Research Article

Gelatin Gel from By-products of Sand Jellyfish (*Rhopilema hispidum*): Physicochemical and Biochemical Characterization

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Abstract
Salted sand-type jellyfish by-products are abundant in collagen, which may be processed into gelatin to decrease food waste. From the production standpoint, various factors affect gel qualities, including raw material used, pretreatment methods, and extraction times. So far, gelatin extracted from desalted sand-type jellyfish by-products (D-SJB) must be adequately characterized. Therefore, this research aimed to characterize gelatin from D-SJB using different pretreatment methods and extraction times. D-SJB was treated with 0.2 M hydrochloric acid (acid method) and extracted for 24 h at 60 °C (SA24), the optimal gelatin extraction condition with the highest gel qualities, while the jellyfish gelatin obtained after D-SJB was treated with pepsin and extracted for 48 h had the lowest gel qualities. The viscosity, gel strength, gelling temperature, and melting temperatures of SA24 were 20.80 cP, 352.22 g, 11.97 °C, and 22.70 °C, respectively. All jellyfish gelatin's gelling and melting temperatures ranged from 6.13−11.97 °C and 15.85−22.70 °C, exhibiting a cold set gel and unstable gels at room temperature. The different pretreatment methods and extraction times during the jellyfish gelatin production resulted in the conversion of amides A, B, I, II, and III, especially the wavenumber of the amide I increased after pepsin pretreatment and increased with longer extraction time. Twenty-one collagen subtypes in bovine, fish, and jellyfish gelatin were analyzed using LC-MS/MS. The collagen alpha-2(I) chain, a key gelatin component, was identified in all gelatins. The research novelty showed the profound characterization results of gelatin gel

produced from D-SJB. However, further experiments will be needed for pilot-scale production to be used in food and non-food applications.

Keywords: Collagen, Extraction, Gelatin, Gel properties, Jellyfish, Pretreatment methods

1 Introduction

Marine gelatin, a protein hydrocolloid, is produced from collagen hydrolysis with alkaline, acid, or enzyme [1]. One of the most intriguing properties of gelatin is a thermoreversible property, giving the gelatin gel melts below body temperature, known as melt-in-mouth properties [2]. Gelatin can be produced from various sources, such as bovine, porcine, and marine products. Marine gelatin has received much attention among these raw materials due to its no biological contaminants, excellent biocompatibility, ease of biodegradation, and certification of certain religions, predominantly Muslim (Halal) and Jewish (Kosher) [3], [4]. Marine gelatins are widely used in food and biomedicine industries as stabilizers, thickeners, emulsifiers, foaming agents, water binders, cryoprotectants, and biopolymers [5]–[8]. Marine gelatins are usually produced from by-products due to the vast quantity of waste from seafood processing at low cost and their similar functional properties to mammalian gelatin [9].

Apart from species of marine, jellyfish is an attractive alternative raw material for producing gelatin food ingredients. Edible jellyfish in Thailand, sand-type jellyfish (*Rhopilema hispidum*), and white-type jellyfish (*Lobonema smithii*) have been consumed as food and exported to various Asian countries [10]. Many by-products of salted jellyfish occur during the trimming process for export. These by-products are abundant in collagen but have minimum fat and cholesterol. Klaiwong *et. al*., [11] reported that sand-type jellyfish (3.22%–4.00%) had a higher collagen content than white-type jellyfish (2.48%–2.80%). In sand-type jellyfish, the amino acids indicating collagen, including glycine, hydroxyproline, and proline, were 55.45, 17.18, and 26.18 mg/100 g samples. In contrast, those of amino acids in white-type jellyfish were 33.79, 12.85, and 17.79 mg/100 g samples, respectively [11]. Therefore, salted sand-type and white-type jellyfish by-products offer great potential as a source of marine gelatin.

However, further research is needed on the characteristics of marine gelatin from sand jellyfish because the physical properties of sand-type jellyfish gelatin have yet to be sufficiently studied, and sophisticated biochemical analyses are still required. Cho *et al*., [12] reported that sand-type jellyfish gelatin exhibited a lower gel strength (31.2 kPa) than other marine and mammalian gelatins. Compared to the previous research, the gelatin qualities of bovine and fish gelatins were higher than those of jellyfish gelatin obtained from white-type jellyfish [13], [14]. Lueyot *et al*., [13] reported that the melting temperature and gel strength of jellyfish gelatin after white-type jellyfish treated with hydrochloric acid (0.2 M HCl) and extracted for 12 h were 20.5 °C and 323.74 g, respectively, displaying unstable gel structure at room temperature. Meanwhile, Charoenchokpanich *et al*., [15] reported that the maximum gel strength of jellyfish gelatin from by-products of white-type jellyfish was 460.02 g when extracted for 24 h at 60 °C.

For gelatin production, factors influencing gelatin's gel properties are differences in raw materials, pretreatment method, extraction temperature, and extraction time [16]. Gelatin qualities may be affected by the differences in raw materials because of differences in amino acid content and collagen type [13], [14]. Lueyot *et al*., [14] reported that jellyfish gelatin after the sample underwent 40 min of sonication and extracted for 4 h at 80 °C gave lower gel strength (447.01 g) than fish gelatin (640.65 g) and bovine gelatin (540.06 g)—the pretreatment methods influence gelatins' overall qualities [17], [18]. The acid pretreatment method produces low molecular weight (MW), quickly destroying gelatin structures and contaminating the environment [17]. The protease (pepsin and trypsin) pretreatment resulted in increased gelatin yield and shorter extraction times, which depended on the types and activities of the enzyme. Additionally, protease enzymes cleave specific peptide bonds, resulting in a wide range of protein molecular weights [17], [19]. Zhang *et al*., [17] reported that the maximum gel strength of gelatin from tilapia skin was 725 g after pretreatment with pepsin. In contrast, the gel strength of tilapia gelatin after pretreatment with 0.05 M acetic acid was 144 g. Jridi *et al*., [18] reported that the cuttlefish skin gelatin had the highest gel strength (197 g) after pretreatment with pepsin (5 units/g of skin). However, the characteristics of sand-type jellyfish

pretreated using acid and pepsin pretreatment and extraction methods have not been systematically studied.

This study aimed to compare the influence of gelatin extraction from salted sand-type jellyfish by-products (umbrella part; S-SJB) using different pretreatment methods (acid and pepsin methods) and extraction times (24 and 48 h). The characteristics of sand-type jellyfish gelatin were compared to commercial gelatins (bovine and fish gelatin). LC-MS/MS determined the differentiation of each gelatin's collagen type and peptide sequences. The findings of this research will provide valuable insights into the potential industrial applications of sand-type jellyfish gelatin, making it a crucial contribution to food science, biotechnology, and marine biology.

2 Materials and Methods

2.1 *Materials*

Salted sand-type jellyfish by-products (S-SJB) were obtained from Chock Dee Sea Products Co., Ltd., Samut Songkhram, Thailand. Commercial bovine gelatin (bovine gelatin) and commercial fish gelatin (fish gelatin) were purchased from JR F&B Co., Ltd., Thailand.

2.2 *Jellyfish gelatin preparation from S-SJB*

Figure 1 provides an overview of this study's gelatin extraction from S-SJB. 1000 g of S-SJB was washed on two cycles for 15 min/cycle using a washing machine [20]. Then, the desalted sand-type jellyfish by-products (D-SJB) were analyzed for chemical composition and extracted into gelatin.

