in 2024, generating substantial quantities of processing by-products, including skins, bones, fins, viscera, and scales, during post-harvest processing [5]. Many of these by-products remain underutilized or are converted into low-value products such as animal feed, fishmeal, fertilizers, and fish oil, despite being rich sources of collagen and other valuable biomaterials [6,7]. Fish scales alone constitute approximately 1–5% of total fish weight and contain up to 60% type I collagen, the most desirable collagen isoform for high-quality gelatin production [8]. Structurally, fish scales are composed of a highly ordered composite of collagen fibers and calcium-deficient hydroxyapatite, resembling mineralized tissues such as bone and teeth, which makes them particularly attractive for functional and biomedical material applications [9,10].

Fish-derived gelatin offers several advantages over mammalian gelatin, including lower immunogenicity, higher solubility in cold water, and lower melting and gelation temperatures, which are beneficial for heat-sensitive food and pharmaceutical applications. Additionally, fish gelatin demonstrates compatibility with bioactive compounds and nanofillers [11], making it suitable for edible films, coatings, and active packaging systems. However, challenges such as lower bloom strength, higher sensitivity of viscosity to pH and temperature, and variability arising from species differences and extraction conditions necessitate systematic characterization and optimization [12].

Among freshwater fish species, Rohu (*Labeo rohita*) is a major carp extensively cultured and consumed across South Asia, generating substantial quantities of scale waste during processing. Previous studies have demonstrated the feasibility of extracting gelatin from Rohu scales and have primarily focused on gelatin extraction and basic physicochemical characterization [1,13]. However, a systematic evaluation integrating physicochemical, functional (textural, foaming, emulsifying, water- and fat-binding), thermal, rheological, and structural characteristics relevant to industrial applications has not yet been reported.

Therefore, the present study aimed to extract gelatin from Rohu (*L. rohita*) scales and comprehensively characterize its physicochemical, thermal, rheological, and functional properties. The findings of this study provide valuable information for the sustainable valorization of Rohu fish scale waste and support the development of alternative non-mammalian gelatin for food, pharmaceutical, packaging, and other industrial applications.

2. Materials and Methods

2.1. Raw Materials

Rohu fish (*L. rohita*) scales were procured from local fish markets in Kawardha, Chhattisgarh, India, and transported under iced conditions to the laboratory of the Department of Fish Harvest and Post-Harvest Technology, LSPN College of Fisheries, Kawardha. The collected scales were thoroughly washed with tap water to remove adhering impurities and subsequently dried in a hot-air oven at 60 °C. The dried scales were then used as the primary raw material for gelatin extraction.

2.2. Gelatin Extraction

Gelatin extraction was carried out using an acid extraction method following the procedure described by He et al. [8] with slight modifications. Dried Rohu fish scales (Figure 1A) were initially subjected to demineralization using 0.5 M hydrochloric acid (Hi-Media Laboratories Pvt. Ltd., Mumbai, India) at a scale-to-solution ratio of 1:10 (*w/v*) for 12 h at room temperature. To ensure efficient mineral removal, the acid solution was replaced at 3 h intervals throughout the demineralization process. Following treatment, the scales were repeatedly washed with distilled water until a neutral pH was attained.

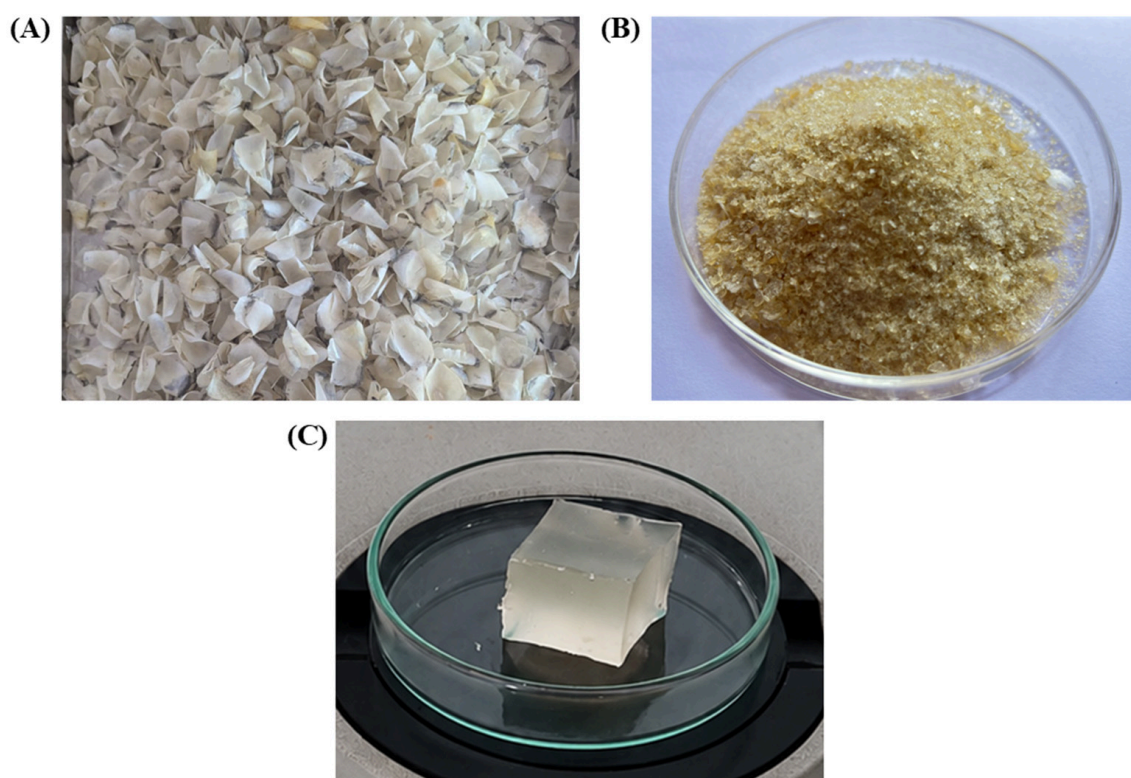


Figure 1. Representative images showing the preparation and appearance of fish scale gelatin: (A) dried fish scales used as the raw material, (B) extracted gelatin in powdered form, and (C) gelatin gel (6.67% (*w/v*); 2 × 2 cm).

The demineralized scales were then mixed with distilled water at a ratio of 1:6 (*w/v*) and extracted in a water bath maintained at 60 °C for 8 h. The extracted solution was filtered through a mesh filter cloth to remove insoluble residues. The filtrate obtained was subsequently dried in a hot-air oven at 70 °C until complete moisture removal to obtain gelatin powder (Figure 1B). Preliminary optimization trials were conducted to

establish suitable demineralization conditions, and the detailed procedure is provided in Appendix A.

2.3. Characterization of Gelatin

2.3.1. Physicochemical Properties

Gelatin Yield

Gelatin yield was determined as the percentage ratio of the dry weight of extracted gelatin to the initial dry weight of dried fish scales following the method of He et al. [8]. Gelatin yield was calculated using the following equation.

$$\text{Yield (\%)} = \frac{m}{M} \times 100 \quad (1)$$

where m represents the mass of the extracted gelatin (g), and M represents the mass of dried fish scales (g).

