# Biopolymer Films from Milkfish Bone Gelatin: A Study of Physicochemical and Mechanical Properties

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## ABSTRACT

**The utilization of milkfish bone as a gelatin source for biopolymers offers an innovative approach that reduces waste and adds value to fishery byproducts. This study aims to characterize gelatin extracted from milkfish bone (*Chanos chanos*) and assess its potential for use in biodegradable film production. Gelatin extraction was conducted using 4.65% hydrochloric acid (HCl) for ~27 h at 89.92 °C, and biodegradable films were prepared via the casting method using 10% (w/v) gelatin and 15% (v/v) glycerol. Comprehensive physicochemical analyses were performed, including measurements of moisture, ash, protein content, pH, freezing/melting points, viscosity, and gel strength. Structural and morphological characteristics were examined through Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM), while mechanical properties, such as tensile strength, elongation at break, and film thickness were also evaluated. The results revealed that milkfish bone gelatin had 7.47% moisture, 84.32% protein, and 1.80% ash content, meeting Indonesian National Standard (SNI) specifications. Additionally, the pH of milkfish bone gelatin (4.39) was lower than that of commercial gelatin (6.25), and it exhibited higher freezing/melting points, but lower viscosity (14.8 cP) and gel strength (9.52 N) than commercial gelatin (15.0 cP, 16.82 N). FTIR spectra confirmed the presence of the gelatin's characteristic spectrum, while the SEM analysis revealed a more porous surface structure than that of the commercial gelatin. The resulting mechanical properties of the milkfish bone gelatin displayed lower tensile strength (1.03 MPa) and elongation at break (151.58%) compared to the films made from commercial gelatin (6.10 MPa, 223.38%). Despite these limitations, the milkfish bone-derived gelatin demonstrates significant potential as a raw material for biodegradable film applications.**

***Keywords-biodegradable; film; gelatin; milkfish bone; waste***

## I. INTRODUCTION

The growing emphasis on sustainable materials in environmental science has underscored the potential of utilizing fishery waste as a source of eco-friendly biopolymers, particularly gelatin derived from fish bones. Gelatin, a natural biopolymer obtained from collagen, is widely used in the food, pharmaceutical, and biodegradable packaging industries [1, 2]. Commercial gelatin is predominantly sourced from mammals, such as cattle and pigs, presenting challenges related to sustainability and the compliance with halal standards [3, 4]. As a result, alternative sources, such as fish bones from low-value species or fish processing byproducts, present a promising solution for the waste reduction and added economic

value of the, otherwise, underutilized products [5, 6]. Moreover, the development of biopolymers from such waste aligns with the global sustainability goals and supports the creation of environmentally responsible packaging materials [7].

Research on biodegradable films made from fish gelatin, whether extracted from skin or bones, has shown encouraging results. For instance, catfish bone gelatin combined with breadfruit starch has been effectively used in film production [8], while trout skin gelatin has demonstrated improved mechanical strength with increased viscosity at lower temperatures [9]. Similarly, films incorporating catfish bone gelatin with chitosan or tapioca starch have exhibited enhanced water resistance and tensile strength [10]. Gelatin from tilapia bones, modified with carrageenan, and from salmon backbones has also proven suitable for film formation [11, 12]. However, challenges remain, particularly with gelatin derived from fish bones, which often exhibits lower gel strength and viscosity, limiting its application in mechanically demanding films [13, 14]. To address these limitations, blending gelatin with other biopolymers has been widely explored to improve the film properties [15]. Moreover, the fish skin gelatin is often superior in quality compared to the bone-derived gelatin, emphasizing the need to investigate alternative sources, such as milkfish bones (*Chanos chanos*) [16].