The extraction of jellyfish gelatin was a meticulous process, following the slightly modified methods from Zhang *et al*. [17]. First, 100 g of D-SJB were agitated in sodium hydroxide (0.05 M; 300 mL) at 4 °C using a precise shaking incubator (WIS-20R, WiseCube, Korea) for 1 h at 150 rpm and then washed at three cycles using a washing machine.

After alkali pretreatment, the sample was treated by two methods: 1) Acid method: The sample was immersed in 0.2 M HCl (200 mL) for 2 h at 150 rpm at 25 °C and washed at three cycles using a washing machine. At the same time, 0.2 M HCl solution was replaced every 60 min. Distilled water (200 mL) was added; 2) Pepsin method: The sample was soaked with 200 mL of distilled water, pre-incubated for 10 min at 95 °C to inactivate the endogenous enzymes, and adjusted to a pH of 2.0. Pepsin enzyme was added with 5 units/g of the sample. After that, the sample was incubated at 150 rpm for 2 h at 37 °C. Subsequently, the enzymatic reaction was stopped for 10 min at 95 °C and then adjusted to a pH of 7.0.

After these pretreatments, the sample was subjected to hot water extraction at 60 °C for 24 and 48 h using a water bath shaker (W350, Memmert, Germany) at 80 rpm. The gelatin solution was filtered through filter paper (Whatman No. 4) and freeze-dried using a freeze dryer (Alpha1−4 LSCplus, Martin Christ, Germany). The obtained freeze-dried jellyfish gelatins were packed in an aluminum foil bag for further analysis.

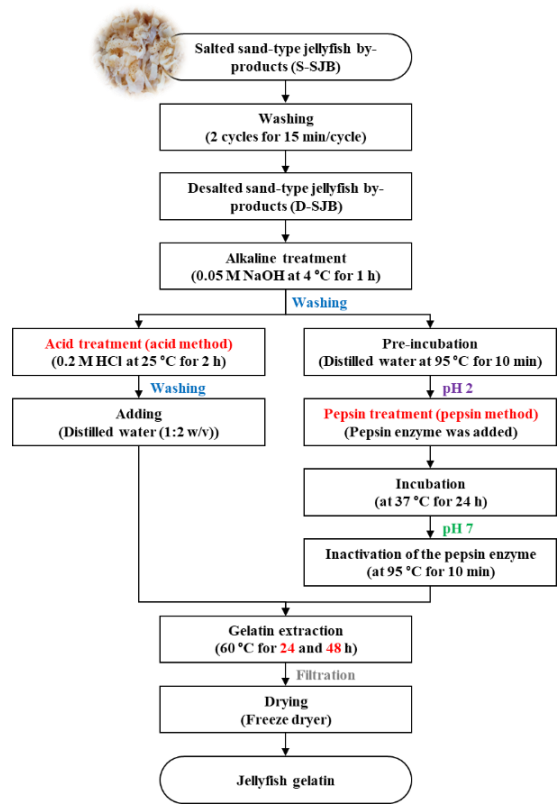

Figure 1: An overview of the jellyfish gelatin extraction from D-SJB with different pretreatment methods and extraction times.

2.3 *Analysis*

2.3.1 Chemical composition and salt content

Protein, ash, moisture, and fat contents of S-SJB and D-SJB were determined according to AOAC methods [21]. The Kjeldahl method determined the total nitrogen content of S-SJB and D-SJB. The nitrogen-to-protein conversion was calculated using a factor of 5.55.

S-SJB and D-SJB were analyzed using a conductivity meter (TDS Meter 308, Systronics, India) for salt content analysis. The standard curve was used to extrapolate the salt percentage [22].

2.3.2 Gelatin yield (%)

The calculation of extraction yield (%) was performed by Equation (1), as shown below:

$$\textit{Gelatin yield}\ (\%) = \left(\frac{M_2}{M_1}\right) \times 100 \quad (1)$$

where M_1 is the weight of the freeze-dried S-SJB (g), and M_2 is the weight of the freeze-dried jellyfish gelatin (g).

2.3.3 Molecular weight of gelatin protein

The molecular weight of jellyfish, bovine, and fish gelatins were compared using SDS-PAGE (Sodium dodecyl sulfate-polyacrylamide gel electrophoresis) following the slightly modified methods from Laemmli [23]. The protein band of each sample was compared to the standard high molecular weight protein marker (PageRuler™ Plus Prestained Protein Ladder, Thermo Fisher Scientific, USA). Briefly, gelatin samples were desalted overnight through dialysis. The gelatin solution (1 mg of protein) was combined with 3X sample buffer in a 2:1 (v/v) ratio and boiled at 95 °C for 3 min. Afterward, the sample (20 μL) was loaded onto a gel (12% separating gel and 5% stacking gel). The electrophoresis process was performed using an applied voltage of 50 V over 4 h. The 0.1% Coomassie Brilliant Blue was used as the staining color and then destained with 20% ethanol and 10% acetic acid.

2.3.4 Viscosity

Jellyfish, bovine, and fish gelatins (6.67%) were analyzed for viscosity using a Brookfield viscometer (DV–II+, Brookfield, USA) at room temperature and a speed of 90 rpm [13].

2.3.5 Gel strength

10 mL of gel solution prepared at the concentration of 6.67% from each jellyfish, bovine, or fish gelatins solution was set in a vial (58 mm in height and 27 mm in diameter) for 18 h at 4 °C. Subsequently, the gelatin samples were subjected to gel strength analysis using a texture analyzer (TA-XT2i, Stable Micro Systems, UK) equipped with a 1.27 cm in diameter (P/0.5R) flat-faced cylindrical Teflon plunger [22].

2.3.6 Thermal stability

The gelatin solutions (6.67%) were subjected to thermal stability analysis for gelling and melting temperature using a rotational rheometer (KNX2312, Malvern Instruments, UK), which was carried out as described by Charoenchokpanich *et al*. [15].

2.3.7 Color

The gelatin solutions (6.67%) were measured using a colorimeter (ColorFlex EZ, Hunter Lab, USA). The color was expressed as three values: L^* represents lightness, a^* stands for red to green, and b^* stands for blue to yellow. The hue angle (h^*) of gelatin was calculated by Equation (2), as shown below:

$$h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (2)$$

2.3.8 FTIR spectroscopic analysis

The commercial and jellyfish gelatins were subjected to infrared spectra using the FTIR spectrometer (Frontier, PerkinElmer, USA). The measurement was carried out with a resolution of ± 4 cm^{-1} in the spectral region of 4000 to 400 cm^{-1}, and four scans per sample.

2.3.9 Identification of gelatin peptide sequence

Before loading the sample into LC-MS/MS, gelatin was initially desalted overnight under dialysis. Then, samples were digested with trypsin for 24 h at 37 °C at a ratio of 1:20, followed by drying using a SpeedVac evaporator (Eppendorf Concentrator Plus, Germany) [13].

