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Valorization of fish scale waste for the synthesis of functional gelatin-based biopolymers

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ABSTRACT

This study investigated the potential of extraction and characterization of bioplastics from *Catla catla* fish scale gelatin as a sustainable alternative to petroleum-based polymers. The extraction process involved NaCl and NaOH treatments, EDTA demineralization, acid solubilization, and thermal hydrolysis. Gelatin was characterized using UV–Visible spectroscopy, Fourier Transform Infrared (FT-IR) spectroscopy, and Scanning Electron Microscopy (SEM). UV-Vis spectroscopic analysis showed absorption peaks below 250 nm, indicating intact peptide bonds. The FTIR spectra displayed amide I and II bands with C–O stretching, confirming protein integrity and plasticization with glycerol. SEM examination revealed the transformation of porous gelatin powder to a smooth biopolymer matrix in the films. The bioplastic demonstrated a pH of 4.72, 5% moisture content, 28% water solubility, and a swelling degree of 32 %, indicating its flexibility and environmental responsiveness. Gel tests confirmed the formation of protein-based gels. Spectral and morphological analyses validated plasticization, supporting the potential of this material for biodegradable packaging and biomedical applications. Thus, the study demonstrates the conversion of fishery waste into biopolymers for use as a sustainable material.

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INTRODUCTION

In recent years, the use of biodegradable materials has increased significantly across multiple industries, such as packaging, agriculture, food, and medicine. Biopolymer blends offer a viable alternative to traditional synthetic polymers such as starch, gelatin, chitosan, alginate, PLA, and PHAs, which are some of the most researched and utilized biopolymers (Akter *et al.,* 2012). Gelatin is a natural biopolymer obtained from the partial hydrolysis of collagen, a structural protein mainly found in animal connective tissues. Gelatin is highly regarded for its outstanding physicochemical characteristics, including biocompatibility, biodegradability, nontoxicity, and excellent film-forming ability, making it extremely useful in the food, pharmaceutical, cosmetic, and biomedical (Gómez-Guillén *et al.,* 2021).

Traditionally, gelatin has been derived from mammalian tissues, especially bovine and porcine bones, skin, and tendons. However, reliance on mammalian sources poses several issues, such as the potential for zoonotic disease transmission, cultural and religious limitations, and environmental sustainability concerns (Zhou *et al.,* 2022). Consequently, there is a growing demand for alternative gelatin sources that can address these challenges while preserving their functional properties.

Fish-scale gelatin possesses a variety of appealing properties, such as strong gel strength, excellent water retention, and outstanding film-forming capabilities, making it ideal for numerous industrial and biomedical applications. Unlike mammalian gelatin, fish gelatin has distinct features, including a lower melting and gelling point, which makes it ideal for applications that require thermal sensitivity, such as encapsulation of bioactive compounds (Fu *et al.,* 2022).

The incorporation of fish-scale gelatin into biopolymer systems has attracted considerable interest owing to its potential in creating sustainable materials and biodegradable packaging solutions. When combined with other biopolymers, such as chitosan, alginate, and polyvinyl alcohol (PVA), fish-scale gelatin can improve the mechanical strength, flexibility, and barrier properties of the resulting materials. Recent research has suggested that gelatin-based films, when combined with natural antimicrobial agents, can act as effective bioactive packaging materials, thereby prolonging the shelf life of perishable goods (Xue *et al.,* 2022).

To further enhance the stability and functionality of fish-scale gelatin-based biopolymers, various chemical modifications such as crosslinking with agents such as glutaraldehyde and genipin have been investigated. These treatments enhance the structural integrity of gelatin films and hydrogels, broadening their application beyond food packaging to include wound dressings, drug delivery systems, and 3D bioprinting materials (Khan *et al.,* 2023).

