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Enhancing the Physicochemical Properties of Tuna Skin Gelatin Through Ultrasound-assisted Green Extraction: A Comparative Study

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ABSTRACT

Background: Gelatin is widely used in food industry and other industries as an emulsifying and emulsifying agent. Ultrasound-assisted extraction (UAE) was used to produce gelatin from tuna skin to enhance its physicochemical properties and extraction efficiency. This study aimed to determine precise method produces gelatin with a better yield, gel strength, viscosity, color and other physicochemical properties.

Methods: Two extraction sequences were compared: ultrasound extraction followed by acid-base extraction (method A) and vice versa (method B). Using both methods, gelatin was extracted from tuna skin, with yields measured, color analyzed by lightness and ΔE and gel strength tested. Viscosity, moisture, protein contents and pH were assessed to understand gelatin quality.

Result: The result showed method B produced higher gelatin yield (13.34%) than method A (4.92%). Method B gelatin had greater gel strength (220 g vs. 200 g), brighter color (lower ÄE) and slightly higher viscosity (10 cP vs. 9 cP). SEM analysis revealed denser microstructure, supporting stronger gels. Significant differences occurred in yield, color and gel strength. Ultrasound-assisted acid-base extraction effectively produced high-quality tuna skin gelatin. This method offers potential applications in food, pharmaceutical and cosmetic industries, providing a sustainable alternative to mammalian gelatin.

Key words: Acid-base extraction, Fish-based gelatin, Gel strength, Physicochemical properties, Tuna skin gelatin, Ultrasound-assisted extraction.

INTRODUCTION

The global fish processing industry, significantly boosts seafood production. Indonesia contributes over 16% of the world's tuna, producing more than 1.2 million tonnes annually, with exports of 198,131 tons valued at 659.99 million USD (PUSDATIN, 2018). Processing generates 30-40% by-products such as skin, bones, fins and entrails, which can be repurposed into valuable products including gelatin, collagen, bioactive peptides and lipids used in food, pharmaceuticals and cosmetics.

Gelatin, obtained through collagen hydrolysis, is valued for its gelling, biodegradable and non-toxic properties (Song *et al*., 2024; Zhang and Wang, 2024). Mammalian sources dominate production, with porcine skin (80%) and bovine hides (15%) (Shah and Yusof, 2014). However, this raises ethical, health and religious issues such as halal certification and prion disease risks (Ahmed *et al*., 2020). Fish-based gelatin, particularly from tuna skin, provides a sustainable alternative, reducing land-based farming emissions (Prajaputra *et al*., 2024) and minimizing seafood waste (Sisa *et al*., 2024). Despite abundant raw material (Li *et al*., 2014), extraction is challenging due to collagen structure and inefficiency of conventional methods.

Recent technologies aim to improve gelatin extraction efficiency and sustainability. Ultrasound-assisted extraction (UAE) employs high-frequency sound waves to break collagen, reducing time, energy and chemical use (Xu *et al*., 2022). UAE yields gelatin with superior gel strength and thermal stability (Derkach *et al*., 2020; Chen *et al*., 2023), making it promising for fish-based applications. UAE can also help increase extraction yields, improved the physical, nutritional and functional properties (Patil *et al*., 2018; Adoni *et al*., 2025). Although less explored than enzymatic hydrolysis and supercritical CO2 extraction, UAE is simpler and more cost-effective. Enzymatic methods improve taste, emulsification and shelf life (Pakbin *et al*., 2022), while CO_2 extraction produces high-purity gelatin with low residues (Idham *et al*., 2023), but both face high costs and complexity.

