

Influence of extraction times on physical and functional properties of gelatin from salted jellyfish by-products

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Abstract. By-products of the marine industry have gained attention for producing valuable food ingredients like gelatin, which might benefit food applications and decrease food waste. Gelatin is the only protein-based food hydrocolloid, mainly used for gelling, viscosity, or emulsifying in the food industry. So far, a number of researchers have reported that by-products of salted jellyfish can produce jellyfish gelatin. The quality of jellyfish gelatin gel depends on several factors including hydrochloric acid pretreatment, extraction temperature, and extraction time. However, the functional properties such as foaming and emulsifying of jellyfish gelatin are not well understood. This research was aimed at investigating the hydrochloric acid pretreatment effect of extraction times (12, 24, and 48 h) at 60 °C on the resulting gelatin's yield, physical, and functional properties. Results showed that jellyfish gelatin's yield, gel strength, and viscosity significantly increased with increasing extraction times. Jellyfish gelatin yields were 2.74-14.07%. The gel strength of jellyfish gelatin extracted for 48 h (325.97±2.84 g) was higher than that of jellyfish gelatins extracted for 12 h (210.46±3.97 g) and 24 h (261.60±3.25 g). All jellyfish gelatins can form gels at 4 °C. Viscosity values of jellyfish gelatin were 23.00-24.50 centipoise. The foaming capacity and foaming stability of jellyfish gelatin were 12.28-17.54% and 10.52-15.78%, respectively. The emulsification activity index of jellyfish gelatin was 13.11-13.30 m²/g, and the emulsification stability index was 39.19-56.42%. As a result, varied gelatin extraction periods influenced jellyfish gelatin's physical and functional properties, indicating that the extended extraction time of 48 h delivered the jellyfish gelatin that can be used as a foaming and emulsifying agent. Therefore, turning the jellyfish by-products into food ingredients like gelatin would increase product values and potential uses in the food and medical applications.

Keyword. Salted jellyfish by-products, Gelatin, Extraction times, Physical properties, Functional properties

1 Introduction

Gelatin is a natural biopolymer derived from the hydrolysis of collagen-producing from acid-base or enzymatic methods. Due to its functional properties, gelatin has been extensively applied in the food, pharmaceutical, medical, and cosmetic industries. Grand View Research [1] reported that by 2027, the global gelatin market would be expected to reach 6.67 billion US dollars. Around 90% of gelatin production on the market is mainly produced from the bone, ligaments, and skin of bovine and porcine, which is higher than gelatin extraction from marine sources [2-3]. However, gelatin production from these mammalian animals has faced certain religious constraints [4]. Therefore, alternative gelatin, especially marine gelatin, has gradually attracted attention in food and pharmaceutical applications.

Furthermore, there are no biological contaminants in marine gelatin, enough to approve product safety.

Gel strength, viscosity, and setting time are the three important functional properties of gelatin [5]. However, typical marine gelatins have lower gel characteristics. Thus, improves marine gelatins comparable to mammalian gelatin sources are needed. Marine gelatin is mainly produced from by-products to decrease food waste and the functional qualities of marine gelatin. Several researchers have reported gelatin extracted from marine by-products such as cold-water fish (chum salmon [6]), warm-water fish (cobia [7], pangasius catfish [8], and tilapia [9-10]), and jellyfish [11-12].

Thailand is one of the top exporters of edible jellyfish, especially jellyfish (*Lobonema smithii*), generally exported in salted jellyfish form to China, Japan, and South Korea. The Ministry of Commerce [13] report presented exports of salted jellyfish in 2021 with a

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value of greater than 559 million baht. Many salted jellyfish by-products occur at processing steps, with irregularly shaped and broken pieces, which have gained increasing attention for gelatin extraction research. The low price of salted jellyfish by-products but rich in collagen protein with minimal fat content [14-18] have potentially been a source for gelatin extraction. The attempts to improve jellyfish gelatin by varying hydrochloric acid (HCl) pretreatment conditions (concentration and time), extraction temperature, and extraction time [11-12, 15-16] have been reported. Charoenchokpanich *et al.* [12] reported that the condition of 2 h HCl pretreatment at 25 °C and 3 h water extraction at 60 °C was insufficient for the jellyfish gelatin process and delivered the low gel strength of 214.47 g. Research on the functional properties of jellyfish gelatin produced from salted jellyfish by-products has been limited. Therefore, this research aimed to investigate the duration of hot water extraction (at 60 °C) varying from 12, 24, and 48 h on jellyfish gelatin's yield, gel properties, and functional properties. The benefit of this work not only provides alternative raw material for gelatin, but also reduce the food waste production as well.

