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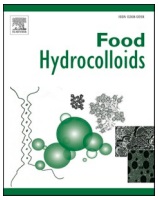

Improvement of gelatine extraction from tuna skin using a eutectic solvent

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ABSTRACT

The growing attention on sustainability in the food industry and waste management has increased interest in the valorisation of fish processing by-products, such as skin, which is rich in collagen and can be processed into gelatine. Traditional methods for gelatine extraction face significant challenges, particularly related to the use of harsh chemicals, environmental concerns, and inefficiencies in protein separation. This study proposes an improved method for gelatine extraction from tuna skin, using a eutectic solvent composed of urea and sodium acetate trihydrate, and also its 65 wt% aqueous solution, as maceration solvents. The method, applied to yellowfin and skipjack tuna skins, overcomes the limitations of previous approaches by reducing the extraction temperature and time, enhancing mass transfer, and improving the functional properties of the extracted gelatine. Compared to traditional extraction techniques and previous eutectic solvent-based methods, this approach avoids the use of aggressive solvents and the complex recovery of protein. The gelatine obtained exhibited improved characteristics, including higher hydroxyproline content, higher molecular weight of polypeptide chains, and better rheological properties (elastic modulus and melting and gelling temperatures). These improvements were more pronounced in the gelatine extracted from yellowfin tuna samples, particularly those pretreated with the aqueous solution of the eutectic mixture at 12 °C. The method also resulted in better thermal stability of the extracted gelatine, as confirmed by TGA analysis. This study highlights the potential of eutectic solvents, and their aqueous solutions, for efficient and sustainable gelatine extraction from fish skin, offering a promising alternative to traditional and less sustainable methods.

1. Introduction

Growing concern about the sustainability of the food industry and the environmental issues related to waste management has increased the interest in the valorisation of by-products through the recovery of added-value compounds from such waste materials (Liu et al., 2023; O'Connor et al., 2021). Together with an enhanced attention to the environmental impact of extraction techniques, this has stimulated the application of novel extraction technologies in food industry (Roy et al., 2023; Sarker et al., 2023). These challenges and opportunities are especially evident in the fish processing industry, which generates large amounts of waste in the form of bones, skin, scales, and viscera (Liu et al., 2025; Rathod et al., 2024). It is estimated that these residues represent over 50 % of the total processed mass, amounting for up to 20 million tonnes per year (Sun et al., 2024). These by-products, while often discarded, are rich in collagen, which can be further processed into gelatine, a high-value compound with numerous industrial applications.

Conventional methods for obtaining gelatine from fish skin face several challenges that not only impact the yield and quality of the final product but also raise concerns related to the solvents typically used in the extraction process: strong acids and alkalis, that can pose significant environmental issues associated to their handling and disposal. Previous studies have proposed the use of eutectic solvents for the recovery of proteins from biomass. For instance, a work by Panić et al. (2021) showed the potential of a choline chloride + ethylene glycol mixture for the valorisation of orange peel waste. Similarly, Lin et al. (2021) demonstrated that the eutectic prepared from choline chloride and levulinic acid can be used to obtain proteins from bamboo shoots, with similar results to those by traditional methods. Regarding the extraction of proteins from fish skin, Batista et al. (2022) obtained biocompatible collagen from blue shark skins using a citric acid + xylitol + water mixture; whereas Silva et al. (2024) employed a urea + propanoic acid mixture to recover collagen hydrolysates from codfish skin. However, all the works published to date use the eutectic mixture as an extraction solvent, making it necessary to recover the protein.

In a previous study (Gallego et al., 2025), we proposed an innovative

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method where a eutectic solvent was used for maceration instead of solubilisation. This approach allowed for avoiding the challenging and inefficient stage of recovering the protein from the eutectic. Specifically, the eutectic mixture of urea and sodium acetate trihydrate (corresponding to a 60:40 molar ratio (Gallego et al., 2023)) was effectively used for the maceration of yellowfin tuna skin, and warm water was used for the subsequent gelatine extraction stage. However, the main drawback of this approach was the need to apply a temperature higher than 31 °C (the eutectic temperature of the mixture) to ensure that the eutectic solvent remained in a liquid state. Temperatures in the pretreatment (maceration) stage should be lower in order to avoid thermal degradation of the protein. Thus, in this work, water is introduced as a third solvent component. As the ternary eutectic composition is not known, water content was selected according to the maximum solubility of the eutectic of urea and sodium acetate trihydrate in water at 12 °C, which corresponds to 65 wt%. This allows for a reduction in the maceration temperature, bringing it closer to traditional methods, as well as a reduction of the viscosity of the solvent (Gullón et al., 2020), enhancing mass transfer. Complementary, with the aim of improving the properties of the resulting gel (Gál et al., 2020), a reduction in the extraction time from overnight in the previous work (Gallego et al., 2025) to 1 h has also been considered herein. The improved method is tested with two different species of tuna (skipjack and yellowfin) to compare the characteristics of the extracted gelatines.

2. Experimental

2.1. Materials

Skins from skipjack tuna (*Katsuwonus pelamis*) and yellowfin tuna (*Thunnus albacares*) were provided by Jealsa Foods S.A.U. (Boiro, Galicia, Spain). Both species were caught in the Atlantic Ocean (FAO area 34) using a combination of purse seine, longline, and pole-and-line fishing methods. After capture, whole fish were placed in containers, covered with saturated brine, and frozen on board at −18 °C within a few hours. The yellowfin tunas were transported to the processing facility, where they were processed for the sale of frozen fish. Keeping the product frozen at all times, the head and fins were removed, and the skin was separated using a blade. In the case of skipjack tunas, they were processed for canned production. The fish were defrosted in water until reaching a temperature between −5 °C and 0 °C. The head and caudal fin were removed, and the skin was separated mechanically using a skinning machine equipped with a cold drum and blade. The skin was then collected and re-frozen. The skin sections used in the present study were specifically obtained from different individuals during the processing stage and were pooled and homogenised to minimise biological variability. Samples were brought to the laboratory under frozen conditions and stored at −20 °C for a maximum of 4 weeks before use.

Sodium acetate trihydrate (>99 wt%) and urea (>99 wt%) were obtained from Scharlau (Sentmenat, Barcelona, Spain). To prepare the eutectic mixture, both chemicals were mixed (urea mole fraction of 0.60) and heated to 60 °C with magnetic stirring until a homogeneous liquid formed. Subsequently, distilled water was added to dilute the eutectic mixture to a concentration of 65 wt%.