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Fish gelatin has strong potential for pharmaceutical and cosmetic applications due to its biocompatibility, biodegradability and non-immunogenic properties, supporting drug delivery, controlled release and safer formulations (Al-Nimry *et al*., 2021; Hussein *et al*., 2023). It also dissolves more easily at low temperatures than mammalian gelatin, although its characteristics vary by species, age and raw material composition (Zhang *et al.*, 2020; Guo *et al*., 2023). This study investigates UAE for extracting gelatin from tuna skin to address existing research gaps. Ultrasound is known to improve protein functionality (Wang *et al*., 2023) and enhance gelatin stability and foaming capacity (Boughriba *et al*., 2022), yet applications using tuna skin remain limited. By comparing UAE with enzymatic and CO_2 methods, this study evaluates the functional properties of tuna skin gelatin and assesses its alignment with Indonesian National Standard and GMIA specifications to strengthen its industrial potential.

MATERIALS AND METHODS

Tuna skin (*Thunnus albacore*) from a local fillet processor was cleaned before extraction due to its high collagen content. NaOH removed non-collagen proteins and CH_3COOH hydrolyzed collagen, producing gelatin with distilled water. Analytical-grade chemicals (Merck) supported pH, moisture, ash and protein analyses. Equipment included flasks, jars, petri dishes, stirrers, filter cloths and blenders. Extraction used an Elmasonic ultrasound system, with freeze-drying for gelatin. Physicochemical tests used Konica Minolta CR-400 (color), VR 3000 Viscometer (viscosity), Texture Analyzer TA-XT Plus (gel strength) and SEM FlexSEM 1000 II (microstructure).

Sample preparation and extraction procedures

Tuna skin was cleaned, washed with distilled water, cut into 1 × 1 cm^2 pieces and stored at -20°C. Two extraction methods combined ultrasound-assisted extraction (UAE) and acid-base treatment. Method A: UAE in water (1:6 b/v, 20 kHz, 750 W, 55°C, 1 h), alkaline treatment with 0.2 M NaOH, then acid extraction with 0.20 M CH_3COOH. Method B: alkaline, acid, then UAE. Extracted gelatin was centrifuged (6000 rpm, 10 min), freeze-dried (-50°C, 120 mBar, 48 h) and powdered for analysis.

Analysis procedures

Yield

The gelatin yield was determined by weighing the final gelatin powder after freeze-drying and comparing it with the initial raw tuna skin weight (AOAC, 2005).

Color

Gelatin powder color was measured using a Konica Minolta CR-400 Chroma Meter for L, a and b values (Cheow *et al.*, 2007). These values indicate Lightness (L*), greenness (-a*), redness (+a*) and blueness/yellowness (±b*). Lighter gelatin is preferred for food and pharmaceutical applications.

Viscosity

The viscosity of gelatin was analyzed following (Rosti and Takagi, 2021). The powder was dissolved in distilled water at 60°C in a shaker bath. After complete dissolution, viscosity was measured using a Brookfield viscometer at 50°C at 10 rpm. Values were multiplied by the spindle conversion factor from the Bookfield manual (Brookfield Engineering Laboratories, 2002) to determine viscosity in centipoises (cP).

Gel strength

Gel strength was determined using a Texture Analyzer TA-XT Plus (Godalming, Surrey GU7 1YL, UK). A 6.67% gelatin solution was gelled at 4°C for 16 h. Bloom strength was measured by applying force until rupture.

Microstructure

Microstructural analysis of gelatin powder was conducted using SEM following Zhou *et al*. (2006). A 0.1 g gelatin sample was mounted on a specimen holder, cleaned and coated with gold or carbon to enhance conductivity. The sample was observed using a FlexSEM 1000 II at 500× magnification, a working distance of 6-10 mm and an accelerating voltage of 20 kV to obtain high-resolution images of the surface morphology.

Moisture content

Moisture content of tuna skin gelatin was determined gravimetrically by oven drying at 100°C for 6 h and expressed as percentage weight loss (AOAC, 2005).

Protein content

Protein content of tuna skin gelatin was analyzed using the Micro-Kjeldahl method (AOAC, 2005). A 0.2 g sample was digested with K_2SO_4, HgO and H_2SO_4 for 1-1.5 h until clear green. After cooling and dilution, the solution was distilled with NaOH, producing NH_3 collected in boric acid. The distillate was titrated with HCl until green turned pink. Total nitrogen content was determined and protein content calculated using the standard formula (AOAC, 2005).

