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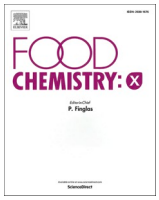

Physiochemical and functional properties of gelatin obtained from frigate mackerel (*Auxis thazard*), skipjack tuna (*Katsuwonus pelamis*), Longtail tuna (*Thunnus tonggol*) and yellowfin tuna (*Thunnus albacares*) skin

Qinghe Yin [a,b,1], Haohao Shi [b,1], Yongqiang Zhao [c], Gang Yu [c], Haohao Wu [e], Guanghua Xia [b,d,*], Tao Yang [a,**]

[a] School of Pharmacy, Hainan Provincial Key Laboratory for Research and Development of Tropical Herbs, Hainan Medical University, Haikou 571199, China
[b] Hainan Engineering Research Center of Aquatic Resources Efficient Utilization in South China Sea, Key Laboratory of Food Nutrition and Functional Food of Hainan Province, Key Laboratory of Seafood Processing of Haikou, School of Food Science and Technology, Hainan University, Hainan 570228, China
[c] Sanya Tropical Fisheries Research Institute, Sanya 572018, China
[d] Collaborative Innovation Center of Provincial and Ministerial Co-Construction for Marine Food Deep Processing, Dalian Polytechnic University, Dalian 116034, China
[e] College of Food Science and Technology, Ocean University of China, Qingdao 266000, China

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ABSTRACT

This study conducted a systematic investigation into the physicochemical and functional properties of gelatin extracted through alkaline methods from the skins of four commercially significant tuna species: frigate mackerel (*Auxis thazard*), skipjack tuna (*Katsuwonus pelamis*), longtail tuna (*Thunnus tonggol*), and yellowfin tuna (*Thunnus albacares*). Comparative analyses revealed notable species-specific variations in gelatin yield, amino acid composition, molecular weight distribution, and functional performance. Notably, yellowfin tuna skin gelatin (YSG) exhibited the highest melting point (28.09 °C), gel strength (271 g), and proline content (14.3 %), along with superior foaming capacity (20.43 %), water retention, and emulsification stability. Molecular weight profiles obtained via SDS-PAGE confirmed the presence of α- and β-chains characteristic of type I collagen, with YSG demonstrating enhanced structural integrity and thermal stability attributed to its elevated proline content. Additionally, Fourier-transform infrared spectroscopy (FTIR) and circular dichroism (CD) analyses indicated stronger hydrogen bonding and preservation of the triple-helix structure in YSG. While longtail tuna yielded the highest extraction rate (21.5 %), skipjack tuna showed the highest protein content (86.7 %). In contrast, frigate mackerel gelatin displayed darker coloration ($\Delta E^* = 53.09$) due to residual pigments. Rheological assessments highlighted YSG's optimal viscoelasticity and melting behavior, aligning with its robust interfacial properties. These findings underscore the potential of yellowfin tuna skin gelatin as a viable alternative to mammalian gelatin in food and biomedical applications, offering enhanced functional performance while valorizing underutilized fishery by-products.

1. Introduction

Frigate mackerel is widely distributed in tropical and subtropical waters all over the world, and is the smallest type of tribe thunnini (Xu, Wang, & Du, 2019). Frigate mackerel is also widely distributed in the South China Sea fishery, according to the latest statistics, the South China Sea small tuna species is about 850,000 tons, of which frigate mackerel accounts for about 30 % (Zhou et al., 2022). Skipjack tuna is of great commercial significance, with an annual harvest exceeding 2 million tons, making up roughly 58 % of the total tuna catch for processing. This species serves as the primary fish utilized in canned tuna production. However, approximately 70 % of this yield is regarded as

* Corresponding author at: Hainan Engineering Research Center of Aquatic Resources Efficient Utilization in South China Sea, Key Laboratory of Food Nutrition and Functional Food of Hainan Province, Key Laboratory of Seafood Processing of Haikou, School of Food Science and Technology, Hainan University, Hainan 570228, China.
** Corresponding authors.
E-mail addresses: xiaguanghua@hainanu.edu.cn (G. Xia), taoyangfood@muhn.edu.cn (T. Yang).
[1] These authors contributed equally to this study.

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by-products, such as heads, skin, intestines, and bones (Pinrattananon et al., 2023). The longtail tuna is the second smallest tuna, and is different from the other tunas, the longtail tuna. Longtail tuna is the second smallest tuna and unlike the other tunas, longtail tuna lives mainly in shallow waters close to the mainland and is rarely fished at the same time as the other tunas and rarely forms large, dense schools, so catches are relatively small, but in recent years it has been slowly becoming a target for the tuna industry (Griffiths, Leadbitter, Willette, Kaymaram, & Moazzam, 2020). Yellowfin tuna has a similar range to the other three species, and is also an economically important species of tuna.

However, the by-products, including skin, viscera, and bones, generated during tuna processing are currently being wasted due to the lack of efficient utilization methods. It is important to highlight that these by-products make up approximately 50 %–70 % of the total tuna yield, with global annual waste exceeding 5 million tons (Wu et al., 2023).In order to solve the above problems, aquatic animal gelatins have been developed to replace the commonly used mammalian gelatins. Extracted mainly from aquatic animal scales, bones and skin, for example, gelatin from seabass and grey mullet scales (Thi Hue, Thi Tu Oanh, Thi Minh Hang, Nguyen Thanh, & Razumovskaya, 2017), tuna bones (Haddar et al., 2012) and from the skin, fins and viscera of *Gadidae* fish have shown excellent gel properties (Zarubin et al., 2021). Therefore, the collection and efficient utilization of these by-products can enhance the utilization of tuna-like resources, while also safeguarding the environment and adding commercial value to the fish processing industry.