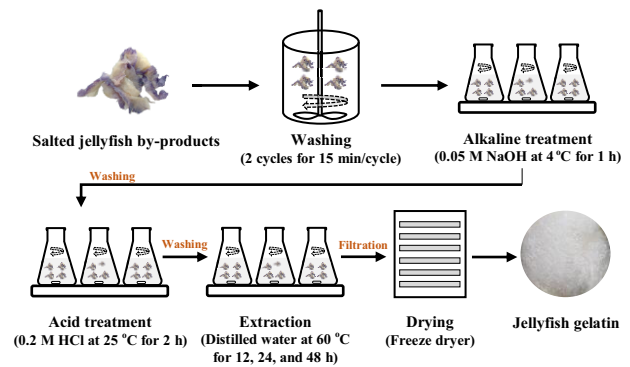


Fig. 1. Process diagram for the preparation of gelatin from salted jellyfish by-products.



Fig. 2. Freeze-dried jellyfish by-products gelatins at various extraction times of 12 (JFG12), 24 (JFG24), and 48 h (JFG48).

2 Materials and methods

2.1 Preparation of jellyfish by-products gelatin

The process diagram for producing jellyfish by-products gelatin is shown in Fig. 1. The salted jellyfish (*Lobonema smithii*) by-products were received from Mahachai Food and Trading Co., Ltd., Samut Sakhon, Thailand. The salted jellyfish by-products were kept at 10 °C and stored in polyethylene (PE) bags until use. A mechanically washing machine briefly performed the wash process at 2 cycles for 15 min per cycle and then drained for 10 min [14, 18].

For gelatin extraction, jellyfish by-products gelatin was prepared according to Lueyot *et al.* [11] and Charoenchokpanich *et al.* [12] with a slight modification. Firstly, the desalted by-products (1 kg) were agitated at 150 rpm and 4 °C for 2 h with 0.5 M sodium hydroxide (NaOH) solution (1:3, w/v) in a shaking incubator (WIS-20R, WiseCube, Korea). The alkali-treated sample was washed with a mechanically washing machine at 3 cycles for 15 min per cycle and then shaken in 0.2 M HCl (1:2, w/v) at a speed of 150 rpm for 1 h at 25 °C. The acid-treated sample was thoroughly washed with a mechanically washing machine at 3 cycles for 15 min per cycle or until neutral pH. The sample was then extracted with distilled water (1:2, w/v) at 60 °C for 12, 24, and 48 h at a speed of 80 rpm in a temperature-controlled water bath shaker (Memmert, Schwabach, Germany) and filtered using a Buchner funnel with Whatman No. 4 filter paper. Finally, the sample was dried with a freeze dryer (GFD5L, Grisriantong, Thailand). The freeze-dried jellyfish by-products gelatins of JFG12, JFG24, and JFG48 were kept in an aluminum foil bag and a desiccator.

2.2 Analysis

2.2.1 Yield

Each freeze-dried jellyfish by-product gelatin sample was weighed and calculated for the yield. The yield was derived from the ratio of the freeze-dried gelatin weight (M_2) to the freeze-dried, salted jellyfish by-products weight (M_1) and calculated using the following equation (1):

$$\text{Yield (\%)} \text{ (dry weight basis)} = (M_2 / M_1) \times 100 \quad (1)$$

2.2.2 Salt (sodium chloride) content

The salt content of the gelatin solution was measured using a conductivity meter (TDS Meter 308, Systronics, India), and the calculated value was extrapolated from the standard sodium chloride 0-1,000 ppm graph [12].

2.2.3 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The preparation of jellyfish by-products gelatin was desalted using a dialysis technique overnight before performing SDS-PAGE. The comparison of protein pattern of jellyfish by-products gelatin was performed using SDS-PAGE according to Laemmli [19] method with a slight modification. The polyacrylamide gel was made up of 5% stacking gel and 12% separating gel. A high molecular weight protein marker (11-250 kDa) was used to estimate the molecular weight of the jellyfish

gelatin protein (PageRuler™ Plus Prestained protein ladder, Thermo Fisher Scientific). All jellyfish by-products gelatin (200 µg protein) samples were heated at 95 °C for 3 min. Then, each cooled sample was loaded onto the gel and subjected to an electrophoresis unit (Mini-slab size electrophoresis system, ATTO, Japan) with a constant current of 50 V in vertical slab gels.

2.2.4 Gel preparation

According to the standard method of GMIA [20], the preparation of gelatin gel (6.67%, w/v) was slightly modified. The freeze-dried jellyfish by-products gelatin was dissolved in deionized water and stirred at 60 °C until completely dissolved. After that, 10 mL of gelatin solution (6.67%) was poured into a vial of 27 mm in diameter and 58 mm in height. The gel sample was kept at 4 °C for 18 before the analysis.

2.2.5 Setting time

The setting time of gelatin was performed as previously described [21] with a slight modification. The jellyfish by-product gelatin solution (6.67%) was prepared using the above method in section 2.2.4. The jellyfish's by-product gelatin in the vial was incubated at 4 °C. During the incubation process, the aluminum needle of 1 mm in diameter and 60 mm in height was inserted into the jellyfish by-products gelatin solution and lifted every 10 s. The setting time of jellyfish by-products gelatin is the amount of time (min) that the needle cannot be separated from the jellyfish by-products gelatin sample.