2.2. Extraction

The skins were defrosted at room temperature (22 ± 2 °C) before removing impurities, primarily meat and scales. They were then rinsed with running tap water, chopped into pieces (~0.5 × ~0.5 cm^2), mixed, packed in bags, and stored at −20 °C until further use. This process was carried out independently for each species to ensure two homogeneous lots that were used throughout the study.

Initially, 10 g of skins were pretreated by maceration with either the pure eutectic solvent at 35 °C (hereinafter referred to as pretreatment A) or with its 65 wt% aqueous solution at 12 °C (hereinafter referred to as pretreatment B), using a solid-to-liquid ratio of 1:3 (w/v). The mixture was mechanically stirred at 30 rpm using a EUROSTAR 20 Digital overhead stirrer (IKA, Staufen, Germany) for 3 h. It was then filtered with a sieve to remove most of the pretreatment solution, and the skins were washed twice with distilled water (using a solid-to-liquid ratio of 1:3 w/v) to remove any traces of the eutectic solvent. The absence of eutectic solvent was verified by confirming that the pH of the washing water reached a neutral value, as both components of the eutectic solvent tend to raise the pH to alkaline levels. After that, the extraction of gelatine was performed in distilled water at 45 °C with a solid-to-liquid ratio of 1:3 (w/v) under mechanical stirring (30 rpm) during 1 h. Both the maceration and the extraction stages were kept thermostated at their respective temperatures using a Julabo F12 circulating bath (Julabo GmbH, Seelbach, Germany). Finally, the liquid phase containing the gelatine was centrifugated using a Digicen 21R centrifuge (Ortoalresa, Madrid, Spain) at 18,000×*g* to remove small fragments of skin, and the clear supernatant was dried in a JP Selecta Conterm 2000209 oven (Selecta, Abrera, Barcelona) at 40 ± 1 °C for 48 h or until constant weight. Each extraction test was done in triplicate.

2.3. Characterisation

2.3.1. Extraction yield

The yield of extraction was calculated based on the weight of dried gelatine and the weight of wet skins before extraction, using the following equation:

$$\textit{Extraction yield}\ (\%) = \frac{\textit{Weight of dried gelatine}\ (g)}{\textit{Weight of wet skin}\ (g)} \times 100 \quad [1]$$

Additionally, an analogous expression was used to calculate the yield of extraction with respect to the weight of dry skin. This approach ensures that the result does not depend on the moisture content of the skin.

2.3.2. Proximate composition

The proximate composition of gelatines was analysed in terms of moisture, protein, organic matter, and ash content. The moisture and ash were determined using gravimetry following the 950.46 and 900.2A AOAC standard methods, respectively (Latimer, 2023). Organic matter was calculated by difference. Total crude protein was calculated as 5.6 × total nitrogen (Mariotti et al., 2008), which was determined by the Kjeldahl method (International Organization for Standarization, 2023) using a Kjeldatherm Digestor and Vapodest 50s distillation system (C. Gerhardt & Co., Königswinter, Germany). All analyses were done in duplicate.

2.3.3. Fourier-transform infrared (FT-IR) spectroscopy

The FT-IR spectra of gelatines were obtained using a 670 IR spectrometer by Varian (Palo Alto, California). The samples were analysed for the presence of different functional groups in the region of 4000-400 cm^{-1} at room temperature. Automatic signals were collected in 32 scans at a resolution of 4 cm^{-1}. Bruker Opus 7.8 data collection software was used to evaluate spectral data.

2.3.4. Amino acid profile

The amino acid profile of the gelatine samples was determined at Centro Tecnolóxico da Carne (San Cibrao das Viñas, Spain) using liquid chromatography, following the AccQ-Tag method with modifications described by Munekata et al. (2020). Briefly, samples were hydrolysed with 6 N HCl for 24 h at 110 °C, and the extracts were derivatised using the AccQ-Tag Ultra Derivatization Kit (Waters, Milford, MA, USA). Separation and identification were performed using HPLC-FL with a Waters 2695 Separations module, equipped with a Waters 2475 Multi Fluorescence detector and a Waters AccQ-Tag Amino Acids C18 analysis column. The Amino Acid Standard H (Thermo Scientific, Rockford, IL, USA) was used for quantification. During hydrolysis, asparagine and glutamine undergo deamidation, resulting in aspartic acid and glutamic

acid, respectively. Therefore, the detected composition reflects the sum of both components. Two independent measurements for each sample were performed for the quantification of the average amino acid contents.

2.3.5. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

To analyse the molecular weight (MW) pattern of gelatine samples, SDS-PAGE was performed following the method described by Laemmli (1970) with minor modifications. Gelatine solutions were mixed with NuPAGE LDS sample buffer 4X (Life Technologies, Carlsbad, CA, USA) and NuPAGE sample reducing agent 10X (Life Technologies) to achieve a final gelatine concentration of 0.2 % w/v. The samples were then heated at 70 °C for 10 min for protein denaturation and loaded into each well of a 4 % stacking and 8 % resolving Bolt Bis-Tris polyacrylamide gel (Life NTechnologies). PageRuler Plus Prestained Protein Ladder (Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania) was loaded along with the samples as protein standard (10–250 kDa). The electrophoresis was carried out at 60 V for the stacking and at 90 V for the resolving gel using a PowerEase 300W power supply (Life Technologies) until the dye front reached the bottom of the gel. After the electrophoresis, the gel was stained using Coomassie brilliant blue R250 (Thermo Fisher, Rockford, IL, USA) for 1 h and then destained in distilled water shaking overnight.

2.3.6. Gel permeation chromatography – size exclusion chromatography (GPC-SEC)

To complement the SDS-PAGE analysis, GPC-SEC was employed for a quantitative assessment of the MW distribution of the gelatine samples. The weight average molecular weight (M_w), number average molecular weight (M_n), and polydispersity index (PDI) were determined using an Agilent 1260 LC system (Agilent Technologies, Santa Clara, CA, USA). The system included a quaternary pump (G1311B), injector (G1329B), column oven (G1316A), and a triple detection setup: refractive index detector (G1362A), diode array detector (G1315C), and dual-angle static light scattering detector (G7800A) (Vázquez et al., 2020).