Gelatin finds wide applications across the food, pharmaceutical, and chemical industries. Reportedly, 70 % of the world's gelatin and collagen is sourced from the skin and bones of pigs and cattle (Zhang et al., 2020). It is worth noting that due to pig and bovine gelatin might be susceptible to contamination with foot-and-mouth disease and bovine spongiform encephalopathy, furthermore, religious dietary restrictions in Judaism, Islam, and Hinduism, which prohibit the consumption of any products derived from pigs and beef that is not ritually slaughtered (Nurilmala, Suryamarevita, Hizbullah, Jacoeb, & Ochiai, 2022). Fish gelatin, when compared to mammalian gelatin, faces certain limitations including inferior rheological properties, a higher cost, and fluctuating market demand, which hinder its broader industrial adoption. Nonetheless, fish gelatin possesses distinct advantages over mammalian gelatin. For instance, it has a lower melting point, making it ideal for food flavoring applications where rapid flavor release is desired, thereby enhancing sensory qualities. Additionally, fish gelatin exhibits superior film-forming and barrier properties. Moreover, there are notable species-specific differences between fish and mammalian gelatin, which have piqued the interest of many researchers in this field. As a result, gelatin derived from fish is a good substitute for mammalian gelatin. The commonly employed extraction methods for fish skin gelatin primarily include alkaline, acid, enzymatic, and combined treatment approaches. Compared to other extraction methods, the alkaline extraction process demonstrates superior efficacy in removing non-collagenous proteins and lipids, thereby enhancing gelatin purity. This technique exhibits broad applicability across various fish species, particularly accommodating both cold-water and warm-water fish varieties. Furthermore, the alkaline method demonstrates the capacity to partially preserve the triple-helical structure of collagen, which contributes significantly to the enhancement of gel strength in the final product.This study systematically investigated the alkaline extraction methodology for isolating gelatin from the skin of four commercially significant tuna species (*Auxis thazard*, *Katsuwonus pelamis*, *Thunnus tonggol*, and *Thunnus albacares*), with a focus on elucidating species-specific variations in their molecular structures (amino acid profiles and polypeptide chain composition) and key physicochemical properties (including gel strength, viscosity, thermal stability, and emulsification capacity). Comparative analyses were conducted to establish correlations between taxonomic classification and functional performance of the derived gelatins, thereby providing insights into their potential industrial applicability and Benchmark against commercial standards to assess industrial viability.

2. Materials and methods

2.1. Material

The species utilized in this study included frigate mackerel (*Auxis thazard*), skipjack tuna (*Katsuwonus pelamis*), longtail tuna (*Thunnus tonggol*), and yellowfin tuna (*Thunnus albacares*). Fish specimens were procured from the Dongmen Seafood Market in Haikou, China, the fish were placed in insulated containers with ice packs and maintained at 4 °C during transportation. All fish were freshly landed on the day of purchase. Upon arrival, the fish skins were meticulously separated, pretreated by scraping off residual scales and adhering muscle tissues, and sectioned into uniform squares (approximately 2 × 2 cm). Processed skins were then flash-frozen at −80 °C and stored for subsequent experiments within 15 days to preserve biochemical integrity. Sodium hydroxide(NaOH , ≥ 97 % , Sigma-Aldrich), ethanol(≥99.5 %, Sigma-Aldrich), pentanol($C_5H_{12}O$, >99.5 %(GC), Macklin), HCl(36–38 %, Xilong Scientific Co., Ltd.), High molecular weight protein markers used in elec-trophoresis control experiments were bought from Beyotime Biotech Inc. Use 4 % to 20 % Future PAGETM protein pre-cast gel (Nanjing Jiancheng Bioengineering Institute, China).

2.2. Gelatin extraction

Fish skin gelatin extraction was done using, with minor adjustments to, the methodology of(Shyni et al., 2014). First, the fish skin was cleansed and then submerged in a solution of 0.1 mol/L sodium hydroxide (1:10, *w/v*) at room temperature to eliminate non-collagenous proteins and fats. The immersion process lasted for 2 h with constant stirring, then every 30 min, the sodium hydroxide solution was changed. The fish skins were then cleaned with distilled water until their pH was neutral, following which they were soaked in a solution of 0.2 mol/L acetic acid (1,10, *w/v*) at 25 °C for 12 h with stirring to further eliminate impurities and make the fish skin fully swollen. Following the acid treatment, the fish skins were cleaned in distilled water to bring them to a pH of neutral. The gelatin was then extracted in distilled water (1,10, w/v) at 60 °C for 12 h while being constantly stirred. Following extraction, after passing the mixed solution through two layers of clean gauze to collect the filtrate, insoluble contaminants were removed by centrifuging the filtrate for 10 min at 10,000 r/min (Thermo Scientific, Dreieich, Germany). The resulting supernatant was then combined with hexane solution to adsorb the oils and fats in the gelatin solution. The mixture was centrifuged again for 10 min at 10,000 r/min after being left to stand for 10 min. The clear liquid of lower layer the was collected and subjected to freeze-drying (Eyel4, Model 1100, Tokyo, Japan) to obtain gelatins from frigate mackerel, Skipjack Tuna, Longtail Tuna, and Yellowfin Tuna skins (FSG, SSG, LSG, and YSG, respectively). It was then kept at room temperature until needed later, packaged in sterile ziplock bags. Based on the ratio of the extracted gelatin's dry weight to the original skin's wet weight, the gelatin yield was computed. The yields of the obtained gelatins were calculated as follows:

$$\text{Gelatin yield} = \frac{W_1}{W_2} \times 100\%$$

W_1 is the weight of gelatin after drying; W_2 is the weight of wet fish skin.

2.3. Gelatin color

Gelatin gels (6.67 %, *w/v*) were prepared by dissolving freeze-dried fish skin gelatin from four species in distilled water at 45 °C until complete dissolution, using standardized glass containers. The resulting homogeneous solution was refrigerated and incubated at 4 °C for 12 h to

facilitate gel maturation. Colorimetric analysis of the gelatin gels was conducted using a high-precision spectrophotometric colorimeter (YS3010, 3nh, China), with triplicate measurements performed for each experimental group to ensure methodological reproducibility.

2.4. Determination of proximate composition

The moisture, ash, crude fat, and crude protein contents of four fish skin gelatins were analyzed using the (AOAC, 1995) method. Moisture content was determined with a moisture meter (HX204, METTLER TOLEDO, Switzerland), ash content by high-temperature combustion method, crude fat content by Soxhlet's method, and crude protein content by Kjeldahl's method with a conversion factor of 5.4 for calculating protein content from nitrogen content.

2.5. Determination of amino acid composition

The amino acid composition was determined following the method described by (Qiu et al., 2019), with modifications to enhance analytical precision and sample compatibility. The material was hydrolyzed under decreased pressure at 110 °C in a sealed ampoule for 24 h after being submerged in a 5 mL solution of 6 mol/L hydrochloric acid. Then 0.2 mL of the hydrolyzed solution was taken and dried by blowing with nitrogen, and after completion of the drying, the solution was diluted with 1 mL of distilled water, and finally filtered through a 0.22 μm membrane into the injection vial. The mixtures were separated and quantified using an equipped high performance liquid chromatograph (Agilent 1100 HPLC, Agilent Ltd., USA). The amino acid composition of fish skin gelatin was characterized by the number of residues per 1000 total residues.