Gelatin pH

The pH of tuna skin gelatin was measured following GMIA (2019). A 6.67 g sample was dissolved in 100/mL water at 60°C, cooled to 25°C and measured with a calibrated pH meter.

Data analysis

The physical and chemical properties of tuna skin gelatin extracted using Method A (ultrasound-assisted extraction with acid-base treatment) and Method B (acid-base treatment with ultrasound-assisted extraction) were evaluated. A t-test at 95% confidence level compared the mean values of yield, moisture, ash, protein content and pH between the two methods.

RESULTS AND DISCUSSION

Yield of gelatin

The gelatin yield from tuna skin extracted using the acid-base method followed by ultrasound treatment (Method B) reached 13.34%, which was significantly higher than that obtained using ultrasound followed by acid–base extraction (Method A) at 4.92% ($p<0.05$). The higher yield in Method B indicates greater extraction efficiency, as gelatin yield reflects the effectiveness of the extraction process. This value is also higher than the 5.4% gelatin yield from Camel's skin obtained using a base treatment with 2% $Ca(OH)_2$ and 1% HCl followed by heating at 60°C for 6 hours (Al-Zoreky *et al*., 2025). The increased yield is attributed to the acid–base treatment, which loosens the collagen matrix, followed by ultrasonic cavitation that accelerates collagen disruption and dissolution. These findings are consistent with reports by Ahmad *et al*. (2018), Rahman and Lamsal (2021) and Hoo *et al*. (2022), which indicate that ultrasound application after initial acid–base treatment enhances collagen extraction efficiency.

Color

Color indicates gelatin quality by showing purity levels. Gelatin from tuna skin using Method A had L* = 75.05, a* = 2.52, b* = 17.28 (ΔE = 24.01), while Method B showed L* = 80.32, a* = 0.46, b* = 7.97 (ΔE = 15.12), indicating higher purity and better contaminant removal. The gelatin's appearance is shown in Fig 1.

Color reflects gelatin purity and marketability. Method B produced higher Lightness (80.32) and lower ΔE (15.12) than Method A (L*: 75.05; ΔE: 24.01) (Table 1), indicating whiter, more purified gelatin with reduced redness and yellowness. Studies (Gunawan *et al*., 2017; Lin *et al*., 2015) note that gelatin color depends on raw materials, processing and impurity removal. Method B's enhanced color results from effective acid-base pretreatment and ultrasound extraction, which facilitate pigment release and collagen breakdown.

Viscosity

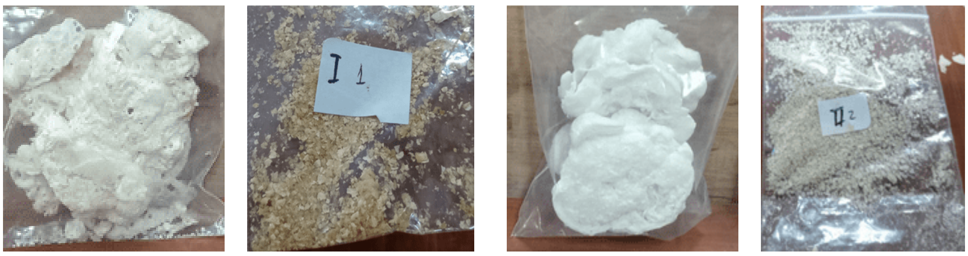
Viscosity is an important parameter determining the functional properties of gelatin in food and pharmaceutical applications. Gelatin extracted using method A had a viscosity of 9 cP, while method B produced 10 cP. Although both values remain below the GMIA standard (15-75 cP), they are closer to the British Standard (15-40 cP). The slightly higher viscosity in method B suggests that ultrasound-assisted extraction contributes to a denser molecular network, improving viscosity and gel strength, although the values are still lower than commercial gelatin. These differences are influenced by factors such as temperature, extraction time, acid concentration and chemical composition, indicating the need for further optimization of extraction conditions.