The development of fish-scale gelatin-based nanocomposites and hybrid materials represents an exciting frontier in biomaterial research. Fish gelatin nanoparticles, for instance, have shown promise as efficient drug delivery vehicles for improving the solubility and bioavailability of poorly water-soluble drugs (Zhang *et al.,* 2024). Additionally, fish-scale-derived gelatin hydrogels are increasingly being recognized for their applications in tissue engineering, where they serve as scaffolds to support cell adhesion and growth (Yang *et al.,* 2023). Waste generated from global fish and shellfish processing poses a significant environmental challenge (Mejia-Saules *et al.,* 2006).

Utilizing this by-product to produce renewable biopolymers offers a dual-purpose solution, contributing to both waste reduction and the development of sustainable products (Gildberg and Stenberg, 2001). Fish scales, in particular, are preferred for gelatin extraction because of their high amino acid content, especially proline, which results in higher gelatin yields than fish skin (Zakaria *et al.,* 2001). Given the increasing global focus on sustainability and plastic waste reduction, fish-scale gelatin-based biopolymers have significant potential

in transforming the packaging industry. Ongoing research into biodegradable and bio-based packaging materials aims to replace conventional synthetic polymers, thereby reducing their environmental impacts. As consumer demand for eco-friendly materials increases, further innovations in fish-scale gelatin-based products are expected to drive progress in this field.

This study aims to address the following gaps: i). Extraction of gelatin from fish scales using optimized extraction techniques ii). The identity of the extracted gelatin was confirmed by UV-Vis spectroscopy, Fourier Transform Infrared (FT-IR) spectroscopy, and Scanning Electron Microscopic (SEM) analyses iii). The surface morphology and structural characteristics of bioplastic films derived from gelatin iv). The physical and chemical properties of gelatin were analyzed to assess its suitability as a biopolymer and v). Synthesis of bioplastics using extracted gelatin.

By combining material extraction with biopolymer synthesis, this research seeks to develop a sustainable and value-added use for fish-scale waste, promoting environmental sustainability, and advancing the development of biodegradable packaging materials.

MATERIALS AND METHODS

Sample collection and preparation

Fish scales were collected from *Catla catla* specimens (average body weight: 100–200 g) obtained from Sitharevu, Dindigul district. The scales were manually detached, placed in polyethylene zip-lock bags and transported to the laboratory on ice. After washing twice with cold water, the scales were air-dried and stored at 5 °C until needed.

Gelatin extraction

Gelatin was extracted as described by Das *et al.* (2017a). The dried scales were treated with 5% NaCl (1:10, w/v) for 30 min with agitation, followed by alkali treatment using 0.4% NaOH (1:10, w/v) for 60 min, with the solution being replaced after 30 min. Demineralization was performed using 0.5 N EDTA (pH 7.66, 1:10, w/v) for 24 h. The scales were then immersed in 0.05 M glacial acetic acid for 24 h. Extraction was performed in a water bath at 70 °C for 1.5 hours with a water-to-solid ratio of 3:1 (v/w). The extract was filtered, dried in a hot air oven at 50 °C, ground into a powder, and stored.

Physicochemical characterization

Gelatin yield was calculated based on the dry weight, as described by Kaewruang *et al.* (2013). pH was measured using a 1% gelatin solution at 25 °C. Moisture content was determined by drying the samples at 90 °C for 18 h (AOAC, 2019). The Biuret test, following Plummer (2017), confirmed the presence of peptide bonds. Gel formation was assessed by heating the sample at 60 °C and then cooling it to 4 °C for 6 h (Kaewruang *et al.,* 2013).

Bioplastic synthesis

Gelatin (0.70 g) was dissolved in 10 ml of warm water (50–60 °C) with continuous stirring. Glycerol was added as the plasticizer. The solution was poured into molds and dried at room temperature to form the bioplastic films (Caroline *et al.,* 2012).

Water solubility and swelling tests

Water solubility was assessed by immersing weighed film samples in distilled water for 24 h at 25 °C followed by drying at 40 °C (Mottalib *et al.,* 2024). The degree of swelling was determined by immersing the dried films in distilled water at 37 °C for 24 h and calculating the weight difference (Mottalib *et al.,* 2024).

Biopolymer characterization

UV-Vis spectroscopy

Gelatin solutions were scanned from 200 to 400 nm using a Shimadzu UV-1800 spectrophotometer after filtering (Patel and Kumar, 2023).