For identification of tryptic peptide samples, LC-MS/MS was a method of choice using an Ultimate 3000 Nano/Capillary LC System (Thermo Scientific, Germany) coupled to Hybrid quadrupole Q-TOF impact II™ (Bruker Daltonics, Germany) equipped with a Nano-captive spray ion source. The dried tryptic peptide samples were dispersed in 0.1% formic acid and injected into a C18 reversed-phase column having 0.075 mm of diameter, 150 mm of length and packed with an Acclaim PepMap® RSLC C18, 3 μm, pore size 100 Å, nanoViper (Thermo Scientific, Germany). Solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in 80% acetonitrile) were used in the analytical column. A 5–55% gradient of solvent B was employed to elute the peptides at a constant flow rate of 300 μL/min over 45 min and a column temperature of 40 °C. Electrospray ionization was carried out at 1.6 kV using the CaptiveSpray. Nitrogen was used as a drying gas at a flow rate of about 50 L/h. Collision-induced-dissociation (CID) product ion mass spectra were obtained using nitrogen gas as the collision gas. Mass spectra (MS) and MS/MS spectra were received at 2 Hz over the range (m/z) of 150 to 2200 (Compass 1.9 software, Bruker Daltonics). The collision energy was adjusted to 10 eV as a function of the m/z value.

The MaxQuant 2.4.10.0 was employed to quantify individual samples bioinformatically, and MS/MS spectra were matched to the UniProt database using the Andromeda search engine [24]. UniProt database provided the protein dataset, which included the collagen alpha-1 chain and collagen alpha-2 chain of jellyfish, bovine, and fish. Potential contaminants that did not correspond to any UPS1 protein were removed from the data set using Perseus software version 2.0.6.0 [25]. Maximum intensities were $\log_2$ transformed. The missing values were imputed and replaced with a constant value of zero [26]. The statistical analyses and visualization of the LC-MS data, including partial least squares-discriminant (PLS-DA) analysis and heatmap, were performed using MetaboAnalyst 6.0 [26].

2.4 *Statistical analysis*

Statistical significance difference (p-value < 0.05) of the results was determined through analysis of variance (ANOVA) and Duncan's multiple range test using Statistical Package for Social Science (SPSS; 22.0).

3 Results and Discussions

3.1 *Chemical composition and salt content*

The protein, ash, and moisture contents of S-SJB (wet weight basis) were 3.04 ± 0.09%, 25.28 ± 0.81%, and 68.82 ± 0.14%, respectively. Meanwhile, the protein, ash, and moisture contents of D-SJB were 3.40 ± 0.29%, 0.65 ± 0.09%, and 95.18 ± 0.17%, respectively (wet weight basis). S-SJB and D-SJB had a fat content of 0.99% ± 0.03%. The salt content of S-SJB and D-SJB was 16.90 ± 0.79% and 1.28 ± 0.03%, respectively. The washing process of salted jellyfish is essential because salted jellyfish consists of salt (sodium chloride), alum, and sodium bicarbonate, especially salt, from the preservation or dehydration process, resulting in salted jellyfish being very salty [20]. In addition, salt affects gelatin quality [27]. Thus, salted jellyfish by-products must be washed before gelatin extraction. Klaiwong *et al*., [11] reported that salted sand-type jellyfish are abundant in collagen protein, which consists of up to 4% collagen. Therefore, salted sand-type jellyfish may be utilized as a raw material for extracting gelatin.

3.2 *Gelatin yield*

The pretreatment with chemical or enzymatic methods and duration time directly affected the extraction yield. Results showed that the pretreatment with the pepsin method gave a higher gelatin yield than the acid method, and the extraction yield increased with increasing extraction time. These findings have practical implications for the optimization of gelatin extraction processes. The extraction yield of jellyfish gelatin is shown in Table 1. The extraction yield of gelatin obtained from D-SJB treated with 0.2 M HCl (acid method) and extracted for 24 (SA24) and 48 (SA48) h at 60 °C ranged from 9.65%−21.21%. The extraction yield of gelatin obtained from D-SJB treated with pepsin (pepsin method) and extracted for 24 (SE24) and 48 (SE48) h at 60 °C ranged from 37.06%−47.98%.

Table 1: Extraction yield of jellyfish gelatins obtained with different pretreatment methods and extraction times.

Gelatin Samples	Extraction Yield* (%)
SA24	9.65 ± 0.49^{d}
SA48	21.21 ± 0.8^{c}
SE24	37.06 ± 0.40^{b}
SE48	47.98 ± 0.33^{a}

*Different superscript letters ($^{a–d}$) within the same column have a significant difference (p-value < 0.05).

The extraction yield of SA48 (21.21%) was lower than that of the white-type jellyfish treated with the acid method and extracted for 48 h, which was 29.73% [15]. However, the extraction yield of the SE48 sample was the highest (47.98%). When D-SJB was hydrolyzed with either hydrochloric acid or pepsin, the collagen triple-helix structure stabilized by hydrogen, and the covalent bonds of the collagen structure were destroyed [28]. The pepsin enzyme pretreatment hydrolyzes inter-molecular and intra-molecular covalent cross-linked bonds [29]. As a result, the helix-to-coil transition increases and converts collagen into soluble gelatin. Sinthusamran *et al*., [30] reported that the collagen triple-helix and the covalent cross-links between collagen molecules are affected by the extraction time and temperature during gelatin extraction, resulting in the extraction yield increased with increasing extraction time.

3.3 *Molecular weight of gelatin protein*

The SDS-PAGE protein profiles of bovine, fish, and jellyfish gelatin are compared and shown in Figure 2. The protein molecular weight (MW) of SA24 and SA48 was 35–180 kDa, whereas the MW of 20–135 kDa was found in SE24 and SE48. Meanwhile, the MW of 25–245 kDa and 48–245 kDa were observed in bovine and fish gelatin, respectively. Figure 2 shows SA24, SA48, SE24, and SE48 samples having α_1-chain (135 kDa) and α_2-chain (113 kDa).

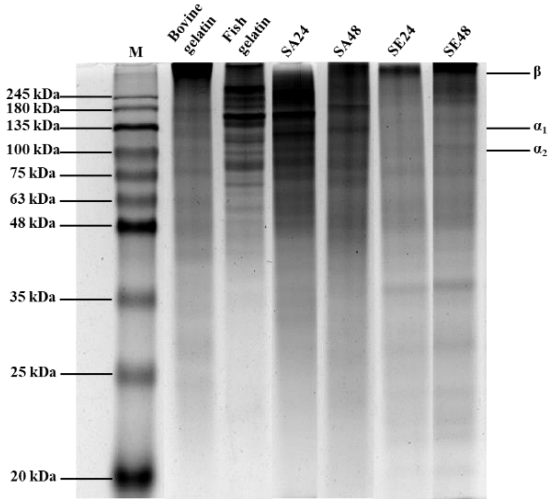

Figure 2: Protein pattern of MW standard (M; Marker), bovine, fish, and jellyfish gelatins obtained with different pretreatment methods and extraction times.

All jellyfish gelatins had a lower MW than bovine and fish gelatins. Focusing on jellyfish gelatin, the gelatin treated with the acid method had a higher MW than the gelatin sample treated with the pepsin method. Pepsin pretreatment cleaved collagen protein chains at specific amino acid chains and was extracted with hot water after a sample was treated with an enzyme, yielding higher numbers of short-chain proteins than HCl pretreatment. The degradation of the α-chains, β-chains, and γ-chains in the significant protein occurred as the extraction temperature increased, resulting in shorter protein chains or increased low MW peptides. The obtained hydrolyzable collagen's interchain cross-links result in gelatin with various MW peptides [31]. The MW distribution, protein structure, and α-chain content in gelatin may influence gelatin's physical properties [32], [33].