2.6. SDS-page

The method was adapted from (S.-Y. Zhang et al., 2022), with minor modifications. Molecular weight distribution of FSG, SSG, LSG, YSG was determined by SDS-PAGE. Future PAGETM protein precast gel (4–20 %) was used, and the samples were dissolved and mixed well with 5 × loading buffer in a 4:1 volume to configure the final concentration of the samples as a protein solution of 2.5 mg/mL, and the precast gel plate was loaded with 10 μL in each well. Electrophoresis was carried out by electrophoresis apparatus (ATTO Corp., Tokyo, Japan) at 80–120 V was performed. After electrophoresis, the plates were stained with Fast Bule protein fast staining solution on a shaker for 30 min and then rinsed with distilled water. Markers were selected from 30 kda ~ 200 kDa.

2.7. Determination of gel strength

The gel strength was assessed by using a freezing force tester (Asahi Sun TS-III, Tianjin Asahi Sun Equipment & Instrument Co. Ltd., China). A 6.67 % (*w/v*) gelatin solution was prepared by dissolving 8 g of lyophilized gelatin in 120 mL of distilled water. The homogenized solution was poured into a 150 mL Bloom jar (inner diameter: 59 mm; height: 85 mm) and incubated at 4 °C for 12 h to facilitate gelation, then incubated at 4 °C for 12 h. The Bloom Jar is placed in a central position below the plunger, then a cylindrical probe of standard radius (P/0.5R) is used to Pierce the sample to a depth of 4 mm. The plunger speed is set at 0.5 mm/s and the gel strength is expressed in (G).

2.8. Circular dichroism (CD) spectra

Prior to testing, a 0.1 % w/v gelatin solution was made by dissolving FSG, SSG, LSG, and YSG in 10 mmol/L sodium phosphate buffer (pH 7.0). Subsequently, the specimens were introduced into quartz cuvettes featuring a 1 mm optical range within circular dichroism (MOS-500, Biologic, France), and the CD spectra were acquired and documented within the far-ultraviolet range spanning 190 to 250 nm, employing a scanning rate of 100 nm/min(Sinthusamran, Benjakul, Hemar, & Kishimura, 2018).

2.9. ATR-FTIR measurements

Structural features and changes in functional groups of FSG, SSG, LSG, YSG were analyzed using an Automatic target recognition (Nicolet™ iS50 FTIR, Thermo Electron, USA) instrument. Infrared analysis was conducted within the range of 400–4000 cm^{-1}, comprising 32 scans cumulatively, to obtain each gelatin sample's FTIR spectra, using a detection resolution of 1 cm^{-1} (J. Xu et al., 2021).

2.10. Flow behavior and thermal properties of gelatin

The viscosity of gelatin was measured according to the method described by (Tan, Karim, Uthumporn, & Ghazali, 2020)Gelatin's rheological characteristics were evaluated with a rheometer (DHR-2, TA Instruments, USA). After 10 min of preheating to 45 °C, a gelatin solution (6.67 %, *w/v*) was applied on a testing platform (60 mm diameter, 1 mm gap). Sample flow curves have been generated as an analysis of shear rate between 1–1000 s^{-1} in a testing temperature range of 25 °C.

Using dynamic temperature scanning rheological testing, the melting and setting temperatures of gelatin were measured. After preparedness for 45 °C, gelatin solutions were moved to a test platform. (60 mm diameter, 1 mm gap). The temperature range for the scanning process was 5–40 °C (heating process) and 40–5 °C (cooling process). The scanning rate was set at 0.5 °C/min, frequency at 60 rad/s, and constant strain at 5 %. The melting or setting point of gelatin is found at a temperature of $\tan\delta = 1$ or $\delta = 45°$(Liao et al., 2021).

2.11. Determination of setting point and setting time

The setting and melting points of gelatin were described according to (Muyonga, Cole, & Duodu, 2004) and with some modifications. The prepared gelatin solution (6.67 %, *w/v*) was transferred to a fine test tube(12 mm × 75 mm)and kept at 4 °C for incubation. Throughout the incubation time, a 1 mm pointer was inserted into the gelatin solution and taken out every 10s. When the pointer and the gelatin sample were unable to be separated, the gelatin had reached its solidification temperature. The time taken for the gelatin to set is setting time.

2.12. Interfacial properties of gelatin

2.12.1. Emulsifying properties

The emulsification bracketing index (EAI) and emulsion stability index (ESI) of FSG, SSG, LSG, YSG solutions were determined using the method of (Pearce & Kinsella, 1978). The gelatin solution (1 g/100 mL, 6 mL) and soybean oil (2 mL) were well combined. Subsequently, 10 mL of the mixture were transferred into a 50-mL centrifuge tube and agitated for 1 min at 15,000 rpm using a high-speed tissue crusher. Following, the emulsions at 0 and 10 min (100 μL) were diluted 100 times using 0.1 % SDS. Following thorough mixing, a spectrophotometer was used to detect the brightness at 500 nm right away. The following equation was used to compute the EAI (m^2/g) and EST (min).

$$EAI = \frac{2.303 \times 2 \times A \times N}{C \times \varphi \times 10000}$$

A: absorbance at 500 nm, N: dilution factor, C: concentration of sample protein in solution, φ: volume fraction of oil phase.

$$ESI = \frac{A_0}{A_0 - A_t} \times \Delta t$$

absorbance at 500 nm, A_0: 0 min absorbance, A_t: t min absorbance.

2.12.2. Foaming properties

Foam expansion capacity (FEC) and foam stability (FS) of gelatin were assessed following the methodology outlined by (Shahidi, Han, & Synowiecki, 1995). A 50 mL centrifuge tube was filled with 10 mL of the sample solution (1 g/100 mL), and subsequently placed under a high-speed tissue masher at room temperature to agitate the gelatin solution (15,000 rpm, 1 min). At 0, 10, and 30 min, the total volume was noted, correspondingly. Using the following equation:

$$FE = \frac{Vt_1}{Vt_0} \times 100$$

$$FS = \frac{Vt_2}{Vt_0} \times 100$$

Vt_0: initial volume, Vt_1: volume following an agitation, Vt_2: volume following a 30-min standing.

2.13. SEM

Samples were processed and pasted on trays for 30 s under reduced pressure gold spraying using a scanning electron microscope Scanning Electron Microscope (S-300n, Tokyo, Japan) magnified 500 times with a 20 kV speeds up voltage.

2.14. Statistical analysis

For every set of samples, at least three parallel experiments were conducted, and the experimental findings were reported as mean ± standard deviation. Using SPSS 23.0 software (Chicago, IL, USA), With a significance level of $p < 0.05$, all the data were examined using one-way assessment of variance (ANOVA) and Duncan's multiple range test.