FT-IR analysis

Thin film slices were analyzed using a Thermo Scientific Nicolet i5 FTIR spectrometer (ATR and transmission modes) in the 4000–400 cm^{-1} range with a 4 cm^{-1} resolution (Campanale *et al.,* 2023; Silva and Green, 2016; Wang and Smith, 2024).

SEM analysis

Films (5 mm × 5 mm) were mounted on aluminum stubs, gold-coated, and imaged using a Tescon Oxford SEM at 5–20 kV (Kim and Lee, 2022; Liu and Zhang, 2023). The surface topography was analyzed using Vega 3 INCA software.

RESULTS AND DISCUSSION

The objective of this study was to produce bioplastics from gelatin extracted from fish scales and to assess their yield, physicochemical characteristics, and functional performance.

Effect of temperature

The gelatin yield was optimized by adjusting the extraction temperature (60°C, 70°C, and 80°C) and skin-to-water ratios (1:2, 1:4, and 1:6). As shown in Fig. 1, higher temperatures and lower skin-to-water ratios significantly enhanced the gelatin yield, reaching a peak of approximately 6.5% at 80°C with a 1:6 ratio. These results corroborate previous studies suggesting that increased temperatures promote collagen hydrolysis (Arnesen and Gildberg, 2007), although excessive heat may compromise gelatin quality (Ahmad and Benjakul, 2011).

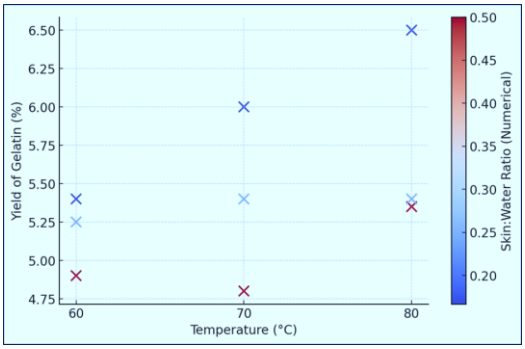


Fig. 1. Effect of temperature and skin to water ratio on gelatin yield

At 60°C, the yields varied from 4.8% to 5.5%, rising to 6.5% at 80°C, which aligns with earlier findings by Jamilah and Harvinder (2002) and Norziah *et al.* (2009) that heat aids solubilization. The impact of dilution was also noticeable; lower skin-to-water ratios resulted in higher gelatin yields due to enhanced collagen hydration and diffusion (Karim and Bhat, 2009; Arnesen and Gildberg, 2007).

Physicochemical analysis

Physicochemical analysis revealed a slightly acidic pH of 4.72 and a moisture content of 5% (Fig. 2). Acidic pH may improve gelling and hydrophobic interactions, which are advantageous for delivering bioactive compounds, as noted by Huang *et al.* (2024). The moisture content contributes to stability and prolongs shelf life, which is consistent with studies on gelatin-based films improved with chitosan and silica sol.

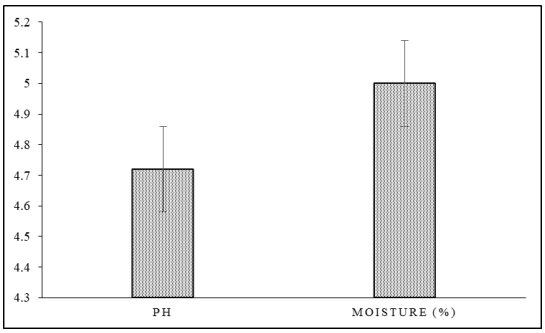


Fig. 2. Physical analysis of gelatin

Characterization of gelatin

Biuret testing confirmed the presence of protein, indicated by a violet color, whereas the gel test demonstrated firm and stable gelation (Fig. 3), which is crucial for biomedical and packaging applications. Bioplastic films made from gelatin and glycerol showed a strong structure and flexibility, confirming fish-scale gelatin as a feasible biopolymer.

