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Comparative study of properties of Rohu and Tilapia scale gelatin with market gelatin

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Abstract
In recent times, significant emphasis has been placed on utilising waste from the fish processing sector to produce value-added products. In this study, gelatin was extracted from the scales of Rohu and Tilapia fish, and its attributes were compared with commercially available gelatin. Rohu scales yielded 48.80% gelatin, while Tilapia scales yielded 37.50%. The proximate analysis of both scale-derived gelatins closely resembled that of market gelatin. Tilapia gelatin exhibited a gel strength of 216 g, close to market gelatin (291 g). The emulsification property and viscosity of scale gelatins mirrored those of market gelatin. However, the foam-forming attribute of scale gelatins surpassed that of market gelatin. Interestingly, as gelatin solution concentration increased, emulsification stability, foam expansion, and foam stability was observed. Whereas emulsification activity exhibited an inverse relationship with gelatin concentration.

Keywords: Gelatin, Rohu, tilapia, bloom strength, viscosity, emulsion

Introduction
Gelatin is a colourless and tasteless protein, comprising of a diverse mixture of water-soluble peptide chains with different molecular weights and lengths. These peptides are obtained from collagen through denaturation caused by heat. Gelatin's nutritional benefits, functional attributes, and capacity to offer a pleasing melt-in-the-mouth experience render it a crucial component in numerous food items. Based on its functional traits, gelatin's applications in food have been classified, where it can be used as a creaming agent, whipping agent, clarifying agent etc.[1-3] Gelatin also finds application in pharmaceutical applications in making of tablets, emulsions, capsules etc. [4]

According to a 2021 press release from the Ministry of Fisheries, Animal Husbandry and Dairying, India ranks as the world's second-largest fish producer, contributing 7.56% to global output [5]. In the fish processing industry, waste is generated during various post-harvest stages, encompassing offal, trimmings, scales, bones, and other unused byproducts. Globally, this sector produces around 100 million metric tonnes of waste, with India alone contributing over 4 million MT [6]. Unfortunately, a significant portion of fish waste is discarded without meaningful utilization. Fish processing byproducts, like skin, bones, fins, and scales, are rich in collagen protein, making them potential sources of gelatin [7]. Scales, resembling bones in structure and composition, consist of collagen fibers, ichthyolepidin, and calcium salt crystals, making them a promising gelatin source [8]. Hence, this study aims to explore the potential of gelatin extracted from Rohu and Tilapia scales and to characterize it's properties.

Materials and method
1. Collection of materials
Rohu (*Labeo rohita*) and Tilapia (*Tilapia mossambica*) fish were selected for this study due to their easy availability. The fish were acquired from the local fish market. Scales from these fish species were removed, thoroughly cleaned, and rinsed to eliminate any blood and debris. Subsequently, the scales were dried under sunlight and stored in sealed plastic zip-lock bags until they were ready for use. Commercial gelatin powder with a Bloom value of 300 g, obtained from Sigma-Aldrich (product code: G-1890), was also included in the study.

2. Extraction of gelatin
Gelatin was extracted using Zakaria and Bakar's method [9] with minor changes. Fish scales were treated with 0.4 (W/V) sodium hydroxide for 4 hours, followed by rinsing to neutralize pH for 1 hour. Afterward, scales were soaked in 0.4 hydrochloric acid (V/V) (100ml) for 4 hours, washed, and treated with five times their water volume. Autoclaving at 15 psi for 3 hours was done, followed by filtration using two layers of sterile muslin cloth. Gelatin film obtained was dried at 50°C and stored in sterile ziplock pouches.

3. Determination of Percentage yield
Percentage yield of gelatin was calculated using the formula:

$\%\ yield$=weight of gelatin/weight of dried scales×100

4. Determination of Protein content
Extracted gelatin was analysed for protein content according to AOAC official methods [10].

5. Determination of gel strength
The bloom strength (a measure of gel strength) was assessed using the method outlined in GMIA official procedures [4]. A 6.67% gel was formed in a water bath at 60°C. The gel was poured into 10 ml plastic cups and refrigerated at 3°C for 17-18 hours. The bloom strength was measured using a Digital Texture Analyser 50N from Parisa Technology.