3. Results and discussion

3.1. Yield and proximate composition

The yields and proximate compositions of gelatin extracted from the four species are summarized in Table 1. Significant differences ($p < 0.05$) were observed in extraction rates, with longtail tuna skin gelatin (LSG) exhibiting the highest yield (21.5 %), followed by yellowfin tuna skin gelatin (YSG) at 18.42 %, consistent with previous reports for tuna-derived gelatins (Nurilmala, Hizbullah, Karnia, Kusumaningtyas, & Ochiai, 2020). In contrast, frigate mackerel (FSG) and skipjack tuna (SSG) showed lower yields (16.79 % and 17.7 %, respectively). These variations are likely attributable to interspecific differences in skin morphology: thicker skins of longtail and yellowfin tuna facilitated enhanced swelling in alkaline-acidic solutions, promoting collagen solubilization, whereas thinner skins (e.g., frigate mackerel) limited extraction efficiency due to incomplete matrix disruption (Gómez-Guillén et al., 2002).

Table 1
Proximate composition and color values of gelatin samples.

Samples	FSG	SSG	LSG	YSG
Yield (%)	16.79 ± 0.51^{c}	17.7 ± 0.08b^{c}	21.5 ± 0.58^{a}	18.42 ± 0.4^{b}
Moisture (%)	11.52 ± 1^{b}	11.81 ± 0.51^{b}	13.65 ± 0.78^{a}	14.62 ± 0.38^{a}
Protein (%)	86.47 ± 0.93^{ab}	86.7 ± 0.63^{a}	84.77 ± 0.92^{bc}	83.39 ± 0.43^{c}
Fat (%)	0.38 ± 0.01^{c}	0.39 ± 0.02^{c}	0.49 ± 0.02^{b}	0.62 ± 0.03^{a}
Ash (%)	1.45 ± 0.1^{a}	0.99 ± 0.07^{b}	1.04 ± 0.09^{b}	1.11 ± 0.11^{b}
L*	57.83 ± 0.44^{c}	64.55 ± 1.72^{b}	66.99 ± 1.45^{ab}	68.10 ± 1.02^{a}
a*	2.38 ± 0.59^{a}	0.41 ± 0.5^{b}	−1.02 ± 0.83^{c}	−0.92 ± 0.64^{c}
b*	38.13 ± 1.22^{a}	33.11 ± 0.81^{b}	23.45 ± 0.55^{c}	10.98 ± 0.13^{d}
ΔE*	53.09 ± 0.73^{a}	45.12 ± 1.71^{b}	36.64 ± 0.9^{c}	29.32 ± 0.98^{d}

Data was expressed as mean value ± standard deviation of triplicates. Different letters in the same row indicate significant differences at $P < 0.05$. FSG: frigate mackerel skin gelatin, SSG: Skipjack tuna skin gelatin, LSG: Longtail tuna and skin gelatin, YSG: Yellowfin tuna skin gelatin.

Proximate analysis revealed that SSG contained the highest protein content (86.7 %), followed by FSG (86.47 %), LSG (84.77 %), and YSG (83.39 %). The elevated protein content in SSG suggests its potential superiority in applications requiring robust gel strength and water-binding capacity, such as confectionery or pharmaceutical capsules (Liao et al., 2021). Conversely, YSG displayed the highest fat content, likely due to yellowfin tuna's larger body size and lipid accumulation in both skin and viscera—a storage pattern distinct from tilapia and squid (Abdelmalek et al., 2016), which primarily deposit lipids in viscera (Shyni et al., 2014). While higher fat content may compromise optical clarity, it could enhance emulsification properties, rendering YSG suitable for emulsion-stabilized food systems(Karim & Bhat, 2009). FSG exhibited the highest ash content (1.45 %), potentially linked to co-extraction of melanin-rich pigments during processing, as evidenced by its darker coloration (ΔE* = 53.09). Residual pigments may restrict FSG's use in applications demanding high transparency, such as edible films or translucent desserts (Gómez-Guillén et al., 2002). These findings highlight the necessity of species-specific selection: SSG's high protein content aligns with gel-based applications, whereas YSG's lipid profile favors emulsified products.

3.2. Color of gelatin

Table 1 shows the L*, a*, b*, and ΔE* of different varieties of fish skin gelatin. In applications related to food, gelatin's color is quite important. While the functional qualities of gelatin are unaffected by its hue, the lighter the gelatin color, the more it reduces the effect on the color of the food, and therefore lighter-colored gelatin will be the first to be selected in food applications. The instrumental results showed that YSG was lighter in color compared to the other three species and was actually white. Compared to the other three fish skin gelatins, the a* and b* of FSG were substantially greater ($p < 0.05$), Furthermore, it is noteworthy that the frigate mackerel had the largest total color difference value, which, combined with the fact that the frigate mackerel skin samples and FSG had the highest ash scores, possibly explained by the fact that the skin color of frigate mackerel is more pigmented, and some of the pigments may have been co-extracted into gelatin leading to a darker gelatin color. The L* of SSG gelatin was significantly lower than that of SSG gelatin extracted with method (Shyni et al., 2014) (75.3 ± 0.6), but higher than the brightness of Yellowfin tuna skin gelatin extracted with method (56.46 ± 0.78)(Abdelmalek et al., 2016). (Karnjanapratum, Sinthusamran, Sae-leaw, Benjakul, & Kishimura, 2017) reported that L* values of gelatin extracted from gelatin of Asian bullfrog skin at 45–75 °C (45.44–48.91) were lower than those of gelatin from four fish skins but the a* values (4.12–6.56) were higher than those of the four fish skin gelatins. According to the above results, the different colors of gelatin may be related to the species and extraction methods. Studies have shown that gelatin extracted from the skin of certain fish species exhibits antioxidant properties, which may be associated with its color. For example, gelatin and derived proteins from yellowfin tuna skin have demonstrated strong antioxidant activity, potentially linked to their specific color and chemical composition(Gál, Mokrejš, Pavlačková, & Janáčová, 2022). Therefore, in the processing of functional foods, selecting fish gelatin with a particular color may enhance the health benefits of the final product.