2.2.6 Determination of gel strength

The gelatin gel sample (6.67%) in a vial from section 2.2.4 was used to determine gel strength using a texture analyzer (TA-XT2i, Stable Micro Systems, UK). The gel strength was performed as previously described [12].

2.2.7 Viscosity

The viscosity of gelatin solution (6.67%) in section 2.2.4 after heating at 60 °C was measured using a Brookfield viscometer (DV - II +, BROOKFIELD, USA) at a speed of 90 rpm and room temperature.

2.2.8 Color measurement

The gelatin gel sample in section 2.2.4 (10 mL) was filled in the glass receptacle and measured using a colorimeter (ColorQUEST 45/0, Hunter Lab, USA). The measured color was expressed as L* (lightness), a* (red-green), and b* (yellow-blue). The hue angle (h*) of gelatin was calculated according to the following equation (2):

$$\text{Hue angle} = \tan^{-1}(b^* / a^*) \quad (2)$$

2.2.9 Foaming properties

The foaming properties of gelatin were estimated as previously described [22] with a slight modification. 20 mL of gelatin solution (1%) was homogenized using a homogenizer (T-25 Ultra-Turrax, IKA Labortechnik, Germany) at speed of 13,000 rpm for 1 min. After that, the mixture was carefully transferred into a measuring cylinder (100 mL) without delay. The sample was then kept at room temperature for 30 min. Finally, the volume was measured. The foaming capacity (FC) and foaming stability (FS) of gelatin were calculated according to the following equation (3) and (4):

$$FC (\%) = [(V_1 - V_0) / V_0] \times 100 \quad (3)$$

$$FS (\%) = [(V_{30} - V_0) / V_0] \times 100 \quad (4)$$

where V_0 = volume before homogenization (mL), V_1 = volume after homogenization (mL), and V_{30} = volume after 30 min (mL).

2.2.10 Emulsifying properties

The emulsion properties of gelatin were determined as previously described [23] with a slight modification. 10 mL of soybean oil was mixed with 6 mL of gelatin solution (1%) and then homogenized using a homogenizer (T-25 Ultra-Turrax, IKA Labortechnik, Germany) at speed of 20,000 rpm for 2 min. The mixed sample was taken out at 0 and 10 min and then mixed with 0.1% sodium dodecyl sulfate (SDS) at a ratio of 1:100 (v/v). The absorbance was measured at 500 nm using a spectrophotometer (SP 830 plus, Metertech, Taiwan). The emulsion activity index (EAI) and emulsion stability index (ESI) of gelatin were calculated according to the following equation (5) and (6):

$$EAI (\text{m}^2/\text{g}) = [(2 \times 2.303 \times A_0 \times N) / (\Phi \times C \times 10,000)] \quad (5)$$

$$ESI (\%) = [A_0 / (A_0 - A_{10})] \times \Delta t \quad (6)$$

where A_0 = absorbance at 500 nm of the sample was kept for 0 min, N = dilution factor, Φ = the volume of oil portion, C = gelatin concentration, A_{10} = absorbance at 500 nm of the sample was kept for 10 min, and Δt = 10 min.

2.3 Statistical analysis

All experiments were performed in triplicate. The results were presented as mean ± standard deviations. Significant differences ($p < 0.05$) between the means values were analyzed by analysis of variance (ANOVA) using Duncan's multiple in the statistical package for social science (SPSS) 22.0.

3 Results and discussions

3.1 Gelatin yield

The gelatin yield of freeze-dried jellyfish by-products gelatins derived from hot water extraction for 12, 24, and 48 h were 2.74%, 4.8 5%, and 14.07% (a dry weight

basis), respectively (Table 1). The gelatin yield of jellyfish by-products gelatin was generally increased as extraction time increased. The jellyfish by-products gelatin yield extracted at 60 °C for 12, 24, and 48 h differed from the previous study. The jellyfish gelatin (type A) pretreated with sulfuric acid (pH 2) solution and extracted at 75 °C for 12 h showed the highest gelatin yield of 39.47% (on a dry weight basis) [15]. The highest gelatin yield of type A gelatin from jellyfish (*Lobonema smithii*) was 40.54% (on a dry weight basis) when pretreated with hydrochloric acid (pH 1) solution and extracted at 75 °C for 12 h [16]. Compared to the same jellyfish by-products, the jellyfish gelatin yield reported in this study was higher than the previous work that showed a gelatin yield of 6.34% (on a dry weight basis) [12], which applied an extraction time of 3 h. It was noted that the longer extraction time than 3 h did increase the gelatin yield. Compared to other marine samples, the highest gelatin yield of blacktip shark and the brown-banded bamboo shark gelatin was 21.17–24.76% and 19.06–22.81% (based on wet weight), respectively, when extracted at 45, 60, and 75 °C for 6 and 12 h [24]. The increased extraction time could destabilize cross-linked proteins at the covalent bonds in the collagen network, resulting in free α -chains or β -chains being liberated from the protein [25]. Consequently, the extraction of gelatin was enhanced, as shown by higher gelatin yield.

