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Comparative effect of ultrasound-assisted extraction configuration method on the physicochemical characteristics of tuna skin gelatin

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Abstract. *Muhtar I, Syahriati, Saleh R, Ahmad I, Ramlah S, Rosniati, Loppies JE. 2026. Comparative effect of ultrasound-assisted extraction configuration method on the physicochemical characteristics of tuna skin gelatin. Asian J Agric 10 (1): g100129. https://doi.org/10.13057/asianjagric/g100129.* Gelatin is a biopolymer widely used in the food industry. The main challenge lies in the low yield and quality of gelatin produced through conventional extraction methods. This study aims to enhance the efficiency and quality of tuna (*Thunnus albacares*) skin gelatin by employing various extraction configurations. Tuna skin obtained from industrial processing by-products is then cleaned, cut uniformly, weighed, and used for pre-treatment and extraction. Tuna skin samples were obtained from processing by-products at PT. Nirvana Niaga Sejahtera from Indonesia in 2025. The extraction methods used include Water Bath Extraction (WBE), Ultrasound-Assisted Extraction Pre-Alkali-Acetic Treatment (UAE-PRAA), and Ultrasound-Assisted Extraction Post-Acetic Treatment (UAE-PSAA). The UAE process was carried out at 175 W power, 25 kHz frequency, and 55°C for 60 minutes. The parameters analyzed included yield, composition (protein, moisture, and ash contents), pH, viscosity, gel strength, and structural characteristics, as determined by Fourier Transform Infrared (FTIR). The results showed that the UAE-PSAA method produced the highest yield (13.34±0.22%), significantly ($p<0.05$) greater than UAE-PRAA (6.60±0.15%) and WBE (5.01±0.12%). The highest protein content was found in WBE (81.39±0.02 g/100g) and UAE-PSAA (80.44±0.28 g/100g), while the lowest moisture content was observed in UAE-PSAA (5.16±0.17 g/100g), indicating better drying efficiency. The lowest ash content (0.62±0.02 g/100g) was also obtained from UAE-PSAA, reflecting higher extraction purity. Gel strength (164.32±0.04 g) and viscosity (10.00±0.05 cP) significantly ($p<0.05$) improved with the UAE-PSAA method. FTIR analysis revealed minor spectral shifts toward lower wavenumbers in the amide regions, indicating molecular rearrangements that improved the functional properties of the extracted gelatin. Overall, the UAE-PSAA configuration significantly ($p<0.001$) enhances yield, physicochemical quality, and molecular stability of tuna skin gelatin, demonstrating strong potential for scalable and sustainable industrial applications.

Keywords: FTIR**,** gelatin extraction, physicochemical properties, ultrasonic-assisted extraction, water bath extraction

INTRODUCTION

Gelatin is a protein-based biopolymer derived from the partial hydrolysis of collagen that has attracted considerable attention for its wide applications in the food, pharmaceutical, cosmetic, and biomedical industries. Traditionally extracted from bovine and porcine sources, gelatin is valued for its excellent gelling, stabilizing, and emulsifying properties (Usman et al. 2025). However, growing concerns over zoonotic diseases, religious restrictions, and sustainability have shifted research toward alternative sources. Fish skin, an abundant and underutilized by-product of seafood processing, represents a promising raw material that supports both waste valorization and environmental sustainability (Gudmundsson and Hafsteinsson 1997; Karim and Bhat 2009).

Despite these advantages, marine-derived gelatin generally exhibits inferior physicochemical properties compared to its mammalian counterpart, largely due to differences in amino acid composition and collagen structure (Diogo et al. 2024). Consequently, there is a pressing need for innovative extraction techniques that enhance yield and quality while maintaining functional integrity. Conventional methods, which depend on acid or alkaline hydrolysis and prolonged thermal treatment, are often inefficient and can cause degradation of gelatin functional properties (Zhang et al. 2025). In response, Ultrasound-Assisted Extraction (UAE) has emerged as a green energy-efficient alternative that promotes mass transfer and cellular disruption via acoustic cavitation, improving extraction yield and preserving protein functionality (Chemat et al. 2017).

Recent studies confirm the potential of ultrasound in enhancing gelatin extraction from fish skin, particularly from tuna, by reducing processing time and increasing yield (Khiari et al. 2017; Ali et al. 2018; Tkaczewska et al. 2018; Ahmad et al. 2018). Although the UAE has been shown to improve rheological and structural properties, comparative investigations focusing on the timing of ultrasonic application in relation to chemical pre-treatment remain very limited. The lack of systematic comparison

between pre-treatment and post-treatment ultrasonic configurations has created uncertainty regarding their respective efficiencies and effects on gelatin characteristics. The main problem addressed in this study is the limited understanding of how pre- and post-treatment ultrasonic systems affect the yield, proximate composition, and physicochemical characteristics of tuna skin gelatin.

The novelty of this research lies in its first systematic comparison of two ultrasound-assisted extraction configurations, Ultrasound-Assisted Extraction Pre-Alkali-Acetic Treatment (UAE-PRAA) and Ultrasound-Assisted Extraction Post-Acetic Treatment (UAE-PSAA), conducted under identical experimental conditions. This study examines how the timing of ultrasonic application relative to chemical pre-treatment influences gelatin yield composition and molecular structure. By integrating these analyses, the research identifies configuration-specific mechanisms that determine extraction efficiency and product quality. The findings provide new insight into how ultrasound configuration can be optimized for scalable and sustainable marine gelatin extraction technologies. Ultrasonication has been reported to alter collagen-based materials by modulating hydrogen bonding triple helix stability and chain length, all of which directly impact gelatin gel strength and viscosity (Kim et al. 2020). Properly optimized ultrasound conditions can improve both yield and quality, whereas excessive sonication can lead to molecular fragmentation and loss of functionality (Taha et al. 2024). Thus, understanding the balance between energy input and structural preservation is crucial for developing effective extraction systems. In addition, optimizing sonication parameters can enhance the functional performance of gelatin in industrial applications by maintaining desirable physicochemical characteristics (Zhang et al. 2024).

Although the UAE has been widely applied for gelatin extraction, there is insufficient comparative data on how different ultrasound configurations influence proximate composition, gel strength, viscosity, and molecular structure as revealed by Fourier Transform Infrared (FTIR) spectroscopy. Addressing this research gap is essential to designing scalable, efficient, and high-quality gelatin extraction technologies from fish by-products. Therefore, this study aims to evaluate and compare the effects of UAE-PRAA and UAE-PSAA systems with conventional Water Bath Extraction (WBE) on the yield, proximate composition (moisture, ash, protein, and pH), gel strength, viscosity, and molecular structure of gelatin derived from tuna skin. By providing a configuration-based analysis, this research offers new insight into the efficiency and mechanisms of ultrasound-assisted extraction, contributing to the advancement of sustainable marine gelatin production within the framework of a circular bioeconomy.