3.3. SDS-PAGE

The molecular weight distribution of four fish skin gelatins analyzed

by SDS-PAGE is shown in Fig. 1. All samples exhibited similar molecular weight profiles, with bands distributed between 120 and 250 kDa, corresponding to β-, α1-, and α2-chains characteristic of typeIcollagen. The presence of α- and β-chains reflects partial degradation of native collagen during extraction, as previously observed in tuna skin gelatin. Notably, the α-chain content—particularly α1-chains—is critical for gelatin's gelation capacity, as higher α-chain proportions correlate with enhanced triple-helix stability and gel strength(Ranasinghe et al., 2022). This aligns with findings in yellowfin tuna gelatin, analogous to gelatins extracted from bluefin tuna (*Thunnus thynnus*) under similar alkaline conditions(Haddar et al., 2012).FSG displayed slightly lower molecular weight than S5G, LSG, and Y5G (Fig. 1), likely due to its higher thermal sensitivity and accelerated degradation during extraction. Similar molecular weight reductions were reported in heat-labile tuna gelatin extracted above 60 °C, underscoring the importance of controlled extraction conditions. The absence of low-molecular-weight bands in all samples confirms effective removal of non-collagenous proteins, consistent with purification protocols for high-purity tuna gelatin.While the band patterns of the four gelatins resembled tilapia skin gelatin extracted at 60 °C (Abuibaid, AlSenaani, Hamed, Kittiphattanabawon, & Maqsood, 2020), they diverged markedly from those of gelatin extracted at 70 °C, highlighting temperature-dependent degradation. Importantly, the molecular weight range (120–250 kDa) overlaps with reported values for skipjack tuna gelatin (135–260 kDa)(Shyni et al., 2014), suggesting shared structural features among pelagic fish species. However, differences in β-chain intensity between our samples and tuna gelatin may arise from interspecies variations in collagen crosslinking (Ahmed, Verma, & Patel, 2020).

3.4. CD spectra

The circular dichroism (CD) spectra of fish skin gelatins from four sources (FSG, SSG, LSG, YSG) are shown in Fig. 2A. All samples exhibited characteristic collagen-like profiles, with a negative peak at 197 nm and a positive peak at 220 nm, suggesting partial retention of the triple-helical conformation. These spectral features closely align with reported CD patterns of yellowfin tuna skin gelatin, where the 220 nm positive peak is attributed to the ordered polyproline II (PP-II) helix structure, a hallmark of collagen-derived gelatin(Lopes, Miles, Whitmore, & Wallace, 2014). The persistence of these peaks indicates limited thermal denaturation during extraction, which is critical for maintaining gelatin's functional properties. Notably, the intensity of the positive peak at 220 nm in FSG was lower than in YSG (Fig. 2A), implying reduced triple-helix content. Similar trends were observed in heat-sensitive skipjack tuna gelatin extracted above 55 °C, where excessive thermal exposure disrupted the PP-II helix (Sinthusamran, Benjakul, & Kishimura, 2013). This correlation underscores the importance of controlled extraction temperatures (<60 °C) to preserve structural integrity(Kaewdang, Benjakul, Prodpran, Kaewmanee, & Kishimura, 2015). Contrary to mammalian gelatins (e.g., bovine), which show complete loss of the 220 nm peak due to helix unwinding (Liu, Nikoo, Boran, Zhou, & Regenstein, 2015), fish gelatins retain partial collagen-like ordering.

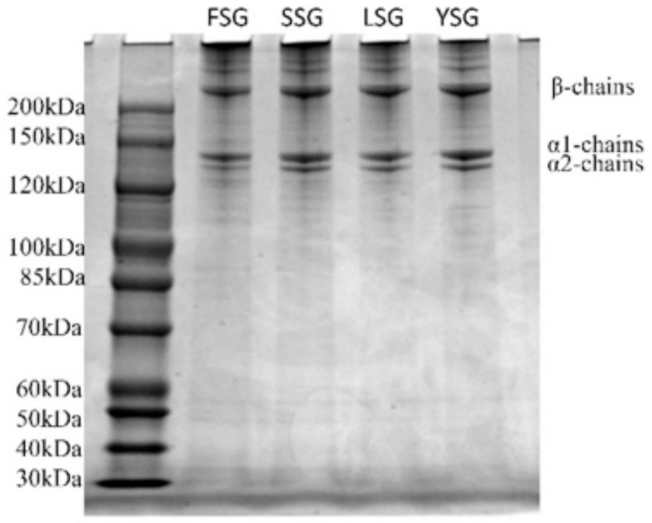

Fig. 1. SDS-PAGE patterns of gelatins of different origin.

3.5. ATR-FTIR measurements

The ATR-FTIR spectra of the four fish skin gelatins (FSG, SSG, LSG, YSG) exhibited characteristic absorption bands associated with collagen-derived proteins (Fig. 2B). All samples displayed Amide A (~3300 cm^{-1}), Amide *B* (~2920 cm^{-1}), Amide *I* (~1630 cm^{-1}), Amide II (~1540 cm^{-1}), and Amide III (~1240 cm^{-1}) bands, consistent with type I collagen secondary structures(Kittiphattanabawon, Benjakul, Sinthusamran, & Kishimura, 2016). Notably, the intensity of the Amide A band (N—H stretching vibrations coupled with hydrogen bonding) was highest in YSG, indicating stronger hydrogen bonding networks within its triple-helix structure(Vázquez et al., 2021). This observation aligns with YSG's superior gel strength (271 g, Table 3) and thermal stability (melting point: 28.09 °C), as enhanced hydrogen bonding contributes to structural rigidity and resistance to thermal denaturation (Gómez-Guillén et al., 2002; Liao et al., 2021). Conversely, FSG showed reduced intensity in the Amide I band (C═O stretching), suggesting partial disruption of α-helical structures, which correlates with its lower molecular weight profile observed in SDS-PAGE (Fig. 1) and diminished functional performance (Table 3). The absorption peak at 1080 cm^{-1}, attributed to C—O stretching vibrations of glycosylated hydroxylysine residues(Petibois, Gouspillou, Wehbe, Delage, & Déléris, 2006), was present in all samples but varied in intensity. This implies species-specific differences in carbohydrate content, potentially influencing water-binding capacity and emulsification properties (Nurilmala et al., 2020). Furthermore, the region between 1460 and 1240 cm^{-1}, associated with C—N stretching and N—H bending modes, confirmed partial preservation of the collagen triple helix in all gelatins(Krishnamoorthi, Ramasamy, Shanmugam, & Shanmugam, 2017). However, YSG exhibited sharper peaks in this region, indicative of higher structural integrity, which supports its outstanding rheological properties (Fig. 2C–E). These findings are consistent with studies on yellowfin tuna gelatin, where stronger hydrogen bonding and intact triple-helix motifs correlate with enhanced functional performance (Nurilmala et al., 2020; Zhang et al., 2020). The FTIR results elucidate structural distinctions among the gelatins that directly align with their functional behaviors. The superior hydrogen bonding and triple-helix stability in YSG, as evidenced by Amide A and Amide I/II band profiles, underpin its high gel strength and thermal resistance, making it a promising candidate for applications requiring robust viscoelasticity. These structural insights complement the amino acid composition (Table 2) and molecular weight data (Fig. 1), providing a comprehensive understanding of species-specific functionality.