Fig. 3. Characterization of fish scale derived gelatin

Properties of bioplastics

Water solubility and swelling behavior were also examined. The bioplastics exhibited 28% solubility and 32% swelling degree (Fig. 4), indicating moderate hydrophilicity. This figure suggests that it performs well in biodegradable food packaging and controlled-

release applications (Sanyang *et al.,* 2016; Sapei *et al.,* 2018). The addition of glycerol reduces solubility by increasing matrix hydrophobicity and enhancing stability under humid conditions (Sionkowska and Płanecka, 2023). The swelling behavior supports the durability of bioplastics and their potential in medical packaging or drug delivery (Mitantsoa *et al.,* 2023). According to Yusril *et al.* (2017), bioplastics with low swelling (0–20%) exhibit strong water resistance and are suitable for moisture-sensitive applications such as electronic packaging. Rahayu *et al.* (2023) observed that moderate swelling (20–50%), as seen in the result of 32% swelling, offers an ideal balance between water absorption and structural stability, making such bioplastics appropriate for biodegradable food packaging and controlled-release systems. Sionkowska and Płanecka (2023) emphasized that glycerol can modulate swelling by altering the matrix hydrophilicity, thus improving performance in humid environments. In contrast, Khan *et al.* (2019) reported that bioplastics with high swelling (>50%) are more suitable for biomedical applications, such as wound dressings and tissue scaffolds, where high fluid uptake is necessary.

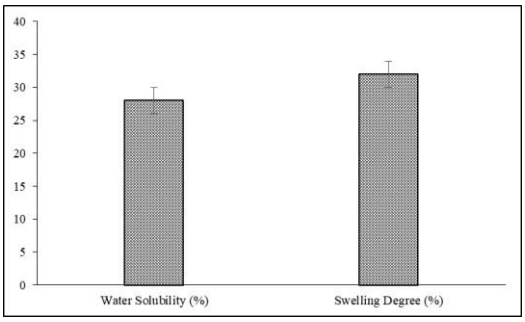


Fig. 4. Water solubility and swelling properties of bioplastic

UV – Vis spectrum analysis

The UV-Vis spectrum of gelatin extracted from fish scales, measured between 200 and 400 nm, displays unique absorption features that highlight its structural soundness, purity, and functional potential for diverse uses. A notable absorption peak around 250 nm, with an absorbance value of approximately 4.213, is linked to π → π* electronic transitions related to amide bonds (-CONH-), and the aromatic amino acids tyrosine, tryptophan, and phenylalanine (Fig. 5) were obtained. This distinct peak suggested that the peptide backbone of gelatin remained largely intact with minimal degradation, confirming the preservation of its native molecular structure (Ahmad *et al.,* 2022; Das *et al.,* 2017b; Nuamsrinuan *et al.,* 2018). The presence of these aromatic residues further supports the retention of crucial functional properties such as gel strength and film-forming ability (Benjakul *et al.,* 2019). In the 250–320 nm range, a gradual decrease in absorbance was observed, characterized by weak bands attributed to n → π* transitions in the carbonyl groups of amide bonds and minor contributions from disulfide bond interactions, indicating limited crosslinking and oxidation (Wulandari *et al.,* 2020; Muyonga *et al.,* 2004; Hosseini *et al.,* 2021). Beyond 350 nm, the spectrum showed minimal absorbance, suggesting the absence of chromophores linked to oxidative degradation, pigments, or Maillard reaction products. This absence confirmed the high purity of gelatin and the effectiveness of the extraction process in minimizing chemical changes or contaminants (Zhang *et al.,* 2018; Jamilah and Harvinder, 2002; Gómez-Guillén *et al.,* 2011).