MATERIALS AND METHODS

Sample preparation

The skin of tuna (*Thunnus albacares*) was obtained from the by-products of processing tuna at that time, collected from a local fish industry, Nirvana Niaga Sejahtera Limited Company in the Makassar Industrial Region, Indonesia. Sample material handling refers to Pezeshk et al. (2022) with slighty modification. As much as 40 kg of fresh tuna skin was immediately (1 h) transferred to the laboratory (about 8 km from the company), washed to remove adhering tissues, descaled, and cut into uniform strips approximately 1×1 cm for the preparation of 78 samples. The samples were stored at -20°C until further use (Nurilmala et al. 2020). Before extraction, the skins were thawed at 4°C, and any residual impurities were removed. The cleaned samples were weighed and used directly in the pre-treatment and extraction stages.

Extraction of gelatin from tuna skin

This study aimed to evaluate the effects of different ultrasonic-assisted extraction configurations, specifically the UAE-PRAA and UAE-PSAA methods, on the yield and quality of gelatin extracted from yellowfin tuna skin. The comparison was carried out against a conventional WBE method, using a consistent extraction protocol to ensure standardization. All experiments were performed in triplicate to ensure reproducibility.

Alkali and acetic treatment

The pre-treatment step was applied uniformly across all extraction methods based on the method by Santiz-Gomez et al. (2019). The cleaned fish skin was subjected to a standardized soaking process involving sequential treatments in alkaline and acidic solutions. Initially, the samples were immersed in 0.2 M NaOH solution at a ratio of 1:6 (w/v) at 25°C with stirring at 125 rpm for 1 hour to remove non-collagenous proteins. Following alkaline treatment, the skins were thoroughly rinsed with distilled water until a neutral pH was achieved. Subsequently, demineralization was conducted using 0.05 M acetic acid at a ratio of 1:6 (w/v) at 25°C with stirring at 125 rpm for 1 hour. After acid treatment, the samples were again washed and neutralized with distilled water to prepare them for extraction.

Gelatin extraction methods

Three extraction methods were employed: WBE, UAE-PRAA, and UAE-PSAA. The bath UAE type (ELMA-Elmasonic EASY 100 H) was performed at 175 W, 25 kHz at 55°C for 60 minutes, with a skin-to-solvent ratio of 1:6 (w/v), using distilled water as the extraction medium (Figure 1).

Conventional water bath extraction

Samples were subjected to alkali treatment (0.2 M NaOH, 1:6 w/v ratio, 25°C, stirred at 125 rpm for 60 min), followed by acetic treatment (0.2 M CH_3COOH, 1:6 w/v ratio, stirred at 125 rpm for 60 min) according to Santiz-Gomez et al. (2019). The pretreated skins were then extracted in deionized water at 55°C for 18 hours in a water bath under continuous stirring (300 rpm).

Ultrasound-assisted extraction pre-alkali-acetic treatment

The samples were first subjected to ultrasound treatment using a bath-type ultrasonic device (Elmasonic EASY 100 H) at 175 W, 25 kHz, and 55°C for 60 minutes with a skin-to-solvent ratio of 1:6 (w/v). After sonication, the samples underwent alkali treatment (0.2 M NaOH, 25°C, stirred at 125 rpm for 60 minutes) and acetic treatment (0.2 M CH_3COOH, stirred at 125 rpm for 60 min) as described by Santiz-Gomez et al. (2019). The treated skins were then extracted in deionized water at 55°C for 18 hours in a water bath.

Ultrasound-assisted extraction post-alkali-acetic treatment

In this configuration, the samples were first treated sequentially with 0.2 M NaOH (1:6 w/v, 25°C, stirred at 125 rpm for 60 min) and 0.2 M CH_3COOH (1:6 w/v, stirred at 125 rpm for 60 min) following Santiz-Gomez et al. (2019). The pretreated skins were then subjected to ultrasound-assisted extraction at 175 W, 25 kHz, and 55°C for 60 minutes, using a skin-to-solvent ratio of 1:6 (w/v), as modified from Kanjanasopa et al. (2017) and Boughriba et al. (2020).

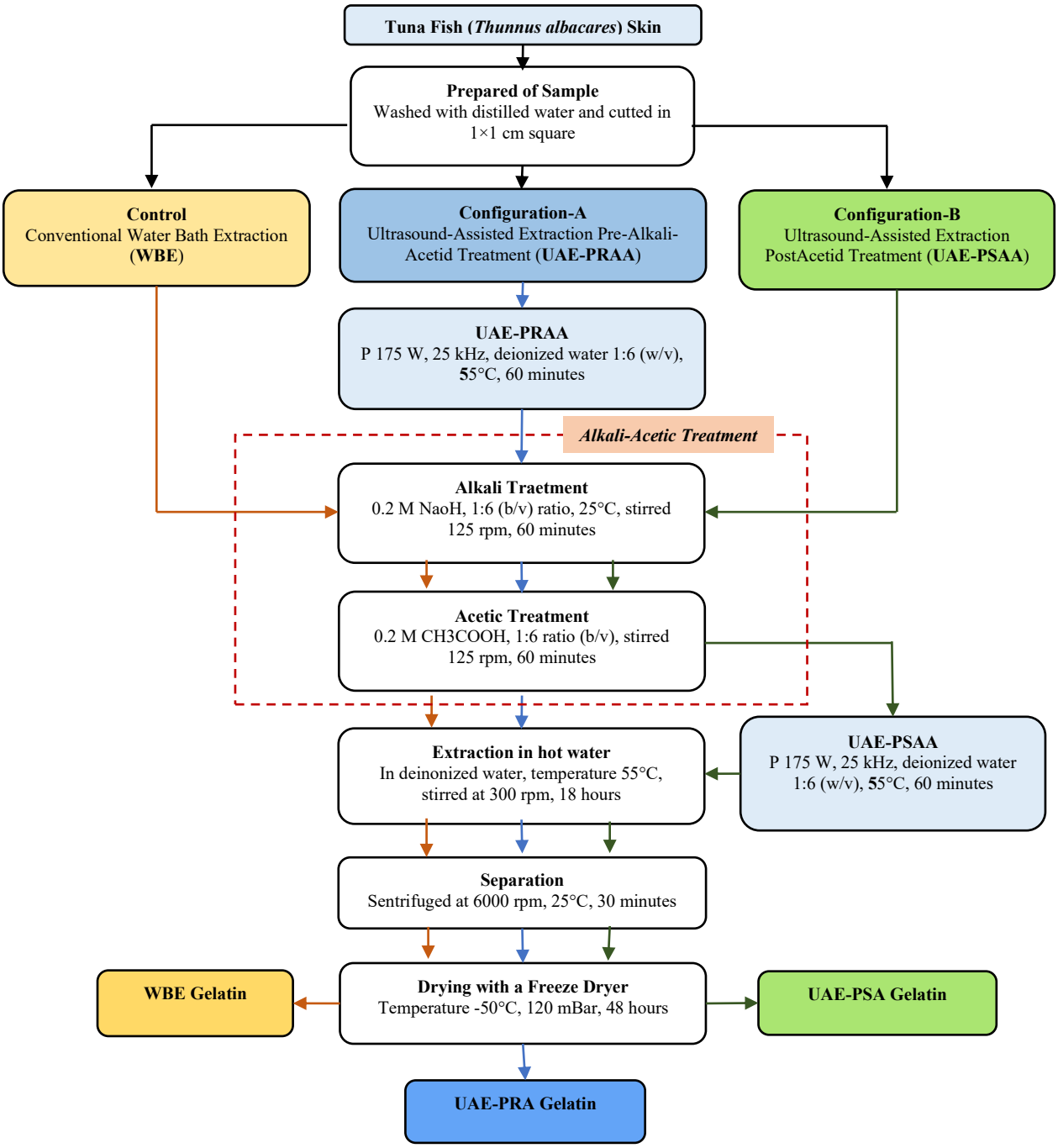

Figure 1. Gelatin extraction process with different ultrasonic-assisted extraction configurations