3.4 *Viscosity*

Viscosity is the physical quality of gelatin, indicating its resistance to flow. Viscosity testing is carried out to determine the gelatin's viscosity level. The viscosity of bovine, fish, and jellyfish gelatin is presented in Table 2. Jellyfish gelatins ranged in viscosity values from 8.42 cP to 20.80 cP. The viscosity of jellyfish gelatin after pretreatment with the acid method (18.55–20.80 cP) was higher than that of the pepsin method (8.42–11.35 cP). The duration of extraction time also affects gelatin's viscosity, which decreases viscosity as extraction times increase. The viscosity of SA24 had the highest (20.80 cP), while SE48 had the lowest (8.42 cP). Compared to commercial gelatins and jellyfish gelatin in previous works, the viscosity of SA24, SA48, SE24, and SE48 was lower than bovine gelatin (28.85 cP), fish gelatin (24.26 cP), and white-type jellyfish gelatin extracted for 24 h at 60 °C (24.45 cP) [15]. Various factors affected the gelatin's viscosity, including raw materials, pretreatment methods, gelatin extraction conditions, and the test instrument's precision and reproducibility [13]. Nevertheless, the high viscosity of jellyfish gelatin after acid pretreatment can be utilized in food and non-food products such as sauces, salad dressings, and film formation to achieve the desired thickness and consistency. In contrast, the low viscosity of jellyfish gelatin after pepsin pretreatment might be used as a suspending, stabilizer, and emulsifier agent. Increasing the concentration of gelatin, the high molecular weight of gelatin, the

molecular size distribution of proteins, the longer collagen protein chain, and the quantity and types of collagens may contribute to the high viscosity of jellyfish gelatin [13], [14]; moreover, these results might be increased biological collagen enhancement. Lueyot *et al*., [14] conducted a comprehensive proteomic analysis using LC-MS/MS to identify jellyfish gelatin peptides and compare them to bovine and fish gelatin. They found the collagen alpha-2(I) chain in all gelatin, which is a specific peptide sequences that affect gelatin qualities; furthermore, they observed distinctiveness in the peptide sequences of jellyfish collagen, which may confirm that collagen of jellyfish is a unique protein. So far, the jellyfish collagen properties need to be better described, and jellyfish collagen should be further studied to understand its full potential, particularly in terms of its usage and consumption.

Table 2: Physical properties of bovine gelatin, fish gelatin, and jellyfish gelatins obtained with different pretreatment methods and extraction times.

Gelatin Properties		Gelatin Samples					
		SA24	SA48	SE24	SE48	Bovine Gelatin	Fish Gelatin
Viscosity* (cP)		20.80 ± 0.68^{c}	18.55 ± 0.02^{d}	11.35 ± 0.78^{e}	8.42 ± 0.16^{f}	28.85 ± 0.12^{a}	24.26 ± 0.23^{b}
Gel strength* (g)		352.22 ± 0.35^{c}	348.38 ± 0.73^{d}	157.41 ± 0.92^{e}	122.00 ± 0.95^{f}	835.42 ± 0.84^{a}	618.32 ± 0.34^{b}
Gelling temperature* (°C)		11.97 ± 0.22^{c}	10.95 ± 0.48^{d}	6.39 ± 0.02^{e}	6.13 ± 0.85^{f}	21.71 ± 0.28^{a}	18.58 ± 0.43^{b}
Melting temperature* (°C)		22.70 ± 0.79^{c}	21.13 ± 0.53^{d}	16.91 ± 0.05^{e}	15.85 ± 0.73^{f}	30.31 ± 0.39^{a}	25.05 ± 0.68^{b}
Color*	*L**	10.85 ± 0.01^{d}	9.77 ± 0.05^{e}	11.62 ± 0.04^{c}	10.54 ± 0.58^{d}	16.98 ± 0.60^{a}	14.02 ± 0.06^{b}
	*a**	0.85 ± 0.23^{a}	0.82 ± 0.06^{a}	0.47 ± 0.13^{b}	0.39 ± 0.03^{bc}	0.14 ± 0.05^{c}	0.78 ± 0.22^{a}
	*b**	1.51 ± 0.11^{d}	1.10 ± 0.12^{e}	1.95 ± 0.02^{c}	1.17 ± 0.13^{e}	4.87 ± 0.12^{b}	5.24 ± 0.18^{a}
	*h** (°)	61.04 ± 6.32^{d}	53.23 ± 4.88^{e}	76.39 ± 3.70^{bc}	71.46 ± 2.56^{c}	88.30 ± 0.64^{a}	81.54 ± 2.15^{ab}

*Different superscript letters ($^{a–f}$) within the same row have a significant difference (p-value < 0.05).

3.5 *Gel strength*

The gel strength of jellyfish, bovine, and fish gelatins (6.67%) is shown in Table 2. Results showed that the gel strength of jellyfish gelatin ranged from 122.00 g to 352.22 g. The samples treated with the acid method exhibited higher gel strength than those treated with the pepsin method; furthermore, the extraction time affected the gel strength of gelatin, which decreased with longer extraction times. The gel strength of SA24 was the highest (352.22 g), while SE48 was the lowest (122.00 g). These results were consistent with the protein pattern. Pretreatment with the pepsin method and the long-duration extraction produced shortened peptide chains instead of long peptide chains (Figure 2). The lower strength of gelatin gel was associated with peptide chains. Ma *et. al*., [34] reported that fragments produced during the protein degradation affected the α-chain's ability to form a gel network. In addition, shorter chains of gelatin protein cannot form a strong inter-junction zone, lowering gelling ability and gel strength [35]. Therefore, the longer protein chain of SA24 can create a more robust gel network, resulting in the highest gel strength. Compared to commercial gelatins, the gel strength of SA24, SA48, SE24, and SE48 was lower than that of fish (618.32 g) gelatin and bovine (835.42 g) gelatin. Compared to jellyfish gelatin in previous studies, the gel strength of SA24 (352.22 g) showed higher strength than that of jellyfish gelatin (323.74 g) after desalted white-type jellyfish by-products soaked with 0.1 M HCl and extracted for 12 h [13]; and jellyfish gelatin (3.4 g) produced from cannonball jellyfish (*Stomolophus meleagris*) after sample immersed with 1.5% citric acid and extracted for 270 min [27]. Meanwhile, the gel strength of SA24 (352.22 g) was lower than that of jellyfish gelatin after the desalted white-type jellyfish by-product's umbrella part was soaked with 0.2 M HCl and extracted for 24 h (460.02 g) [15]. Therefore, raw materials, pretreatment methods, and extraction times may affect the gel strength of gelatin from D-SJB.

3.6 *Thermal stability*

Thermal stability is a significant parameter for measuring gelatin gel's gelling and melting temperature. A gelatin solution forms a compact gel matrix upon cooling by forming three-dimensional networks through hydrogen bonds between the gelatin molecular chains [36]. In contrast, heating causes the resulting gelatin to lead to the reverse process. The gelling and melting temperatures of bovine, fish, and jellyfish gelatins are presented in Table 2. The gelling temperature of jellyfish gelatins obtained by different pretreatment methods and extraction times ranged from 6.13−11.97 °C, whereas the melting temperature of jellyfish gelatins ranged from 15.85−22.70 °C. SA24, SA48, SE24, and SE48 had lower gelling and melting temperatures than bovine (21.71 and 18.58 °C) and fish (30.31 and 25.05 °C) gelatins. The SA24

sample showed the highest gelling (11.97 °C) and melting (22.70 °C) temperatures. However, all jellyfish gelatins' gelling and melting temperatures were lower than commercial gelatins. After treatment with the pepsin method, jellyfish gelatin showed lower thermal stability than the acid method; moreover, the thermal stability decreased with increasing gelatin extraction time. The lower thermal stability may be related to the lower MW distribution (Figure 2). The presence of lower MW peptides formed by enzymatic hydrolysis during pretreatment and increased gelatin extraction time resulted in short molecular chains, reducing the possibility of forming hydrogen bonds and decreasing the gelling and melting temperature [36]. Cao *et al.*, [29] reported that numerous low MW peptides in the gelatin samples after pretreatment with pepsin may influence the gelatin gel network formation. Therefore, the thermal stability of jellyfish gelatin was determined by pretreatment conditions and extraction times.