6. Determination of viscosity
The viscosity of gelatin was measured with a modified version of the method by Davis and colleagues [11]. A 6.67% gelatin solution's viscosity was assessed at 60°C using the Ostwald viscometer. The viscosity was reported in cP (centipoise).

7. Determination of Emulsification activity
The emulsification capability of the gelatin derived from fish scales was examined employing a method adapted from Pearce and Kinsella [12]. Ten milliliter of mustard oil was combined with either 30 ml of 0.1% gelatin solution or 30 ml of 0.2% gelatin solution. This mixture was homogenized at 8000 rpm for 2 minutes using a Remi RQ-127A homogenizer to form an oil-in-water emulsion. From the bottom of the emulsion sample, 50 µl was taken out and diluted 100 times using a 0.1% SDS solution, both at 0 and 10 minutes. The mixture was then vortexed. The absorbance of this blend was estimated using a UV-Vis spectrophotometer at a wavelength of 500 nm. Subsequently, the EAI (Emulsifying Activity Index) and ESI (Emulsion Stability Index) were computed using the formulas given below:

$$EAI\left(\frac{m^2}{g}\right) = \frac{2 \times 2.303 \times A_{500} \times D}{L \times ø \times c}$$

Where A_{500} is sample absorbance at 500nm, D is the factor of dilution, L is cuvette diameter (m) , ø is content of oil in emulsion , c is concentration of protein (g/m^3).

$$ESI\ (minutes) = \frac{A_0}{A_0 - A_{10}} \times \Delta t$$

Where A_0 is sample absorbance at 0 minute, A_{10} is sample absorbance at 10 minute, Δt is time interval (10 minute)

8. Determination of foaming property
Gelatin solutions at 0.1%, 0.2%, concentrations were used to measure foam expansion (FE) and stability (FS) following a method given by Shahidi [13]. Using Remi RQ-127A homogenizer, 25 ml of each gelatin solution was homogenized for 2 minute at room temperature (25°C ± 1 °C). The resulting foam was promptly transferred in a measuring cylinder (50ml), and the total volume was measured at 0 and 30 minutes. The foaming expansion and the foam stability was calculated by the formulas given below:

$$FE\ (\%) = \frac{VT - V0}{V0} \times 100$$

$$FS\ (\%) = \frac{Vt - V0}{V0} \times 100$$

Where V0 is initial volume (ml) of solution, VT is total volume (ml) after whipping, Vt = total volume (ml) after 30mins.

Result and discussion
1. Percentage yield
A greater percentage yield indicates a production process that is more efficient and cost-effective. In comparison, Rohu scales exhibited a higher yield (48.80%) than tilapia scale gelatin (which measured 37.50 %). The variation in gelatin yields across fish species can be attributed mainly to differences in collagen content, skin composition, and the skin matrix. In this present investigation, the yield obtained from Rohu scales exceeded the results reported in a prior study concerning Rohu's skin and scales (11.78 ± 0.20, 7.21 ± 0.12) [14]. Additionally, the yield obtained from tilapia scales was higher than the yield from scales (16%), bones (5%), and skin (5.39%) of black tilapia [9, 15]. These variations can be attributed to differences in extraction methods, processing techniques, and the specific conditions employed in each study [16-18].

2. Protein Content
Both Rohu and Tilapia gelatins displayed high protein values (86.47% and 90.72%, respectively). These protein contents were comparable to market gelatin (91.66%). The protein content derived from the skin of Tilapia was determined to be 81.16% [19] and scales of Rohu was 65.85% [14]. The protein content in the scale gelatin of both fish species in our study was higher than the values reported by the mentioned authors.

3. Bloom strength
Gelatin has a remarkable ability to create stable gels by forming hydrogen bonds with water molecules which is denoted as Bloom strength/gel strength. Among the gelatins studied, Market gelatin displayed the highest bloom value at 291.33 g, while Tilapia scale gelatin exhibited a notable bloom value of 216.33 g. In contrast, Rohu scale gelatin did not exhibit gelling properties. The gel strength of Tilapia scale gelatin was higher than that of Tilapia skin gelatin 180.76 g [15], but it was lower than the gel strength of Tilapia skin gelatin 263g [20]. Fish gelatin's gel strength has been documented across a wide range, from 124 g to 426 g [21]. Though Rohu scale gelatin belongs to non-gelling category in our study, it can be used in items that demand lower gelling temperatures or in chilled product [22].