Determination of Protein Content and Ash

Protein and ash contents were determined according to the Association of Official Analytical Chemists (AOAC) [14] methods. Protein content was analyzed by the Kjeldahl method using a conversion factor of 5.55, while ash content was determined by incineration in a muffle furnace.

Color

The color parameters (L^* , a^* , and b^*) of gelatin powder and gelatin gel (6.67% w/v) were measured using a HunterLab Color Analyzer (ColorFlex EZ, Hunter Associates Laboratory, Inc., Reston, VA, USA). The gelatin samples were finely powdered and placed in a 1.25 inch glass sample port with a black cover. Prior to measurement, the instrument was properly standardized according to the manufacturer's instructions. Here, L^* represents lightness, a^* indicates redness/greenness, and b^* denotes yellowness/blueness.

Determination of Melting Temperature, Setting Temperature, and Setting Time

The melting temperature, setting temperature, and setting time of gelatin were determined according to the method described by Ninan et al. [15] with slight modifications. Gelatin solutions (6.67%, w/v) were prepared by dissolving gelatin in distilled water, and 5 mL aliquots were transferred into test tubes. The tubes were sealed with parafilm and heated in a water bath maintained at 60 °C for 15 min to ensure complete dissolution. The samples were subsequently cooled rapidly in ice-chilled water and conditioned at 4 °C for 16–18 h to facilitate gel formation.

For melting temperature determination, five drops of a mixture containing 75% chloroform and 25% red dye were carefully placed onto the surface of the gelatin gel. The test tubes were then immersed in a water bath maintained initially at 10 °C, and the temperature was increased gradually at a rate of 1 °C/min. The temperature at which the dye droplets began to move freely through the gel matrix was recorded as the melting temperature.

For the determination of setting temperature, freshly prepared gelatin solutions (6.67%, w/v) were transferred into test tubes and placed under refrigerated conditions. The sample temperature was monitored at 30 s intervals using a thermometer. The temperature at which the gelatin solution ceased to flow from the thermometer tip was recorded as the setting temperature.

For setting time determination, gelatin solutions prepared under similar conditions were refrigerated at 4 °C. A glass rod was inserted into the gelatin solution and lifted at 15 s intervals. The time required for gel formation, indicated by the point at which the gelatin no longer detached from the rod upon lifting, was recorded as the setting time.

FTIR

The FTIR spectra of gelatin powder were obtained under ambient conditions using an FTIR spectrometer (Nicolet iS50, ThermoFisher Scientific, Waltham, MA, USA) operated in ATR (Attenuated Total Reflectance) mode, in the range of 4000–600 cm^{-1} , with a resolution of 4 cm^{-1} and a total of 32 scans.

SDS-PAGE

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis of the extracted gelatin was performed following the method described by Elavarasan et al. [16]. Dried gelatin (200 mg) was dissolved in 9.8 mL of 5% SDS solution and heated in a boiling water bath for 30 min to ensure complete solubilization. The solution was subsequently cooled and centrifuged at $9000 \times g$ for 15 min at room temperature to remove insoluble materials. The supernatant was mixed with sample buffer at a ratio of 1:1 (*v/v*). The sample buffer consisted of 0.5 M Tris–HCl (pH 6.8), 4% SDS, 20% glycerol, and 10% β -mercaptoethanol. The resulting mixture was heated in a boiling water bath for 90 s, cooled, and stored under frozen conditions until further analysis.

Prior to electrophoresis, the samples were thawed, and 5 μL aliquots were loaded onto polyacrylamide gels consisting of a 7.5% resolving gel and a 4% stacking gel. Electrophoresis was carried out at a constant voltage of 90 V using a Mini Protein II electrophoresis unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). Following separation, protein bands were stained using 0.05% (*w/v*) Coomassie Brilliant Blue G-250 prepared in 8% (*v/v*) acetic acid and subsequently destained with 8% (*v/v*) acetic acid to enhance band visualization. A prestained protein ladder (MBT092-100LN, HiMedia, Maharashtra, India) was used as a molecular weight marker.

2.3.2. Functional Properties

Solubility

Gelatin solubility was determined following the method described by He et al. [8]. Gelatin solutions (2 mg/mL) were centrifuged at 5000 rpm for 20 min at 4 °C, and the protein concentration in the resulting supernatant was determined using the biuret method. Solubility was calculated as the percentage ratio of the protein concentration in the supernatant to the initial protein concentration using the following equation:

$$S (\%) = \frac{c}{C} \times 100 \quad (2)$$

where *S* represents solubility (%), *c* denotes the protein concentration in the supernatant (mg/mL), and *C* represents the initial protein concentration of the gelatin solution (mg/mL).

Gel Strength and Texture Profile Analysis (TPA)

Gel strength

The gel strength was determined according to the British Standard 757:1975 method [17], with slight modifications, using a Texture Analyser, (Model TA.XTplus, Stable Microsystems, Surrey, England) equipped with Exponent Lite software version 3.0.5.0 (Stable Microsystems, Surrey, UK). A 6.67% (*w/v*) gelatin solution was prepared by dissolving 7.50 g of gelatin in 105 mL of distilled water in 100 mL beakers. The mixture was swirled thoroughly and allowed to stand at room temperature for 1 h to facilitate hydration and swelling of the gelatin. Subsequently, the beakers were transferred to a water bath maintained at 65 °C for 25 min to ensure complete dissolution of the gelatin, with occasional swirling during heating. After dissolution, the samples were cooled at room temperature for

15 min and then placed in a refrigerator maintained at 5 ± 1 °C for 16–18 h prior to gel strength analysis.

For gel strength analysis, the gelatin samples were set in 100 mL beakers and positioned centrally beneath a 5 mm plunger probe attached to a Texture Analyser equipped with Exponent Lite software (Stable Microsystems, Surrey, UK). The analysis was performed using a 5 kg load cell at a crosshead speed of 0.5 mm/s.

Texture profile analysis (TPA)

Textural properties, including hardness, adhesiveness, springiness, cohesiveness, chewiness, gumminess, and resilience, were determined using a Texture Analyser (Model TA.XTplus, Stable Microsystems, Surrey, England) equipped with Exponent Lite software (Stable Microsystems, Surrey, UK), a 75 mm compression plate, and a 50 kg load cell. Gelatin gel samples were prepared in 100 mL beakers following the modified British Standard 757:1975 [17] method. The gels were cut into cubes (2×2 cm) (Figure 1C), and five cubes from each sample were subjected to Texture Profile Analysis (TPA) at room temperature. The samples were compressed to 40% of their original height under the following conditions: pre-test speed of 1 mm/s, post-test speed of 5 mm/s, test distance of 8 mm, and trigger force of 5 g.

Foaming Properties

Foaming capacity (FC) and foam stability (FS) of gelatin samples were determined according to the method described by Cho et al. [18]. Gelatin (1 g) was dispersed in 50 mL of distilled water and allowed to swell at room temperature. The swollen gelatin was subsequently dissolved at 60 °C, and foam was generated by homogenizing the solution at 10,000 rpm for 5 min using a handheld homogenizer (DLAB D-160, DLAB Scientific Co., Ltd., Beijing, China). The homogenized sample was immediately transferred into a 250 mL graduated measuring cylinder for foam volume measurement.