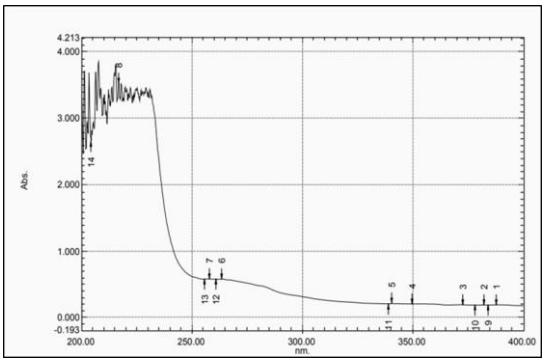
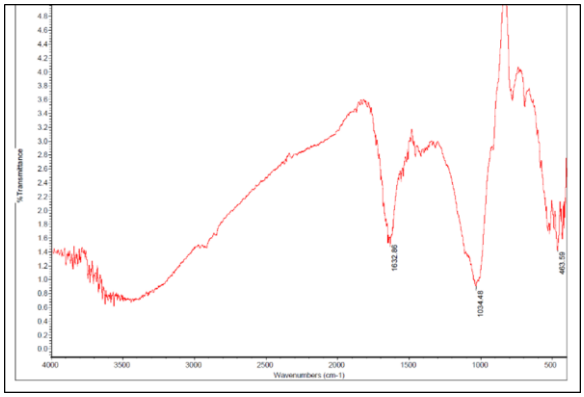
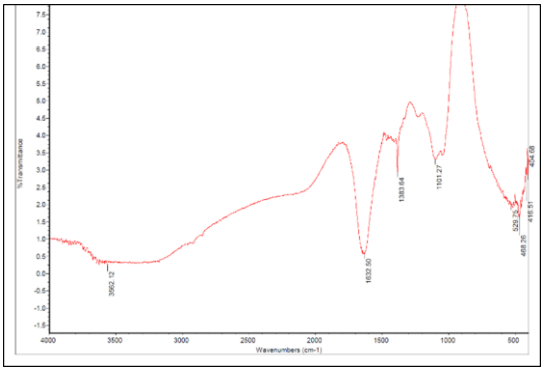


Fig. 5. UV-Vis spectral analysis of fish scale derived gelatin for purity and structural validation

Overall, these spectral characteristics demonstrate that gelatin derived from fish scales maintains its molecular integrity and is suitable for application in the food, pharmaceutical, and biomaterial industries because of its preserved gelling strength, flexibility, and film-forming capacity (Zhou and Regenstein, 2004; Karim and Bhat, 2009; Mahmood *et al.,* 2020).



a. Gelatin powder



b. Bioplastics

Fig. 6. FT-IR spectrum of gelatine powder derived from fish scales and bioplastics

FT-IR analysis

Fourier Transform Infrared (FTIR) spectroscopy offers valuable insights into the differences between gelatin derived from fish scales and their bioplastic counterparts, emphasizing significant structural changes. FT-IR spectral analysis provides compelling evidence for the successful transformation of fish scale-derived gelatin into a bioplastic, revealing both the retention of the core protein structure and the emergence of new chemical interactions. In the raw gelatin spectrum (Fig. 6), a prominent peak at 1632.86 cm^{-1} corresponds to the Amide I band (C=O stretching), indicative of peptide linkages from collagen. Additional peaks at 1034.48 cm^{-1} (C–O stretching) and 463.59 cm^{-1} (skeletal bending or mineral content) confirm the proteinaceous and partially mineralized nature of the material. Upon synthesis into bioplastic, the FT-IR spectrum retains the Amide I peak at 1633.50 cm^{-1}, demonstrating structural integrity of the polypeptide backbone, but new peaks at 1333.64 cm^{-1} and 1101.27 cm^{-1} appear, which are attributed to C–N and C–O stretching vibrations (Fig. 5). These changes suggest successful plasticization and possible crosslinking, likely due to the incorporation of additives, such as glycerol or citric acid. A broad O–H stretching band at 3562.12 cm^{-1} indicates enhanced hydrogen bonding, leading to increased hydrophilicity and flexibility of the bioplastic matrix. Peaks in the lower fingerprint region (529.75, 470.51, 468.26, and 404.68 cm^{-1}) point to structural reorganization or interactions with residual mineral phases. These results agree with the findings of Jridi *et al.* (2022) and Nascimento *et al.* (2023), who reported similar spectral shifts in gelatin films treated with plasticizers. Studies by Gómez-Estaca *et al.* (2009) and Souza *et al.* (2020) also confirmed that new vibrational modes in the 1100–1300 cm^{-1} range correspond to enhanced flexibility and molecular mobility, while Martucci and Ruseckaite (2009) linked increased O–H bonding to improved water interaction and biodegradability. Furthermore, Tajik *et al.* (2021) emphasized that the residual mineral content can influence the mechanical behavior of protein films, supporting the presence of low-frequency bands in the current spectra. These FTIR observations indicate that the transformation of gelatin to bioplastic is not merely physical, but involves chemical modifications through hydrogen bonding, esterification, and potential ionic interactions. Such modifications tailor essential properties, such as tensile strength, moisture resistance, and degradation potential, making the material suitable for eco-friendly applications in the packaging, agriculture, and biomedical sectors. This study supports a growing body of literature advocating the valorization of marine byproducts into sustainable biomaterials, as highlighted by Santos *et al.* (2022) and Kaewprachu *et al.* (2018), affirming the potential of fish-scale-derived gelatin as a renewable source for high-performance biodegradable plastics. These spectral changes confirmed the preservation of the proteinaceous nature of gelatin in the raw material and its successful transformation into a more flexible and mechanically stable bioplastic, primarily through plasticizer-mediated structural reorganization.