3.6. Determination of amino acid composition

Table 2 displays the amino acid composition of the four fish skin gelatins. The composition of gelatin varies depending on the source, with glycine and proline being the predominant amino acids, consistent with findings on yellowfin tuna skin gelatin(M. Nurilmala, Jacoeb, & Dzaky, 2017). The viscosity of gelatin correlates with the polypeptide chain length, where longer chains generally exhibit higher viscosity. Amino acid content also significantly influences the melting point and gel strength of gelatin, as observed in tuna and rohu fish gelatins(Ninan, Ynudheen, Joshy, & Yousuf, 2013). As we all know, glycine, alanine, proline and hy-droxyproline are four of the most abundant amino acids

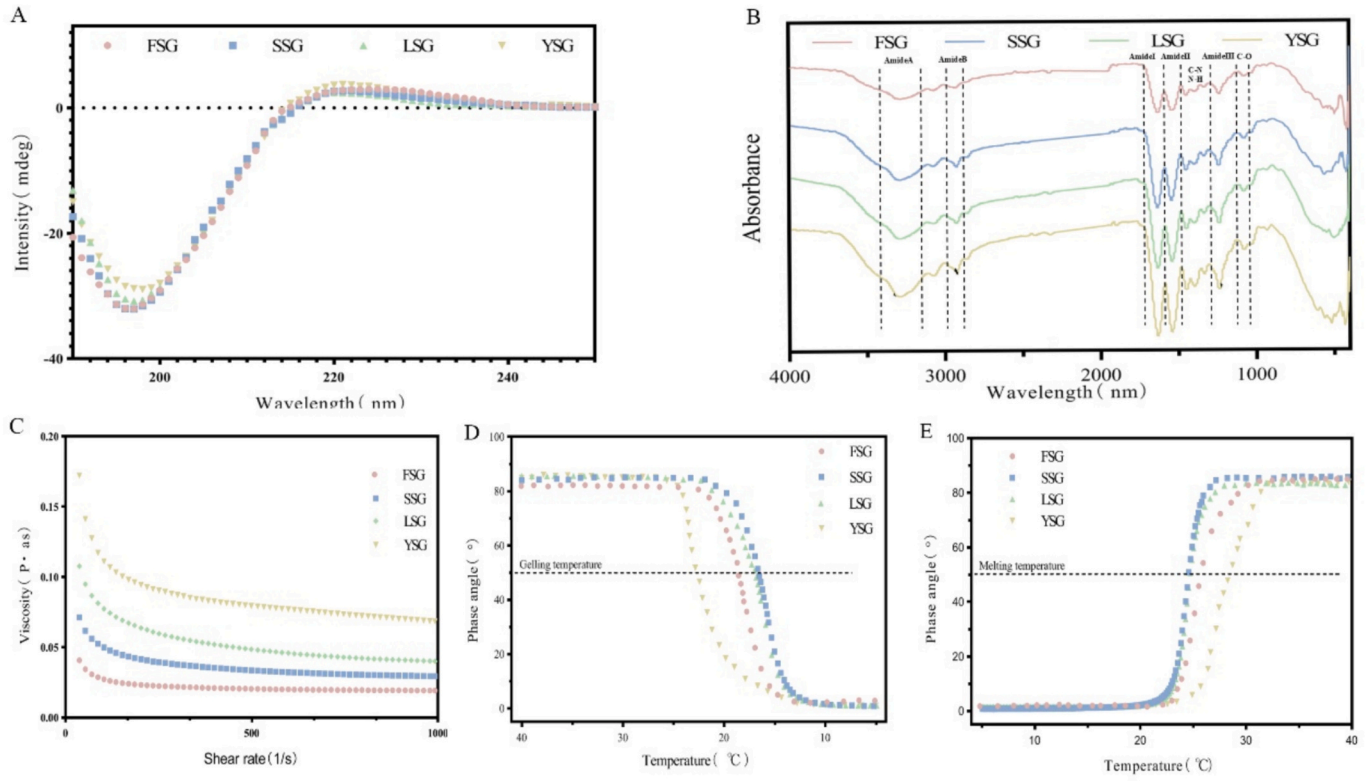

Fig. 2. CD spectra of gelatins of different origin(A), ATR of gelatins of different origin(B),Viscosity variation with shear rate of gelatins of different origin(C),Gelling temperature (D) and melting temperature (E) of gelatins of different origin.

Table 2
Amino acid content (Residues/1000 residues) of gelatin samples extracted at different origin.

Amino acid	FSG	SSG	LSG	YSG
ASP	58	57	61	58
Thr	33	32	32	32
Ser	37	36	41	40
Glu	106	103	111	105
Gly	211	203	211	208
Ala	114	117	125	113
Val	23	22	26	25
Met	18	17	6	11
Ile	14	14	14	13
Leu	29	28	31	30
Tyr	6	6	8	7
Phe	23	24	26	24
His	18	17	22	18
Lys	4	39	41	41
Arg	95	96	103	92
Pro	128	135	140	145

in gelatin. The samples had an imino acid content of 17.96–19.75 % of protein. (Benjakul, Kittiphattanabawon, & Regenstein, 2012) found that hydroxyproline directly participates in gel formation by acting as a hydrogen donor, enabling hydrogen bonding with hydrogen acceptors from adjacent chains. In fact, factors such as fish species, living environment, and habitat temperature play a crucial role in determining the proline and hydroxyproline content. For example, gelatin extracted from tropical animal tissues has been found to be rich in proline. Since tilapia is a tropical fish, a relatively high proline content (128–145 residues/1000 residues) has been observed in tuna(Ali, Kishimura, & Benjakul, 2018). This content is lower than that found in unicorn leatherjacket fish skin (174–179 residues/1000 residues) and reef grouper skin (173–180 residues/1000 residues) but higher than the 107 residues/1000 residues reported for tuna gelatin by J. Gómez-Estaca and the 127 residues/1000 residues found in bovine gelatin(Gómez-Estaca, Montero, Fernández-Martín, & Gómez-Guillén, 2009).

3.7. Gel strength values of gelatin samples

Gel strength, a critical quality indicator of gelatin, reflects its viscoelasticity and thermal stability. As shown in Table 3, the gel strength values of frigate mackerel (FSG), skipjack tuna (SSG), longtail tuna (LSG), and yellowfin tuna (YSG) skin gelatins were 218 g, 229 g, 230 g, and 271 g, respectively. Notably, YSG exhibited the highest gel strength (271 g) among the four fish gelatins, approaching the performance of poultry skin gelatin (260 g at 60 °C) and even certain

Table 3
Functional properties of gelatin samples.