Foaming capacity was expressed as the percentage ratio of foam volume to the initial liquid volume. Foam stability was determined as the percentage of foam volume retained after 30 min relative to the initial foam volume immediately after homogenization.

Water-Holding and Fat-Binding Capacities

Water-holding capacity (WHC) and fat-binding capacity (FBC) of gelatin samples were determined according to the method described by Cho et al. [18]. Gelatin (1 g) was transferred into a pre-weighed centrifuge tube, and the combined weight was recorded. For the determination of WHC and FBC, 50 mL of distilled water and 10 mL of sunflower oil (Fortune, AWL Agri Business Limited, Ahmedabad, Gujarat, India) were added separately to the gelatin samples, respectively. The mixtures were allowed to stand at room temperature for 1 h with intermittent mixing using a vortex mixer for 5 s at 15 min intervals to facilitate hydration and interaction of gelatin with water or oil.

Following incubation, the samples were centrifuged at $450 \times g$ for 20 min using a cooling centrifuge (Sorvall ST 8R, Thermo Fisher Scientific, USA). The supernatant was carefully decanted, and the centrifuge tubes were inverted at an angle of 45° and drained on filter paper for 30 min to remove unbound water or oil. WHC and FBC were calculated as the ratio of the final weight of gelatin after draining to the initial dry weight of gelatin and expressed as a percentage (wt.%) of dry gelatin.

Emulsion Properties

Emulsion capacity

Emulsion capacity (EC) of gelatin at different concentrations was determined according to the emulsification method described by Yasumatsu et al. [19], with slight

modifications. Gelatin solutions were prepared at concentrations of 0.5, 1.0, 1.5, and 2.0% (*w/v*) by dispersing gelatin powder in distilled water. The dispersions were heated at 45–50 °C under continuous stirring until complete dissolution and subsequently cooled to room temperature.

For each concentration, 10 mL of gelatin solution was mixed with 10 mL of sunflower oil (Fortune, AWL Agri Business Limited, Ahmedabad, Gujarat, India) and homogenized at 12,000 rpm for 1 min using a high-speed homogenizer to obtain an oil-in-water emulsion. The emulsions were immediately transferred into graduated centrifuge tubes and centrifuged at 3500 rpm for 5 min. Following centrifugation, the total height of the sample and the height of the emulsified layer were measured. EC was calculated as the percentage ratio of the height of the emulsified layer to the height of the whole layer in the centrifuge tube.

$$EC (\%) = \frac{\text{Height of emulsified layer}}{\text{Height of whole layer}} \times 100 \quad (3)$$

Emulsion stability

Emulsion stability (ES) was determined using emulsions prepared during the emulsion capacity analysis. The emulsions were heated in a water bath at 80 °C for 30 min. The heating conditions were adopted from the published method of Yasumatsu et al. [19]. In the present study, this standardized heat treatment was used to evaluate the ability of the gelatin-stabilized emulsion to resist heat-induced destabilization before centrifugation and subsequently cooled to room temperature. The samples were then centrifuged at 3500 rpm for 5 min, and the height of the remaining emulsified layer was recorded. ES was expressed as the percentage ratio of the height of the remaining emulsified layer to height of the whole layer in the tube.

$$ES (\%) = \frac{\text{Height of remaining emulsified layer}}{\text{Height of whole layer in the tube}} \times 100 \quad (4)$$

Rheological Property (Viscosity)

A 6.67% (*w/v*) gelatin solution was prepared by dissolving gelatin in distilled water and heating at 60 °C following the method of Tabarestani et al. [20]. Viscosity was measured at 60 °C, which is above the melting temperature of the extracted gelatin (23.38 °C), to ensure that the measured value represents the viscosity of the gelatin solution rather than that of the gel, thereby allowing comparison with previous studies using similar measurement conditions. Viscosity was determined using an Ostwald viscometer maintained at 60 °C. The flow time of distilled water and the gelatin solution through the viscometer was recorded, and viscosity was expressed in centipoise (cP). The density and viscosity of distilled water at 60 °C were considered as 0.9832 g/cm³ and 0.466 mPa·s (cP), respectively, based on standard water property data [21]. The viscosity of the gelatin solution was calculated using the following equation [22]:

$$\frac{\eta_2}{\eta_1} = \frac{d_2 \times t_2}{d_1 \times t_1} \quad (5)$$

where η_1 and η_2 represent the viscosities of distilled water and the gelatin solution, respectively; d_1 and d_2 represent the densities of distilled water and the gelatin solution (g/cm³), respectively; and t_1 and t_2 denote the flow times of distilled water and the gelatin solution (s), respectively.

2.4. Statistical Analysis

All experiments were conducted in triplicate unless otherwise stated, and the results are expressed as the mean $\pm$ standard deviation (SD). Statistical analyses were performed using SPSS software (Version 23, IBM Corp., Armonk, NY, USA). Statistical analysis was conducted only for emulsion capacity and emulsion stability data obtained at different gelatin concentrations (0.5–2.0%, *w/v*). Differences among means were evaluated by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test for post hoc comparison when significant differences were detected. Statistical significance was considered at $p < 0.05$. For all other physicochemical, thermal, rheological, and functional parameters, only a single optimized gelatin sample was evaluated; therefore, inferential statistical analysis was not applicable. Although comparisons with the published literature were included to provide scientific context, formal statistical comparisons were not performed because differences in fish species, raw materials, extraction conditions, analytical methodologies, and experimental designs among studies make direct statistical comparisons of means inappropriate and potentially misleading.

3. Results and Discussion

3.1. Physicochemical Properties

3.1.1. Gelatin Yield

Gelatin yield is an important indicator of extraction efficiency and economic feasibility. In the present study, gelatin extracted from Rohu scales showed a yield of 21.86% (Table 1), calculated based on the dry weight of the dried fish scales used as the starting material. This yield is comparable to the previously reported values of 25% and 24% for Rohu scale gelatin [1,13]. However, higher yields of 48.80% from Rohu scales and 37.50% from tilapia scales have also been reported [23]. Lower yields were observed in lizardfish scales (10.6%), tilapia skin (19.3%), and sea bass scales (18.49%) [24–26]. Variations in gelatin yield among studies may be attributed to differences in fish species, collagen content, pretreatment, and extraction conditions, including acid concentration, extraction temperature, extraction time, and demineralization efficiency, all of which influence collagen swelling, solubilization, and gelatin recovery [27,28]. In the present study, demineralization with 0.5 M HCl effectively removed the mineral phase of Rohu scales, facilitating collagen swelling and improving the accessibility of collagen fibers for subsequent hot-water extraction. Furthermore, extraction at 60 °C for 8 h promoted sufficient collagen denaturation and solubilization into gelatin while minimizing excessive degradation of collagen-derived polypeptides, thereby contributing to the gelatin yield obtained under the selected extraction conditions [27]. The gelatin yield of 21.86% obtained under the selected extraction conditions therefore represents the proportion of gelatin recovered relative to the initial dry weight of the dried fish scales. Although the residual material was not characterized in the present study, previous studies have indicated that the residue remaining after gelatin extraction contains residual mineral components, along with unextracted collagen and other insoluble organic constituents. This residual fraction can be further valorized for the recovery of hydroxyapatite, collagen-derived products, and other value-added biomaterials, thereby supporting the sustainable utilization of fish-processing by-products [10,29–31].

