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Extraction and evaluation of high-quality gelatin from *Scomberomorus commerson* bone residues in compliance with the INSO 3474 standard

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Abstract

The present study aimed to extract and characterize gelatin (GEL) from the bone residues of *Scomberomorus commerson*, a significant fishery by-product. A systematic extraction procedure was employed, involving alkaline deproteinization, EDTA-based decalcification, and lipid removal using butyl alcohol, followed by hydrothermal hydrolysis. The extracted GEL achieved a yield of 24% (on a dry weight basis). Structural and morphological analyses (FTIR, UV-Vis, XRD, and FE-SEM) confirmed that the primary protein structure of collagen was effectively preserved and converted into GEL. Quality evaluation according to the INSO 3474 standard revealed that the GEL possessed favorable sensory attributes, including a light yellow color and negligible odor, alongside excellent gelation performance. Although this study successfully validated the physicochemical quality of the GEL, further assessments regarding microbial safety and heavy metal content are required for commercial applications. In conclusion, *Scomberomorus commerson* bone residues represent a sustainable and cost-effective raw material for producing high-quality edible GEL, offering a promising solution for waste management in the seafood industry.

Keywords Bone residues; Gelatin extraction; Physicochemical characterization; Food-grade.

1. Introduction

Gelatin, the denatured form of collagen, is produced through partial hydrolysis of collagen extracted from animal skins, bones, and connective tissues. As a versatile, biodegradable, and abundant biopolymer, it is widely utilized across the biomedical, food, pharmaceutical, and cosmetic industries [1]. In the food sector, gelatin serves as a functional ingredient in confectionery, dairy products, and meat formulations, and has increasingly been studied for its role in developing low-carbohydrate food products for diabetic individuals [2]. Historically, commercial gelatin production has relied

on mammalian sources, specifically porcine and bovine skins and bones. However, the market for mammalian gelatin faces limitations due to food-safety concerns, such as bovine spongiform encephalopathy (BSE), and strict religious dietary restrictions, such as those observed in Judaism, Islam, and Hinduism [3]. This has driven global demand for alternative, culturally inclusive sources, positioning seafood processing by-products as a sustainable and high-quality reservoir of collagen.

Fish processing generates substantial waste, which can constitute up to 75% of the total fish weight after filleting. While fish skins and bones are often discarded, they



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contain significant levels of high-quality collagen that can be upcycled into value-added gelatin [4]. While gelatin extraction from fish skin is well-documented, studies focusing specifically on fish bone-derived gelatin remain limited, despite fish bones often being preferred for their high amino acid content (notably proline), which enhances extraction efficiency and functional properties [5].

The properties of gelatin are intrinsically linked to the raw material and the extraction protocol. Effective bone gelatin extraction requires a multi-step process, beginning with the removal of non-collagenous proteins and minerals—most crucially, the decalcification of bone matrix to expose the collagen network. Although various methods exist, using specialized agents like EDTA for mineral removal and dilute acetic acid for hydrothermal hydrolysis has been shown to yield high-quality gelatin while preserving its structural integrity [6].

Despite the potential of fish bone-derived GEL, there remains a need to establish optimized, replicable protocols that meet international and local quality standards. This study aims to bridge this gap by extracting and characterizing high-quality gelatin from the bone residues of *Scomberomorus commerson*, collected from the Persian Gulf. The research objectives include optimizing the extraction process using a combination of alkaline treatment, EDTA-based decalcification, and hydrothermal hydrolysis, followed by a comprehensive characterization of the structural and functional properties of the product. Finally, the quality of the resulting GEL is evaluated against the Iranian National Standard (INSO 3474 [7]) to determine its viability for food-grade applications.

2. Materials and methods

2.1. Materials

Bone residues of *Scomberomorus commerson* were collected from the shores of the Persian Gulf. All chemicals, including NaOH, EDTA, butyl alcohol, and acetic acid, were of analytical grade and obtained from commercial suppliers. Deionized water was used in all experimental procedures.