Table 1. Physicochemical properties of jellyfish by-products gelatin.

Properties	Sample			
	JFG12	JFG24	JFG48	
Gelatin yield* (%)	2.74±0.36 ^c	4.85±0.58 ^b	14.07±0.53 ^a	
Conductivity ^{ns} (ms/cm)	0.32±0.01	0.32±0.01	0.32±0.01	
NaCl ^{ns} (ppm)	113.01±0.01	113.01±0.01	113.01±0.01	
Setting time* (min)	43.57±1.29 ^a	23.67±1.15 ^b	20.03±0.06 ^c	
Gel strength* (g)	210.46±3.97 ^c	261.60±3.25 ^b	325.97±2.84 ^a	
Viscosity ^{ns} (cP)	23.00±1.00	24.00±1.73	24.5±0.87	
<i>L</i> *	29.43±0.01 ^c	31.29±0.03 ^b	37.16±0.01 ^a	
Color*	<i>a</i> *	1.32±0.02 ^c	1.57±0.01 ^a	1.428±0.03 ^b
	<i>b</i> *	1.23±0.03 ^c	5.10±0.04 ^b	6.92±0.16 ^a
	<i>h</i> *	46.88±0.99 ^a	17.09±0.03 ^b	11.66±0.18 ^c

*Different superscript letters in the same row indicate a significant difference (p<0.05).

^{ns}The samples were not significantly different (p≥0.05).

3.2 Conductivity and salt content

The conductivity of JFG12, JFG24, and JFG48 samples was 0.32 ms/cm, and sodium chloride (NaCl) was 113.01 ppm (Table 1). The different extraction times did not significantly affect jellyfish by-products gelatin's

conductivity and salt content. The salt content of jellyfish by-products gelatin in this study showed lower than in other research. Hydrolyzed salted, dried cannonball jellyfish (*Stomolophus meleagris*) gelatin (H-SDJ) showed a salt content of 56.61% remaining in the sample [26]. This might be due to the unsuccessful removal of salt from the washing and soaking step. The presence of electrolytes has directly influenced low gelatin gel quality. Sow and Yang [27] reported that the addition of 1.5% NaCl into fish gelatin effect reduced gel strength because of loss in molecular order in secondary protein structure. As a result, excess salt content must be first removed prior before continuing further extraction steps.

3.3 Protein pattern

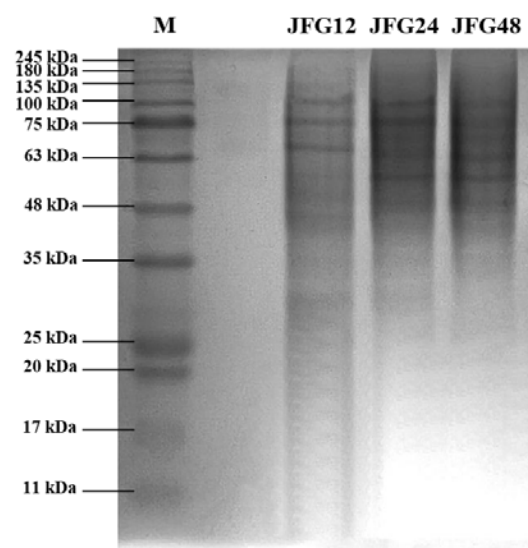


Fig. 3. Protein pattern of jellyfish by-products gelatins (M) molecular weight standard and jellyfish by-products gelatin extracted at different times of 12 h (JFG12), 24 h (JFG24), and 48 h (JFG48).

The protein pattern of jellyfish by-products gelatin is presented in Fig. 3. The protein patterns of JFG12, JFG24, and JFG48 samples found protein having a molecular weight of 65–125 kDa, 56–125 kDa, and 52–125 kDa, respectively. Moreover, the α -chain was observed in all samples, which had MW of 80–125 kDa [28]. The physical and functional properties of gelatins are influenced by their molecular weight distribution, structure, and subunit makeup, which includes α -chain and low-molecular-weight protein fragments. The recovered proteins' molecular weight distribution differed slightly from gelatins extracted with a longer extraction time. The degradation of gelatin's major protein components (α -, β - and γ -chain) became more prominent as the extraction time increased, thereby increasing low molecular weight peptides. The hydrolysis process may affect the molecular weight of the extracted jellyfish by-products gelatin, which contributes to the splitting of the peptide chains as the extraction temperature increases [29]. The inter-chain cross-links of collagen were hydrolyzed during gelatin

extraction, resulting in gelatin with varying molecular weight peptides [30]. The low content of α -chain in gelatin may affect the physical and functional properties of gelatin [31].