Table 1. Yield, proximate composition, and thermal properties of gelatin extracted from Rohu (*L. rohita*) scales.

Parameter	Values
Yield (%)	21.86 ± 0.41
Protein (%)	88.37 ± 0.59
Ash (%)	1.5 ± 0.01
Melting temperature (°C)	23.38 ± 0.54
Setting temperature (°C)	20.4 ± 0.10
Setting time (s)	660 ± 1.17

Values are expressed as mean ± SD ($n = 3$).

3.1.2. Protein and Ash Content

The protein content of gelatin extracted from Rohu fish scales was 88.37% (Table 1), indicating the successful recovery of collagen-derived proteins during the extraction process. This value is comparable to those reported for fish scale gelatins from other species. For instance, Asiamah et al. [32] reported protein contents of 94.90% for tilapia scale gelatin, while Sreeja et al. [13] reported a protein content of 86.4% for Rohu scale gelatin. Similarly, Jakhar et al. [33] observed a protein content of 87.66% in Rohu scale gelatin. The slight variations in protein content among fish-derived gelatins may be attributed to differences in fish species, biological factors such as sex, age, feeding habits, and seasonal variations, as well as processing conditions, including extraction, pretreatment, and drying methods. Furthermore, moisture removal during drying can increase the relative proportion of protein in the final gelatin product [34,35].

The ash content of Rohu scale gelatin was 1.5% (Table 1), indicating effective removal of minerals during the demineralization process. This value was comparable to those reported for tilapia scale gelatin (2.05%) [32], Rohu scale gelatin (2.10%) [13] and Rohu scale gelatin (1.18%) [33]. Differences in ash content are generally associated with variations in the mineral composition of the raw material and the efficiency of demineralization treatments. The low ash content obtained in the present study indicates that treatment with 0.5 M HCl effectively dissolved and removed the calcium phosphate-rich mineral matrix of the fish scales before gelatin extraction, thereby reducing the residual inorganic content in the final product [28]. The ash content obtained in the present study was below the maximum limit of 2.0% recommended for food-grade gelatin by the Gelatin Manufacturers of Europe [36], indicating its suitability for food applications.

3.1.3. Color

The color characteristics of Rohu scale gelatin powder and gel are presented in Table 2. The gelatin powder exhibited L^* , a^* , and b^* values of 75.85, 1.14, and 14.85, respectively. The high L^* value indicates a bright and light-colored product, suggesting effective removal of pigments and non-collagenous materials during extraction [16]. The L^* value was higher than that reported for Nile tilapia scale gelatin (65.95) [32] and tiger tooth croaker head gelatin (70.69) [16]. The positive a^* value indicated slight redness, while the relatively high b^* value reflected moderate yellowness, which may be associated with residual pigments, lipid oxidation products, and mild browning reactions during drying.

Table 2. Instrumental color characteristics of gelatin powder and gelatin gel prepared from Rohu (*L. rohita*) scales.

Sample	L^*	a^*	b^*
Gelatin powder	75.85 ± 0.005	1.14 ± 0.01	14.85 ± 0.005
Gelatin gel	21.19 ± 0.594	-1.14 ± 0.22	1.87 ± 0.54

* Values are expressed as mean $\pm$ SD ($n = 3$).

Following gel formation, the L^* value decreased to 21.19, while the a^* value shifted from a positive to negative value (-1.14), and the b^* value decreased to 1.87. Similar color changes have been reported for fish gelatins, including tiger tooth croaker gelatin gel, which exhibited L^* , a^* , and b^* values of 51.30, 2.03, and 12.36, respectively [16]. The lower lightness (L) and yellowness (b) of the gelatin gel may be attributed to changes in light absorption and scattering within the hydrated gel matrix resulting from the formation of a three-dimensional gelatin network [37], thereby altering the optical properties of the gelatin gel. Although color influences consumer acceptance and visual appearance, it has little effect on the functional properties of gelatin [38]. The color characteristics indicate that Rohu scale gelatin possesses acceptable visual quality for food and industrial applications.

3.1.4. Melting Temperature, Setting Temperature and Setting Time

The thermal properties of Rohu scale gelatin, including melting temperature, setting temperature and gelling time, are presented in Table 1. The extracted gelatin exhibited a melting temperature of 23.38°C , a setting temperature of 20.4°C and a gelling time of 660 s. These parameters reflect the gel-forming ability and thermal stability of gelatin. The melting temperature obtained in the present study was within the reported range for fish gelatins (11 – 28°C) and was higher than the 18.9°C reported for Rohu skin gelatin by Ninan et al. [15], while being comparable to the 23.6°C reported for Rohu scale gelatin by Sreeja et al. [13]. Similarly, the setting temperature was close to the values reported for Rohu scale gelatin (18.2°C) [13] and grass carp scale gelatin (20.5°C) [39]. The gelling time of 660 s was longer than those reported for tiger tooth croaker gelatin (366 s) [16] and grass carp gelatin (68.6 s) [39], but considerably shorter than the 30 min required for balsa and tilapia gelatins to gel at 4°C [40]. Variations in thermal properties among fish gelatins may be attributed to differences in molecular characteristics, molecular weight distribution, collagen integrity, and extraction conditions. These factors influence the thermal stability and gel-forming behavior of gelatin [40–42]. In the present study, the relatively higher melting and setting temperatures, together with the moderate gelling time, suggest the preservation of sufficient collagen-derived high-molecular-weight components capable of forming stable intermolecular junctions within the gelatin network, thereby enhancing its thermal stability and gel-forming ability.

3.1.5. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of gelatin extracted from Rohu scales is presented in Figure 2. Characteristic absorption bands corresponding to the proteinaceous structure of gelatin were observed, confirming the successful conversion of collagen into gelatin during the extraction process. The broad absorption band at 3280 cm^{-1} was assigned to O–H and N–H stretching vibrations (amide A), which are associated with hydrogen-bonded hydroxyl groups and peptide linkages in gelatin [1,43,44]. The absorption bands observed at 3073 and 2937 cm^{-1} correspond to amide B and asymmetric stretching vibrations of CH_2 groups, respectively, indicating the presence of aliphatic amino acid residues [43].