Elution was carried out with an aqueous mobile phase of 0.15 M ammonium acetate and 0.2 M acetic acid (pH 4.5) at a flow rate of 1 mL/min. A 100-μL sample volume was injected onto a set of four Proteema columns (PSS GmbH, Mainz, Germany): a precolumn (5 μm, 8 × 50 mm), and three analytical columns with pore sizes of 30 Å (5 μm, 8 × 300 mm), 100 Å (5 μm, 8 × 300 mm), and 1000 Å (5 μm, 8 × 300 mm), all maintained at 30 °C. The detectors were calibrated using a polyethylene oxide standard (MW = 106 kDa, PI = 1.05) from PSS GmbH. Absolute molecular weights were calculated using a refractive index increment (*dn/dc*) of 0.19 (Meyer & Morgenstern, 2003). Both gelatine samples and standard solutions were prepared at 2 mg/mL.

2.3.7. Thermal characterisation

Thermal stability and decomposition behaviour of gelatines were determined using a Q500 thermogravimetric analyser (TA Instruments, New Castle, DE, USA). Gelatine samples (ca. 5–20 mg) were heated from room temperature to 800 °C at a rate of 5 °C/min. Nitrogen gas (99.999 %, Nippon Gases Iberia, Madrid, Spain) was used as both the balance purge gas (40 mL/min) and the sample purge gas (60 mL/min). The resulting thermograms were analysed with the Universal Analysis 2000 software by TA Instruments.

2.3.8. Rheological behaviour

The dynamic rheological properties of the gelatine solutions were measured using an Anton Paar MCR 102 modular compact rheometer (Anton Paar, Graz, Austria) equipped with a cone-plate geometry (1° cone angle, 50 mm cone diameter, 0.098 mm gap), following the small amplitude oscillatory shear (SAOS) methodology (Steffe, 1996). The experiments were performed according to the protocol by Fernández-Díaz et al. (2001) with slight modifications. Briefly, a 6.67 wt% gelatine solution was prepared at 45 °C and cooled to room temperature before measurements. Rheological properties were assessed at a fixed frequency of 1 Hz and a strain of 10 %, within the linear viscoelastic region previously determined by strain sweep tests. Temperature sweeps were performed from 2 to 30 °C and back to 2 °C, at a rate of 1 °C/min. The changes in storage modulus (G′), loss modulus (G″), and phase angle (tan $\delta = G''/G'$) were recorded as a function of temperature.

2.4. Statistical analysis

Statistical analyses were carried out according to the specific requirements of each characterisation procedure, including extraction yield, proximate composition, and amino acid profile. Data were evaluated using one-way analysis of variance (ANOVA) to determine the presence of significant differences between treatments. Statistical significance was considered at $p < 0.05$. All analyses were conducted using IBM SPSS Statistics version 27.0.1.0 (IBM Corp., Armonk, NY, USA).

3. Results and discussion

3.1. Tuna skins

The proximate composition of yellowfin and skipjack tuna skins, provided by the supplier, is shown in Table 1. The statistical analysis revealed significant ($p < 0.05$) interspecies differences. Skipjack skins showed higher moisture content (55.4 ± 1.3 %) than yellowfin (47.4 ± 0.2 %), which influences the wet basis proportions of the remaining components. Protein content was significantly higher in yellowfin tuna on both wet (34.0 ± 2.0 %) and dry basis (65.0 ± 3.8 %) compared to skipjack (30.3 ± 0.8 % and 58.8 ± 1.6 %, respectively), suggesting a greater potential for gelatine extraction. In contrast, skipjack skins had significantly more lipids (11.3 ± 1.3 % wet, 21.9 ± 2.5 % dry). Regarding the ash content, it was similar between species (~9.9 % wet, ~19 % dry), indicating comparable mineral levels. Overall, both skin types present suitable compositions for gelatine production, although species-specific differences may affect processing requirements and yield.

3.2. Extraction yield

The extraction process significantly affects both the yield and the properties of the gelatines (Gong et al., 2024; Karim & Bhat, 2009; Li et al., 2024). The extraction yield for the four experiments (coded as: YFA for the yellowfin tuna using pretreatment A, YFB for the yellowfin tuna using pretreatment B, SJA for the skipjack tuna using pretreatment A, and SJB for the skipjack tuna using pretreatment B) was calculated both on wet and dry bases of fish skin to avoid distortion of results due to variations in moisture content. The yield of each extraction is represented in Table 2.

As observed in Table 2, all the yields obtained in this study fall within the middle of the typical yield range, which spans from 6 to 19 g of gelatine per 100 g of wet skin (Karim & Bhat, 2009). For yellowfin tuna, no statistically significant difference ($p > 0.05$) was found between

Table 1
Composition of tuna skins used in this work in wet and dry basis. Values are expressed as mean ± standard deviation of three replicates.

Composition	Content (g/100 g of wet skin)		Content (g/100 g of dry skin)	
	Yellowfin tuna	Skipjack tuna	Yellowfin tuna	Skipjack tuna
Moisture	47.4 ± 0.2	55.4 ± 1.3	–	–
Protein[a]	34.0 ± 2.0	30.3 ± 0.8	65.0 ± 3.8	58.8 ± 1.6
Lipid	8.5 ± 0.7	11.3 ± 1.3	16.3 ± 1.3	21.9 ± 2.5
Ash	9.8 ± 0.9	9.9 ± 0.8	18.7 ± 1.7	19.2 ± 1.6

[a] Determined by the Kjeldahl method. The protein content was calculated as the product of total nitrogen times the factor 5.6.

Table 2
Gelatine extraction yields. Values are the average of three replicates ± standard deviation.

Sample	Yield (g gelatine/100 g skin)	
	Wet skin basis	Dry skin basis
YFA	11 ± 1	20 ± 2
YFB	14 ± 2	25 ± 3
SJA	11 ± 1	24 ± 2
SJB	13 ± 2	29 ± 3

yields using the eutectic solvent at 35 °C (YFA: 11 ± 1 g/100 g wet skin) and those using the 65 wt% eutectic solution at 12 °C (YFB: 14 ± 2 g/100 g wet skin). Similarly, for skipjack tuna, yields for SJA (11 ± 1 g/100 g wet skin) and SJB (13 ± 2 g/100 g wet skin) did not differ significantly ($p > 0.05$). The slightly higher extraction values observed with pretreatment B in Table 2 could be attributed to decreased solvent viscosity, which favours mass transfer, and reduced protein solubility at lower temperatures, minimising protein loss during pretreatment. However, these differences were not statistically significant under the conditions tested.