This study aims to characterize the physicochemical properties of gelatin extracted from milkfish bones (*Chanos chanos*) and evaluate its potential for use in biodegradable films. The extraction was performed using an optimized acid-based method. Comprehensive analyses were carried out to assess the proximate composition, viscosity, gel strength, and microstructural characteristics through FTIR, and SEM. Furthermore, the produced gelatin films underwent testing for their mechanical properties, including tensile strength, elongation, and thickness. For the comparative analysis, commercially available fish gelatin powder was used to benchmark the performance of the milkfish bone-derived gelatin.

## II. MATERIALS AND METHODS

### A. Materials

The milkfish bones used in this study were obtained from the Teaching Factory of the Sidoarjo Marine and Fisheries Polytechnic. For the comparative analysis, commercial gelatin was obtained from Kuro fish gelatin powder via the Shopee Marketplace (Indonesia). To ensure consistency and reliability in the experimental procedures, additional reagents, including glycerol (Sigma-Aldrich, Spain), hydrochloric acid (HCl), and distilled water, were employed.

### B. Gelatin Extraction

Gelatin was extracted from milkfish bones using an acid hydrolysis method adapted from the procedure outlined in [17]. The bones were first immersed in a 4.65% HCl solution, which facilitated the breakdown of collagen into gelatin. This pre-treatment was conducted over a period of 26 h and 55 min to ensure a sufficient acid penetration and collagen denaturation. The extraction process was carried out at a controlled temperature of 89.92 °C, a critical parameter that enhances the collagen solubilization while maintaining the functional integrity of the resulting gelatin.

### C. Edible Film Preparation

Edible films were prepared using the casting method. A gelatin solution was formulated by dissolving 10% (w/v) gelatin in distilled water, with 15% (v/v) glycerol added as a plasticizer to improve the film flexibility. The mixture was heated to 60 °C and stirred continuously for 30 min to ensure homogeneity and complete dissolution. Once a uniform solution was achieved, 25 mL of the solution was poured into a 90 mm diameter petri dish. The films were then dried at room temperature for 48 h to allow solvent evaporation and film formation. This protocol was applied uniformly to both the milkfish bone gelatin and the commercial Kuro gelatin, ensuring consistent processing conditions across all samples.

### D. Physicochemical Characterization of Gelatin

Physicochemical characterization of the extracted gelatin was carried out on core compositional parameters, including moisture content, ash content, and crude protein. These parameters were determined in strict accordance with the Association of Official Analytical Chemists (AOAC) guidelines [18], ensuring the reliability and comparability of the data. Functional properties, including the pH, freezing point, melting point, viscosity, and gel strength, were evaluated following the procedures outlined in [4], with each test performed thrice to minimize variability and ensure statistical robustness. In addition, the chemical structure of the gelatins was analyzed using FTIR, as described in [19], providing insights into the molecular bonds and functional groups present. Additionally, SEM was deployed following the method described in [20] to investigate the surface morphology and network architecture of the gelatin. Collectively, these techniques enabled an evaluation of both the chemical and physical properties of the gelatin.

### E. Mechanical and Physical Properties of Edible Films

The mechanical properties of the gelatin films, tensile strength, elongation percentage, and thickness, were evaluated following the method described in [21]. For testing, each film sample was cut into 5×5 cm sections, clamped horizontally, and subjected to a 1 kg load. The tensile strength was determined as the maximum force $F$ applied before the film ruptured, divided by the cross-sectional area $A$, as expressed in:

$$Tensile\ Strength = \frac{F}{A} \quad (1)$$

Elongation is calculated by dividing the difference between the increased final film length $FFL$ after rupture and the initial film length $IFL$, as expressed in:

$$Elongation\ (\%) = \frac{FFL - IFL}{IFL} \cdot 100\% \quad (2)$$

The thickness of the edible film is measured using a micrometer screw gauge with an accuracy of 0.001 mm at five different points on the film. The average of these values was used to ensure an accurate representation of the film thickness.

Additionally, pH measurements of the gelatin film solutions were conducted prior to drying, following the protocol in [22]. A calibrated pH meter was used, with calibration performed using standard buffer solutions. The electrode was dried with tissue paper before being immersed in the film solution. After stabilization, the pH value was recorded to assess the acidity level of the gelatin solution, which can influence the film formation and stability.