3.4 Physical properties

3.4.1 Setting time

The setting time required to develop jellyfish by-products gelatin gel at 4 °C is shown in Table 1. All jellyfish by-products gelatin (6.67%) solution samples can form a gel at 4 °C. The setting time decreased as the extraction time increased. The shortest setting time was for the JFG48 sample, while the longest time was for the JFG12 sample. However, the setting time of jellyfish by-products gelatin was longer than commercial bovine gelatin, commercial fish gelatin, and other work. The setting time of commercial bovine and fish gelatin was 10.37 ± 0.05 and 12.00 ± 1.73 min, respectively. The shortest setting time of tilapia gelatin solution was 0.87 min when extracted at 45 °C for 6 h at pH 5 [32]. For jellyfish by-products gelatin, using a more extended time during the extraction may cause the triple helix structure of jellyfish collagen to denature [10], resulting in increased helix-to-coil transition and converted to soluble gelatin and high gelling ability. On the other hand, the shortened extraction time may be caused by crude gelatin, resulting in taking a long time to form a reasonable gel network arrangement.

3.4.2 Gel strength

The gel strength of gelatin is one of the essential physical properties that influence the textural quality of food, pharmaceutical, and cosmetic products. The gel strength of JFG12, JFG24, and JFG48 samples is shown in Table 1. The gel strength of jellyfish by-products gelatin was in the range of 210.46-325.97 g after being extracted at 60 °C for 12, 24, and 48 h. The gel strength of jellyfish by-products gelatin was increased as extraction time increased. This result is consistent with the trend corresponding to the setting time of gelatin. The JFG48 sample showed the highest gel strength of 325.97 g. The gel strength of all jellyfish by-products gelatin gel in this study was lower than that of commercial bovine gelatin (358.62 g) [12]. However, the gel strength of commercial fish gelatin (306.11 g) was lower than that of the JFG48 sample. Previous research has found that jellyfish by-products gelatin has lower gel strength due to differences in collagen type, the alpha and beta chain ratio in the gelatin, and the gelatin peptides of the alpha chain [11]. The lowest gel strength of jellyfish (*Lobonema smithii*) gelatin was 108.36 g [15]. The bloom of hydrolyzed salted, dried cannonball jellyfish gelatin (H-SDJ) (6.67%) was 3.4 g [26], which is lower than that of other research because H-SDJ had a high salt content. The salt may affect the gel strength because Na^+ and Cl^- may interfere with hydrogen bonds with the carbonyl oxygen of an adjacent peptide group, resulting in lower gelling properties. Compared to the

same jellyfish by-products (*Lobonema smithii*), the gel strength of the JFG48 sample was higher than previously studied. The jellyfish gelatin produced from by-products had the gel strength of 223.61-323.74 g [11-12] but the value was still lower than that of marine gelatin from the previous work. The highest gel strength value of tilapia gelatin was 382.3 g when extracted at 45 °C for 6 h [32]. Therefore, compared to the gel strength from other studies, factors of raw material, salt content, pretreatment conditions, extraction temperature, and extraction times influenced collagen conversion into gelatin, thereby affecting its quality.

3.4.3 Viscosity

Viscosity is another significant factor in gel formation. The viscosity of jellyfish by-products gelatin is shown in Table 1. The viscosity of jellyfish by-products gelatin was in a range of 23.00-24.50 cP. The viscosity of all jellyfish by-products gelatin was higher than that of those previously studied. Lueyot *et al.* [11] reported that the highest viscosity of jellyfish by-products gelatin was 7.73 cP when extracted at 60 °C for 12 h. The different extraction times did not significantly affect viscosity. The viscosity of commercial bovine and fish gelatin was 27.00 ± 1.73 cP and 24.00 ± 1.00 cP, respectively. These results were slightly different from other studies, which reported that the viscosity of commercial bovine gelatin was 9.8 cP [2]. However, the viscosity of all jellyfish by-products gelatin samples was lower than that of commercial bovine gelatin. In contrast, the viscosity of commercial fish gelatin showed no significant differences from all jellyfish by-products gelatin samples. Compared to other gelatins, the viscosity of all jellyfish by-products gelatin samples is within an acceptable range of 2.0-13.0 cP [2, 33]. The viscosity is influenced by several parameters, including raw material sources, extraction conditions, gelatin gel preparation, and the test instrument's precision and repeatability [11].