2.2. Extraction of gelatin (GEL) powder

Bone residues were soaked in a 1 M NaOH solution for 24 h to remove non collagenous proteins, followed by repeated washing with distilled water and subsequent lyophilization. The deproteinized bones were decalcified using 0.5 M EDTA (pH 7.4) for 48 h. The obtained residue was then treated with a 10% butyl alcohol solution for 24 h to eliminate remaining fats, washed again with distilled water, and lyophilized. Subsequently, the dried samples were soaked in 0.5 M acetic acid for 72 h, neutralized to pH 6, and autoclaved at 100 °C (20 psi) for 1 h

to enhance collagen hydrolysis and GEL formation. The upper pale yellow liquid phase was collected by decantation and centrifuged at 4000 rpm for 15 min to remove insoluble particles. The clarified supernatant was then dried in a vacuum oven at 40 °C, collected, and ground into a fine powder.

2.2. Characterization of GEL

Phase identification and crystallinity of the GEL samples were analyzed by X-ray diffraction (XRD) using a Bruker D8 Advance diffractometer (Germany) with Cu K α radiation ($\lambda = 0.15406$ nm). Functional group characterization was performed via Fourier-transform infrared (FTIR) spectroscopy (Thermo Nicolet 6700) within the range of 400–4000 cm⁻¹. Morphological features were examined using field-emission scanning electron microscopy (FE-SEM). Finally, UV–Vis absorption spectra were recorded in the 200–400 nm range using a UV-8500 II spectrophotometer (Techcomp).

3. Results and discussion

3.1. Extraction yield and structural characterization of GEL powder

The extraction of GEL from the bone residues of *Scomberomorus commerson* was successfully carried out using a modified method based on previous literature [8]. The systematic extraction procedure involved the removal of non-collagenous proteins through alkaline treatment (NaOH), followed by decalcification using an EDTA solution. Subsequently, lipid residues were eliminated using butyl alcohol. Finally, a hydrothermal hydrolysis process was employed to convert the collagen matrix into GEL.

The GEL extraction yield was calculated at 24% (on a dry weight basis). This result is highly encouraging and compares favorably with the yields reported in the existing literature for similar fish species [9, 10]. The structural and morphological characteristics of the extracted GEL were evaluated as follows:

The FTIR spectrum of the extracted GEL in the range of 400–4000 cm⁻¹ is presented in Fig. 1a. The broad band at 3329 cm⁻¹ is attributed to the N–H and O–H stretching vibrations, reflecting the protein structure and moisture content. Absorption bands at 3067 and 2935 cm⁻¹ correspond to C–H stretching modes, while the intense peak at 1655 cm⁻¹ arises from both the bending of water molecules and the C=O stretching of Amide I. Furthermore, the band at 1535 cm⁻¹ is assigned to Amide II, resulting from a combination of N–H bending and C–N stretching vibrations. Additional bands at 1450 and 1333 cm⁻¹ correspond to the symmetric and asymmetric bending vibrations of methyl groups, and the band at 1238 cm⁻¹ rep-