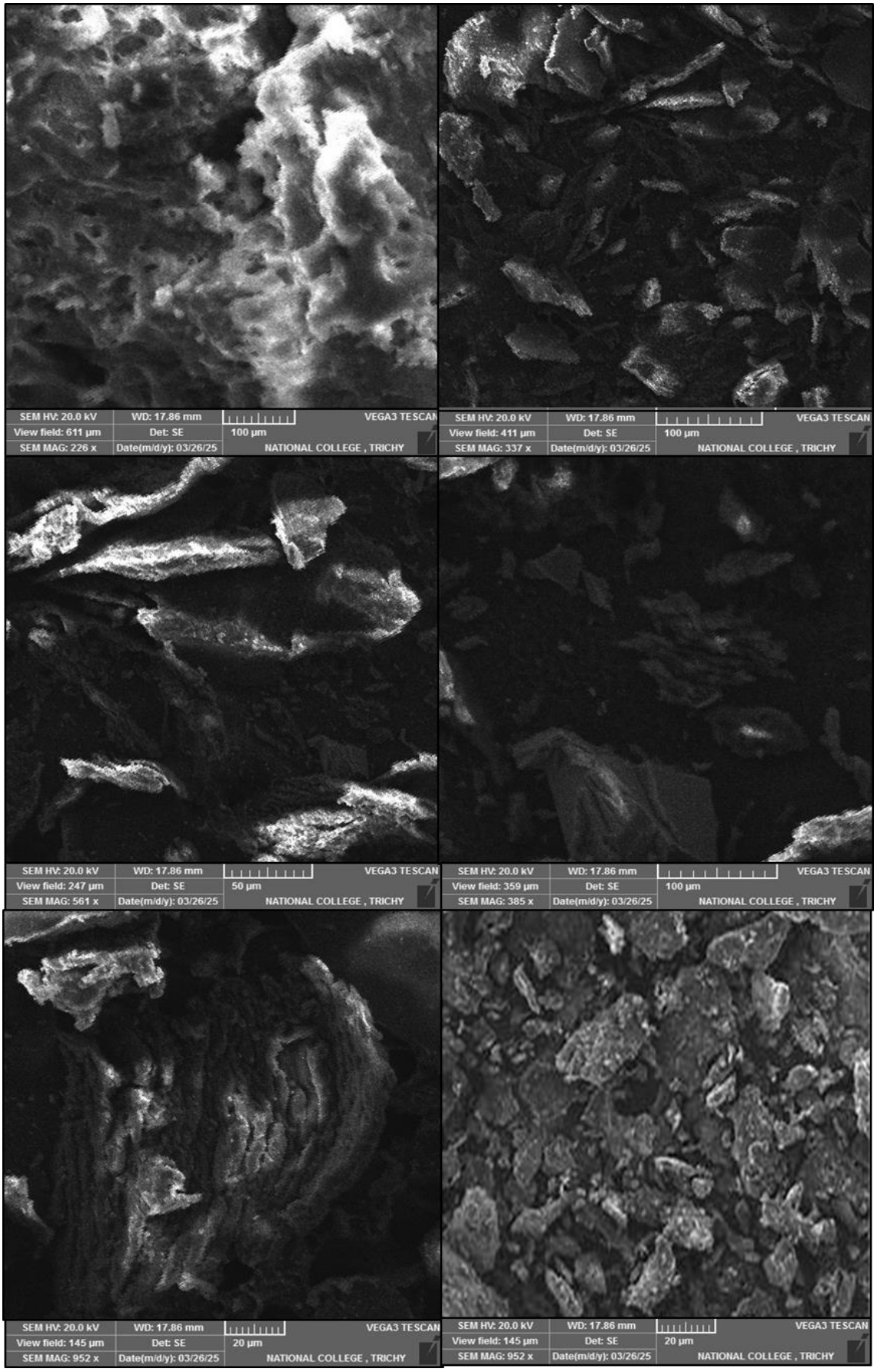


Fig. 7. SEM photograph of gelatine powder and bioplastics

SEM analysis

Scanning Electron Microscopy (SEM) analysis revealed significant morphological differences between gelatin powder and its derived bioplastics, highlighting the structural transformation induced by the plasticization process (Fig. 7). The SEM micrographs of the gelatin powder show a rough, flaky, and irregular surface with jagged edges and a highly porous matrix. These features are typical of protein-based materials dried through evaporation, which results in microcracks, voids, and brittle structures with fragmented particles (Patel *et al.,* 2022; Zhang *et al.,* 2023). The particles were loosely aggregated owing to hydrophilic interactions, reflecting low compactness and poor mechanical integrity (Niu *et al.,* 2021). High magnification reveals crystalline-like flakes and fractured surfaces, further emphasizing the brittleness of gelatin and its limited structural cohesion (Singh *et al.,* 2022). In contrast, bioplastics exhibit a smooth, compact, and homogeneous surface morphology with significantly reduced porosity and enhanced density resulting from the incorporation of plasticizers such as glycerol (Benjakul *et al.,* 2021; Li *et al.,* 2023).

These plasticizers fill the intermolecular voids and facilitate cross-linking, forming a continuous polymer matrix with improved flexibility and mechanical stability. Some SEM images also display dispersed micro-droplets, indicative of phase separation caused by glycerol migration, which enhances elasticity and reduces the brittleness (Zhang *et al.,* 2024).