3.4.4 Color

The color of gelatin is one of the factors that affect food product preference. The color of jellyfish by-products gelatin is presented in Table 1. JFG12, JFG24, and JFG48 gel (6.67%) samples showed that the lightness (L^*) increased as extraction time increased. The hue value of JFG12, JFG24, and JFG48 gel samples was 46.88 ± 0.99 , 17.09 ± 0.03 , and 11.66 ± 0.18 , respectively. The hue value of jellyfish by-products gelatin gel decreased significantly with increasing extraction time. The color of jellyfish by-products gelatin showed light to dark brown, as shown in Fig. 4. These results were different from the previous study, which reported a hue value of jellyfish gelatin in the range of 32.43-33.05 after being extracted at 60 °C for 3 h; the sample was then dried using a tray dryer at 60 °C for 3 days [12]. The intense brown color of jellyfish by-products gelatin gel could be due to the color of the raw material, pretreatment condition, extraction temperature, extraction time, drying, and Maillard reaction, in which

reducing sugars react with a free amino acid at the appropriate temperature during jellyfish by-products gelatin drying [30].

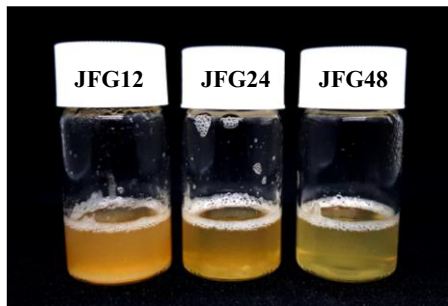


Fig. 4. Jellyfish gelatins gel (6.67%) extracted at different times of 12 h (JFG12), 24 h (JFG24), and 48 h (JFG48).

3.5 Functional properties

3.5.1 Foaming properties

The foaming properties of gelatin are widely used in dessert products, especially soft, light, and porous products, such as bakery products, meringue, and marshmallows [16]. The foaming capacity (FC) and foaming stability (FS) of jellyfish by-products gelatin are presented in Table 2.

Table 2. Functional properties of jellyfish by-products gelatin.

Properties	Sample		
	JFG12	JFG24	JFG48
FC* (%)	12.28±1.52 ^b	16.67±1.52 ^a	17.54±1.52 ^a
FS* (%)	10.53±0.00 ^b	12.28±1.52 ^b	15.79±2.63 ^a
EAI* (m ² /g)	13.30±0.02 ^a	13.12±0.01 ^b	13.11±0.02 ^b
ESI* (%)	56.42±0.39 ^a	44.32±0.14 ^b	39.19±2.50 ^c

*Different superscript letters in the same row indicate a significant difference (p<0.05).

The foaming capacity of JFG12, JFG24, and JFG48 samples were in the range of 12.28-17.58%. The foaming capacity of jellyfish by-products gelatin was generally increased as extraction time increased. A similar result was reported in type A gelatin from jellyfish (*Lobonema smithii*) [16]. The foaming capacity of commercial bovine and fish gelatin samples was 20.17±4.02% and 19.29±1.52%, respectively. All jellyfish by-products gelatin samples had slightly lower foaming capacity than commercial bovine, and fish gelatin due to the migration of shorter peptides to the air-water interface [34]. During foam formation, the higher foaming capacity is associated with readily the absorbed molecule of gelatin that can migrate rapidly in the gas-liquid interface. The foaming stability of JFG12, JFG24, and JFG48 samples was 10.52-15.78% which was lowered than those of commercial bovine and fish gelatin samples exhibiting 17.54±4.02% and

14.91±1.52%, respectively. The foaming stability of jellyfish by-products gelatin decreased as extraction time increased. Foaming qualities are affected by the degree of interaction between protein molecules and the existence of hydrophobic regions [35]. The results suggested that extended extraction time increased the foaming properties of jellyfish by-products gelatin. The jellyfish by-products gelatin could function as a foaming agent in food applications.

3.5.2 Emulsifying properties

Gelatin can function as an emulsifier that plays an essential role in the stabilization of emulsion in the food and pharmaceutical industry. In this investigation, the emulsion was made using soybean oil, water and jellyfish by-products gelatin as an emulsifier. The emulsion activity index (EAI) and emulsion stability index (ESI) of jellyfish by-products gelatin are shown in Table 2. The emulsion activity index of JFG12, JFG24, and JFG48 samples were 13.11-13.30 m²/g, and the emulsification stability index was 39.18-56.42%. The emulsion activity index of commercial bovine and fish gelatin was 13.91±0.22 m²/g and 13.72±0.13 m²/g, respectively, and the emulsification stability index was 58.34±0.64% and 52.63±1.22%, respectively. The EAI and ESI of the JFG12 sample were higher than that of any jellyfish by-products gelatin (JFG24 and JFG48 samples), but lower than commercial bovine and fish gelatin. The EAI and ESI of jellyfish by-products gelatin were decreased as extraction time increased. The degradation of jellyfish collagen might be due to the generation of short-chain peptides during long extraction times. The increased short-chain peptides yield more charged groups, which are more hydrophilic and tend to adsorb in the water phase, resulting in a decrease in encapsulated oil droplets. The longer peptide chains might have a stronger film surrounding the oil, resulting in enhanced emulsion stability [32]. Emulsification stability depends on the rate of reaching equilibrium surface tension, steric stabilization, volume and surface viscosity, and electrical repulsion between droplets [36]. Thus, the effect of extraction times plays a vital role in producing jellyfish gelatin with good emulsifying properties.