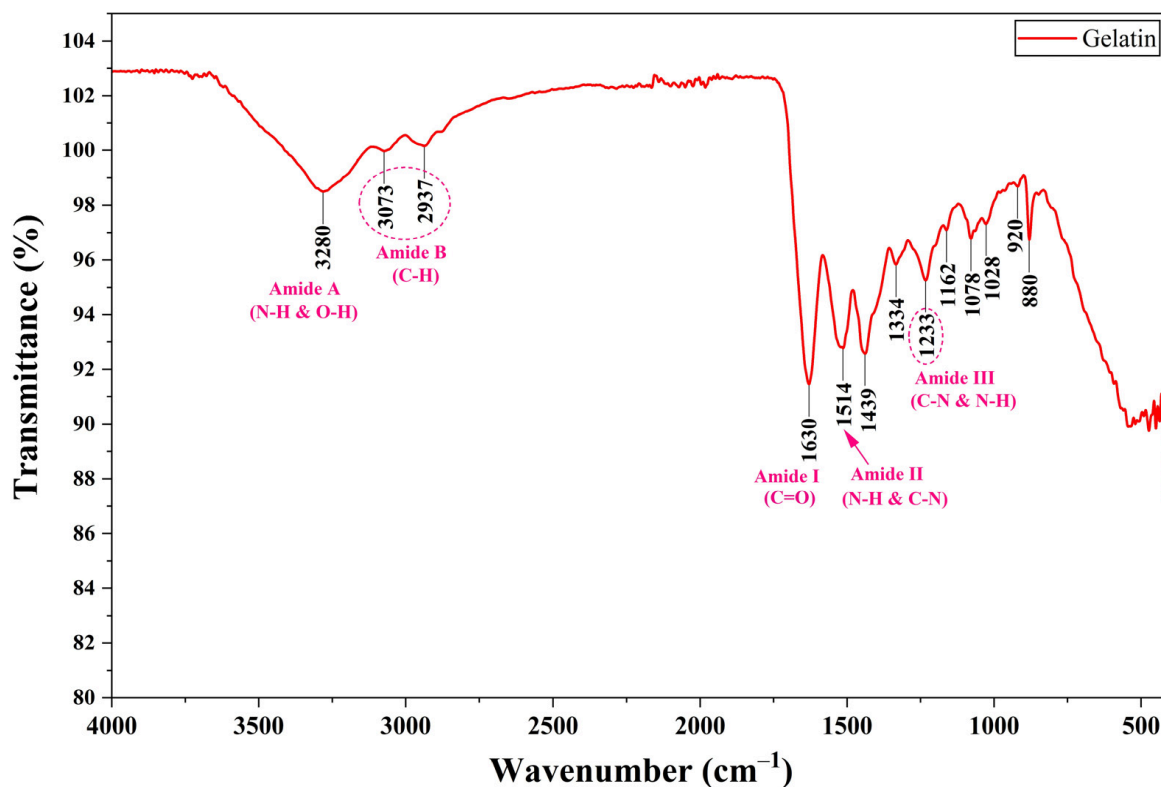


Figure 2. FTIR spectrum of gelatin extracted from Rohu (*L. rohita*) fish scales.

A prominent absorption peak was detected at 1630 cm^{-1} , corresponding to the amide I region, which is primarily attributed to $\text{C}=\text{O}$ stretching vibrations of peptide bonds [1,43,44]. The amide I band is highly sensitive to protein secondary structure and is considered one of the most important indicators of gelatin integrity. The peak observed at 1514 cm^{-1} was assigned to the amide II band, arising from N-H bending coupled with C-N stretching vibrations [1]. The amide I and amide II bands are characteristic of gelatin and indicate the preservation of the peptide backbone following extraction.

The absorption band at 1233 cm^{-1} was attributed to the amide III region, which is associated with C-N stretching and N-H deformation vibrations [1]. The presence of amide III further supports the existence of collagen-derived polypeptide chains in the extracted gelatin. Additional peaks observed at 1439 and 1334 cm^{-1} were related to CH_2 bending and wagging vibrations [43], while bands at 1162 , 1078 , and 1028 cm^{-1} corresponded to C-O stretching and skeletal vibrations of amino acid residues. Minor absorption bands below 1000 cm^{-1} were associated with out-of-plane bending vibrations and fingerprint region characteristics of gelatin molecules.

The FTIR profile was consistent with those reported for fish-derived gelatins, confirming the successful extraction of gelatin from Rohu scales while preserving its characteristic functional groups [1,13,45].

3.1.6. SDS-PAGE

The SDS-PAGE profile of gelatin extracted from Rohu fish scales (Figure 3) exhibited three major protein bands corresponding to the β -chain ($>245\text{ kDa}$, retained near the top of the gel), $\alpha 1$ -chain ($\sim 100\text{--}140\text{ kDa}$), and $\alpha 2$ -chain ($\sim 70\text{--}110\text{ kDa}$), which are characteristic molecular components of type I collagen-derived gelatin. The prominent β -component observed at higher molecular weight represents intermolecularly cross-linked dimers of α -chains, suggesting that a substantial proportion of collagen molecules remained structurally intact during extraction. The clear presence of $\alpha 1$ and $\alpha 2$ bands indicates that the extraction

process effectively disrupted the native collagen triple-helix structure while preserving the primary polypeptide chains. Similar electrophoretic patterns have been reported for fish scale gelatin from tilapia, Hilsha, bighead carp, and carp scales, confirming the typical molecular architecture of fish gelatin [8,46]. The retention of α - and β -chains is considered an indicator of high-quality gelatin because these fractions contribute significantly to gel network formation, viscoelasticity, and thermal stability [47,48]. Moreover, the absence of pronounced low-molecular-weight fragments below 75 kDa indicates limited peptide chain degradation and minimal over-hydrolysis during processing. Excessive hydrolysis can cause extensive fragmentation of α -chains, reduce molecular integrity and impair the ability of gelatin molecules to form stable three-dimensional gel networks, thereby weakening their functional and gelling properties [46]. Therefore, the SDS-PAGE results demonstrate the successful extraction of structurally preserved gelatin from Rohu fish scale waste, highlighting its potential as a sustainable source of high-value gelatin for food, pharmaceutical, biomedical and biodegradable packaging applications.

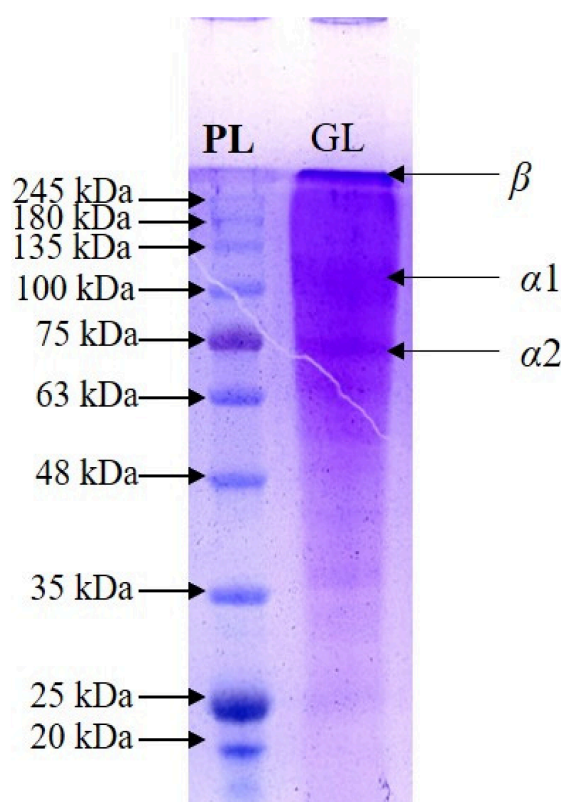


Figure 3. SDS-PAGE electrophoretic pattern of Rohu (*L. rohita*) scale gelatin.

