

Effect of hydrochloric acid extraction on yield and gel properties of gelatine from salted jellyfish by-products

Wiriya Charoenchokpanich¹, Pratchaya Muangrod¹, Vilai Rungsardthong¹, Savitri Vatanyoopaisarn¹, Benjamaporn Wonganu², Sittiruk Roytrakul³ and Benjawan Thumthanaruk^{1,*}

¹Department of Agro-Industrial Food and Environmental Technology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand

²Department of Biotechnology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand

³National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency (NSTDA), Pathum Thani, Thailand

Abstract. Salted jellyfish by-products have collagen protein that is mainly sold for animal feed at a low price. The change of jellyfish by-products into a food ingredient like gelatine could benefit food applications and reduce food waste. Indeed, jellyfish gelatine production is a time-consuming process that includes alkaline pre-treatment, acid pre-treatment, hot water extraction, and drying. Reduced times of acid pre-treatment and water extraction might deliver different gel properties. Therefore, this research aimed to investigate the effect of hydrochloric acid (HCl) pre-treatment on the gel quality of resultant gelatine. Desalted jellyfish by-products were immersed in 0.5 M sodium hydroxide at 4°C for 1 h and then were acid-treated by varying HCl concentrations (0.1, 0.2, and 0.3 M) at 25°C for 2 h. After that, samples were extracted at 60°C for 3 h and dried at 60°C for 3 days. Results showed that gelatine yield significantly increased with increasing HCl concentration. Gelatine yield were 2.97±0.97%, 5.60±1.01%, and 6.34±1.08%, after extraction with 0.1, 0.2, and 0.3 M HCl, respectively. Gel strength generally decreased as HCl concentration increased. Gel strength values were in the range of 118.89-223.60 g. The colour of jellyfish gelatine showed light to dark brown with no differences in Hue values. Thus, the short duration of HCl pre-treatment for 2 h and hot water extraction for 3 h was insufficient for the jellyfish gelatine process.

Keyword. Salted jellyfish by-products, Gelatine, Pre-treatment, Yield, Gel strength

1 Introduction

Gelatine is a protein-type hydrocolloid widely utilised in food industries, functioning as gelling, thickening stabilising, foaming, emulsifying, water binding, and crystal growth modifying [1-3]. Gelatine is derived from the denaturation of collagen [4]. Commercial gelatines are mainly produced from bovine bone and porcine skin [5]. However, bovine and porcine gelatine have faced some constraints related to religious issues and biological contaminants [6]. Therefore, alternative gelatine derived from marine animals, especially from fish, has been sold and substituted for bovine and porcine gelatines.

Many marine animals have been researched as alternative gelatine sources, especially from the by-products of a particular industry. Interestingly, the functional qualities of marine gelatine can be comparable to mammalian gelatine to some extent [7]. However, fish gelatine generally has lower gel strength than commercial bovine and porcine gelatines [8]. To date, several studies have reported gelatine extracted from marine by-products, including clown featherback [8], blacktip shark [9], brown-banded bamboo shark [9],

salmon [10], tuna [11], and squid [12]. In general, gelatine production is a time-consuming process depending on the type of raw material, type of chemicals, duration time used for the alkaline and acid pre-treatments, water extraction, and drying. However, the previous studies took a long time for acid extraction ranging from 6 to 12 h. No study has been used to shorten the time for acid extraction, which could minimise the duration time of gelatine production.

Apart from marine species, jellyfish, which main protein is collagen and consists of little fat and cholesterol [13-15], have gained much attention for gelatine extraction. Jellyfish-based foodstuff is generally known as delicacies in Asian countries [16] which has recently gained attention in European countries [17] due to its unique jelly, crunchy texture, and its richness in collagen protein [16, 18]. As a consequence, the demand and preference for jellyfish as a portion of food has increased worldwide. The fresh jellyfish has been preserved with salt chemicals by professional fishermen and jellyfish companies. Salted jellyfish by-products are generated from various stages of the dehydration process [19]. In general, most salted jellyfish by-products have been sold for feed meals at a low price [19]. Research on

* Corresponding author: benjawan.t@sci.kmutnb.ac.th

jellyfish gelatine has been reported with a lower yield and gel strength than commercial gelatines (bovine and fish gelatine) [13-15, 20]. In addition, the drawback of jellyfish gelatine is not being able to retain the gel structure at room temperature [15, 20]. Upon jellyfish gelatine research (Fig. 1), chemicals used for pre-treatment and the extraction condition of temperature and time [12, 13, 20] affecting gelatine quality and yield have been reported elsewhere. The jellyfish gelatine extracted with 0.1 M HCl at 60°C for 12 h showed a maximum gel strength of 323.74 g [20]. According to the previous result, during the gelatine extraction, factors of the acid pre-treatment step and hot water extraction for a particular time could influence collagen extractability [21]. Therefore, this research aimed to investigate the reduced time process of gelatine extraction by the varying conditions of HCl concentrations (0.1, 0.2, and 0.3 M) for only 2 h and hot water extract at 60°C for only 3 h on yield and gel quality of the resultant gelatine.