4 Conclusions

Jellyfish by-products may be a promising source for gelatin production, which reduces food waste from the salted jellyfish industry. The factor of extraction time directly impacts jellyfish gelatin quality. The resulting molecular weight distribution and the triple helix content of gelatin influence the physical and functional properties of jellyfish by-products gelatin. All jellyfish gelatins derived from different extraction times showed lower gel strength values than commercial bovine gelatin. In conclusion, the proper condition for producing jellyfish gelatin with the highest gel strength of 325.97±2.84 g was 60 °C for 48 h. The foaming and emulsifying properties of jellyfish gelatin were similar to

commercial bovine and fish gelatin. Thus, jellyfish by-products gelatin may be used in food applications due to the gelation gel, foam formation, and emulsification qualities.

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References

1. Grand View Research, <https://www.grandviewresearch.com/press-release/global-gelatin-market> (2020)
2. L. Lin, J. M. Regenstein, S. Lv, J. Lu, S. Jiang, An overview of gelatin derived from aquatic animals: Properties and modification, *Trends in Food Science & Technology*, 68 (2017): 102-112
3. J. Tkaczewska, M. Morawska, P. Kulawik, M. Zająca, Characterization of carp (*Cyprinus carpio*) skin gelatin extracted using different pre-treatments method, *Food Hydrocolloids*, 81 (2018): 169-179
4. P. Kittiphattanabawon, S. Benjakul, S. Sinthusamran, H. Kishimura, Gelatin from clown featherback skin: extraction conditions, *LWT*, 66 (2016): 186-192
5. T. Huang, Z. Tu, X. Shangguan, X. Sha, H. Wang, L. Zhang, N. Bansal, Fish gelatin modifications: A comprehensive review, *Trends in Food Science & Technology*, 86 (2019): 260-269
6. Y. Liu, L. Xia, H. Jia, Q. Li, W. Jin, X. Dong, J. Pan, Physicochemical and functional properties of chum salmon (*Oncorhynchus keta*) skin gelatin extracted at different temperatures, *Journal of the Science of Food and Agriculture*, 97, 15 (2017): 5406-5413
7. R. S. G. Silva, S. F. Bandeira, L. A. A. Pinto, Characteristics and chemical composition of skins gelatin from cobia (*Rachycentron canadum*), *LWT*, 57, 2 (2014): 580-585
8. F. Mahmoodani, V. Sanaei Ardekani, S. F. See, S. M. Yusop, A. S. Babji, Optimization and physical properties of gelatin extracted from pangasius catfish (*Pangasius sutchi*) bone, *Journal of Food Science and Technology*, 51, 11 (2014): 3104-3113
9. T. Zhang, R. Sun, M. Ding, L. Tao, L. Liu, N. Tao, X. Wang, J. Zhong, Effect of extraction methods on the structural characteristics, functional properties, and emulsion stabilization ability of *Tilapia* skin gelatins, *Food Chemistry*, 328 (2020): 127114
10. B. Wonganu, Application of gelatin derived from waste tilapia scales to an antibiotic hydrogel pad, *E3S Web of Conferences*, 141 (2020): 03004
11. A. Lueyot, V. Rungsardthong, S. Vatanyoopaisarn, P. Hutangura, B. Wonganu, P. Wongsangasri, S. Charoenlappani, S. Roytrakul, B. Thumthanaruk, Influence of collagen and some proteins on gel properties of jellyfish gelatin, *PLOS One*, 16, 6 (2021): e0253254
12. W. Charoenchokpanich, P. Muangrod, V. Rungsardthong, S. Vatanyoopaisarn, B. Wonganu, S. Roytrakul, B. Thumthanaruk, Effect of hydrochloric acid extraction on yield and gel properties of gelatine from salted jellyfish by-products, *E3S Web of Conferences*, 302 (2021): 02009
13. Ministry of Commerce, http://www.ops3.moc.go.th/infor/MenuComTH/stru1_export/export_topn_report.asp (2021)
14. W. Charoenchokpanich, V. Rungsardthong, S. Vatanyoopaisarn, B. Thumthanaruk, Y. Tamaki, Salt reduction in salted jellyfish (*Lobonema smithii*) using a mechanical washing machine, *Science, Engineering and Health Studies*, 14, 3 (2020): 184-192
15. P. Chancharern, N. Laohakunjit, O. Kerdchoechuen, B. Thumthanaruk, Extraction of type A and type B gelatin from jellyfish (*Lobonema smithii*), *International Food Research Journal*, 23, 1 (2016): 419-424
16. U. Rodsuwan, B. Thumthanaruk, O. Kerdchoechuen, N. Laohakunjit, Functional properties of type A gelatin from jellyfish (*Lobonema smithii*), *International Food Research Journal*, 23, 2 (2016): 507-514
17. S. Cho, J.R. Ahn, J.S. Koo, S.B. Kim, Physicochemical properties of gelatin from jellyfish *Rhopilema hispidum*, *Fisheries and Aquatic Sciences*, 17, 3 (2014): 299-304
18. P. Muangrod, V. Rungsardthong, S. Vatanyoopaisarn, Y. Tamaki, E. Kuraya, B. Thumthanaruk, Effect of wash cycle on physical and chemical properties of rehydrated jellyfish by-products and jellyfish protein powder, *Science, Engineering and Health Studies*, 15 (2021): 21030004
19. U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature*, 227, 5259 (1970): 680-685
20. GMIA, *Standard methods for the sampling and testing of gelatins* (Gelatin Manufacturers of America, Inc., New York, 1986)
21. J.H. Muyonga, C.G.B. Cole, 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*, 86, 3 (2004): 325-332
22. S.K. Sathe, D.K. Salunkhe, Functional properties of the great northern bean (*Phaseolus vulgaris* L.) proteins: Emulsion, foaming, viscosity, and gelation properties, *Journal of Food Science*, 46, 1 (1981): 71-81