3.7 *Color*

The color of bovine, fish, and jellyfish gelatin is shown in Table 2; the gels of gelatins are shown in Figure 3. The lightness of SA24, SA48, SE24, and SE48 (9.77−11.62) was lower than that of bovine (16.98) and fish (14.02) gelatins. The lightness of the gelatin treated with pepsin enzyme (10.54−11.62) was higher than that of the gelatin treated with HCl (9.77−10.85).

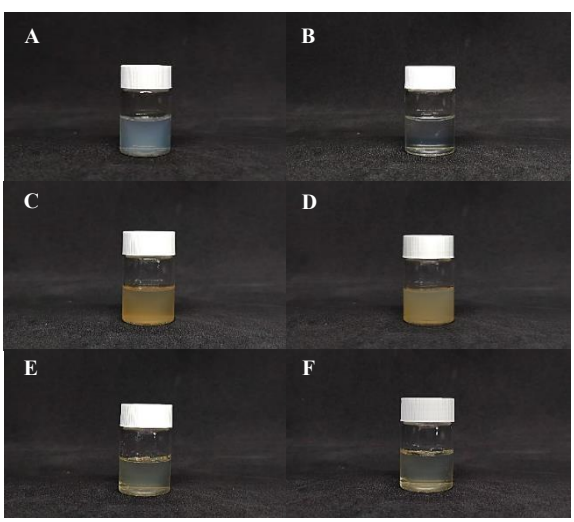

Figure 3: 6.67% of gelatin gel (A) bovine gelatin, (B) fish gelatin, (C) SA24, (D) SA48, (E) SE24, and (F) SE48.

The hue angle (h^*) is related to the variation of redness and yellowness. The h^* of bovine and fish gelatin was 88.30° and 81.54°, while the h^* of jellyfish gelatin ranged from 53.23−76.39°. The h^* of SA24 and SA48 was higher than that of SE24 and SE48. The bovine and fish gelatin gel showed light yellow and light white gel. The gel of jellyfish gelatin appeared dark brown because of a non-enzymatic browning reaction at high temperatures during gelatin extraction (Maillard reaction) [13], [37]. The dark brown of jellyfish gelatin gel may influence its use in food (cheese, yogurt, jelly, and ice cream) and non-food (skin cream, edible film, and hydrogel) products, potentially altering product color or reducing its visual appeal to consumers.

3.8 *FTIR*

The patterns of amide (I, II, III, A, and B) in jellyfish, bovine, and fish gelatin were analyzed, as presented in Figure 4, while the locations of FTIR peaks are shown in Table A1. Gelatin samples include five different FTIR regions: 3400−3440 cm^{-1} (amide A), ~2900 cm^{-1} (amide B), 1600−1700 cm^{-1} (amide I), 1550−1600 cm^{-1} (amide II), and 1220−1330 cm^{-1} (amide III). Amide A corresponds to the stretching vibrations of N−H, while amide B corresponds to the =C−H and NH_3^+ asymmetric stretching vibrations. The amide A band in jellyfish, bovine, and fish gelatin ranged from 3318.46−3414.78 cm^{-1}, while amide B ranged from 2920.43−2935.95 cm^{-1}. The amide A position was shifted to around 3300 cm^{-1} or lower, indicating the participation of the peptide's N−H group in hydrogen bonds, which may be a carbonyl group of the peptide chain [38].

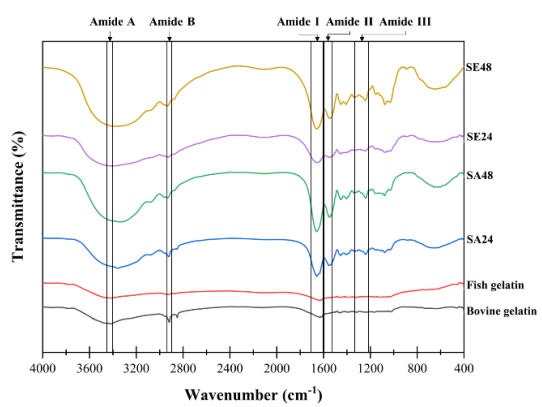

Figure 4: FTIR spectra of bovine gelatin, fish gelatin, and jellyfish gelatins obtained with different pretreatment methods and extraction times.

Amide I appeared in the spectrum between 1600 and 1700 cm^{-1}, related to the stretching vibrations of C=O (carbonyl group). Amide I band in SU24, SU48, SE24, and SE48 samples was detected at 1650.34, 1653.57, 1658.92, and 1660.43 cm^{-1}, respectively. In the amide I band, the wavenumber of jellyfish gelatin after acid pretreatment (1650.34−1653.57 cm^{-1}) was lower than that of jellyfish gelatin after enzyme pretreatment (1658.92−1660.43 cm^{-1}). Furthermore, jellyfish gelatins (1650.34−1660.43 cm^{-1}) had a higher wavenumber of amide I than bovine gelatin (1618.47 cm^{-1}) and fish gelatin (1626.68 cm^{-1}). The wavenumber of the amide I increased after pepsin pretreatment and increased with increasing extraction time. Collagen protein was denatured into gelatin solution, which may be due to the pepsin pretreatment and increased extraction time, which changed the molecules' structure from an alpha-helical to a random coil [39]. The peak wavenumber of amide II in jellyfish, bovine, and fish gelatin ranged from 1452.34−1461.38 cm^{-1}. The amide II band is usually more responsive to hydration than protein structural changes [40]. The amide III band of 1239.87–1240.76180 cm^{-1} was observed in jellyfish, bovine, and fish gelatins. Amide III includes a combination of peaks representing C−N stretching vibrations, N−H deformation from amide linkages, and absorptions from the CH_2 group's wiggle vibrations in proline side chains and the glycine backbone [38].

3.9 *Identification of jellyfish gelatin peptides*

The difference in the gel qualities of jellyfish gelatin derived with different pretreatment methods and extraction times may be explained by differences in collagen types and peptide sequences of gelatin. LC-MS/MS was utilized to identify, separate, and quantify the level of collagen alpha-1 and alpha-2 proteins in jellyfish, bovine, and fish gelatin. Collagen alpha-1 and collagen alpha-2 proteins determined in bovine, fish, and jellyfish gelatin are shown as a heatmap (Figure 5), and their amino acid sequences are shown in Table A2. PLS-DA was used to demonstrate the separation between different groups of identified collagens. Moderate separation between the identified collagens in the bovine gelatin and those in the jellyfish gelatin was derived with varying pretreatment and extraction times. In contrast, there was an overlap between the jellyfish gelatin (Figure 6). Figure 6 showed that the collagen profile of SA24 was most similar to fish gelatin, followed by SA48, SE24, and SE48, respectively. Meanwhile, the collagen profile of jellyfish gelatin after pretreatment with the acid method (SA24 and SA48) was different from the collagen profile of jellyfish gelatin after pretreatment with the pepsin method (SE24 and SE48) (Figure 6). The levels of collagen alpha-1 and collagen alpha-2 protein in all jellyfish and commercial gelatins differed, which may be due to differences in raw materials, pretreatment methods, and extraction times. Other research supported that the differences in the quantity and type of collagen peptides, the ratio of α-chains to β-chains, and the composition of amino acid influenced gelatin functionalities [13], [15].