	FSG	SSG	LSG	YSG
Bloom (g)	218 ± 2^{c}	229 ± 6.25^{b}	230 ± 3^{b}	271 ± 4.73^{a}
Setting Time at 4 °C (s)	168.67 ± 7.77^{a}	176 ± 4^{a}	168 ± 1.73^{a}	154.67 ± 4.51^{b}
Melting temp (°C)	25.29 ± 0.17^{b}	24.95 ± 0.7^{b}	24.96 ± 0.5^{b}	28.09 ± 1.45^{a}
Gelling temp (°C)	17.28 ± 0.94^{b}	15.86 ± 0.54^{b}	16.84 ± 0.52^{b}	19.08 ± 0.85^{a}
FA (%)	15.37 ± 0.4^{d}	16.93 ± 0.21^{c}	18.93 ± 0.4^{b}	20.43 ± 0.59^{a}
FS (%) 30 min	$10.270.3^{d}$	11.23 ± 0.49^{c}	15.13 ± 0.41^{b}	16.37 ± 0.35^{a}
EAI (m^2/g)	27.81 ± 3.79^{a}	24.9 ± 4.11^{a}	24.2 ± 5.37^{a}	23.87 ± 5.12^{a}
ESI (%)	21.23 ± 0.35^{a}	22.4 ± 0.56^{a}	22.36 ± 0.41^{a}	23 ± 0.26^{a}

Values are given as mean ± standard deviation of triplicate. Values with the same superscript letters within a column are not significantly different ($p < 0.05$).

commercial mammalian gelatins (bovine gelatin: 200–300 Bloom) (Kuan, Nafchi, Huda, Ariffin, & Karim, 2017). However, as documented in prior studies, mammalian gelatins (porcine: 466.4 g; rabbit: 457.57 g) generally surpass fish-derived gelatins in gel strength due to their higher proline and hydroxyproline, which stabilize triple-helix structures via hydrogen bonding with water and carbonyl groups (Chandra & Shamasundar, 2015; Oslan, Shapawi, Mokhtar, Noordin, & Huda, 2022). Despite this disparity, the gel strength range of the fish gelatins in this study (218–271 g) aligns with applications requiring moderate elasticity, such as dairy products (yogurt stabilization), confectionery (gummy candies), and edible films(Sinthusamran, Benjakul, & Kishimura, 2014). For instance, commercial fish gelatin with a Bloom strength of 250–300 g is widely used in halal/kosher markets as a mammalian gelatin substitute (Nurilmala et al., 2020). YSG's performance (271 g) positions it as a viable candidate for such applications, particularly where ethical or religious restrictions limit mammalian gelatin use.

3.8. Melting point, setting point and setting time of gelatin

The setting and melting temperatures of gelatin obtained by rheometer measurements are shown in Fig. 2D and Fig. 2E. The setting and melting temperatures of the extracted gelatin were 16.55–26.66 °C and 25.41–27.95 °C, respectively, and the phase angle of the rheological curves of the obtained gelatin from fish skin decreased dramatically during the cooling process. The decrease in temperature leads to the formation of numerous three-dimensional networks between gelatin molecular chains via hydrogen bonding, resulting in the creation of a compact gel matrix. The solidification point 16.29 and melting point 24.55 of SSG were similar to the reported solidification point 18.7 and melting point 24.2 of SSG (Shyni et al., 2014), but there was a large discrepancy with (Tumerkan, Cansu, Boran, Mac Regenstein, & Ozogul, 2019) reported solidification point 22 and melting point 31 of SSG, which may be related to the difference in the extraction method of gelatin. The setting temperatures of YSG and the reported melting temperatures are in good agreement with the melting temperatures of yellowfin tuna gelatin previously reported by (Gurunathan, Mohanty, & Nayak, 2015) The freezing and melting temperatures of YSG compared to the other three fish skin gelatins were higher, but the freezing and melting points of cowhide gelatins, goatskin and camel skin gelatins were significantly higher than those of most fish gelatins (Abuibaid, AlSenaani, Hamed, Kittiphattanabawon, & Maqsood, 2020; Almeida & da Silva Lannes, 2013; Mad-Ali, Benjakul, Prodpran, & Maqsood, 2017), Generally speaking, gelatin from cold-water fish has a lower freezing point than gelatin from warm-water fish. The differences in molecular weight and the relative amount of α- and β-chain components among gelatins from different species could be the cause of the variances in gelling temperatures (Reategui-Pinedo et al., 2022), It has been claimed that sub amino acids like proline and hydroxyl proline, which form hydrogen bonds and stabilize the triple helix structure, are substantial contributors to gel strength, however this is due to differences in the quantity of these sub amino acids. From the results of amino acid analysis of the four fish skin gelatins, it can be seen that YSG has the highest content of proline and thus can form more hydrogen bonds, thus increasing the stability of the gelatins, and from the infrared analysis of the four gelatins, it can also be seen that YSG generates the most chemical bonds. Gelatin gels melted at higher temperatures have been reported to provide better texture when consumed (Sinthusamran et al., 2014) and the gel can be maintained at room temperature for a longer period of time, which ultimately increases the application options for gelatin.

3.9. Flow behavior of gelatin

The Fig. 2C. shows the variation of the flow behavior of the four fish skin gelatins with shear rate at room temperature. All gelatin samples have a tendency to become less viscous as the shear rate rises. This is attributed to the disruption of intermolecular protein forces under high shear, leading to an elevation in free water content and consequently a reduction in viscosity.

3.10. Interfacial properties of gelatin

The Table 3. displays the EAI and ESI of fish skin gelatin that was isolated from several species. The emulsifying properties of gelatin enable it to form stable emulsion systems in food products, making it particularly important in the production of emulsified foods such as ice cream, milkshakes, and mayonnaise(Hasibuan, Harianto, & Baga, 2023). Gelatin's emulsifying action enhances the texture and mouthfeel of foods, resulting in a smoother and more uniform consistency. Foaming ability refers to gelatin's capacity to generate and stabilize foam, a characteristic that is highly beneficial in the preparation of baked goods such as cakes, cookies, and bread. By promoting foaming, gelatin increases the volume and softness of food products, thereby improving their appearance and texture(Rozhno, Podobiy, & Yurchak, 2016). The EAI values of the four species of fish skin gelatin were not significantly different, and all of them were in the range of 23.87–27.81 ($p < 0.05$). The increase in EAI may be due to the fact that the gelatin protein of FSG was denatured more than the other three species of fish skin gelatin, which may produce more short peptides, this could enhance the solubility of gelatin and expedite its migration to and localization at the oil-water interface, thereby improving its performance. The decrease in ESI may be due to the inability of shorter peptides to form a stronger cohesive film around the oil droplets (Kaewruang, Benjakul, & Prodpran, 2013). Moreover, longer chains might be able to create a robust coating surrounding the oil droplets, enhancing the emulsion's stability and contributing to ESI. The trend of the change of EAI and ESI shows that EAI shows a negative correlation to ESI, which means that a larger EAI will have a smaller ESI. Proteins' capacity to move, adsorb, and reorient at the air-water interface often influences FA and FS (Maqsood et al., 2019). Through interactions between water, oil, and other components, functional qualities like as water retention and fat-binding capability are closely related to texture. FS and FS are shown in Table shown, the four fish skin gelatins again showed no significant differences. (Amagliani & Schmitt, 2017) reported that proteins that adsorb quickly, foam to form viscoelastic membranes, and unfold and reorient at interfaces have better foaming ability.