### *F. Statistical Analysis*

Statistical evaluations were performed using an independent t-test to ascertain the significance of the differences between the gelatin and edible film groups, with a significance level set at $\alpha = 0.05$. All statistical analyses were executed in Microsoft Excel, and the results were reported as mean ± standard deviation.

## III. RESULTS AND DISCUSSION

### *A. Physicochemical Characterization of Gelatin*

Figure 1 presents the resulting commercial gelatin (Kuro) alongside the milkfish bone gelatin. Table I lists the resulting physicochemical properties of both gelatins alongside the corresponding SNI specifications.

Significant differences were observed in the water content, ash content, pH, freezing point, and gel power, indicating compositional and structural variations between the two gelatin sources. However, no significant differences were found in the protein content, melting point, and viscosity, suggesting comparable thermal stability and solubility. Furthermore, both gelatin samples met the SNI requirements, which specify a maximum moisture content of 16%, a maximum ash content of 3.25%, and a protein content range of 84–90% [23]. These results indicate that the milkfish bone gelatin has the potential to serve as an alternative to the commercial fish gelatin, provided that its functional properties are further optimized.

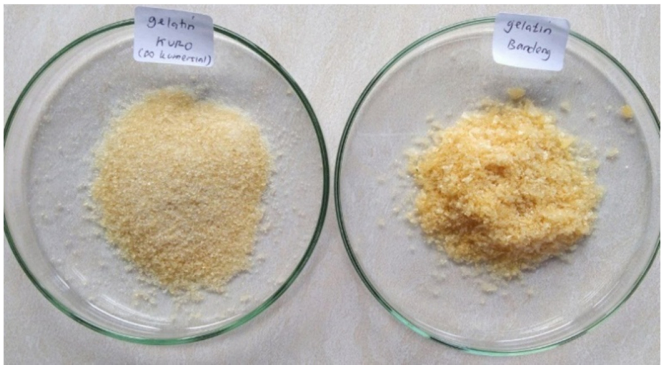


Fig. 1. Kuro gelatin (left) and milkfish bone gelatin (right).

The lower pH observed in milkfish bone gelatin, attributed to the acid extraction method, aligns with the typical acidic profile of the acid-extracted gelatin (pH ~4.85) [24]. This is consistent with earlier studies showing that the acid-treated gelatin generally exhibits lower pH values than the alkali- or enzyme-treated counterparts [25]. The acidic nature of milkfish gelatin may affect its solubility, gelation behavior, and overall stability, potentially limiting its compatibility with certain biopolymers.

TABLE I. PHYSICOCHEMICAL PROPERTIES OF MILKFISH BONE BONE GELATIN (*CHANOS CHANOS*) AND KURO GELATIN

| Characteristics | Milkfish bone gelatin | Kuro gelatin | SNI |
|---|---|---|---|
| Water content (%) | 7.47±0.24[a] | 10.25±0.15[b] | Max. 16 |
| Ash content (%) | 1.80 ±0.04[a] | 1.11±0.21[b] | Max. 3.25 |
| Protein content (%) | 84.32 ±0.96[a] | 85.30 ±1.09[a] | 84-90 |
| pH | 4.39±0.17[a] | 6.25±0.06[b] | - |
| Freezing point (°C) | 10.18±1.33[a] | 7.8±1.64[b] | - |
| Melting point (°C) | 36.28±1.70[b] | 34.92±2.34[b] | - |
| Viscosity (cP) | 14.8±0.84[c] | 15±0.70[c] | 2.5 – 5.5 |
| Gel power (N) | 9.52±1.03[a] | 16.82±3.48[b] | - |

Values in the same row with the same superscript letter are not significantly different ($p > 0.05$). Different superscript letters in the same row indicate a statistically significant difference.