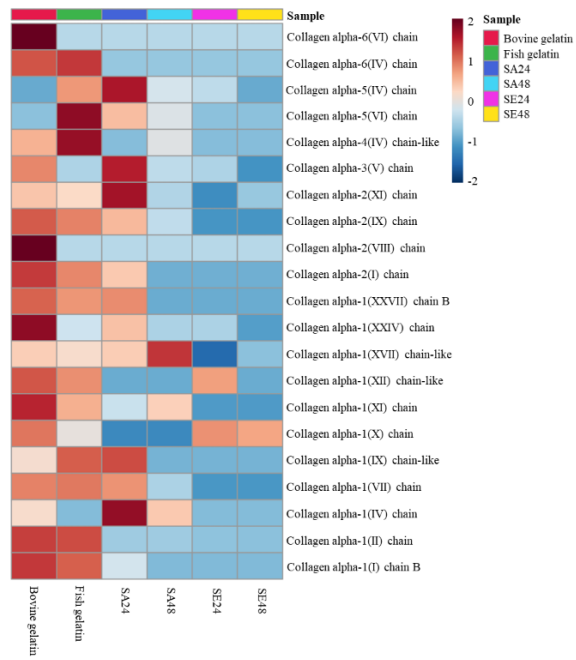

Figure 5: Heat maps of protein types in bovine, fish, and jellyfish gelatins.

Twenty-one collagen subtypes were identified in bovine, fish, and jellyfish gelatins. Bovine gelatin, fish gelatin, SA24, SA48, SE24, and SE48 identified 19, 18, 15, 13, 8, and 6 subtypes, respectively. Number of collagen subtypes detected was lower in fish gelatin than in bovine gelatin. Collagen subtypes observed in bovine, fish, and all jellyfish gelatins were similarly identified in at least three peptides, including the collagen alpha-1(II) chain, collagen alpha-1(VII) chain, and collagen alpha-2(I) chain, while the collagen alpha-2(I) chain is a specific peptide sequences that affect gelatin qualities [13]. However, quantities of collagen alpha-2(I) chain in jellyfish

gelatins were lower than in bovine and fish gelatins (Figure 5). These results were consistent with viscosity, thermal stability, and gel strength, demonstrating lower jellyfish gelatin properties than bovine and fish gelatin.

These results presented unique amino acid sequence verification and detection of multiple collagen subtypes from jellyfish gelatin when gelatin extraction from D-SJB with different pretreatment methods and extraction times (Table A2). Furthermore, protein subtypes and peptide quantities in jellyfish gelatin are compared to bovine and fish gelatins to understand the gelatin properties resulting from protein types, which may help improve the functionality of gelatin.

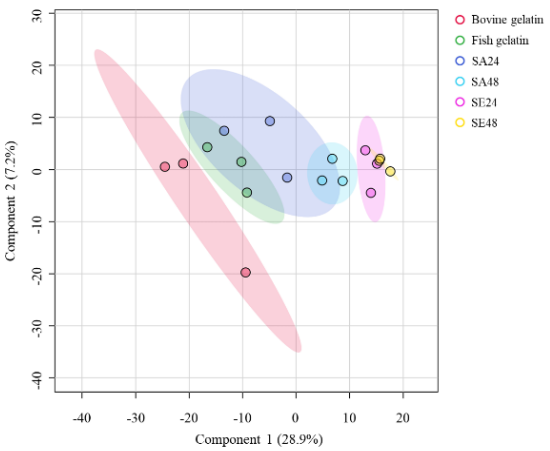

Figure 6: PLS-DA of protein in bovine, fish, and jellyfish gelatins. SA24 was from the extraction of acid methods, 60 °C for 24 h; SA48 was from the extraction of acid methods, 60 °C for 48 h; SE24 was from the extraction of pepsin methods, 60 °C for 24 h; SE48 was from the extraction of pepsin methods, 60 °C for 48 h.

4 Conclusions

D-SJB can produce gelatin using different pretreatment methods and extraction times. The jellyfish gelatin after pretreatment of D-SJB with acid method followed by gelatin extraction with hot water for 24 h at 60 °C (SA24) showed the highest gel properties, especially gel strength (352.22 g), while the gelatin's gel properties after sample treated with pepsin and extracted for 48 h showed lowest. All jellyfish gelatins from D-SJB can form a gel at a temperature higher than 4 °C, whereas the jellyfish gelatins had a higher melting temperature than 15 °C. Compared to the two extraction methods, the gelatin obtained from the pepsin method had lower gel properties than the acid method, and the gelatin's gel properties decreased with increased extraction times. From proteomic analysis, jellyfish, bovine, and fish gelatins were similarly found in at least three peptides, including collagen alpha-2(I) chain (GDPGPPGL). Quantities of collagen alpha-2(I) chain in jellyfish gelatins were lower than in fish and bovine gelatins, affecting the lower gel properties. Therefore, further research on scaling up gelatin extraction and production from D-SJB and applications of jellyfish gelatin obtained from D-SJB in food or biomedical fields will be needed.

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Author Contributions

W.C.: conceptualization, investigation, data analysis, methodology, formal analysis, research design, data curation, visualization, validation, and writing an original draft; B.T. and S.R.: conceptualization, methodology, formal analysis, validation, writing-reviewing and editing, project administration, funding acquisition, and supervision; P.M., S.C., and B.W.: methodology; V.R. and F.C.: validation, and writing-reviewing and editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] A. Hamzeh, S. Benjakul, T. Sae-Leaw, and S. Sinthusamran, "Effect of drying methods on gelatin from splendid squid (*Loligo formosana*) skins," *Food Bioscience*, vol. 26, pp. 96–103, Dec. 2018, doi: 10.1016/j.fbio.2018.10.001.

[2] S. Kasapis, "Developing minced fish products of improved eating quality: An interplay of

instrumental and sensory texture," *International Journal of Food Properties*, vol. 12, pp. 11–26, 2009, doi: 10.1080/10942910802252171.

[3] P. Kittiphattanabawon, S. Benjakul, S. Sinthusamran, and H. Kishimura, "Gelatin from clown featherback skin: Extraction conditions," *LWT - Food Science and Technology*, vol. 66, pp. 186–192, Mar. 2016, doi: 10.1016/j.lwt.2015.10. 029.

[4] I. Milovanovic and M. Hayes, "Marine gelatine from rest raw materials," *Applied Sciences*, vol. 8, no. 12, Nov. 2018, Art. no. 2407, doi: 10.3390/ app 8122407.

[5] L. M. Kasankala, Y. Xue, Y. Weilong, S. D. Hong, and Q. He, "Optimization of gelatin extraction from grass carp (*Catenopharyngodon idella*) fish skin by response surface methodology," *Bioresource Technology*, vol. 98, pp. 3338–3343, Dec. 2017, doi: 10.1016/j.bior tech.2006.03.019.