3.2. Functional Properties

3.2.1. Solubility

The solubility of Rohu scale gelatin was 87.49% (Table 3). Solubility is an important functional property that influences the application of gelatin in food and pharmaceutical products and is influenced by protein structure, molecular characteristics, and molecular weight distribution [8]. The solubility obtained in the present study was slightly lower than that reported for carp scale gelatin (92.23%) and bighead carp scale gelatin (92.07%) [8]. Similarly, Ai-Temimi et al. [22] reported higher solubility values of 98.33% and 97.47% for carp and grass carp gelatins, respectively. The slightly lower solubility observed in the present study may be attributed to differences in collagen source and extraction conditions, including acid concentration, extraction temperature, extraction time, and pH, which influence collagen denaturation and the molecular characteristics of gelatin. Fur-

thermore, variations in molecular weight distribution and subunit composition can alter protein–water interactions, thereby affecting gelatin solubility [28,49,50]. Despite being slightly lower than values reported for some other fish gelatins, the solubility of Rohu scale gelatin was sufficiently high, indicating favorable functional properties for potential food and industrial applications.

Table 3. Functional and rheological properties of gelatin extracted from Rohu (*L. rohita*) fish scales.

Parameter	Values
Solubility (%)	87.49 ± 1.48
Gel strength (g)	182.82 ± 11.22
Viscosity (cP)	6.56 ± 0.21
WHC (%)	169.7 ± 2.08
FBC (%)	188.50 ± 11.11
Foaming capacity (%)	193.33 ± 6.11
Foaming stability (%)	77.65 ± 1.84

Values are expressed as mean ± SD ($n = 3$).

3.2.2. Gel Strength and TPA

Gel Strength

The gel strength of gelatin extracted from Rohu scales was 182.82 g (Table 3), indicating good gel-forming ability and the formation of a stable gel network. The value is comparable to the values reported for tilapia gelatin (180.76 g) [51] and Rohu skin gelatin (188.6 g) [39]. However, it was considerably lower than the gel strengths reported for carp scale gelatin (643.28 g) and bighead carp scale gelatin (658.16 g) [8]. Differences in gel strength among fish gelatins may be attributed to variations in collagen source, pretreatment, and extraction conditions, molecular weight distribution, collagen integrity, and intermolecular interactions [16,52,53]. These factors collectively influence collagen solubilization, gel network formation, and the resulting gel strength. The measured gel strength is also consistent with the SDS–PAGE profile of the extracted gelatin, which showed distinct α - and β -chain components. The preservation of these high-molecular-weight collagen-derived polypeptides suggests limited collagen degradation during extraction and is likely to facilitate the formation of intermolecular junction zones that strengthen the three-dimensional gel network during gelation [28,49,54]. Thus, the SDS–PAGE findings provide structural evidence supporting the moderate gel strength and good gel-forming ability of the Rohu scale gelatin observed in the present study. The gel strength obtained in the present study falls within the range reported for many commercial fish gelatins, indicating its potential suitability for food, pharmaceutical, and packaging applications.

TPA

The texture profile characteristics of Rohu scale gelatin are presented in Table 4. The gelatin exhibited a hardness of 427.55 g, adhesiveness of −28.25 g·s, springiness of 0.982, cohesiveness of 0.946, gumminess of 404.29 g, chewiness of 396.71 g, and resilience of 0.870. Hardness reflects the strength of the gel structure during compression and is closely associated with gel strength. The observed hardness value indicates the formation of a firm and stable gel network, supporting the good gel-forming ability of the extracted Rohu scale gelatin.

Table 4. Texture profile of gelatin extracted from Rohu (*L. rohita*) fish scales.

Parameters	Value
Hardness (g)	427.55 ± 53.45
Adhesiveness (g·s)	−28.24 ± 15.87
Springiness	0.98 ± 0.03
Cohesiveness	0.94 ± 0.01
Gumminess (g)	404.29 ± 48.10
Chewiness (g)	396.71 ± 47.01
Resilience	0.87 ± 0.04

Values are expressed as mean ± SD ($n = 5$).

Springiness is related to the elasticity of the gel and its ability to recover its original shape after deformation. The springiness value (0.982) was higher than those reported by Chandra et al. [55] for porcine gelatin (0.943), *Catla catla* (0.925), *Cirrhinus mrigala* (0.903), and Rohu skin gelatin (0.846), indicating superior elastic recovery. Higher springiness values generally require greater mastication energy [56]. Similarly, the cohesiveness value (0.946) exceeded the range reported by Chandra et al. [55] (0.648–0.913) and was slightly higher than the values reported for fish (*Coregonus peled*, carp and bighead carp) scale gelatins (0.71–0.91) [8], suggesting a highly integrated and stable gel network.

The gumminess (404.29 g) and chewiness (396.71 g) values were higher than those reported for Rohu skin gelatin [55], indicating greater resistance to deformation and mastication. High gumminess is generally associated with increased hardness and cohesiveness [57,58]. The resilience value (0.870) was higher than that reported for Rohu skin gelatin (0.770) and comparable to *Catla catla* (0.885) [55], demonstrating good elastic recovery after compression. These favorable textural properties may be attributed to strong intermolecular interactions and effective three-dimensional network formation within the gelatin matrix [8,55].

3.2.3. Water-Holding Capacity and Fat-Binding Capacity

The WHC and FBC of Rohu scale gelatin were 169.7% and 188.5%, respectively (Table 3). These properties are important for determining the ability of gelatin to retain moisture and interact with lipids in food systems. WHC is associated with the ability of the gelatin network to retain water through protein–water interactions and hydrogen bonding, whereas FBC depends on interactions between lipids and hydrophobic domains within the protein structure [15,59,60]. The WHC obtained in the present study was lower than the values reported for *Channa lucius* (967%), *Puntius bulu* (777.33%), and *Cyprinus carpio* (312%) [61]. Previous studies have reported that variations in WHC may be associated with hydrophilic amino acid residues and hydroxyproline content [15,62]. A lower content of hydrophilic amino acids, particularly hydroxyproline, may reduce the ability of gelatin to retain water through hydrogen bonding. Therefore, the comparatively lower WHC of Rohu scale gelatin compared with that of *Channa lucius* may be associated with a lower proportion of hydrophilic amino acid residues and/or a greater degree of collagen denaturation [62,63]. Although amino acid composition was not determined in the present study, this explanation remains hypothetical and is supported by previous studies. In addition, differences in collagen source and extraction conditions may influence the molecular characteristics and water-binding behavior of gelatin, thereby contributing to variations in WHC.

The FBC of Rohu scale gelatin was lower than that reported for *C. lucius* (207%) but higher than those observed for *P. bulu* (98.33%) and *C. carpio* (37.66%) [61]. Previous studies have reported that FBC is influenced by the exposure of hydrophobic domains and

nonpolar side chains involved in lipid binding [15,61]. Differences in the degree of collagen denaturation may also influence the exposure of hydrophobic regions responsible for lipid binding, thereby contributing to variations in FBC [15,62]. Although this was not evaluated in the present study, this explanation remains hypothetical and is consistent with previous studies. Similarly, differences in collagen source and extraction conditions may influence the molecular characteristics and lipid-binding behavior of gelatin, thereby contributing to variations in FBC. The relatively high FBC observed in the present study suggests that Rohu scale gelatin has good oil-binding ability and may be suitable for applications in fat-containing food products.