Finally, when compared to the previous work using pure eutectic solvents with longer extraction times (Gallego et al., 2025), a clear and statistically significant decrease in extraction yield was observed: from 27–33 to 11–14 g of dried gelatine per 100 g of wet skin ($p < 0.05$), highlighting the strong effect of extraction time on process yield.

3.3. Proximate composition

The proximate composition of the yellowfin and skipjack tuna gelatines extracted with the two different pretreatments is shown in Table 3. As observed, crude protein content was very similar in all samples: no significant differences ($p > 0.05$) were found between samples YFA, YFB and SJA, and only a slightly lower, but statistically significant ($p < 0.05$), value was detected in sample SJB. Nevertheless, the crude protein content of all gelatines extracted in this work is consistent with the usual values for fish gelatines (from 81 to 94 %), as reported by previous studies (Kanwate & Kudre, 2022; Pranoto et al., 2011; Sousa et al., 2017). Regarding the ash content, the gelatines extracted from yellowfin tuna show significantly higher values than those extracted from skipjack tuna. These results are consistent with the literature, as the ash content of the gelatine extracted from skipjack tuna typically ranges from 0.5 to 2 % (Shyni et al., 2014; Zhang et al., 2022) while for the case of yellowfin tuna it ranges from 1.1 to 3.7 % (Pranoto et al., 2011; Sousa et al., 2017). No significant differences ($p > 0.05$) were found between the samples with different pretreatment conditions. Finally, the moisture content of samples YFA, YFB, and SJA was significantly lower than that of sample SJB. This result may be related to the higher yield observed in SJB (Table 2), potentially indicating that the extraction conditions for SJB favour greater water retention in the gelatine matrix. Nevertheless, the moisture content in all samples was quite below typical ranges (6–11 %) reported for fish gelatines (Pranoto et al., 2011; Shyni et al., 2014; Sousa et al., 2017; Zhang et al., 2022), yet complies with the standard regulations for edible gelatines, which set a limit of 15 % (Gelatine Manufacturers of Europe, 2023) to ensure stability. The composition of these four gelatines is quite similar to those obtained after maceration with pure eutectic solvent followed by overnight extraction with warm water (Gallego et al., 2025), with the only noticeable difference corresponding to the reduction in moisture (from 8.1-9.2 % to 1.6–3.4 %) and an increase in organic matter content.

Table 3
Composition of gelatine samples. Values are the average of two replicates ± standard deviation.

Sample	Content (g/100 g sample)			
	Moisture	Ash	Organic matter	Crude protein
YFA	1.6 ± 0.1	3.1 ± 0.3	95.3 ± 0.4	85.1 ± 0.3
YFB	1.6 ± 0.2	3.2 ± 0.5	95.2 ± 0.4	85.3 ± 0.2
SJA	1.6 ± 0.9	1.7 ± 0.6	96.7 ± 0.4	85.5 ± 0.3
SJB	3.4 ± 0.3	1.6 ± 0.2	95.0 ± 0.5	83.3 ± 0.5

3.4. FT-IR spectra

The infrared spectra of yellowfin tuna and skipjack tuna gelatines are shown in Fig. 1. As can be noted, all samples present similar patterns, exhibiting the most characteristic peaks consistent with the presence of functional groups typically found in protein structures.

A broad and intense peak is found at ~3300 cm^{-1}, corresponding to the O-H groups of carbohydrates and water, and to the N-H stretching bonds of the protein. This peak, typically associated to amide A region, is significantly flatter in the spectra of gelatines from skipjack tuna. This, together with a shift of the maximum intensity from 3307 cm^{-1} in yellowfin tuna samples to 3269 cm^{-1}, suggests a higher hydrolysis grade in skipjack tuna gelatines (Kristoffersen et al., 2020; Muyonga et al., 2004; Shahvalizadeh et al., 2021). This band partially overlaps with the amide B peak, with a maximum observed at ~2950 cm^{-1}, which is associated with C-H stretching vibrations (Li et al., 2024; Sow & Yang, 2015). The amide I region, corresponding to C=O stretching vibrations, is the most important in the characterisation of the secondary structure of proteins. In this study, the peak reaches its maximum at around 1635 cm^{-1} in all samples, and is commonly associated with contributions from β-sheet structures (Hermida-Merino et al., 2022; Sow & Yang, 2015). Meanwhile, the amide II band appears with a maximum at 1529 cm^{-1} in the case of yellowfin tuna gelatines, and is likely associated with the N-H bending and C-H vibrations in the peptide backbone (Ji et al., 2020; Kong & Yu, 2007). In the case of skipjack tuna gelatines this peak is shifted to 1571 cm^{-1} and is much more intense than the amide I peak, unlike in yellowfin tuna samples. This can be attributed to structural changes in protein conformation caused by hydrolysis, which enhances hydrogen bonding and exposes more N-H groups. These changes increase the prominence and intensity of the amide II band and shift it to higher frequencies due to alterations in the local environment around the amide band (Ji et al., 2020; Koochakzaei & Sabaghian, 2023). Amide III is associated with the stretching vibration of C-N and N-H deformation from amide bonds, and its maximum is found at around 1225 cm^{-1} in all samples. This location has been associated with the presence of some molecular disorder, probably related to the loss of the triple helix structure (Kumar et al., 2018; Sinthusamran et al., 2014).

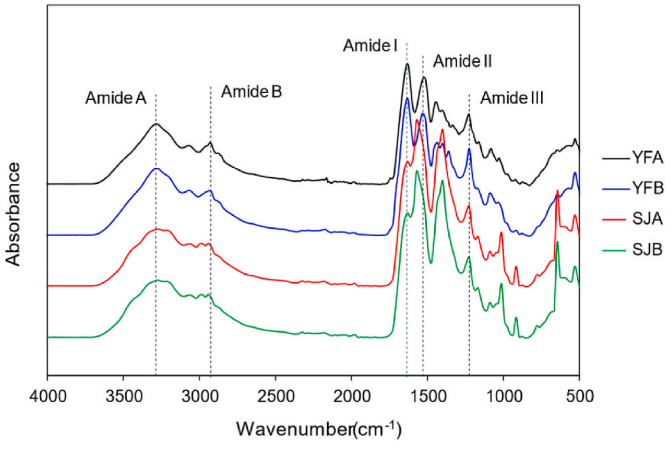

Fig. 1. FT-IR spectra of extracted gelatines. Vertical dotted lines correspond to the wavenumbers of the characteristic peaks of amides.