4. Viscosity
Following bloom strength, viscosity stands as the second most crucial attribute of gelatin, contributing to its application in various fields. Market gelatin exhibited the highest viscosity of 7.32 cP (Figure 1). Whereas the viscosity of Tilapia scale gelatin (6.8 cP) closely resembled that of Market gelatin. Black tilapia skin gelatin exhibited viscosities of 7.12 cP, 6.9 cP and 3.8 cP in different studies [15, 23, 24]. Variations in viscosity can be ascribed to the organization of molecular weights among polypeptide chains within gelatin samples [4]. Hence, both skin and scales are equally potential for use in products where viscosity plays a major role.

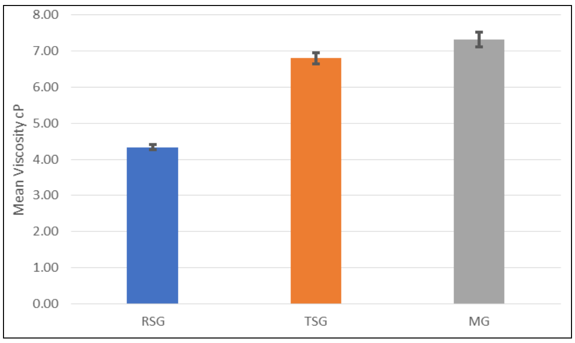
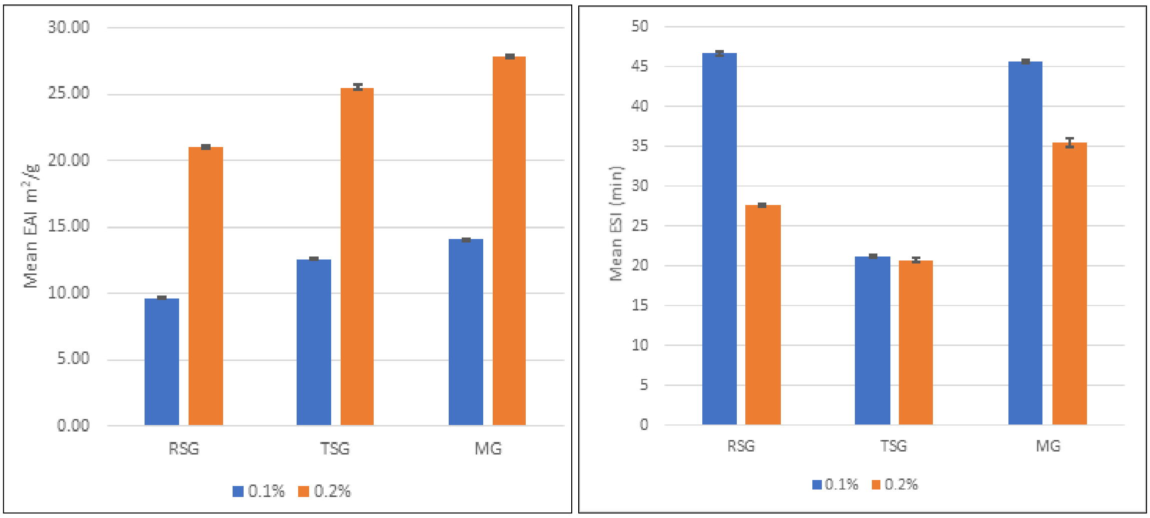


Fig 1: Mean viscosity (cP) of Rohu, Tilapia scale gelatin and Market gelatin

5. Emulsifying capacity and stability
The emulsification property of gelatin holds significance as it plays a role in creating and sustaining the stability of oil-in-water emulsions. Two aspects of emulsification property

are emulsification activity index (EAI) and emulsification stability index (ESI). The emulsification activity index in this study was found to be higher for 0.1% gelatin solutions compared to 0.2% solutions (depicted in Figure 2 [A]). Gelatin derived from scales showed similarity for EAI to market gelatin. Tilapia scale gelatin exhibited emulsion activity indices of 25.54 m2/g and 12.58 m2/g at concentrations of 0.1% and 0.2%, respectively. These values surpassed those reported in a different study (5.76 m2/g and 2.19 m2/g, respectively) at equivalent concentrations [9]. Similarly, Rohu scale gelatin exhibited higher emulsion activity (21.03 m^2/g) than gelatin from Rohu skin gealtin (0.55 ml/mg) skin at 0.1% concentration [25].