Gel strength

Gel strength measures gelatin firmness and texture. Method A produced gelatin with 200 g gel strength, while method B achieved 220 g. Method B's stronger gel strength suggests acid-base extraction with ultrasound enables better collagen cross-linking. This is consistent with the findings of Hasdar *et al*. (2024), which found that the combination of acetic acid and ultrasound pretreatment was very effective in increasing gelatin strength. Gel strength is an essential parameter determining gelatin's functional properties in food and pharmaceutical applications. Method B produced higher gel strength than method A, indicating that sequential acid-base extraction with ultrasound enhances cross-linking and yields stronger gels. Based on Bloom degree, gelatin is classified as low (<100 g), medium (100-200 g) and high (>200 g) (Usman *et al*., 2021; Adnan *et al*., 2024). Higher Bloom values improve stability, controlled release (Kuai *et al*., 2020), scaffold strength (Roldán *et al*., 2024) and food texture (Obas *et al*., 2021). Determining factors include extraction method (Okur *et al*., 2020), chemical composition (Ahmad *et al*., 2021), molecular weight (Wang, 2024) and processing conditions (Zhang *et al*., 2021). SEM showed a denser structure in method B, supporting Li *et al*. (2024) that ultrasound enhances molecular integrity through cross-linking.

Microstructure

SEM analysis revealed distinct microstructures in the gelatin samples. Method A produced a porous, sponge-like structure with large cavities, while method B showed a denser structure



(a) (b)

Fig 1: Color appearance of tuna skin gelatin sheets and powder extracted by (a) ultrasound-acid-base and (b) acid-base-ultrasound method.

with smaller cavities (Fig 2). Method B's more uniform network contributed to better gel strength and texture, aligning with findings that ultrasound extraction enhances network compactness (He *et al.,* 2021; Wang *et al.*, 2023).

SEM analysis showed clear microstructural differences between methods. Method A produced porous gelatin with larger cavities, while method B yielded denser, compact gelatin with smaller cavities, resulting in higher gel strength. Gelatin with strong gels typically shows compact structures (Sae-leaw *et al*., 2016). Cross-linking enhances collagen triple helix formation, strengthening networks (Zuev *et al*., 2024). Method B's compact structure improved gel strength, confirming Li *et al*. (2024) that ultrasound-assisted extraction creates denser gelatin than heat extraction, producing superior texture and functional properties.

Table 1: Gelatin color value of tuna skin (*Thunnus albacore)* from ultrasound extraction.

Color value	Extraction method A	Extraction method B
L* (Lightness)	75.05	80.32
a* (Redness)	2.52	0.46
b* (Yellowness)	17.28	7.97
ΔE	24.01	15.12

Note:

A: Ultrasound extraction followed by acid-base extraction.

B: Extraction with acid-base followed by extraction with ultrasound.

Table 2: Chemical characteristics of tuna skin gelatin (*Thunnus albacore*) extracted using ultrasound.

Chemical characteristics	Extraction method A	Extraction method B
Moisture content (%)	2.02±0.07	5.16±1.34
Protein (%)	64.99±4.91	80.44±1.40
pH	5.72±0.14	5.99±0.21

Note:

A: Ultrasound extraction followed by acid-base extraction.

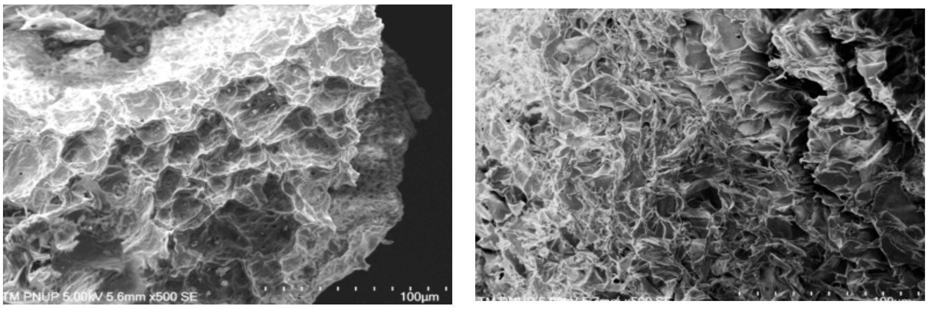
B: Extraction with acid-base followed by extraction with ultrasound.