In terms of thermal properties, the milkfish bone gelatin exhibited higher freezing and melting points compared to the Kuro gelatin. These thermal differences are influenced by both the source of collagen and extraction conditions, which impact the resulting gel network structure [26]. A higher melting point implies improved thermal stability, which is advantageous for applications in high-temperature food processing and encapsulation technologies where heat resistance is critical.

In terms of viscosity, the milkfish bone gelatin displayed a slightly lower value compared to the commercial Kuro gelatin. Lower viscosity typically indicates a less dense molecular structure or reduced molecular weight, allowing the gelatin to flow more easily. This characteristic can significantly influence its application in food formulations, coatings, and pharmaceutical products, where a precise control of viscosity is essential for achieving the desired texture, consistency, and processability [27]. A lower viscosity also facilitates easier handling and mixing, making it suitable for liquid-based formulations, such as beverages, emulsions, and soft gels. However, in some applications, higher viscosity may be desirable for improving the textural and gelling properties of gelatin-based products.

Gel strength, a key determinant of firmness and textural performance, was significantly lower in milkfish bone gelatin than in Kuro gelatin. Nonetheless, it exceeded that of the pink ear emperor fish skin gelatin, as reported in [28]. This suggests that the milkfish gelatin may be well suited for applications demanding softer, more flexible textures, such as gummy candies, soft capsules, and biodegradable films. The gel strength is highly influenced by the amino acid composition and processing conditions, which govern the formation of intermolecular bonds [16]. Improvements in gel strength could be achieved through process optimization or the addition of functional biopolymers, like polysaccharides or proteins.

### *B. Gelatin Structure Analysis*

The FTIR spectra of milkfish bone gelatin and commercial Kuro gelatin (Figure 2) confirmed the presence of characteristic infrared absorption bands corresponding to Amide A, B, I, II, and III [29]. These bands correspond to specific molecular vibrations within the gelatin structure, indicating the

preservation of its proteinaceous composition. The absorption bands were identified within the typical spectral ranges: Amide A (3478–3310 $cm^{-1}$), Amide B (2935–2915 $cm^{-1}$), Amide I (1658–1653 $cm^{-1}$), Amide II (1575–1480 $cm^{-1}$), and Amide III (1240–1234 $cm^{-1}$). The Amide A band, which represents N-H stretching related to hydrogen bonding in the gelatin structure, was observed at 3287.42 $cm^{-1}$ for milkfish bone gelatin and 3294.55 $cm^{-1}$ for Kuro gelatin. These values indicate similar hydrogen bonding networks in both samples, suggesting a preserved triple-helix structure.

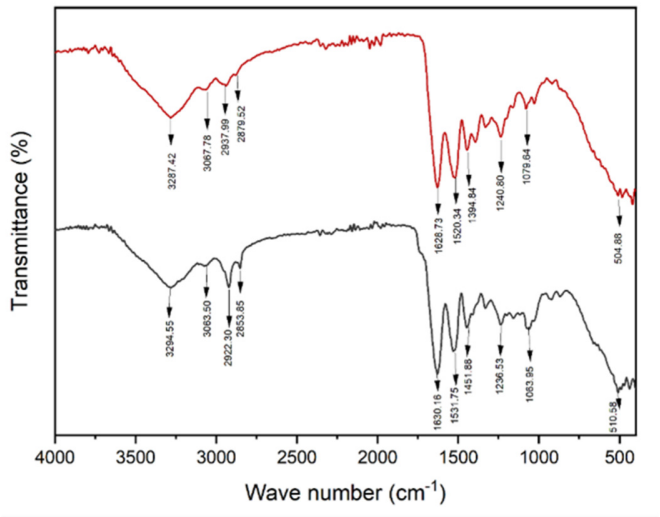


Fig. 2. FTIR on milkfish bone gelatin (red) and Kuro gelatin (black).