3.2.4. Foaming Properties

The FC and FS of Rohu scale gelatin were 193.33% and 77.65%, respectively (Table 3). Foaming properties determine the suitability of gelatin for aerated food products by enhancing foam formation and stability. The FC obtained in the present study was lower than the values reported for *P. bulu* (613%), *C. lucius* (600%), and *C. carpio* (406.67%) [61]. Similarly, Sharma and Phanse [23] reported a higher FC for tilapia scale gelatin (242.67%) compared with Rohu scale gelatin. However, the FC was higher than that reported for black tilapia scale gelatin (60%) and bone gelatin (25%) [64].

The FS value of Rohu scale gelatin (77.65%) was lower than that reported for *P. bulu* (83.42%) but higher than *C. lucius* (73.60%) and *C. carpio* (28.30%) [61]. It was also lower than the FS reported for tilapia scale gelatin (170.67%) [23]. FC depends on the ability of gelatin molecules to rapidly adsorb at the newly formed air–water interface, where they undergo unfolding and molecular rearrangement to form a cohesive viscoelastic interfacial film. This film reduces surface tension, stabilizes air bubbles, and enhances both foam formation and foam stability [65]. Variations in FC and FS among fish gelatins may therefore be attributed to differences in collagen source, pretreatment and extraction conditions, molecular characteristics, and molecular weight distribution, which influence interfacial adsorption and film-forming behavior [66,67]. Although species-specific differences may partly explain the higher FS reported for tilapia scale gelatin, differences in experimental methodology, including homogenization conditions, mixing intensity, protein concentration, aeration procedure, and other analytical parameters, may also have contributed to the observed variation because these factors strongly influence interfacial film formation and foam stability [66,68]. Therefore, direct comparison of absolute FC and FS values among different studies should be interpreted with caution because differences in both extraction conditions and analytical methodologies can substantially influence the measured foaming properties. Since FS reflects protein–protein interactions within the matrix, the results indicate moderate foaming properties of Rohu scale gelatin [69].

3.2.5. Emulsion Properties

The emulsifying properties of Rohu scale gelatin exhibited a concentration-dependent increase, as shown in Figure 4. Emulsion capacity (EC) increased from 38.61% at a 0.5% gelatin concentration to 44.11% at 2.0%, while emulsion stability (ES) increased from 36.62% to 42.15%. Significant differences ($p < 0.05$) were observed among some concentration levels, with the highest values recorded at a 2.0% gelatin concentration. The progressive increase in EC and ES with increasing gelatin concentration suggests enhanced adsorption of gelatin molecules at the oil–water interface. Proteins act as emulsifiers by reducing interfacial tension and forming protective films around dispersed oil droplets [70]. At higher concentrations, the greater availability of gelatin molecules at the interface likely contributed to the formation of stronger interfacial films and improved droplet stabilization, resulting in enhanced emulsion formation and stability [16,71].

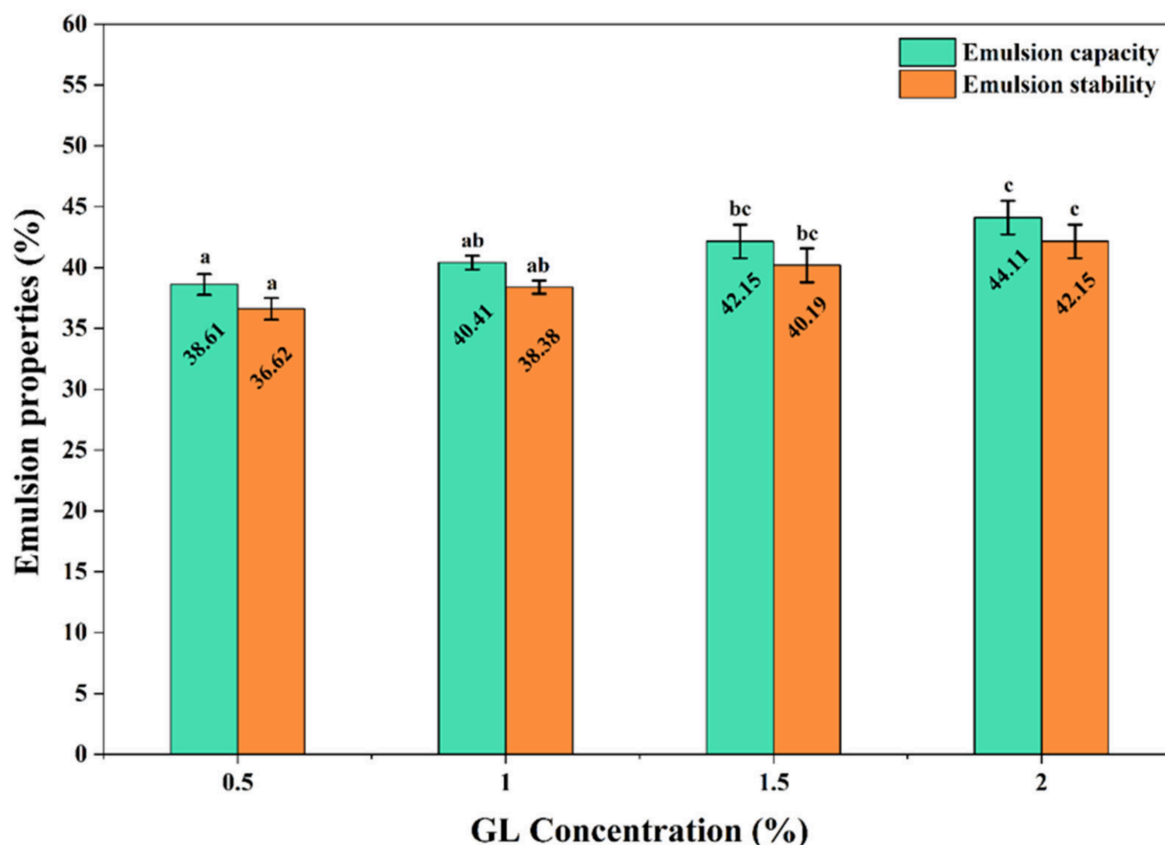


Figure 4. Emulsion capacity (EC) and emulsion stability (ES) of Rohu (*L. rohita*) scale gelatin at different concentrations (0.5–2.0%, *w/v*). Values are mean $\pm$ SD ($n = 2$). Different letters indicate significant differences ($p < 0.05$). GL—Gelatin.