3.5. Amino acid profile

The amino acid composition is essential for assessing the quality and functionality of the extracted gelatines. The amino acid profile of the gelatines studied in this work is shown in Table 4.

The amino acid sequence of the gelatine macromolecule can be described by a repetitive unit with the general formula Gly-X-Y, where "Gly" refers to glycine and X and Y are mostly occupied by proline and hydroxyproline, respectively. This is in accordance with the results of Table 4, since glycine is the most abundant amino acid in all samples (~23 %), with no significant differences ($p > 0.05$) between species or between type of pretreatment.

Total pyrrolidine amino acids (proline and hydroxyproline) content is crucial for the functionality of the gelatines, as they have a critical role in the stabilisation of the triple helix and thus affect the properties of the resulting gel, especially hydroxyproline due to its capacity to build hydrogen bonds (Gómez-Guillén et al., 2002; Piez & Gross, 1960). Statistical analysis showed no significant differences ($p > 0.05$) in the content of these amino acids among samples, regardless of pretreatment or species. Hydroxyproline values ranged from 8.77 to 9.56 g/100 g total amino acids, while proline content varied between 9.42 and 10.18 g/100 g total amino acids. Although some authors suggest that the hydroxyproline content is influenced by the extraction conditions (Nikoo et al., 2013), the results indicate that neither the extraction method nor the tuna species significantly affected the levels of the key amino acids responsible for the stabilisation of the triple helix structure. As shown in Table 4, the total pyrrolidine amino acid content of yellowfin and skipjack tuna gelatines was approximately 19 and 18 %, respectively. These results are in agreement with the amino acid content of gelatines reported in previous studies conducted on the same tuna species, where the glycine content ranged from 22 to 34 %, and the proline + hydroxyproline content varied between 17 and 19 % (Jamili et al., 2019; Nurilmala et al., 2022; Qiu et al., 2019; X. R. Yang et al., 2019). Comparing these results with those obtained using the pure eutectic and prolonged extraction time (Gallego et al., 2025), it can be observed that the hydroxyproline content has significantly ($p < 0.05$) increased from ~7 % to ~9 %. Since the content of this amino acid is commonly used as an indicator of gelatine quality (Cho et al., 2005; Sato et al., 1991), this suggests that the protocol reported in the present work improves the outcome of the process in terms of gelatine quality.

Regarding the other amino acids, high levels of alanine (~10 %), arginine (~9 %), and glutamine + glutamic acid (10–11 %) were also found, which aligns with the expected ranges for gelatines derived from warm water fish species (Atma, 2017; Derkach, Voron'ko, Kuchina, & Kolotova, 2020; Hassan et al., 2025). The only amino acids that showed significant ($p < 0.05$) differences in content across the different samples were cysteine (with YF gelatines exhibiting slightly lower levels than SJ samples) and tyrosine (sample SJB presented a lower content compared to the other three samples). In terms of nutritional composition, the content of essential amino acids (see Table 4) was similar across all samples, ranging from 26 % to 28 %. This composition, which appears to be independent of both maceration and species, is consistent with the findings of Alves et al. (2022), who reported values between 26 % and 29 % for codfish-derived gelatines, and with those of Vázquez et al. (2021), who observed a EAA/TAA ratio of 26 %–28 % in salmon skin gelatines.

Table 4
Amino acid content (expressed in g of amino acid/100 g of total amino acids) of yellowfin tuna and skipjack tuna gelatines. Values are represented as mean of two replicates ± standard deviation.[a]

	Concentration (g/100 g total amino acids)			
Amino acid	YFA	YFB	SJA	SJB
Alanine	10.30 ± 0.15	9.94 ± 1.44	10.23 ± 1.77	10.25 ± 0.48
Arginine	9.04 ± 0.24	9.14 ± 1.31	8.82 ± 1.52	8.89 ± 0.01
Aspartic acid[a]	5.63 ± 0.05	5.33 ± 0.84	5.81 ± 1.00	5.82 ± 0.33
Cysteine	0.32 ± 0.00	0.31 ± 0.02	0.35 ± 0.00	0.41 ± 0.05
Glutamic acid[b]	10.49 ± 0.08	9.93 ± 1.52	10.94 ± 1.89	11.05 ± 0.59
Glycine	22.91 ± 0.65	23.22 ± 3.16	22.67 ± 3.76	23.15 ± 0.49
Histidine	1.42 ± 0.05	1.42 ± 0.21	1.36 ± 0.22	1.26 ± 0.05
Isoleucine	1.23 ± 0.03	1.25 ± 0.19	1.34 ± 0.25	1.17 ± 0.03
Leucine	2.72 ± 0.05	2.76 ± 0.42	2.97 ± 0.54	2.66 ± 0.08
Lysine	3.61 ± 0.01	3.44 ± 0.54	3.93 ± 0.64	4.06 ± 0.24
Methionine	1.84 ± 0.05	1.92 ± 0.25	1.55 ± 0.24	1.61 ± 0.02
Hydroxyproline	8.77 ± 0.29	9.22 ± 1.13	9.21 ± 1.24	9.56 ± 0.12
Phenylalanine	2.11 ± 0.06	2.24 ± 0.27	1.99 ± 0.30	1.67 ± 0.03
Proline	10.08 ± 0.16	10.18 ± 1.27	9.42 ± 1.56	9.45 ± 0.28
Serine	3.69 ± 0.12	3.70 ± 0.56	3.46 ± 0.61	3.37 ± 0.06
Threonine	3.12 ± 0.08	3.22 ± 0.46	3.43 ± 0.59	3.40 ± 0.01
Tyrosine	0.41 ± 0.02	0.44 ± 0.07	0.36 ± 0.06	0.23 ± 0.00
Valine	2.32 ± 0.04	2.34 ± 0.35	2.17 ± 0.39	1.99 ± 0.06
EAA/TAA[c] (%)	27.41 ± 0.25	27.74 ± 1.61	27.56 ± 1.64	26.70 ± 0.39
NEAA/TAA[d] (%)	72.59 ± 0.63	72.27 ± 5.18	72.44 ± 5.14	73.29 ± 1.05

[a] Including asparagine, which is deamidated during the hydrolysis process to aspartic acid.
[b] Including glutamine, which is deaminated during the hydrolysis process to glutamic acid.
[c] EAA/TAA: ratio essential amino acids/total amino acids.
[d] NEAA/TAA: ratio non-essential amino acids/total amino acids.