3.11. SEM

The results of the scanning electron microscopy, as displayed in the above Fig. 3, showed that the microstructures of the four fish skin gelatins were lamellar, with a looser structure and larger matrix voids, and the FSG and SSG gelatins had more structural folds under the same magnification, while the LSG and YSG surfaces were smoother. Gel strength of gelatin is generally influenced by the arrangement and interactions of protein molecules inside the gel matrix (Benjakul, Oungbho, Visessanguan, Thiansilakul, & Roytrakul, 2009). Thus LSG and YSG protein molecules are more tightly packed than FSG and YSG protein molecules.

4. Conclusion

This study comprehensively analyzed the functional properties of gelatins extracted from four fish by-products: frigate mackerel, skipjack tuna, longjack tuna, and yellowfin tuna skin. While minor differences were observed in extraction yields, interfacial properties, and molecular weight distributions among the four sources, all exhibited promising functional characteristics, supporting their potential as sustainable alternatives to mammalian gelatin. Notably, yellowfin tuna skin gelatin (YSG) demonstrated superior performance in key metrics, including higher gel strength and enhanced thermal stability, aligning with its elevated imino acid content. However, YSG still underperforms

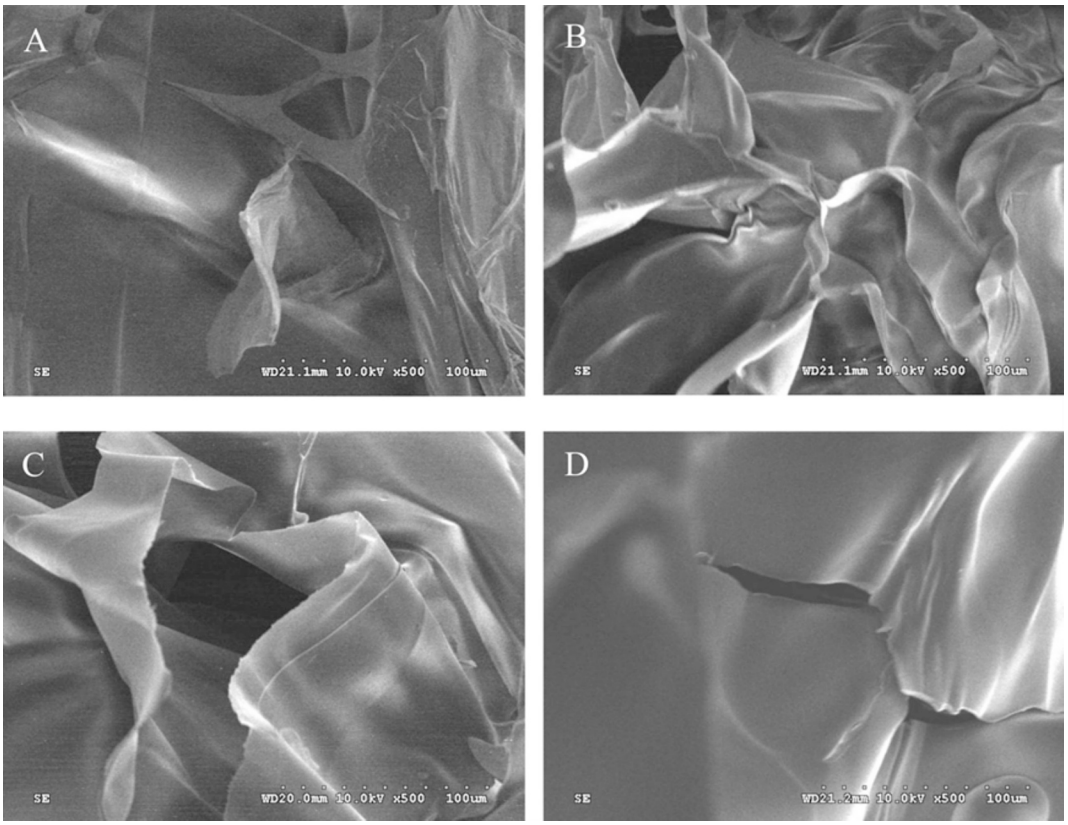

Fig. 3. SEM patterns of gelatins of different origin. A: FSG(frigate mackerel skin gelatin), B:SSG(Skipjack tuna skin gelatin),C: LSG(Longtail tuna and skin gelatin), D: YSG(Yellowfin tuna skin gelatin).

compared to mammalian gelatin in critical aspects. For instance, its gel strength (271 g) remains significantly lower than porcine gelatin (466.4 g) and bovine gelatin (457.57 g), primarily due to lower imino acid content. Additionally, fish gelatins generally exhibit reduced viscosity and melting points, which may limit their application in high-temperature processing. These limitations necessitate targeted optimizations, such as enzymatic crosslinking or hybrid formulations with plant-based stabilizers, to bridge the performance gap. Despite these challenges, YSG shows exceptional promise in niche applications, including cold-set desserts, bioactive packaging, and low-heat dairy products, where its moderate gel strength and biocompatibility are advantageous. To validate broader industrial applicability, future studies should focus on scaling extraction protocols, cost-benefit analysis, and long-term stability testing under commercial conditions.In conclusion, yellowfin tuna skin represent a viable and eco-friendly source of gelatin for specific sectors of the food and pharmaceutical industries. While not a universal replacement for mammalian gelatin, their tailored use, coupled with ongoing process refinements, could significantly reduce reliance on traditional animal-derived counterparts.

CRediT authorship contribution statement

Qinghe Yin: Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Haohao Shi:** Writing – review & editing, Visualization, Funding acquisition, Conceptualization. **Yongqiang Zhao:** Writing – review & editing, Resources, Conceptualization. **Gang Yu:** Writing – review & editing, Resources, Conceptualization. **Haohao Wu:** Writing – review & editing, Visualization, Funding acquisition, Conceptualization. **Guanghua Xia:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Tao Yang:** Writing – review & editing, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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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Data availability

Data will be made available on request.

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