Separation and drying

After extraction, all gelatin solutions were centrifuged at 6000 rpm and 25°C for 30 minutes using a Hettich ROTOFIX 32A centrifuge to remove insoluble materials. The supernatant was then freeze-dried at -50°C and 10 mBar for 48 hours to obtain gelatin powder, which was stored at -18°C until further analysis.

Yield

The extraction yield was determined based on the following formula:

$$\textit{Yield}\ (\%) = \frac{\textbf{Weight of freeze dried material (g)}}{\textbf{Wet weight of gelatin (g)}} \times \mathbf{100}$$

Proximate analysis

The physicochemical properties of gelatin, including yield, moisture, ash, and protein content, were measured following AOAC (2005) standard procedures. Moisture and ash contents were determined using oven drying and muffle furnace methods, respectively. Protein content was measured by the Kjeldahl method.

pH

The pH was assessed using a portable pH meter (PHB-4 PH Meter Automatic, Tiongkok). The electrode was ensured to be in good condition and not dry. the electrode was dipped into a ph 7 buffer, which represented the neutral point. The reading was allowed to stabilize for approximately 1–2 minutes. The calibrate button was pressed to set the ph 7 reading as the zero point. calibration was continued using a ph 4 buffer. The calibrated ph meter electrode was then immersed into a 1% (w/v) gelatin solution at room temperature until a stable reading was obtained, and the value was recorded. All measurements were performed in triplicate

Gel strength and viscosity

The method described corresponds to the GMIA standard for Gelatin gel strength testing. Gel strength was measured following the British Standard Method BS 757:1975 using TA.XT.PLUS.TA texture analysis. Gelatin solutions (6.67%, w/v) were made up and poured into standard glass bloom jars (150 ml capacity). The filled Bloom jars are chilled in a water bath at 10±0.1°C for 17±1 h. Gel strength was determined using a texture analyzer equipped with a standard 0.5-inch diameter plunger. The force required (in g-force) to penetrate the gel to a depth of 4 mm was recorded as gel strength. The gel strength measurements were performed in triplicate.

Viscosity was measured using a Brookfield viscometer at 60°C with spindle No. 1 at 60 rpm. Prepare a water bath and set the temperature to 60°C. Ensure the temperature of the standard liquid is the same as the water bath. The appropriate spindle for the standard solution was installed, and the instrument was positioned for measurement. The spindle was immersed in the standard solution placed in a beaker within a water bath. The measured value was compared with the corresponding standard viscosity. When the results deviated from the acceptable tolerance range, the viscometer was adjusted or recalibrated. All viscosity measurements were conducted in triplicate.

FTIR spectroscopy

The molecular structure of the gelatin samples was analyzed using a spectrometer (INVENIO FT-IR Research Spectrometers, Germany) through FTIR analysis. A sample of 1 mg of freeze-dried gelatin powders were mixed with 100 mg of KBr (1:100 w/w ratio) and ground until smooth in a mortar. The mixture was added with solvent until homogeneous, then formed into transparent thin pellets, and was free of bubbles. The pellets are then placed on the FTIR testing table. The sample was and scanned within the range of 4000-500 cm^{-1} at a resolution of 4 cm^{-1}. the main peaks analyzed included amide I (1600-1700 cm^{-1}), amide II (1500-1550 cm^{-1}), amide III (1200-1300 cm^{-1}), amide A (3300-3400 cm^{-1}), and amide B (2920-2940 cm^{-1}), which correspond to C=O stretching, N-H bending, and c-n stretching vibrations.

Statistical analysis

All experimental data were expressed as mean (±) standard deviation. Statistical differences between treatments were analyzed using one-way ANOVA followed by Tukey's honest significant difference (HSD) post hoc test to identify significant differences ($p<0.05$). Before ANOVA, data normality and homogeneity of variance were verified using the Shapiro-Wilk and Levene tests, respectively. In cases where the normality assumption was violated, non-parametric analysis was carried out using the Kruskal-Wallis test. Tukey's HSD test was then applied to determine pairwise differences among treatment means. Statistical analyses were conducted using SPSS version 26.0.

RESULTS AND DISCUSSION

Yield of gelatin

The gelatin yields obtained from tuna skins by WBE and US-assisted extractions configuration (UAE-PRAA and UAE-PSAA) are tabulated in Table 1. The gelatin yield was higher ($p<0.05$) in the UAE-PSAA (13.34±0.22%) and UAE-PRAA (6.60±0.15%) compared to WBE (5.01±0.12%). The higher yield in the UAE application occurs by acoustic cavitation-enhanced mass transfer that improves the solubilization of gelatin in yellowfin tuna skin collagen systems. Gelatin extraction using ultrasound in UAE-PRAA and UAE-PSAA shows 1.3 and 2.7 times, respectively, increase compared to conventional methods (WBE). This finding was in accordance with Boughriba et al. (2020), who conducted research on the use of ultrasound-assisted extraction (25 kHz, 25°C, 30 min) followed by an alkali and acid process and found 1.26 times increase in yield compared to without ultrasound treatment at the beginning. This process shows similarities with the results from UAE-PRAA, which produces 1.3 times, slightly higher yield increase. Likewise, Pezeshk et al. (2022) found that extraction with alkali and acid treatment prior to ultrasound-assisted extraction (20 kHz,

5°C, 25 min) increased yield by 1.26 times compared to without ultrasound treatment, similarly configuration with UAE-PSAA which produced a yield 2.7 times that of without ultrasound. It has been concluded that the yield in the UAE-PSAA configuration is possibly higher because the alkaline stage with NaOH effectively removes non-collagen proteins, fats, and pigments, followed by an acid stage using HCl, which effectively develops collagen tissue and weakens cross-links so that collagen is more easily hydrolyzed when extracted with ultrasound.

Proximate composition

The proximate composition of gelatin extracted from yellowfin tuna skin under different extraction configurations is presented in Table 1. The results indicate significant differences ($p<0.05$) in all measured parameters, protein, moisture, and ash content among the three methods.