Chemical composition

The moisture content of gelatin from method A was 2.02%, while Method B produced 5.16% (Table 2), a significant difference ($p<0.05$). Both values complied with SNI 8622-2018 (≤12%) and FAO/WHO standards (≤18%), but fell below GMIA's recommended 8-15% for food gelatin. Moisture content influences shelf life, texture and quality; excessive levels reduce lightness and cause off-flavors. Method B's higher value is linked to acid-base extraction with ultrasound, enhancing collagen hydration and water-holding capacity, consistent with Li *et al*. (2024). These findings indicate process differences affect gelatin quality and that drying optimization may be needed for standard compliance.

In Table 2, the protein content of gelatin from method B reached 80.44%, significantly higher than that of method A which was 64.99% ($p<0.05$). This value approached the GMIA standard of 84-90% (GMIA, 2012), indicating greater efficiency in collagen extraction and conversion to gelatin. The higher protein level in method B resulted from sequential acid-base extraction combined with ultrasound treatment, which enhanced collagen solubilization. Protein content reflects final gelatin quality since gelatin is derived from collagen (Asmudrono *et al*., 2019; Gerungan *et al*., 2019). These findings align with previous studies (Khushboo, 2023; Ata *et al.*, 2023) confirming a direct correlation between protein levels and extraction efficiency.

The pH values of gelatin from methods A and B were 5.72 and 5.99 (Table 2), with no significant difference ($p>0.05$). Both values met the SNI 8622-2018 and GMIA standards of pH 3.8-7.5, indicating suitability for industrial use. Gelatin pH affects viscosity, gel strength, solubility and stability (Feng *et al*., 2023; Wang *et al*., 2023). Method B's slightly higher pH (5.99) was associated with increased viscosity (10 cP) and gel strength (220 g), consistent with previous studies (Wang *et al*., 2023; Chen *et al*., 2023). Although the difference was minimal, the near-neutral pH values support good functional and application potential.



A B

(a) excitation and extraction with acid-base followed by extraction with ultrasound method (b) at 500x magnification.

Fig 2: Microstructure of tuna skin gelatin (*Thunnus albacore*) obtained by ultrasound extraction followed by acid-base extraction method.

Implications and limitations

This study's findings have important implications for gelatin extraction from fish skin. Method B, combining acid-base and ultrasound extraction, produces gelatin with higher yield, better colour and improved gel strength-crucial properties for food, pharmaceutical and cosmetic applications. However, the viscosity in both methods fell below GMIA standards, requiring parameter optimization. Further research into alternative techniques and industrial scale-up is needed to maintain consistent quality and yield.

Future research directions

Future studies should explore how ultrasonic power, temperature and time affect gelatin yield and quality, while developing enzymatic and sustainable extraction methods to enhance efficiency, environmental and economic sustainability.

CONCLUSION

Ultrasound-assisted extraction enhances tuna skin gelatin properties. Comparing ultrasound-acid-base (method A) and acid-base-ultrasound (method B), the latter consistently outperformed. Method B yielded higher output (13.34% vs. 4.92%), stronger gel, better color and compact microstructure. Although viscosity fell short of GMIA standards, method B produced stable gelatin for industrial use. This method effectively converts tuna skin, a sustainable by-product, into high-quality gelatin with potential in food, pharmaceuticals and cosmetics.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

The Committee of Experimental Animal Care approved all animal procedures for experiments and handling techniques.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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