The present study observed higher ESI values in 0.2% gelatin solutions (Figure 2 [B]). Specifically, Rohu scale gelatin at 0.2% concentration displayed the highest ESI of 46.64±0.29 minutes, which was higher than market gelatin, indicating its potential as an effective emulsion stabilizer. The study's findings also highlighted that increasing gelatin concentrations led to elevated ESI values, a result consistent with other studies [26, 27] on Mackerel, Blue whiting bone, and Unicorn leatherjacket fish gelatin. The emulsifying properties of gelatin can be influenced by factors like amino acid composition, polypeptide chain length, molecular weight distribution, and extraction methods [28].

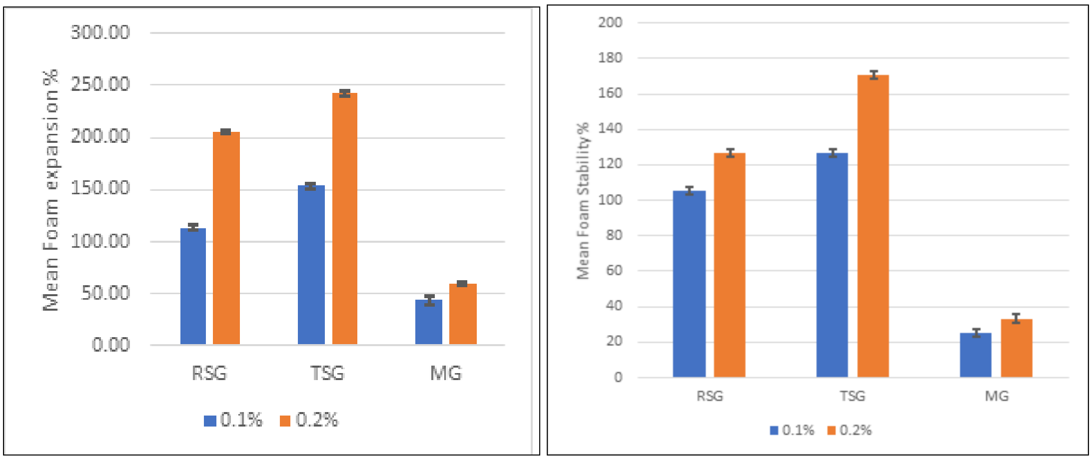


A B

Fig 2: Emulsification Activity of Rohu, Tilapia scale gelatin and Market gelatin. [A] Emulsion activity index (m^2/g), [B] Emulsion stability Index (mins)

6. Foaming properties

Foaming properties refer to a protein's ability to form stable foam structures when mixed with air or other gas phases. The foam expansion increased with increasing concentration. Highest foaming capacity was shown by Tilapia scale gelatin (242.67%), followed by Rohu scale gelatin (205.33%). Market bovine gelatin showed 60% Foam expansion (Figure 3). The results indicated that both the scale gelatins showed better foam expansion in comparison to market gelatin. Similar trend was observed for foam stability where stability increased with increasing concentration. Tilapia scale gelatin showed the highest foam stability (170.67%) and the lowest was shown by market gelatin (33.33%). This aligns with the findings where it was noted that higher concentrations of cuttlefish gelatin led to raised values of foam expansion and foam stability [29]. Foams that contain protein at higher concentrations, exhibited increased density and improved stability due to the increase in interfacial film thickness [30]. The foam's stability is impacted by a range of factors, encompassing the speed at which surface tension equilibrium is reached, viscosity levels, the effect of spatial hindrance stabilization, and the electrostatic repulsion between opposing sides of the foam structure [31].



A B

Fig 3: Foaming capacity of Rohu, Tilapia scale gelatin and Market gelatin. [A] Foam Expansion (%), [B] Foam stability (%)

Conclusion

The findings of this study suggest that the waste scales from Rohu and Tilapia fish can serve as viable sources for gelatin extraction. The gelatins obtained from these scales exhibited properties similar to commercially available gelatin. In terms of foaming properties, the gelatin from fish scales outperformed the market gelatin. Overall, the functional properties of Tilapia scale gelatin were comparable to those of the market gelatin.

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