Protein content remained high in both WBE and UAE-PSAA samples, with values of 81.39±0.02 and 80.44±0.28 g/100g, respectively. In contrast, UAE-PRAA gelatin showed lower protein concentration (64.99±0.32 g/100g), potentially due to incomplete collagen hydrolysis caused by mechanism of combining alkaline pre-treatment with subsequent acetic acid exposure after ultrasound promotes chain fragmentation and loss during washing, reducing the protein fraction in the final gelatin (Dang and Chau 2016). In contrast, the protein content in the UAE-PSAA configuration is higher indicated extractability of acid-soluble collagen with high yield because non-collagenous proteins, fats, and pigments have been removed beforehand, and acid treatment weakens cross-links so that collagen is more easily hydrolyzed (Shaik et al. 2021).

Moisture content was lowest in UAE-PSAA gelatin (5.16±0.17 g/100g) and compare with in UAE-PRAA (7.02±0.21 g/100g) and WBE (8.42±0.24 g/100g). The reduced moisture in UAE-PSAA samples suggests better dehydration and structural consolidation during freeze-drying, likely attributed to the more complete extraction and denser gel matrix. Ultrasound-enabled processing generally yields cleaner extracts and a matrix that dries more efficiently, leading to lower final moisture. This aligns with reports that process optimization balances extraction severity and product quality (Cho et al. 2005; Montero and Acosta 2020). Conversely, cavitation from ultrasound can cause gelatin structures to become more fragmented, increasing their water-binding capacity (Wang et al. 2024).

Ash content was significantly reduced in UAE-PSAA samples (0.62±0.02 g/100g), followed UAE-PRAA (4.00±0.05 g/100g) and WBE (4.41±0.03 g/100g). Low ash content in UAE-PSAA indicating improved purification and reduced mineral contamination, whereas higher ash values in WBE may reflect less effective removal of inorganic residues during the pre-treatment or extraction process. For low-ash range reported for high-quality tuna skin gelatin for hard capsule applications. Tighten washing (neutralization) controls in WBE and UEA-PRAA to push ash toward ≤1%, a common benchmark for food-grade gelatin (Nurilmala et al. 2020).

pH of gelatin

In terms of pH (Table 2), all extracted gelatin samples exhibited slight acidity (Table 2). The pH of WBE (6.30±0.03) was higher ($p<0.05$), UAE-PRAA (5.70±0.05) and UAE-PSAA (5.30±0.04) have shown no significant difference ($p<0.05$). Initial ultrasound treated breaks down collagen structures, allowing acid residues to be more easily absorbed, resulting in a lower pH in UAE-PRAA compare with WBE. The decrease in pH in UAE-PSAA is likely caused by the effect of ultrasonic waves helping to break down collagen that has reacted with acid and maximizing the release of H^+ ions into the solution. The effect is freer H^+ ions in the solution, causing the pH to become lower (Hasdar et al. 2024). The lower pH of UAE-PRAA is closer to the stable gelatin pH (5.5-6.5) for food and pharmaceutical purposes, so it is highly considered in the extraction process to produce gelatin that balances yield and quality (Cho et al. 2005). Research indicates that ultrasound treatment can enhance the solubility of gelatin at specific pH levels, while potentially affecting its stability through cavitation effects, which can induce the aggregation or disaggregation of gelatin molecules (He et al. 2022).

Table 1. Extraction yield, moisture, protein, water, and ash contents of tuna skin gelatin

Parameter	Extraction method	Mean±SD	p-value
Yield (% w/w)	WBE	5.01±0.07a	0.001
	UAE-PRAA	6.60±0.07b	
	UAE-PSAA	13.34±0.10c	
Protein (g/100g)	WBE	81.39±0.08a	0.001
	UAE-PRAA	64.99±0.07b	
	UAE-PSAA	80.44±0.07c	
Water (g/100g)	WBE	8.42±0.08a	0.001
	UAE-PRAA	7.02±0.08b	
	UAE-PSAA	5.16±0.04c	
Ash (g/100g)	WBE	4.41±0.06a	0.001
	UAE-PRAA	4.00±0.07b	
	UAE-PSAA	0.62±0.03c	

Note: WBE: Water Bath Extraction, UAE-PRAA: Ultrasound-Assisted Extraction Pre-Alkali-Acetic Treatment, UAE-PSAA: Ultrasound-Assisted Extraction Post-Alkali-Acetic Treatment. Values are presented as mean ± SD

Table 2. pH of tuna skin gelatin

Parameter	Extraction method	Mean±SD	p-value
pH	WBE	6.29±0.02a	0.027
	UAE-PRAA	5.70±0.04b	
	UAE-PSAA	5.30±0.03c	

Note: WBE: Water Bath Extraction, UAE-PRAA: Ultrasound-Assisted Extraction Pre-Alkali-Acetic Treatment, UAE-PSAA: Ultrasound-Assisted Extraction Post-Alkali-Acetic Treatment. Values are presented as mean ± SD

Viscosity and gel strength

The rheological properties of the gelatin samples are presented in Table 3. The UAE-PSAA method produced gelatin with the highest viscosity (10.00±0.05 cP) and gel strength (164.32±0.04 g), significantly outperforming both WB and UAE-PRAA ($p<0.05$). The WB method exhibited moderate gel strength (165.29±0.19 g) but low viscosity (4.00±0.01 cP), whereas UAE-PRAA resulted in both low gel strength (10.06±0.08 g) and viscosity (4.00±0.01 cP). This is consistent with the results that gelatin extraction with ultrasound treatment after acid treatment produces high viscosity and gel strength (due to relatively good molecular weight distribution) compared to initial ultrasound treatment followed by acid treatment (Li et al. 2024). This interpretation is consistent with the reported study that the efficiency of ultrasonic treatment can affect the physicochemical parameters of gelatin, including yield, viscosity, and gel properties (Kim et al. 2020).

FTIR spectral characteristics

Structural integrity of the gelatin extracted by different methods was assessed using FTIR spectral analysis (Table 4). The major absorption bands observed were Amide I (C=O stretching), Amide II (N-H bending), Amide III (C-N stretching), Amide A (N-H stretching), and Amide B (CH_2 asymmetric stretching), which are characteristic of gelatin and collagen-based materials. The FTIR spectra showed distinct shifts in peak positions, particularly in the Amide I and III bands, for UAE-PSAA gelatin compared with other methods. For instance, the Amide III peak for UAE-PSAA appeared at 1235.93 cm^{-1}, indicating possible preservation or modification of the triple helix structure due to ultrasonic cavitation. These spectral shifts suggest molecular rearrangements that may explain the improved gel strength and viscosity observed in the UAE-PSAA samples. FTIR spectroscopic analysis has been widely used to study the functional groups and the changes in the secondary structure of gelatin samples (Muyonga et al. 2004; Renuka et al. 2019).

In order to understand the nature of changes that may occur as a result of different pre-treatments applied to skins, FTIR spectroscopic analysis was applied, and the results are depicted in Figure 2. For all samples, the Amide I band was observed within the typical range of 1600-1700 cm^{-1}. However, a slight shift was noted in UAE-PSAA gelatin, which showed a peak at 1635.97 cm^{-1}, compared to 1639.52 cm^{-1} in UAE-PRAA. This shift may indicate subtle changes in the triple-helix structure or hydrogen bonding due to the intense sonication during UAE-PSAA processing.