At higher magnifications, the bioplastic revealed film- and fiber-like structures, suggesting the successful formation of a flexible biopolymer network (Patel *et al.,* 2021). Unlike gelatin powder, bioplastics exhibit fewer fractures and a more cohesive surface, indicating superior durability and resilience under mechanical stress (Rahman *et al.,* 2020). Overall, the SEM analysis confirmed the morphological transition from a brittle, porous gelatin matrix to a structurally stable, flexible bioplastic, validating its potential for biodegradable and eco-friendly applications.

CONCLUSION

This study successfully demonstrated the development and characterization of bioplastics synthesized from fish-scale-derived gelatin, highlighting their potential as a sustainable alternative to synthetic plastics. The bioplastic films exhibited enhanced structural, mechanical, and functional properties, including improved plasticization, flexibility, and stability. Analytical techniques such as UV-Vis spectroscopy, FTIR, SEM, and physicochemical evaluations confirmed the robustness of the bioplastic, with moderate water solubility, swelling capacity, and consistent gelation properties. The bioplastic also maintained a neutral pH, balanced moisture content, and demonstrated biodegradability, making it suitable for eco-friendly applications in the food packaging, agriculture, and biomedical fields. This study underscores the feasibility of utilizing fish-scale waste for sustainable bioplastic production, promoting waste valorisation, and reducing plastic pollution. Future research could focus on optimizing bioplastic formulations, enhancing their durability, and evaluating their performance in real-world applications.

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