3.6. Molecular weight distribution

Together with the amino acid profile, the MW distribution of the polypeptide chains that conform gelatine plays an essential role in the physical properties of the resulting gel. Therefore, all the gelatines obtained in this work were analysed by SDS-PAGE and GPC-SEC.

As shown in Fig. 2, all samples present heterogeneous patterns, with several bands distributed across a wide range of MW. These patterns are very similar to those obtained for fish gelatines extracted in previous works (Ruan et al., 2023; Yu et al., 2023, 2024). However, the

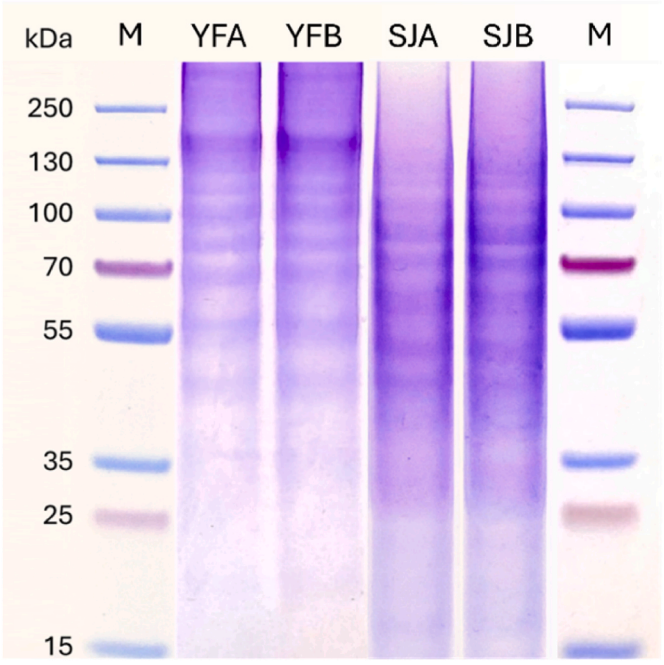

Fig. 2. SDS-PAGE patters of yellowfin and skipjack tuna gelatines extracted after different pretreatments. The code M refers to the prestained protein ladder marker (MW from 15 to 250 kDa).

electrophoresis patterns of the analysed gelatines show significant differences between samples from different species: the MW distribution of the gelatines derived from yellowfin tuna is notably higher than that of the gelatines from skipjack tuna. YFA and YFB samples exhibit most of their bands from 70 kDa to ~150 kDa, which could be indicative of the presence of the residual α_1 and α_2 chains of collagen (Alves et al., 2022). In contrast, SJA and SJB gelatines only show lower MW bands, mainly below ~80 kDa. This is consistent with what was already suggested in section 3.3 on the analysis of the FTIR spectra: the gelatines derived from skipjack tuna undergo a higher degree of hydrolysis than those from yellowfin tuna, resulting in the shift of the polypeptide chains to lower molecular weights. Since the skins of both species were subjected to identical pretreatment, extraction, and drying processes, the only possibility is that this higher degree of hydrolysis is due to the inherent characteristics of the skin itself.

Regarding the differences between the gelatines extracted with different pretreatments, a small difference in band intensity can be observed: in both cases, the samples extracted after applying pretreatment B (12 °C, 65 wt% eutectic solvent solution) show slightly more intense bands at higher molecular weights than those from pretreatment A (35 °C, 100 % eutectic solvent). This is possibly due to the fact that a more aggressive pretreatment leads to a greater degradation of the polypeptide chains (Kittiphattanabawon et al., 2016; H. Yang et al., 2008).

The gelatines were also examined by GPC-SEC for a more precise, quantitative analysis of their MW. All data are listed in Table 5. By comparing the differences between species, GPC-SEC confirms the hypothesis suggested in the SDS-PAGE and FTIR analyses: the gelatines derived from skipjack tuna are much more hydrolysed than those from yellowfin tuna, as both their M_w and M_n are significantly lower (22–25 kDa versus 93–98 kDa in the case of M_w, and 11–12 kDa versus 50–55 kDa in the case of M_n). Although the differences are much less significant, there are also some variations between the gelatines obtained after different pretreatments: in both species, the gelatines extracted with pretreatment B show slightly higher M_w and M_n values compared to those from pretreatment A, as previously suggested in the SDS-PAGE analysis. A high PDI is observed in all cases, which is consistent with the fact that all polypeptide chains, exhibiting significantly varying molecular weights, fall within the same distribution range. This aligns with the use of PDI as a measure of the breadth of the molecular weight distribution (Shrivastana, 2018).

An indicative distribution of the molecular weight profiles determined by GPC is shown in Fig. S1 (supplementary material) and the area percentages of each region are summarised in Table S1 (supplementary material). In the case of YF gelatines, less than 5 % of the total area could be attributed to β-chains residues, around 43–47 % to α-chains and about 50 % to a range of varied peptides. This distribution differs considerably in SJ gelatines, without presence of β-chains, lower percentage of α-chains (10–13 %), and higher level of peptides (87–90 %).

When comparing these results with the gelatines obtained using the pure eutectic during maceration and a prolonged extraction time (Gallego et al., 2025), some differences can again be observed. In contrast to this study, three GPC-SEC regions could be identified in the gelatines obtained in the previous work. However, in that case, the higher molecular weight regions (associated with the presence of α chains and β-dimers) accounted for only 18–36 % of the total polypeptide chains. The remaining chains, between 64 % and 82 %, corresponded to chains with a molecular weight of approximately 26–30 kDa. In this study, although these regions could not be distinguished, a significantly higher M_w value was obtained, which should positively impact the properties of the resulting gel.

Table 5
Molecular weight of gelatines extracted in this work. Values are represented as mean of two replicates ± standard deviation.

Sample	M_n[a] (kDa)	M_w[b] (kDa)	PDI[c]
YFA	50.4 ± 4.3	93.6 ± 1.3	1.9
YFB	54.3 ± 1.6	97.7 ± 0.7	1.8
SJA	12.3 ± 1.0	22.9 ± 1.3	1.9
SJB	11.3 ± 0.5	24.7 ± 1.6	2.2

[a] M_n: number average molecular weight.
[b] M_w: weight average molecular weight.
[c] PDI: polydispersity index.

3.7. Thermal characterisation

The proximate composition, amino acid profile and MW distribution of gelatines also influence their thermal stability, which is crucial to determine their potential applications (Martins et al., 2018).