FTIR spectra of gelatins and their peak positions are demonstrated in Table 4 and Figure 2, respectively. A similar pattern in FTIR spectra exhibiting major peaks at the amide region with characteristic amide I, amide II, amide III, amide A, and B bands among all gelatin samples was observed. The Amide II band also exhibited a similar trend, with a peak at 1538.76 cm^{-1} in UAE-PSAA, compared to 1531.08 cm^{-1} in UAE-PRAA. Such shifts are often associated with altered molecular conformation and interactions among peptide chains. The Amide III band, associated with the C-N stretch and N-H bending, shifted from 1234.43 cm^{-1} (UAE-PRAA) to 1273.80 cm^{-1} (UAE-PSAA), suggesting potential unfolding of the protein chains or realignment of secondary structures.

Amide A and B bands were also slightly shifted in UAE-PSAA, recorded at 3290.81 cm^{-1} and 2922.74 cm^{-1}, respectively, compared to 3291.14 cm^{-1} and 2923.14 cm^{-1} in UAE-PRAA samples. Although these differences are subtle, they collectively suggest that ultrasonic treatment may lead to minor structural rearrangements without significantly denaturing the protein backbone. Overall, the FTIR data support the hypothesis that UAE-PSAA preserves or enhances molecular integrity more effectively than the bath or conventional methods. This is consistent with the observed improvements in gel strength and viscosity, indicating a strong relationship between molecular structure and functional performance.

Table 3. Viscosity and gel strength of tuna skin gelatin

Parameter	Extraction method	Mean±SD	p-value
Viscosity (cP)	WBE	4.00±0.01a	0.001
	UAE-PRAA	4.00±0.08a	
	UAE-PSAA	10.00±0.05b	
Gel Strength (g, bloom)	WBE	165.29±0.19a	0.001
	UAE-PRAA	10.06±0.08b	
	UAE-PSAA	164.32±0.04c	

Note: WBE: Water Bath Extraction, UAE-PRAA: Ultrasound-Assisted Extraction Pre-Alkali-Acetic Treatment, UAE-PSAA: Ultrasound-Assisted Extraction Post-Alkali-Acetic Treatment. Values are presented as mean ± SD

Table 4. FTIR spectra peak positions of gelatin from tuna skin gelatin

Parameter	Extraction method		
	WBE	UAE configuration	
		UAE-PRAA	UAE-PSAA
Amide I	1639.52	1635.97	1635.97
Amide II	1531.68	1538.76	1539.33
Amide III	1234.43	1237.97	1235.93
Amide A	3291.14	3290.81	3271.71
Amide B	2923.17	2922.74	2931.12

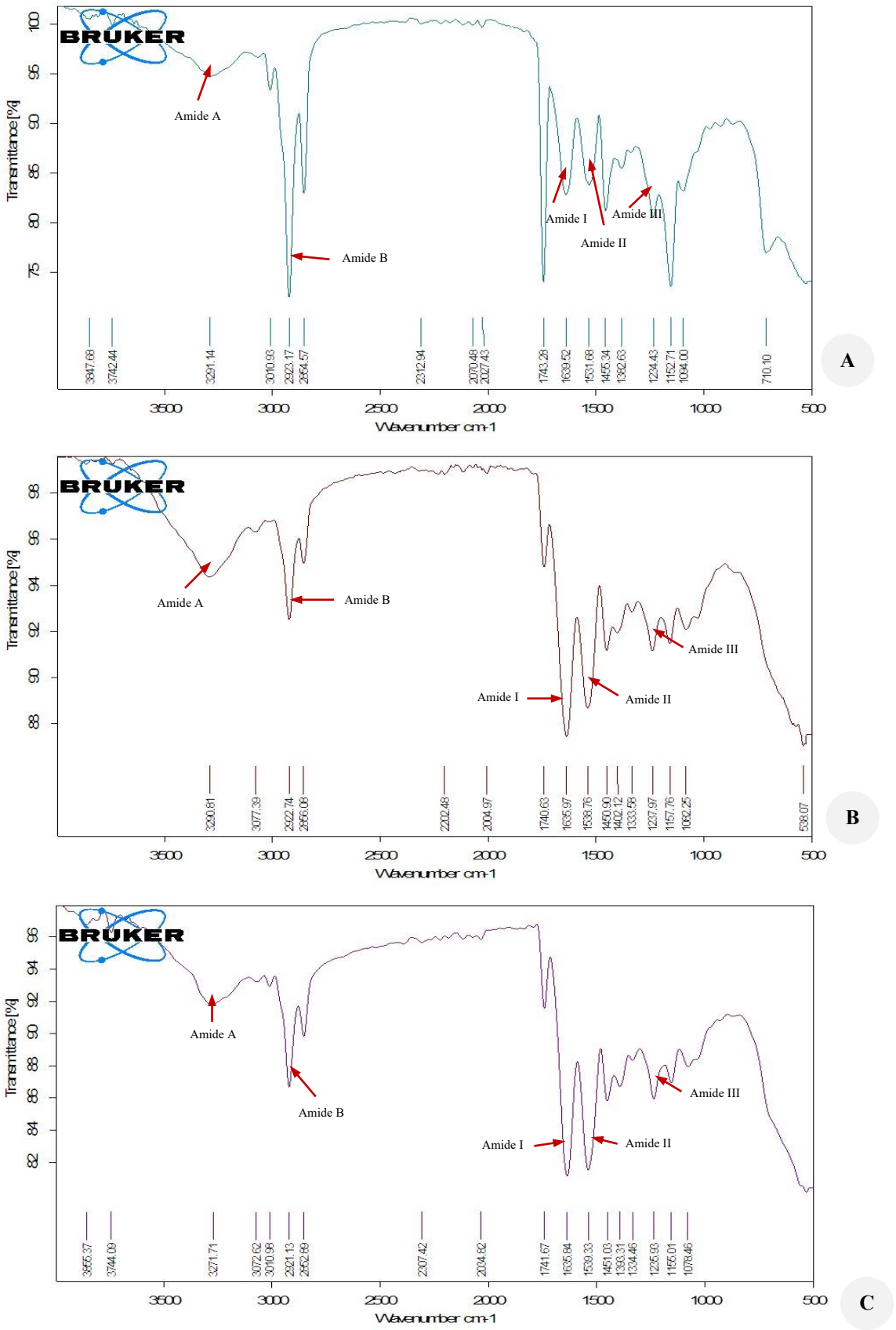

Figure 2. FTIR spectra of *T. albacore* skin gelatins. A. WBE, B. UAE-PRAA, C. UAE-PSAA

Discussion

Enhancement of gelatin yield

The present study clearly demonstrated that UAE-PSAA outperformed both UAE-PRAA and WBE methods in terms of gelatin yield. The increase from 5.01% in WBE and 6.60% in UAE-PRAA to 13.34% in UAE-PSAA is consistent with previous reports indicating that intense acoustic cavitation induced by ultrasonics facilitates deeper penetration and efficient disintegration of the collagenous matrix (Chemat et al. 2017; Kim et al. 2020). The UAE-PSAA delivers energy directly into the extraction medium, generating localized high-pressure and temperature zones that promote cell rupture and enhance solvent diffusion. This physical phenomenon, known as transient cavitation, appears critical for maximizing collagen solubilization and hence gelatin recovery. While UAE-PRAA also showed modest improvements over WBE, the indirect and less intense nature of cavitation in configuration may limit its capacity to achieve full extraction. Prior research has highlighted that insufficient sonication intensity may lead to incomplete matrix breakdown, resulting in lower yields and under-processed raw materials. Therefore, the greater yield obtained with UAE-PSAA confirms the hypothesis that the method offers a more effective means for extracting gelatin from structurally dense raw materials such as fish skin.