23. K.N. Pearce, J.E. Kinsella, Emulsifying properties of proteins: Evaluation of a turbidimetric technique, *Journal of Agricultural and Food Chemistry*, 26, 3 (1978): 716-723
24. P. Kittiphattanabawon, S. Benjakul, W. Visessanguan, F. Shahidi, Comparative study on characteristics of gelatin from the skins of brownbanded bamboo shark and blacktip shark as affected by extraction conditions, *Food Hydrocolloids*, 24, 2-3 (2010): 164-171
25. S. Sinthusamran, S. Benjakul, H. Kishimura, Characteristics and gel properties of gelatin from skin of seabass (*Lates calcarifer*) as influenced by extraction conditions, *Food Chemistry*, 152 (2014): 276-284
26. P.G. Chiarelli, R.B. Pegg, G.D. Kumar, K.M. Solval, Exploring the feasibility of developing novel gelatin powders from salted, dried cannonball jellyfish (*Stomolophus meleagris*), *Food Bioscience*, 44 (2021): 101397
27. L.C. Sow, H. Yang, Effects of salt and sugar addition on the physicochemical properties and nanostructure of fish gelatin, *Food Hydrocolloids*, 45 (2015): 72-82
28. V. Gudipati, *Chapter 3. Fish Gelatin: A Versatile Ingredient for The Food and Pharmaceutical Industries in Marine Proteins and Peptides: Biological Activities and Applications*, edited by S.K. Kim (Wiley-Blackwell, Oxford, UK, 2013), pp. 271–295
29. N.M. Sarbon, F. Badii, N.K. Howell, Preparation and characterisation of chicken skin gelatin as an alternative to mammalian gelatin, *Food Hydrocolloids*, 30, 1 (2013): 143-151
30. P. Kaewruang, S. Benjakul, T. Prodpran, S. Nalinanon, Physicochemical and functional properties of gelatin from the skin of unicorn leatherjacket (*Aluterus monoceros*) as affected by extraction conditions, *Food Bioscience*, 2 (2013): 1-9
31. A. Erge, Ö. Zorba, Optimization of gelatin extraction from chicken mechanically deboned meat residue using alkaline pre-treatment, *LWT*, 97 (2018): 205-212
32. W. Liao, Y. Zhu, Y. Lu, Y. Wang, X. Dong, G. Xia, X. Shen, Effect of extraction variables on the physical and functional properties of tilapia gelatin, *LWT*, 146 (2021): 111514
33. E.T.A. Tümerkan, Ü. Cansu, G. Boran, J.M. Regenstein, F. Özoğul, Physicochemical and functional properties of gelatin obtained from tuna, frog and chicken skins, *Food Chemistry*, 287 (2019): 273-279
34. H. Kchaou, N. Benbettaied, M. Jridi, M. Nasri, F. Debeaufort, Influence of Maillard reaction and temperature on functional, structure and bioactive properties of fish gelatin films, *Food Hydrocolloids*, 97 (2019): 105196
35. K. Elavarasan, V.N. Kumar, B.A. Shamasundar, Antioxidant and functional properties of fish protein hydrolysates from fresh water carp (*Catla catla*) as influenced by the nature of enzyme, *Journal of Food Processing and Preservation*, 38, 3 (2014): 1207–1214
36. F. Ravera, K. Dziza, E. Santini, L. Cristofolini, L. Liggieri, Emulsification and emulsion stability: The role of the interfacial properties, *Advances in Colloid and Interface Science*, 288 (2021): 102344

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