The thermograms obtained by thermogravimetric analysis (TGA), in Fig. 3, show the profile of weight loss (as a percentage of the initial sample mass) with increasing temperature. In all cases, thermal decomposition of gelatine occurs in two different stages. The first step represents a loss mass of ~20 % and takes place between room temperature and ~150 °C. Since gelatine has a highly hydrophilic character, this step is likely associated with the loss of adsorbed water during sample handling and storage (Martins et al., 2018), as well as the water bound to proteins through hydrogen bonding (Valcarcel et al., 2021). After this, a more pronounced step starts near 200 °C, corresponding to a mass loss of ~60 % of the total mass. This is in agreement with Martins et al. (2018), who suggested that this decrease is related to the loss of amino acids during gelatine decomposition together with the loss of structurally bound water (Vázquez et al., 2021).

Finally, the presence of a minimum at 600–700 °C in the derivative curves might be interpreted as the start of a third decomposition step resulting from the total decomposition of the gelatine network (Hermida-Merino et al., 2022; Mishra et al., 2011). Table 6 presents, for all four samples, the onset decomposition temperature (T_{onset}) and the temperature of the maximum decomposition rate (T_{max}) of the two first, nitid decomposition stages, as well as the mass loss associated with each of these steps, and the final residue at 800 °C (temperature at which the TGA runs were concluded).

As shown in Table 6, all the values obtained during the TGA analyses are very similar across the different samples, although some small differences can be noted. Regarding the gelatines from different species, it can be observed that the decomposition temperatures, both T_{onset} and T_{max}, are slightly higher (by 3–7 °C) in the yellowfin tuna samples than in the skipjack tuna samples, likely due to their lower degradation during the extraction process. On the other hand, when comparing samples from the same species subjected to different pretreatments, the samples extracted after pretreatment B are slightly more stable (around 4–7 °C) than those after pretreatment A, likely due to the longer length of the polypeptide chains (Correia et al., 2013).

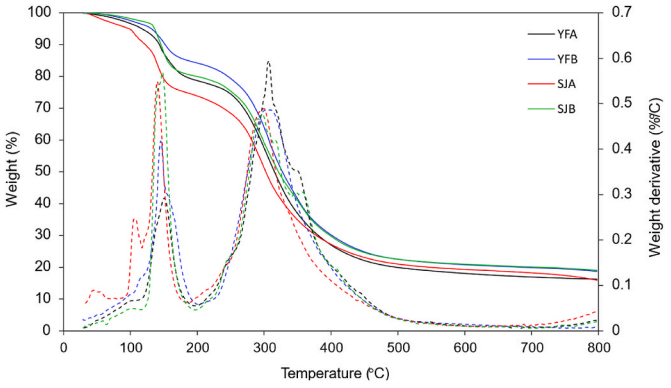

Fig. 3. TGA curves (solid lines), and their derivatives with temperature (dashed lines), for the gelatines extracted from yellowfin tuna and skipjack tuna skins.

Table 6
T_{onset}, T_{max}, and mass loss (with respect to the initial sample mass) associated with each of the two main decomposition steps identified using TGA in the thermal characterisation of the gelatines. The final residue at 800 °C is also included.

Sample	Decomposition step	T_{onset} (°C)	T_{max} (°C)	Mass loss (%)	Residue at 800 °C (%)
YFA	1st	127	146	21.3	16.2
	2nd	264	311	82.1	
YFB	1st	131	158	19.8	19.7
	2nd	262	268	78.9	
SJA	1st	124	140	25.2	15.9
	2nd	262	301	80.8	
SJB	1st	124	151	15.6	18.7
	2nd	270	307	79.4	

Finally, it is worth noting a significant increase in the T_{onset} and T_{max} values, ranging from 10 to 20 °C in both decomposition stages, compared to the TGA studies conducted on the gelatines obtained with the pure eutectic and overnight extraction stage (Gallego et al., 2025). This suggests that the new, less aggressive pretreatment improves the thermal stability of the gelatines, thus expanding their range of applicability.

3.8. Rheological behaviour

Gelatine-derived gels exhibit a complex behaviour influenced by both their viscosity and elasticity. The rheological properties of these gels are crucial to determine their potential applicability, as they provide essential insights into their texture, stability, and performance under different conditions (Ahmed, 2017). In this work, this behaviour was studied through the variation of the storage modulus (G') and loss modulus (G") across a range of temperatures for aqueous solutions of the obtained gelatines. These variations are presented in Fig. 4.

As shown in Fig. 4a, all samples exhibit similar trends during the heating process: at low temperatures the storage modulus remains consistently higher than the loss modulus, indicating a predominantly elastic behaviour. As the temperature increases, the gelatine molecules break their initial ordered triple-helix structure, returning to a disordered, coil-like conformation. These structural changes result in a decrease in both G' and G", pointing to the weakening of the three-dimensional network. At a certain temperature, both curves intersect, and the samples transition from elastic-dominated to viscous-dominated behaviour, marking the end of the melting process and the transition to liquid state (Ahmed, 2017; Kokol et al., 2021). This temperature is referred to as the melting temperature (T_{melt}).

In contrast, Fig. 4b shows the opposite behaviour: during the cooling process, the samples remain in a liquid-dominated state until they reach a certain temperature. Below this point, both moduli begin to increase, indicating the formation of a more rigid network as the temperature decreases. This increase is a result of the formation of junction zones and the reinforcement of the gel network through hydrogen bonding, van der Waals forces, self-assembly, and hydrophobic interactions (Da Silva et al., 2015). As the temperature continues to drop, G' becomes greater than G", meaning the transition from a liquid-dominated phase to a solid-dominated phase. The crossover point of the two moduli curves marks the gelling point (T_{gel}), where the gelatine undergoes gelation and forms a more stable, semi-solid structure (Ahmed, 2017; Huang et al., 2017).

Although the samples of both species exhibit similar behaviours, there are significant differences between them, as shown in Table 7. First, it is worth noting that the values of G′ and G″ at lower temperatures are significantly higher in the samples from yellowfin tuna than in the samples from skipjack tuna. Additionally, the difference between both curves is also significantly greater in the former case. Since both the value of G′ and the difference between G' and G" are directly related to the strength of the gel (Chenite et al., 2001; Magami & Williams, 2018), it can be concluded that the yellowfin tuna gelatines produce much stronger gels than the skipjack tuna gelatines. In fact, it can be remarked that in the SJA and SJB gels the difference between G′ and G″ is less than one order of magnitude, indicating a not fully formed gel even though the elastic behaviour predominates over the viscous one (Morris et al., 2012).