Functional superiority and protein integrity

Among ultrasonic configurations, a particularly noteworthy finding was the preservation of high protein content in the UAE-PSAA gelatin (80.44 g/100 g), comparable to that of the WBE method (81.39 g/100 g), but with a considerably higher yield. The highest gelatin yield (13.34%) with gel strength (164.32 bloom) comparable to the control (WBE, 165.29 bloom), while maintaining high protein content (80.44%) and highest viscosity (10 cP). This suggests that the PSAA ultrasonic condition enhanced extraction efficiency without causing excessive protein fragmentation. This is reinforced by the FTIR test results on UAE-PSAA, which show an indication of modification of the triple helix structure due to ultrasonic cavitation, improved gel strength, and viscosity. In contrast, UAE-PRAA resulted in a severe reduction in gel strength (10.06 bloom) accompanied by lower protein content (64.99%), indicating possible structural degradation and loss of gel-forming capability under this ultrasonic condition. Stronger gel strength is associated with higher molecular weight distribution and a better-preserved triple-helix structure, key features responsible for water-holding capacity and elasticity in gelatin gels. These findings align with those reported by Cuevas-Acuña et al. (2020), who found that high-intensity ultrasound enhanced gelatin's mechanical properties by improving collagen fiber breakdown without causing denaturation.

Functional superiority of gelatin produced under different extraction configurations was evaluated primarily through gel strength and pH as key quality indicators for food applications. The control treatment (WBE) exhibited the highest gel strength (165.29 bloom) with a near-neutral pH of 6.29, indicating optimal gel-forming capacity and favorable acidity for food-grade gelatin. Notably, the ultrasonic-assisted extraction configuration UAE-PSAA achieved a comparable gel strength (164.32 bloom), demonstrating that this ultrasonic condition was able to maintain the gel network formation potential at a level equivalent to WBE. However, UAE-PSAA produced gelatin with a slightly lower pH (5.30), suggesting a more acidic product environment, potentially influenced by pre-treatment conditions or enhanced release of acidic components during ultrasonication. Despite this shift, the pH remained within an acceptable range for food gelatin, supporting its applicability in food systems requiring stable gelation properties. In contrast, UAE-PRAA resulted in a drastic reduction of gel strength (10.06 bloom) accompanied by a pH of 5.70, indicating that this ultrasonic configuration was not favorable for preserving gel functionality. The extremely low gel strength suggests disruption of gelatin chain integrity and weakened intermolecular interactions, which ultimately limited gel formation. Overall, UAE-PSAA demonstrated superior functional performance among ultrasonic treatments by sustaining high gel strength while maintaining an acceptable pH profile for food applications. The pH range of commercial gelatin products is 5.43-5.85 (Ham et al. 2023), and the typical pH range for commercial food-grade gelatin is between 5.0 and 6.0, though the specific pH can vary depending on the raw material and manufacturing process (ChemKnock 2020).

Moisture and ash content as indicators of gelatin purity

Mineral content and processing conditions significantly affect the purity, stability, and physical characteristics of gelatin, which determine its quality and potential applications in the food and non-food industries (Kanwate and Kudre 2022). The standard moisture content for pharmaceutical-grade gelatin must be less than or equal to 12-14%**,** while for food-grade gelatin, the typical range is between maximun 12% (LanPu Group 2025). Whereas the standard ash content for pharmaceutical-grade gelatin must be less than or equal to about 1%**,** while for food-grade gelatin industrial, the typical range is between maximun 3.25% (GMIA 2012). Based on the results of the study in Table 1, different ultrasonic configuration treatments had a significant effect on the water content and ash content of gelatin ($p<0.05$). The results demonstrated that gelatins obtained from treatments WBEA and UAE-PRA exhibited moisture contents of 5.01% and 6.60%, respectively, which were below the required range for pharmaceutical applications (12-14%) and the maximum limit for food applications (≤12%), while their ash contents (4.41% and 4.00%) exceeded the acceptable limits for both pharmaceutical (≤1%) and food-grade gelatin (≤3.25%). In contrast, treatment UAE-PSA produced gelatin with a moisture content of 13.34%, complying with pharmaceutical standards but slightly exceeding the food-grade requirement, and an ash content of 0.62%, which met both pharmaceutical and food specifications. Overall, treatment UAE-PSAA yielded the most suitable gelatin quality for pharmaceutical applications, whereas treatments WBE and UAE-PRAA

require further process optimization, particularly in mineral removal and moisture adjustment, to achieve compliance with industrial quality standards.

Structural modifications observed by FTIR analysis

Fourier transform infrared spectroscopy confirmed the presence of characteristic amide peaks in all gelatin samples, signifying successful extraction of gelatinous proteins. However, slight shifts in Amide I, II, and III bands in UAE-PSAA samples indicate structural modifications induced by ultrasonic cavitation. Notably, the Amide III band in UAE-PSAA was recorded at 1273.80 cm^{-1}, compared to 1234.43 cm^{-1} in UAE-PRAA, suggesting a rearrangement or partial reformation of secondary structures. These spectral changes support the notion that the UAE-PSAA method may facilitate selective cleavage of weaker bonds while preserving core structures critical for functional behavior. This is consistent with the mechanistic explanation offered by Kim et al. (2020), who observed that controlled sonication could enhance hydrogen bonding and α-chain alignment, resulting in structurally robust gelatin.

The downshift of the Amide I band (from 1639.52 to 1635.97 cm^{-1}) indicates changes in C=O stretching vibrations and hydrogen bonding patterns, implying a partial transition from an ordered triple-helix to a more flexible random coil configuration. This structural adaptation can enhance the mobility of gelatin chains, facilitating better network formation during gelation. Similarly, the slight upshift observed in the Amide II region (1531.08 to 1538.76 cm^{-1}) reflects increased N-H bending vibrations associated with peptide chain flexibility and improved hydration capacity. The marked shift of Amide III toward higher wavenumbers (1273.80 cm^{-1}) suggests partial refolding and reorganization of the polypeptide backbone, which strengthens molecular interactions and contributes to higher gel strength and viscosity.