The EC values obtained in the present study were lower than those reported for tiger-toothed croaker skin gelatin (55.70%) and pink perch gelatin (47.50%) but were comparable to the range reported for several fish bone gelatins (35.50–40.50%) [72]. Differences in emulsifying properties among gelatins may be attributed to variations in amino acid composition, molecular weight distribution, and extraction conditions, which influence interfacial activity and emulsion stabilization [73]. The comparatively lower EC observed in the present study at the highest gelatin concentration (2.0%, *w/v*), compared with tiger-toothed croaker skin gelatin and pink perch gelatin, may be associated with differences in molecular weight distribution arising from collagen hydrolysis during gelatin extraction. It is possible that Rohu scale gelatin may have contained a relatively greater proportion of low-molecular-weight peptide fractions, which stabilize emulsions less effectively than high-molecular-weight peptides [54,69]. Previous studies have shown that shorter peptide chains exhibit higher mobility and migrate more rapidly to the oil–water interface, facilitating rapid adsorption during emulsion formation [54]. However, high-molecular-weight peptides contribute more effectively to ES by forming thicker, stronger, and more cohesive interfacial films around oil droplets [69]. Although the SDS–PAGE profile confirmed the presence of well-preserved α_1 -, α_2 -, and β -chain components, this technique provides only qualitative information and does not quantify the proportion of low-molecular-weight peptide fractions. Therefore, differences in the extent of collagen hydrolysis during gelatin extraction may result in variations in molecular weight distribution, with a greater proportion of low-molecular-weight peptide fractions forming thinner and less cohesive interfacial films, thereby providing weaker steric stabilization of oil droplets and reducing emulsion stability [74]. Furthermore, low-molecular-weight peptide fractions may reduce the viscos-

ity of the continuous phase, thereby increasing droplet mobility and collision frequency, which promotes droplet flocculation and coalescence [75,76]. Although the molecular weight distribution of Rohu scale gelatin was not determined in the present study, differences in the extent of collagen hydrolysis during extraction may partially explain why its EC remained lower than that of tiger-toothed croaker skin gelatin and pink perch gelatin despite the concentration-dependent increase in EC.

3.2.6. Rheological Property (Viscosity)

Viscosity is one of the most important commercial properties of gelatin after gel strength, as it influences its processing and application characteristics [77]. The viscosity of gelatin extracted from Rohu fish scales was 6.56 cP (Table 3). This value was comparable to those reported for other fish-derived gelatins, including 7.12 cP for black tilapia and 3.20 cP for red tilapia gelatin [51], as well as 7.07 cP for grass carp gelatin [39]. Higher viscosities of 10.53 and 8.30 cP have been reported for tiger-toothed croaker skin and bone gelatins, respectively [72]. Variations in viscosity among gelatins may be attributed to differences in collagen source, pretreatment, and extraction conditions, molecular weight distribution, peptide polydispersity, and the arrangement of polypeptide chains within the gelatin structure, whereas viscosity measurements are further influenced by gelatin concentration, temperature and pH [16,61,78,79]. In the present study, viscosity was measured at 60 °C, which is above the melting temperature of the extracted gelatin (23.38 °C), ensuring that the gelatin remained in the sol state, where the measured value represents the viscosity of the gelatin solution rather than that of the gel, thereby allowing direct comparison with previous studies using similar measurement conditions. At temperatures above the melting temperature, gelatin exists predominantly as mobile random-coil chains [80]. As the temperature decreases toward the melting and gelation temperatures, these random coils progressively renature into triple helices, which act as junction zones [81–83]. The progressive formation of triple-helical junction zones leads to the development of a three-dimensional gel network, increasing intermolecular interactions and resistance to flow, thereby resulting in higher viscosity at lower temperatures [80,84]. The viscosity obtained in the present study indicates that Rohu scale gelatin possesses suitable flow properties and supports its potential application in food, pharmaceutical, and packaging systems.

4. Study Limitations

Although the present study demonstrated the feasibility of producing gelatin from Rohu fish scales with desirable physicochemical, thermal, rheological, structural, textural, and functional properties, systematic optimization of extraction parameters using statistical design approaches, such as Response Surface Methodology (RSM), could further improve gelatin yield and quality and should be investigated in future studies. Additionally, the present study did not include direct experimental comparisons with commercially available gelatin or gelatin extracted from other freshwater fish species. Furthermore, only a single optimized gelatin preparation was evaluated; therefore, statistical comparisons were limited to parameters measured under multiple experimental conditions (emulsion capacity and emulsion stability). Although comparisons with previously published studies were included to provide scientific context, formal statistical comparisons with literature values were not performed because substantial differences in raw materials, extraction conditions, and analytical methodologies make direct statistical comparisons inappropriate. Consequently, these literature comparisons should be interpreted qualitatively rather than statistically. Future studies should incorporate commercially available gelatin and gelatin from other freshwater fish species as control groups to enable a comprehensive evaluation of the performance of Rohu scale gelatin under identical experimental conditions. Fur-

thermore, the stability of raw fish scales and the extracted gelatin under different storage conditions and durations was not evaluated. Future studies should investigate the effects of storage on key quality attributes, including gel strength, solubility, and color, to establish the shelf life and storage stability of both the raw material and the final gelatin product. Finally, the functional properties of the extracted gelatin were evaluated only under fixed experimental conditions. Future studies should investigate the effects of different pH and temperature conditions on the functional properties of Rohu scale gelatin to better support its application in diverse food systems.

5. Conclusions

This study demonstrated the feasibility of utilizing Rohu (*L. rohita*) scale waste as a valuable raw material for gelatin production. The extracted gelatin exhibited desirable physicochemical, thermal, rheological, and functional characteristics, indicating its suitability for a range of food and related industrial applications. Structural characterization confirmed the successful conversion of collagen into gelatin while preserving the characteristic protein functional groups. The observed gel-forming ability, water- and fat-binding properties, foaming performance, and emulsifying behavior further highlight the functional potential of the extracted gelatin. The findings emphasize the potential of transforming an underutilized fish-processing by-product into a value-added biomaterial, thereby contributing to sustainable waste management and resource utilization within the fisheries sector. Rohu scale gelatin may serve as a promising non-mammalian alternative to conventional gelatin sources, particularly for applications where consumer preferences, religious considerations, and sustainability concerns are important. The study supports the development of fish scale-based gelatin as a commercially relevant biopolymer and promotes the valorization of fish-processing waste through the production of high-value functional ingredients.

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Appendix A

Preliminary trials were conducted to determine the demineralization conditions required for effective mineral removal from Rohu scales prior to gelatin extraction. Rohu scales (500 g) were subjected to demineralization using 0.5 M HCl at a solid-to-solution

ratio of 1:10 for 12 h with occasional stirring. To maintain demineralization efficiency, the acid solution was replaced every 3 h. Changes in the pH of the soaking solution and the ash content of the scales were monitored throughout the treatment period (Table A1).

The initial ash content of the scales was 22.5%, indicating a high mineral content. During demineralization, the pH of the soaking solution increased from 1.2 to 3.1 after 3 h, reflecting the dissolution of mineral components into the acid medium. A progressive reduction in ash content was observed with increasing treatment time, decreasing to 5.3% after 6 h and further to 1.5% after 12 h. These results demonstrated effective mineral removal and indicated that 12 h of demineralization with 0.5 M HCl was sufficient to obtain adequately demineralized scales for subsequent gelatin extraction.

Following demineralization, the scales were washed with tap water until a near-neutral pH (6.0–7.0) was attained and then mixed with distilled water at a ratio of 1:6 (*w/v*). Gelatin extraction was subsequently carried out in a water bath at 60 °C for 8 h with occasional stirring. The extract was filtered through muslin cloth and dried in a hot-air oven at 70 °C to obtain gelatin.

Table A1. Changes in pH of the demineralization medium and ash content of Rohu (*L. rohita*) scales during demineralization with 0.5 M HCl.

Demineralization Time (h)	Initial pH of Fresh HCl Solution	pH After Soaking	Ash Content (%)
0	1.20	–	22.5
3	1.20	3.10	ND
6	1.78	2.80	5.3
9	1.40	2.80	ND
12	1.09	2.30	1.5

ND = Not determined.

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