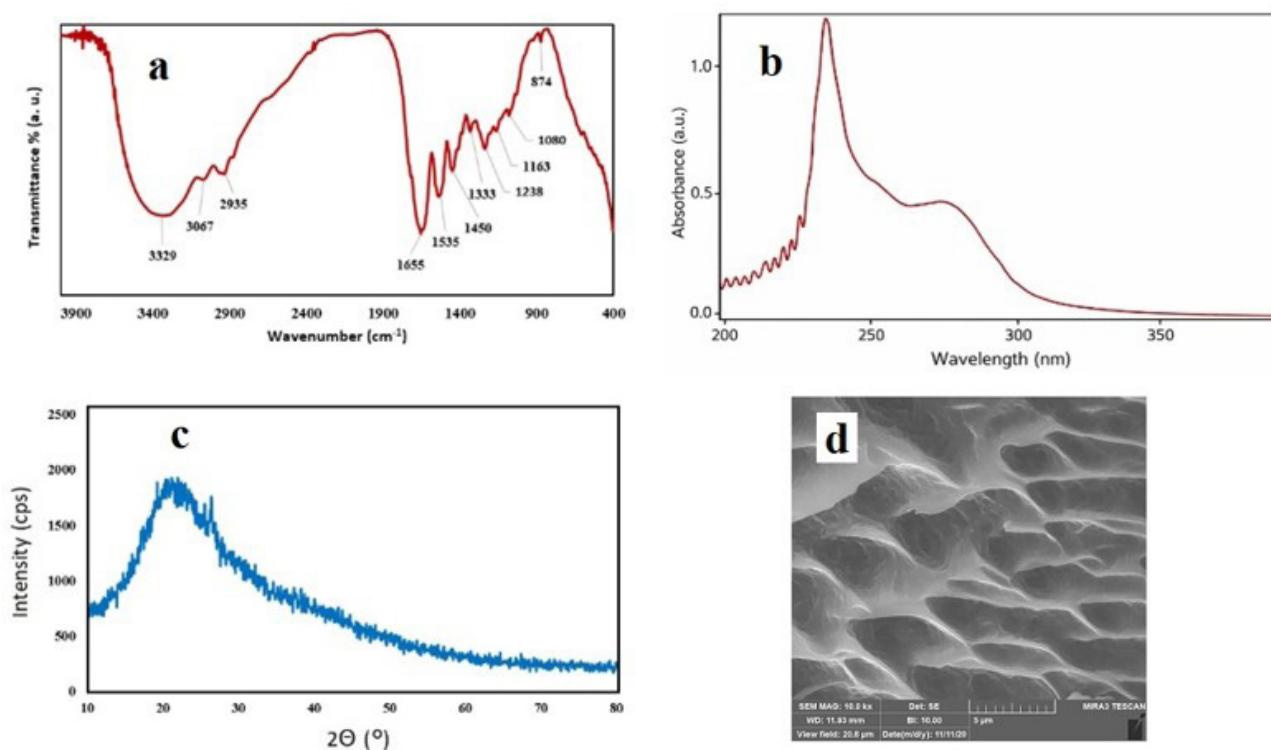


Fig. 1 FT-IR spectra (a), UV-Vis absorption spectra (b), XRD pattern (c), and FE-SEM image (at magnification of 5 μm) (d) of GEL powder

resents Amide III. Finally, the peaks at 1163, 1080, and 874 cm^{-1} are associated with the N-H bending mode and C-O and C-C stretching vibrations, respectively [9, 11]. The UV-Vis absorption spectrum (200–400 nm) is illustrated in Fig. 1b. An absorption peak observed at 224 nm is attributed to the $\pi \rightarrow \pi^*$ transitions of peptide bonds, confirming the proteinaceous nature of the extracted GEL [9]. Additionally, the broad shoulder observed between 250 and 300 nm corresponds to the absorption of aromatic amino acids, specifically tyrosine, within the protein backbone [12].

The XRD pattern of the GEL powder (Fig. 1c) exhibits a broad diffraction peak centered around $2\theta \approx 20^\circ$, which is characteristic of an amorphous structure and suggests the presence of residual triple-helical collagen fragments. Finally, the FE-SEM micrograph (Fig. 1d) reveals a rough yet structurally stable morphology for the extracted GEL. The presence of these characteristic amide bands in the FTIR spectrum, combined with the successful identification of peptide bonds in the UV-Vis analysis, confirms that the primary structure of the collagen was effectively preserved. Furthermore, the amorphous nature observed in the XRD pattern is a typical characteristic of high-quality GEL. These structural findings indicate that the extracted GEL possesses the essential features

required for food-grade applications; therefore, its quality indices were further evaluated in compliance with the INSO 3474 standard in the following section.