Interestingly, the Amide A and B bands also exhibited minor downshifts in UAE-PSAA samples, indicating increased flexibility or conformational changes in peptide backbones. These structural rearrangements, though subtle, are closely related to the enhanced rheological behavior of UAE-PSAA gelatin, where improved hydrogen bonding and chain alignment promote stronger intermolecular cross-linking. Such modifications, while subtle, are likely responsible for the improved rheological properties observed. This underscores the value of FTIR as a tool for correlating molecular structure with functional outcomes in gelatin research.

Configuration-specific considerations in UAE technology

The comparative nature of this study highlights the importance of choosing the appropriate UAE configuration for collagen extraction to produce gelatin. While both UAE-PRAA and UAE-PSAA systems utilize acoustic cavitation, the delivery method drastically influences extraction outcomes. Two extraction configurations were evaluated to determine the functional superiority of ultrasonic-assisted gelatin production. The configuration in which ultrasonication was applied prior to alkali-acid pre-treatment (UAE-PRAA) resulted in low yield (6.60%), reduced protein content (64.99%), and an extremely low gel strength (10.06 bloom), indicating poor gel-forming performance. In contrast, applying alkali-acid pre-treatment prior to ultrasonication (UAE-PSAA) significantly improved the extraction outcome, yielding gelatin (13.34%) with higher protein content (80.44%), markedly higher gel strength (164.32 bloom), and higher viscosity (10 cP). This difference has been reported in other studies on plant-based and marine hydrocolloids, suggesting a generalizable trend across various biomaterial types (Chemat et al. 2017). This is consistent with the latest comparative study showing that the acid/base pre-treatment sequence followed by ultrasonication produces gelatin with higher gel strength and protein content compared to the reverse sequence, due to more efficient collagen network development prior to intensive ultrasonic cavitation (Syahriati et al. 2026). Moreover, while ultrasonic UAE-PSAA are generally more effective, it also carry risks of localized overheating and requires precise control to avoid excessive degradation. The findings highlight that the timing of ultrasound application after the alkali-acetic treatment is the main determinant of extraction efficiency and gelatin quality. This configuration-specific sequence represents the key novelty of the study, confirming that UAE-PSAA yields superior structural enhances extraction efficiency while preserving gel-forming functionality compared to other methods.

In conclusion, this study demonstrated that UAE-PSAA is a highly effective method for producing high-quality gelatin from yellowfin tuna skin. Compared to both the UAE-PSAA and WBE methods, UAE-PSAA significantly increased gelatin yield and improved key physicochemical attributes such as protein content, moisture, ash, and pH. Most notably, gelatin obtained via UAE-PSAA exhibited superior functional properties, including enhanced gel strength and viscosity, indicating better preservation of the protein's structural integrity. FTIR spectral analysis further revealed subtle yet important structural modifications in UAE-PSAA treated gelatin, supporting the hypothesis that intense cavitation from UAE-PSAA systems promotes efficient collagen breakdown without extensive denaturation. These findings confirm that UAE-PSAA systems offer a configuration-specific advantage in marine gelatin extraction, providing a scalable, efficient, and sustainable alternative to conventional methods. This study contributes to the growing body of research supporting the application of non-thermal technologies in biopolymer processing. The results suggest that UAE-PSAA can be adopted for industrial-scale production of marine-based gelatin with improved quality and yield. Future work may focus on optimizing sonication parameters, evaluating bioactivity, and assessing economic feasibility in commercial settings.

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REFERENCES

Ahmad T, Ismail A, Ahmad S, Khalil K, Leo T, Awad E, Imlan J, Sazili A. 2018. Effects of ultrasound-assisted extraction in conjunction with the aid of actinidin on the molecular and physicochemical properties of bovine hide gelatin. Molecules 23 (4): 730. https://doi.org/10.3390/molecules23040730.

Ali AMM, Kishimura H, Benjakul S. 2018. Physicochemical and molecular properties of gelatin from skin of golden carp (*Probarbus jullieni*) as influenced by acid pre-treatment and prior ultrasonication. Food Hydrocoll 82: 164-172. https://doi.org/10.1016/j.foodhyd.2018.03.052.

Association of Officiating Analytical Chemists (AOAC) International. 2005. Official Methods of Analysis of AOAC International. 18th Edition. AOAC International, Gaithersburg.

Boughriba S, Souissi N, Jridi M, Li S, Nasri M. 2020. Thermal, mechanical and microstructural characterization and antioxidant potential of *Rhinobatos cemiculus* gelatin films supplemented by titanium dioxide doped silver nanoparticles. Food Hydrocoll 103: 1-43. https://doi.org/10.1016/j.foodhyd.2020.105695.

British Standards Institution. 1975. Methods for Sampling and Testing Gelatin (Physical and Chemical Methods). BS 757:1975. British Standards Institution, London.

Chemat F, Rombaut N, Sicaire AG, Meullemiestre A, Fabiano-Tixier AS, Abert-Vian M. 2017. Ultrasound-assisted extraction of food and natural products: Mechanisms, techniques, combinations, protocols, and applications-A review. Ultrason Sonochem 34: 540-560. https://doi.org/10.1016/j.ultsonch.2016.06.035.

ChemKnock. 2020. Fish Gelatin. ChemKnock Chemical Business Platform. https://chemknock.com.

Cho SM, Gu YS, Kim SB. 2005. Extracting optimization and physical properties of yellowfin tuna (*Thunnus albacares*) skin gelatin compared to mammalian gelatins. Food Hydrocoll 19 (2): 221-229. https://doi.org/10.1016/j.foodhyd.2004.05.005.

Cuevas-Acuña DA, Arias-Moscoso JL, Torres-Arreola W, Cadena-Cadena F, Valdez-Melchor RG, Chaparro-Hernandez S, Santacruz-Ortega, Ruiz-Cruz S. 2020. High-intensity ultrasound pulses effect on physicochemical and antioxidant properties of tilapia (*Oreochromis niloticus*) skin gelatin. Appl Sci 10 (3): 1004. https://doi.org/10.3390/app10031004.

Dang MN, Chau TH. 2016. Effect of ultrasound on pre-treatment of tuna skin for gelatin production. Vietnam J Sci Technol 54 (4A): 55-62. https://doi.org/10.15625/2525-2518/54/4a/11978.

Diogo GS, Pirraco RP, Reis RL, Silva TH. 2024. From its nature to its function: Marine-collagen-based-biomaterials for hard tissue applications. Tissue Eng Part B Rev 30 (3): 299-314. https://doi.org/10.1089/ten.teb.2023.0077.

Gelatin Manufacturers Institute of America (GMIA). 2012. Gelatin Handbook. GMIA. https://nitta-gelatin.com.

Gudmundsson M, Hafsteinsson H. 1997. Gelatin from cod skins as affected by chemical treatments. J Food Sci 62 (1): 37-39. https://doi.org/10.1111/j.1365-2621.1997.tb04363.x.

Ham YK, Noh SW, Lee JH, Yang NE, Choi YS, Kim HW. 2023. Optimization of gelatin extracting condition from korean native black goat skin and quality comparison with commercial gelatin. Food Sci Anim Resour 43 (1): 61-72. https://doi.org/10.5851/kosfa.2022.e58.