[6] P. Kittiphattanabawon, S. Benjakul, W. Visessanguan, and F. Shahidi, "Cryoprotective effect of gelatin hydrolysate from blacktip shark skin on surimi subjected to different freeze-thaw cycles," *LWT*, vol. 47, no. 2, pp. 437–442, Jul. 2012, doi: 10.1016/j.lwt.2012.02.003.

[7] L. Wang, H. J. Zhang, X. Liu, Y. Liu, X. Zhu, X. Liu, and X. You, "A physically cross-linked sodium alginate-gelatin hydrogel with high mechanical strength," *ACS Applied Polymer Materials*, vol. 3, pp. 3197–3205, May 2021 doi: 10.1021/acsapm.1c00404.

[8] Z. Zhu, S. Ling, J. Yeo, S. Zhao, L. Tozzi, M. J. Buehler, F. Omenetto, C. Li, and D. L. Kaplan, "High-strength, durable all-silk fibroin hydrogels with versatile processability toward multifunctional applications," *Advanced Functional Materials*, vol. 28, Mar. 2018, Art. no. 1704757, doi: 10.1002/adfm.201704757.

[9] T. Huang, Z. C. Tu, X. Shangguan, X. Sha, H. Wang, L. Zhang, and N. Bansal, "Fish gelatin modifications: A comprehensive review," *Trends in Food Science & Technology*, vol. 86, pp. 260–269, Apr. 2019, doi: 10.1016/j.tifs. 201 9.02.048.

[10] M. Omori and E. Nakano, "Jellyfish fisheries in southeast Asia," *Hydrobiologia*, vol. 451, pp. 19–26, May 2001, doi: 10.1023/A:1011879821323.

[11] T. Klaiwong, P. Hutangura, S. Rutatip, P. Wongsa-Ngasri, and B. Thumthanaruk, "Comparative properties of pepsin hydrolyzed jellyfish protein from salted jellyfish," *Journal of Agricultural Science and Technology B*, vol. 4, no. 7, pp. 555–564, 2014.

[12] S. Cho, J. R. Ahn, J. S. Koo, and S. B. Kim, "Physicochemical properties of gelatin from jellyfish *Rhopilema hispidum*," *Fisheries and Aquatic Sciences*, vol. 17, no. 3, pp. 299–304, Sep. 2014, doi: 10.5657/FAS.2014.0299.

[13] A. Lueyot, V. Rungsardthong, S. Vatanyoopaisarn, P. Hutangura, B. Wonganu, P. Wongsa-Ngasri, S. Charoenlappanit, S. Roytrakul, and B. Thumthanaruk, "Influence of collagen and some proteins on gel properties of jellyfish gelatin," *PLoS ONE*, vol. 16, no. 6, Jun. 2021, Art. no. e0253254, doi: 10.1371/journal. pone.0253254.

[14] A. Lueyot, B. Wonganu, V. Rungsardthong, S. Vatanyoopaisarn, P. Hutangura, P. Wongsa-Ngasri, S. Roytrakul, S. Charoenlappanit, T. Wu, and B. Thumthanaruk, "Improved jellyfish gelatin quality through ultrasound-assisted salt removal and an extraction process," *PLoS ONE*, vol. 17, Nov. 2022, Art. no. e0276080, doi: 10.1371/journal.pone.0276080.

[15] W. Charoenchokpanich, P. Muangrod, S. Roytrakul, V. Rungsardthong, B. Wonganu, S. Charoenlappanit, F. Casanova, and B. Thumthanaruk, "Exploring the model of cefazolin released from jellyfish gelatin-based hydrogels as affected by glutaraldehyde," *Gels*, vol. 10, no. 4, Apr. 2024, Art. no. 271, doi: 10.33 90/gels10040271.

[16] U. Rodsuwan, B. Thumthanaruk, O. Kerdchoechuen, and N. Laohakunjit, "Functional properties of type A gelatin from jellyfish (*Lobonema smithii*)," *International Food Research Journal*, vol. 23, pp. 507–514, 2016.

[17] T. Zhang, R. Sun, M. Ding, L. Tao, L. Liu, N. Tao, L. Liu, N. Tao, X. Wang, and J. Zhong, "Effect of extraction methods on the structural characteristics, functional properties, and emulsion stabilization ability of *Tilapia* skin gelatins," *Food Chemistry*, vol. 328, Oct. 2020, Art. no. 127114, doi: 10.1016/j.foodchem.20 20.127114.

[18] M. Jridi, R. Nasri, I. Lassoued, N. Souissi, A. Mbarek, A. Barkia, and M. Nasri, "Chemical and biophysical properties of gelatins extracted from alkali-pretreated skin of cuttlefish (*Sepia officinalis*) using pepsin," *Food Research International*, vol. 54, no. 2, pp. 1680–1687, Dec. 2013, doi: 10.1016/j.foodres.2013.09.026.

[19] M. Jridi, N. Souissi, A. Mbarek, G. Chadeyron, M. Kammoun, and M. Nasri, "Comparative study of physico-mechanical and antioxidant properties of edible gelatin films from the skin of cuttlefish," *International Journal of Biological Macromolecules*, vol. 61, pp. 17–25, Oct. 2013, doi: 10.1016/j.ijbiomac.2013.06.040.

[20] W. Charoenchokpanich, V. Rungsardthong, S. Vatanyoopaisarn, B. Thumthanaruk, and Y. Tamaki, "Salt reduction in salted jellyfish (*Lobonema smithii*) using a mechanical washing machine," *Science, Engineering and Health Studies*, vol. 14, no. 3, pp. 184–192, Sep. 2020, doi: 10.14456/sehs.2020.17.

[21] AOAC, *Official Method of Analysis: Association of Analytical Chemists*, 19th ed., Washington, D.C.: AOAC International, 2012.

[22] W. Charoenchokpanich, P. Muangrod, V. Rungsardthong, S. Vatanyoopaisarn, B. Wonganu, S. Roytrakul, and B. Thumthanaruk, "Effect of hydrochloric acid extraction on yield and gel properties of gelatine from salted jellyfish by-products," in *E3S Web of Conferences*, 2021, Art. no. 02009.

[23] U. K. Laemmli, "Cleavage of structural proteins during the assembly of the head of bacteriophage T4," *Nature* vol. 227, pp. 680–685, Aug. 1970, doi: 10.1038/227680a0.

[24] S. Tyanova, T. Temu, and J. Cox, "The MaxQuant computational platform for mass spectrometry-based shotgun proteomics," *Nature Protocols*, vol. 11, pp. 2301–2319, Dec. 2016, doi: 10.1038/nprot.2016.136.

[25] S. Tyanova, T. Temu, P. Sinitcyn, A. Carlson, M. Y. Hein, T. Geiger, M. Mann, and J. Cox, "The Perseus computational platform for comprehensive analysis of (prote) omics data," *Nature Methods*, vol. 13, pp. 731–740, Sep. 2016, doi: 10.1038/nmeth.3901.

[26] Z. Pang, G. Zhou, J. Ewald, L. Chang, O. Hacariz, N. Basu, and J. Xia, "Using MetaboAnalyst 5.0 for LC-HRMS spectra processing, multi-omics integration and covariate adjustment of global metabolomics data," *Nature Protocols*, vol. 17, pp. 1735–1761, Aug. 2022, doi: 10.1038/s41596-022-00710-w.

[27] P. G. Chiarelli, R. B. Pegg, G. D. Kumar, and K. M. Solval, "Exploring the feasibility of developing novel gelatin powders from salted, dried cannonball jellyfish (*Stomolophus meleagris*)," *Food Bioscience*, vol. 44, Dec. 2021, Art. no. 101397, doi: 10.1016/j.fbio.2021.101397.