Moreover, the T_{melt} of the yellowfin tuna samples (21–23 °C) are significantly higher than those of the skipjack tuna samples (12–13 °C). In the case of the T_{gel}, those of yellowfin samples are in the range 14–16 °C, whereas for the skipjack tuna samples they could not be detected. This is consistent with the MW distribution of the samples, as a larger size of polypeptide chains leads to higher T_{melt} and T_{gel} values, as well as higher G' and G" values (Duthen et al., 2018; Enrione et al., 2020; He et al., 2024). This can also be observed when comparing the

Table 7
Melting temperature (T_{melt}) and gelling temperature (T_{gel}) of aqueous solutions of the gelatines. (n.d. = non detected).

Sample	T_{melt} (°C)	T_{gel} (°C)
YFA	21.9	14.7
YFB	22.7	15.8
SJA	12.2	n.d.
SJB	13.1	n.d.

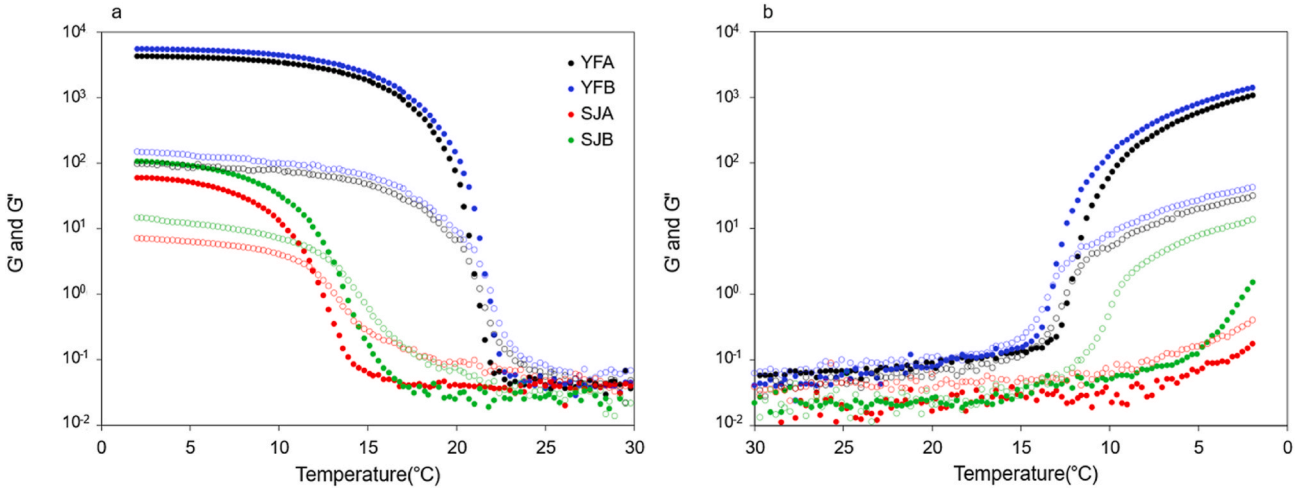

Fig. 4. Variation of G' (filled markers) and G" (unfilled markers) of aqueous gelatine solutions with temperature: heating ramp (a) and cooling ramp (b).

samples from pretreatment A to those from pretreatment B: both the curves and the values of T_{melt} and T_{gel} for YFB and SJB are respectively higher than those for YFA and SJA.

Finally, it is worth highlighting a significant improvement compared to the gels from the previous work (Gallego et al., 2025), in which pure eutectic solvent was used during maceration stage and an overnight extraction with warm water was carried out. Taking the YFB sample as a reference, the maximum value of G′ has increased from 1.5 kPa to 5.5 kPa, and T_{melt} and T_{gel} have increased from 19.4 °C to 22.7 °C and from 11.0 °C to 15.8 °C, respectively.

4. Conclusions

In this study, an improved method for gelatine extraction from fish skin was developed. To achieve this, two extraction approaches were tested: (A) maceration with a eutectic mixture of urea and sodium acetate trihydrate at 35 °C (slightly above the melting temperature of the pure eutectic mixture), followed by gelatine extraction with water at 45 °C for 1 h, and (B) maceration with an aqueous solution of the eutectic mixture (65 wt%) at 12 °C, also followed by gelatine extraction with water at 45 °C for 1 h. Both methods were successfully applied to two tuna species: yellowfin and skipjack.

In comparison to maceration with the eutectic solvent at 35 °C and extraction with water at 45 °C overnight, the reduced extraction time resulted in lower yields, but the gelatines obtained showed improved properties: the amino acid profile revealed a higher content of hydroxyproline, and the molecular weight distribution (analysed by SDS-PAGE and GPC-SEC) showed a higher molecular weight of the polypeptide chains. These two factors were reflected in the rheological behaviour of the corresponding gels, where an increase in both the elastic modulus and the melting and gelling temperatures was observed. This improvement was also reflected in the thermal stability of the samples (analysed by TGA).

While the above results were evident in the two samples obtained from yellowfin tuna skin, they were slightly better in the sample pretreated with the aqueous eutectic solution compared to the sample pretreated with the pure eutectic. This is likely due to two factors: on the one hand, the lower temperature of pretreatment B makes it less aggressive, causing less protein degradation; on the other hand, the addition of water reduces the viscosity of the solvent and enhances mass transfer, thereby facilitating the pretreatment.

Regarding the differences between species, the skipjack tuna samples underwent considerably more degradation than the yellowfin tuna samples: the degree of hydrolysis of the polypeptide chains was much higher, leading to a significant reduction in molecular weight and much worse rheological properties.

Compared to traditional extraction processes, this method eliminates the use of harsh chemicals such as strong acids or bases. In contrast to other recently proposed methods that involve solubilising fish skin in eutectic solvents, the one reported herein avoids the challenging separation of protein and solvent.

In summary, this study demonstrates the potential of the eutectic solvent constituted by urea and sodium acetate trihydrate as effective agent in processing fish skins to obtain added-value components, with the addition of water to this eutectic being an attractive possibility for extracting high-quality gelatine.

CRediT authorship contribution statement

Cristina Gallego: Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **José Antonio Vázquez:** Writing – review & editing, Investigation, Formal analysis. **Héctor Rodríguez:** Writing – review & editing, Supervision, Conceptualization. **Ana Soto:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2025.111789.

Data availability

No data was used for the research described in the article.

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