Hasdar M, Nalinanon S, Sriket C. 2024. Impact of pre-treatment with acid and ultrasound on the production and characteristics of goat skin gelatin. Curr Res Nutr Food Sci 12 (2): 887-907. http://dx.doi.org/10.12944/CRNFSJ.12.2.32.

He L, Han L, Wang Y, Yu Q. 2022. Appropriate ultrasonic treatment improves the production of antioxidant peptides by modifying gelatin extracted from yak skin. Intl J Food Sci Technol 57 (9): 5897-5908. https://doi.org/10.1111/ijfs.15912.

Kanjanasopa D, Somwong B, Srisawat T, Thitithanakul S, Sontikul Y, Choengthong S. 2017. Callus induction and somatic embryogenesis from cultured zygotic embryo of *Eleiodoxa conferta* (Griff.) Burr., an edible native plant species in Southern Thailand. Walailak J Sci Technol 14 (11): 875-882.

Kanwate BW, Kudre TG. 2022. Impact of different extraction conditions on yield, physicochemical and functional characteristics of gelatin from *Labeo rohita* swim bladder. Food Sci Biotechnol 31 (10): 1277-1287. https://doi.org/10.1007/s10068-022-01121-z.

Karim AA, Bhat R. 2009. Fish gelatin: Properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocoll 23 (3): 563-576. https://doi.org/10.1016/j.foodhyd.2008.07.002.

Khiari Z, Rico D, Martin-Diana AB, Barry-Ryan C. 2017. Valorization of fish by-products: Rheological, textural and microstructural properties of mackerel skin gelatins. J Mater Cycles Waste Manag 19 (1): 180-191. https://doi.org/10.1007/s10163-015-0399-2.

Kim SI, Kim E, Aghasa A, Hwang S. 2020. Shift in bacterial diversity in acidogenesis of gelatin and gluten seeded with various anaerobic digester inocula. Bioresour Technol 306: 123158. https://doi.org/10.1016/j.biortech.2020.123158.

LanPu Group. 2025. Pharmacopoeial Quality Standards for Pharmaceutical-grade Gelatin. LanPu Group. https://www.lanpugroup.com.

Li Y, Ma Z, Yan Qi, Cao D, Yuan R, Wang J, Lu S. 2024. Effect of low-frequency ultrasound-assisted acid extraction on gel properties and structural characterization of sheep's hoof gelatin. Intl J Biol Macromol 271 (2): 132701. https://doi.org/10.1016/j.ijbiomac.2024.132701.

Montero M, Acosta OG. 2020. Tuna skin gelatin production: Optimization of extraction steps and process scale-up. CyTA J Food 18 (1): 580-590. https://doi.org/10.1080/19476337.2020.1801849.

Muyonga JH, Cole CGB, Duodu KG. 2004. Fourier Transform Infrared (FTIR) spectroscopic study of acid soluble collagen and gelatin from skins and bones of young and adult nile perch (*Lates niloticus*). Food Chem 86 (3): 325-332. https://doi.org/10.1016/j.foodchem.2003.09.038.

Nurilmala M, Adinugraha SC, Jacoeb AM, Susilawati S, Ochiai Y. 2020. Evaluation of the properties of tuna skin gelatin as a hard capsule material. Fish Sci 86 (5): 917-924. https://doi.org/10.1007/s12562-020-01457-7.

Pezeshk S, Rezaei M, Abdollahi M. 2022. Impact of ultrasound on extractability of native collagen from tuna by-product and its ultrastructure and physicochemical attributes. Ultrason Sonochem 89: 106129. https://doi.org/10.1016/j.ultsonch.2022.106129.

Renuka V, Ravishankar CNR, Zynudheen AA, Bindu J, Joseph TC. 2019. Characterization of gelatin obtained from unicorn leatherjacket (*Aluterus monoceros*) and reef cod (*Epinephelus diacanthus*) skins. LWT 116: 108586. https://doi.org/10.1016/j.lwt.2019.108586.

Santiz-Gomez MA, Mazorra-Manzano MA, Ramírez-Guerra HE, Scheuren-Acevedo SM, Navarro-García G, Pacheco-Aguilar R, Ramírez-Suárez JC. 2019. Effect of acid treatment on extraction yield and gel strength of gelatin from whiptail stingray (*Dasyatis brevis*) skin. Food Sci Biotechnol 28 (3): 751-757. https://doi.org/10.1007/s10068-018-0514-y.

Shaik MI, Chong JY, Sarbon, NM. 2021. Effect of ultrasound-assisted extraction on the extractability and physicochemical properties of acid and pepsin soluble collagen derived from sharpnose stingray (*Dasyatis zugei*) skin. Biocatal Agric Biotechnol 38: 102218. https://doi.org/10.1016/j.bcab.2021.102218.

Syahriati, Muhtar I, Saleh R, Loppies EJ, Rosniati, Ramlah S. 2026. Enhancing the physicochemical properties of tuna skin gelatin through ultrasound-assisted green extraction: A comparative study. Asian J Dairy Food Res 1: 1-7. https://doi.org/10.18805/ajdfr.DRF-605.

Taha A, Mehany T, Pandiselvam R, Siddiqui SA, Mir NA, Malik MA, Sujayasreel OJ, Alamurum KC, Khanashyamn AC, Casanovao F, Xu

X, Pan S, Hu H. 2024. Sonoprocessing: Mechanisms and recent applications of power ultrasound in food. Crit Rev Food Sci Nutr 64 (17): 6016-6054. https://doi.org/10.1080/10408398.2022.2161464.

Tkaczewska J, Morawska M, Kulawik P, Zając M. 2018. Characterization of carp (*Cyprinus carpio*) skin gelatin extracted using different pre-treatments method. Food Hydrocoll 81: 169-179. https://doi.org/10.1016/j.foodhyd.2018.02.048.

Usman M, Ishaq A, Regenstein JM, Sahar A, Aadil R M, Sameen A, Khan MI, Alam A. 2025. Valorization of animal by-products for gelatin extraction using conventional and green technologies: A comprehensive review. Biomass Convers Bioref 15 (22): 28355-28367. https://doi.org/10.1007/s13399-023-04547-5.

Wang L, Ma Y, Shen R, Zhang L, He L, Qu Y, Ma X, Ma G 1, Guo Z, Chen C, Li H, Kong X. 2024. Effect of ultrasonic treatment on the physicochemical properties of bovine plasma protein-carboxymethyl cellulose composite gel. Foods 13 (5): 732. https://doi.org/10.3390/foods13050732.

Zhang S, Zhao D, Yin L, Wang R, Jin Z, Xu H, Xia G. 2025. Physicochemical and functional properties of yanbian cattle bone gelatin extracted using acid, alkaline, and enzymatic hydrolysis methods. Gels 11 (3): 186. https://doi.org/10.3390/gels11030186.

Zhang W, Li M, Chen J, Chen Y, Liu C, Wu X. 2024. A review of modified gelatin: Physicochemical properties, modification methods, and applications in the food field. J Agric Food Chem 72 (38): 20705-20721. https://doi.org/10.1021/acs.jafc.4c03194.