[28] B. Wonganu, "Application of gelatin derived from waste tilapia scales to an antibiotic hydrogel pad," in *E3S Web of Conferences*, 2020, Art. no. 03004.

[29] S. Cao, Y. Wang, L. Xing, W. Zhang, and G. Zhou, "Structure and physical properties of gelatin from bovine bone collagen influenced by acid pretreatment and pepsin," *Food and Bioproducts Processing*, vol. 121, pp. 213–223, May 2020, doi: 10.1016/j.fbp.2020.03.001.

[30] S. Sinthusamran, S. Benjakul, and H. Kishimura, "Characteristics and gel properties of gelatin from skin of seabass (*Lates calcarifer*) as influenced by extraction conditions," *Food Chemistry*, vol. 152, pp. 276–284, Jun. 2014, doi: 10.1016/j.foodchem.2013.11.109.

[31] P. Kaewruang, S. Benjakul, T. Prodpran, and S. Nalinanon, "Physicochemical and functional properties of gelatin from the skin of unicorn leatherjacket (*Aluterus monoceros*) as affected by extraction conditions," *Food Bioscience*, vol. 2, pp. 1–9, Jun. 2013, doi: 10.1016/j.fbio.2013.03.002.

[32] A. Erge and Ö. Zorba, "Optimization of gelatin extraction from chicken mechanically deboned meat residue using alkaline pre-treatment," *LWT*, vol. 97, pp. 205–212, Nov. 2018, doi: 10.1016/j.lwt.2018.06.057.

[33] P. Díaz-Calderón, E. Flores, A. González-Muñoz, M. Pepczynska, F. Quero, and J. Enrione, J. "Influence of extraction variables on the structure and physical properties of salmon gelatin," *Food Hydrocolloids*, vol. 71, pp. 118–128, Oct. 2018, doi: 10.1016/j.foodhyd.2017.05.004.

[34] Y. Ma, X. Zeng, X. Ma, R. Yang, W. Zhao, "A simple and eco-friendly method of gelatin production from bone: One-step biocatalysis," *Journal of Cleaner Production*, vol. 209, pp. 916–926, Feb. 2019, doi: 10.1016/j.jclepro.2018.10.313.

[35] V. Normand, S. Muller, J. C. Ravey, and A. Parker, "Gelation kinetics of gelatin: A master curve and network modeling," *Macromolecules*, vol. 33, no. 3, pp. 1063–1071, Feb. 2000, doi: 10.1021/ma9909455.

[36] W. Liao, Y. Zhu, Y. Lu, Y. Wang, X. Dong, G. Xia, and X. Shen, "Effect of extraction variables on the physical and functional properties of

tilapia gelatin," *LWT*, vol. 146, Jul. 2021, Art. no. 111514, doi: 10.1016/j.lwt.2021.111514.

[37] H. Kchaou, N. Benbettaieb, M. Jridi, M. Nasri, F. Debeaufort, "Influence of Maillard reaction and temperature on functional, structure and bioactive properties of fish gelatin films," *Food Hydrocolloids*, vol. 97, Dec. 2019, Art. no. 105196, doi: 10.1016/j.foodhyd.2019.105196.

[38] B. Yesiltas, C. Robert, H. O. Petersen, F. Jessen, F. Ajalloueian, M. A. Mohammadifar, C. Jacobsen, J. J. Sloth, G. Jakobsen, and F. Casanova, "Gelatin from saithe (*Pollachius virens*) skin: Biochemical characterization and oxidative stability in O/W emulsions," *Marine Drugs*, vol. 20, no. 12, Dec. 2022, Art. no. 739, doi: 10.3390/md20120739.

[39] J. H. Muyonga, C. G. B. Cole, and K. G. Duodu, "Fourier transform infrared (FTIR) spectroscopic study of acid soluble collagen and gelatin from skins and bones of young and adult Nile perch (*Lates niloticus*)," *Food Chemistry*, vol. 86, no. 3, pp. 325–332, Jul. 2004, doi: 10.1016/j.foodchem.2003.09.038.

[40] S. Benjakul, K. Oungbho, W. Visessanguan, Y. Thiansilakul, and S. Roytrakul, "Characteristics of gelatin from the skins of bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*," *Food Chemistry*, vol. 116, no. 2, pp. 445–451, Sep. 2009, doi: 10.1016/j.food chem.2009.02.063.

Appendix

Table A1: Peak wavenumbers of bovine, fish, and jellyfish gelatins.

Region	Peak Wavenumber (cm^{-1})					
	SA24	SA48	SE24	SE48	Bovine Gelatin	Fish Gelatin
Amide A	3359.51	3339.55	3324.23	3318.46	3414.78	3406.57
Amide B	2923.80	2935.70	2928.27	2935.95	2920.43	2931.36
Amide I	1650.34	1653.57	1658.92	1660.43	1618.47	1626.68
Amide II	1454.76	1452.34	1456.90	1452.51	1461.38	1457.25
Amide III	1240.57	1240.41	1240.38	1239.87	1242.07	1240.76

Table A2: Protein names of bovine, fish, and jellyfish gelatins.

Protein Names	Peptide Sequences	SA24	SA48	SE24	SE48	Bovine Gelatin	Fish Gelatin
Collagen alpha-1(I) chain B	AAGATGFP	✓				✓	✓
Collagen alpha-1(II) chain	AKGSAGAP	✓	✓	✓	✓	✓	✓
Collagen alpha-1(IV) chain	EQGLPGPDG	✓	✓			✓	
Collagen alpha-1(VII) chain	ACGGSGPD	✓	✓	✓	✓	✓	✓
Collagen alpha-1(IX) chain-like	GEQGPPGEMGPPG	✓				✓	✓
Collagen alpha-1(X) chain	PQGPPGPAGYS			✓	✓	✓	✓
Collagen alpha-1(XI) chain	EEDSYYYEYPYYDDADAKPL EPTT	✓	✓			✓	✓
Collagen alpha-1(XII) chain-like	GESMPLAGEEKTTLSD			✓		✓	✓
Collagen alpha-1(XVII) chain-like	DYYLFWEA	✓	✓		✓	✓	✓
Collagen alpha-1(XXIV) chain	DCQREISD	✓	✓	✓		✓	✓
Collagen alpha-1(XXVII) chain B	GGKGGAGR	✓				✓	✓
Collagen alpha-2(I) chain	GDPGPPGL	✓	✓	✓	✓	✓	✓
Collagen alpha-2(VIII) chain	LPCGGWNCECAF					✓	
Collagen alpha-2(IX) chain	VGKPGGKG	✓	✓			✓	✓
Collagen alpha-2(XI) chain	LVKAQAGLQAFL	✓	✓		✓	✓	✓
Collagen alpha-3(V) chain	LDPDNGTELE	✓	✓	✓		✓	✓
Collagen alpha-4(IV) chain-like	APGCPGGGP		✓			✓	✓
Collagen alpha-5(VI) chain	LVYGPAGP	✓	✓				✓
Collagen alpha-5(IV) chain	EPGDTFDGYYF	✓	✓	✓			✓
Collagen alpha-6(IV) chain	GFCACDGG					✓	✓
Collagen alpha-6(VI) chain	KFVKAFISSV					✓	

"✓" stands for the existence of peptide.