3.2. Evaluation of Selected Quality Indices of Extracted GEL

According to the INSO 3474 standard, the color of solid GEL typically varies from pale yellow to dark brownish-yellow. Organoleptic properties, including odor and taste, serve as primary quality indicators; the presence of any undesirable or off-odors is generally indicative of inferior quality or suboptimal extraction conditions.

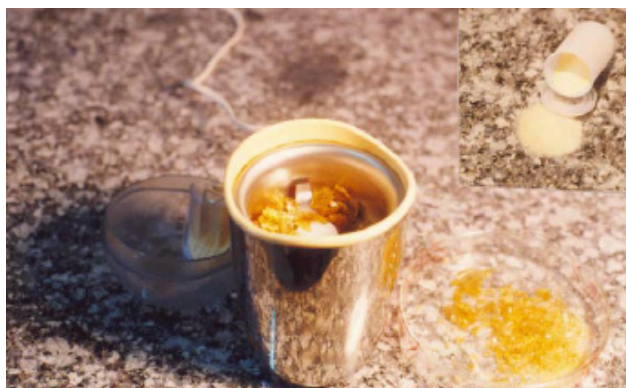
In this study, the extracted GEL was recovered as light yellow thin sheets, which were subsequently ground into a fine yellow powder (Fig. 2). The final product exhibited a negligible fishy or acidic odor, meeting the acceptable sensory criteria. Regarding its functional properties, the GEL demonstrated significant hygroscopicity, with a swelling capacity of approximately four times its original weight. Although insoluble in cold water, the product formed a stable, relatively firm gel that underwent a reversible phase transition back to a liquid state upon reheating, confirming its high-quality gelation performance.

For qualitative identification, a 1% (w/v) GEL solu-

Table 1. Selected physicochemical parameters of GEL According to the INSO 3474 Standard

Quality indices	Standard range	Result
Moisture % (w/v)	< 15.0	7.0
pH	3.8-7.6	6.0
Total ash % (w/v)	< 2.0	1.5
Sulfur dioxide (mg/kg)	< 50	22.0
Nitrogen in dry matter % (w/v)	> 15.0	25.0

tion reacted with copper sulfate and sodium hydroxide to produce a characteristic purple color, confirming the presence of peptide bonds through the Biuret reaction. These physicochemical attributes were found to be in full compliance with the specifications outlined in the INSO 3474 standard. Additional physicochemical parameters and their comparison with the standard values are summarized in Table 1. All experimental measurements were performed in triplicate, and the resulting data were subjected to statistical analysis using analysis of variance (ANOVA).

**Fig. 2** Thin, light yellow sheets of extracted GEL before grinding.

4. Conclusion

This study demonstrated that the bone residues of *Scomberomorus commerson* can be successfully valorized as a promising raw material for GEL production, providing a relatively high extraction yield of about 24% (on a dry weight basis). The structural and morphological characterization of the extracted GEL using UV-Vis, FTIR, XRD, and FE-SEM analyses confirmed that the primary protein structure of collagen was effectively preserved and converted into GEL. The FTIR spectrum revealed the presence of characteristic amide bands, while the UV-Vis results confirmed the proteinaceous nature of the product through peptide-bond absorption. The XRD pattern indi-

cated an amorphous structure, and the FE-SEM micrographs showed a rough, microporous morphology, which is favorable for hydration and gel formation.

Furthermore, the extracted GEL met the sensory and physicochemical requirements specified in the INSO 3474 standard for food-grade GEL, including acceptable color, negligible fishy or acidic odor, and good gelation performance. It should be noted that the present study focused primarily on physicochemical and structural characterization. To fully validate this GEL for commercial food-grade applications, further investigations—including microbial safety, heavy metal analysis, and compliance with Halal certification standards—are essential and recommended as future work.

In conclusion, GEL derived from *S. commerson* bone residues is a cost-effective and environmentally friendly alternative source of edible GEL, with considerable potential for use in the food industry.

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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