The Amide B band, attributed to asymmetric $CH_2$ stretching, was detected at 2937.99 $cm^{-1}$ for the milkfish bone gelatin and 2922.30 $cm^{-1}$ for the Kuro gelatin. The Amide I band, which is associated with C=O stretching and provides insights into secondary protein structures, was observed at 1628.73 $cm^{-1}$ for the milkfish bone gelatin and 1630.16 $cm^{-1}$ for the Kuro gelatin. Differences in the peak position may indicate variations in the helical or sheet structure of the gelatin. The Amide II and Amide III bands, representing N-H bending and C-N stretching, also exhibited minor shifts in wavenumber, reflecting differences in the hydrogen bonding interactions and peptide bond environments. These spectral variations indicate differences in the protein structure and intermolecular bonding, which can influence the functional properties of gelatin, including its gel strength, viscosity, and thermal behavior [30]. Figure 3 presents SEM images of milkfish bone gelatin and commercial Kuro gelatin.

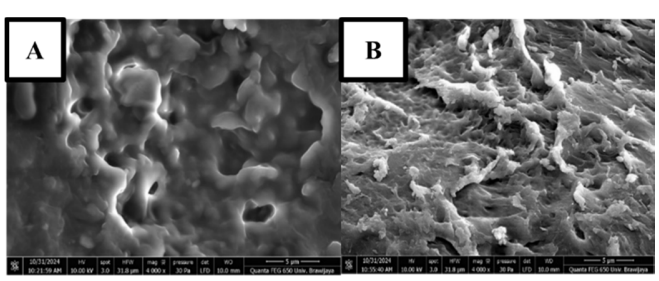


Fig. 3. SEM analysis: (a) milkfish bone gelatin, and (b) Kuro gelatin.

The SEM analysis revealed that the milkfish bone gelatin exhibited a non-homogeneous surface with a highly porous structure (~5 µm), whereas the Kuro gelatin displayed a more compact and uniform morphology. This high porosity in milkfish bone gelatin is associated with lower gel strength compared to the Kuro gelatin, as indicated in Table I. These properties suggest that the milkfish bone gelatin may be advantageous for applications requiring flexibility, such as edible films, although structural reinforcement may be necessary for more demanding uses. The sponge-like morphology of milkfish bone gelatin resembles that of the croaker fish skin gelatin, as reported in [31], suggesting that similar extraction and processing conditions can lead to comparable microstructures across the fish species. In contrast, the denser, layered, and fibrillar structure of the Kuro gelatin implies a stronger molecular cohesion and better protein-protein interactions [32]. The more cohesive network and lower porosity in Kuro gelatin suggest enhanced hydrogen bonding, which supports greater mechanical stability, an essential trait for biopolymer-based packaging applications [33].

### *C. Characterization of Edible Film*

Figure 4 presents the resulting edible milkfish bone and Kuro gelatin films. Table II lists the measured mechanical and physical properties of both edible films.

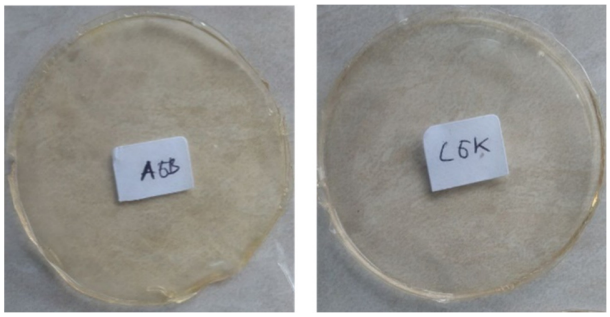


Fig. 4. Edible milkfish bone gelatin film (left), and Kuro gelatin film (right).

TABLE II. CHARACTERISTICS OF MILKFISH BONE GELATIN FILM AND COMMERCIAL KURO GELATIN FILM

| No. | Characteristic | Milkfish bone gelatin | Kuro gelatin |
|---|---|---|---|
| 1 | Tensile strength (MPa) | 1.03 ±0.16[a] | 6.10±0.77[b] |
| 2 | Percentage of elongation (%) | 151.58±14.77[a] | 223.38±31.40[b] |
| 3 | Thickness (mm) | 0.29±0.027[a] | 0.33±0.006[b] |
| 4 | pH | 4.97 ±0.30[a] | 7.51±0.0.14[b] |

Values in the same row with the same superscript letter are not significantly different ($p > 0.05$). Different superscript letters in the same row indicate a statistically significant difference.

The Kuro gelatin film demonstrated superior mechanical properties, with a tensile strength of 6.10 ± 0.77 MPa, significantly higher than that of the milkfish bone gelatin film, which had a tensile strength of 1.03 ± 0.006 MPa. This difference suggests that the structural integrity of the Kuro gelatin film is more stable and resistant to external forces. Its enhanced mechanical performance is likely attributed to its higher collagen quality or a more optimized extraction and processing method, as noted in [8]. Both films meet the

Japanese Industrial Standard (JIS) minimum tensile strength requirement of 0.392 MPa [28]. The Kuro gelatin film also exhibited a higher elongation percentage (223.38 ± 31.40%) compared to the milkfish bone gelatin film (151.58 ± 14.77%). This indicates that the Kuro gelatin film is more flexible and can stretch further before breaking, which is a desirable property in packaging applications [34]. The elongation values of both films met the JIS standard, which classifies elongation below 10% as poor and above 50% as good [28].

The milkfish bone gelatin film exhibited a thinner structure (0.29 ± 0.027 mm) than the Kuro gelatin film (0.33 ± 0.006 mm). Although thicker films generally offer better barrier protection, they may compromise flexibility, necessitating a balanced approach based on the intended use.

The pH of the milkfish bone gelatin film was 4.97 ± 0.30, whereas the Kuro gelatin film had a higher pH of 7.51 ± 0.14. The acidic nature of the milkfish bone gelatin may influence its stability and interactions with food products, while the higher pH of the Kuro gelatin could provide better stability under various storage conditions and influence the taste and aroma of packaged products [35]. The pH of edible films has been reported to impact their physical and mechanical properties, as well as their interactions with food components [36]

Overall, the comparison of physicochemical characteristics between gelatin films derived from milkfish bone and Kuro gelatin indicates that Kuro gelatin has advantages in terms of tensile strength, elongation percentage, and a more neutral pH, making it more suitable for food packaging applications. However, gelatin from milkfish bones also holds significant potential, particularly in terms of sustainability and the utilization of local resources. Further modifications, such as the incorporation of biopolymers, could enhance the functional properties of edible gelatin films [2]. The combination of fish and poultry gelatin for sachet oil packaging in the food industry has been proposed [37]. The potential use of gelatin from fish bone waste supports the innovations in biodegradable biopolymer materials.

## IV. CONCLUSION

This study explored the potential of gelatin extracted from milkfish bones, a commonly underutilized fishery byproduct, as a raw material for biodegradable edible films. The physicochemical and mechanical properties of the milkfish bone gelatin were systematically compared with those of the commercial fish gelatin (Kuro). The results indicated that the milkfish bone gelatin met the Indonesian National Standard (SNI) for the moisture and protein content but exhibited lower pH, viscosity, and gel strength. Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) confirmed the presence of characteristic gelatin functional groups and revealed a more porous microstructure in the milkfish gelatin, which contributed to its reduced mechanical strength. Films produced from milkfish bone gelatin demonstrated a lower tensile strength and elongation than those from commercial gelatin, though they still satisfied the minimum industrial requirements. These findings validate the feasibility of the milkfish bone gelatin as an alternative biopolymer source, supporting the sustainability goals and promoting circular economy practices in the fishery sector.

This is one of the few studies to characterize edible films derived specifically from milkfish bone gelatin, integrating both physicochemical and microstructural analyses. Future research should focus on enhancing the film performance through cross-linking, blending with other biopolymers, or incorporating functional additives. Such advancements could broaden the applicability of this biopolymer in food packaging while reinforcing the efforts toward waste valorization and environmental conservation.

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