2 Materials and methods

2.1 Preparation of desalted jellyfish by-products

Salted jellyfish by-products (*Lobonema smithii*) were obtained from Mahachai Food and Trading Co., Ltd., Samut Sakhon, Thailand. The by-products were stored in polyethylene bags, transported to the lab at King Mongkut's University of Technology North Bangkok, Thailand, and kept refrigerated until use. For the experiment, the jellyfish material was prepared with slight modifications [19, 22]. First, the material was mechanically washed with water by a washing machine at a ratio of 1:10 (w/v) at 2 cycles. Each cycle was set for 15 min. The sample was then drained for 10 min and ready for the next step of gelatine production.

2.2 Preparation of jellyfish gelatine

Fig. 1 displays the production of jellyfish gelatine. The gelatine production was modified according to the method of Lueyot et al. [20]. In this study, the gelatine preparation consists of multi-steps, including alkaline pre-treatment, washing, acid pre-treatment, washing, water extraction, and drying. Firstly, 1 kg of the desalted jellyfish by-products was continuously stirred with 0.05 M NaOH solution in the ratio of 1:3 (w/v) at 4°C for 1 h using a shaking incubator (WIS-20R, WiseCube, Korea) at 150 rpm. The sample was washed with tap water until neutral pH (6.5-7.0). The sample was agitated in HCl solution in a ratio of 1:2 (w/v) at 25°C for 2 h at a speed of 150 rpm. Three different HCl conditions of 0.1, 0.2, and 0.3 M were compared at this step. The HCl solution was changed every 60 min. Next, the swollen sample was thoroughly washed with tap water until the sample had neutral pH. After that, the sample was extracted with distilled water at 60°C for 3 h with a sample to water ratio of 1:2 (w/v) in a temperature-controlled water bath shaker (Memmert, Schwabach, Germany) at a speed of

100 rpm. Then, the sample was filtered using a Buchner funnel with Whatman No. 4 filter paper. Finally, the sample was dried with a tray dryer (ED 400, Binder, U.S.A.) at 60°C for 3 days. The obtained jellyfish gelatine samples were calculated for yield and subjected to further analysis. Each experiment was performed in triplicate.

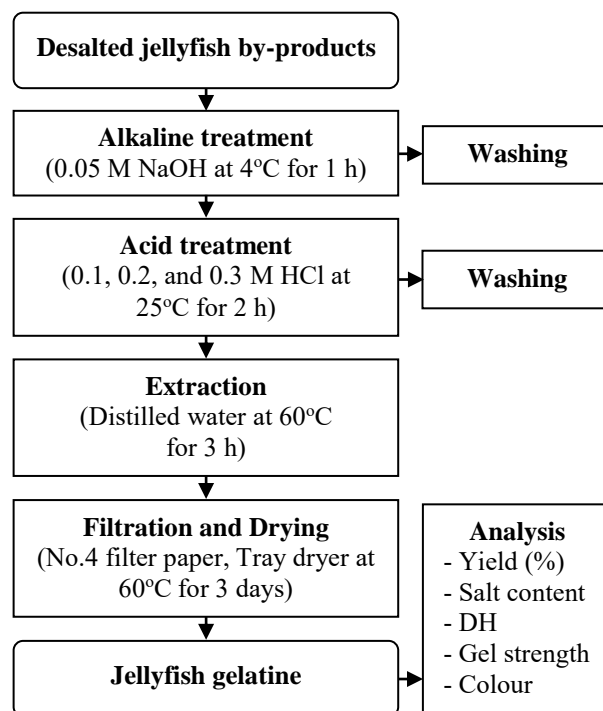


Fig. 1. Gelatine extraction from jellyfish.

2.3 Analysis

2.3.1 Chemical composition

The chemical composition of desalted jellyfish by-products was determined protein, ash, fat, and moisture content according to the AOAC standard methods [23]. The protein content was determined by the Kjeldahl method by using a factor of 5.55 to convert the nitrogen value to protein [23, 24]. The ash content, fat content, and moisture content were determined using the muffle furnace method, the Soxhlet extraction method, and the oven drying method, respectively [23].

2.3.2 Yield

The gelatine yield was calculated for all the treatments on a dry weight basis based using the following eq (1):

$$Yield (\%) = (M_2 / M_1) \times 100 \quad (1)$$

where M_1 = Weight of the oven-dried salted jellyfish (g) and M_2 = Weight of the oven-dried gelatine (g)

2.3.3 Salt content

The salt (sodium chloride) content of jellyfish gelatine solution after filtration (200 mL) was measured with a

conductivity meter (TDS Meter 308, Systronics, India). The calculation of the percentage of sodium chloride was extrapolated from the standard curve.

2.3.4 Degree of hydrolysis

The sample used for DH determination was the sample before drying, followed by the previous report [25]. The jellyfish gelatine solution after filtration (125 μ L) was mixed with 1 mL of 0.01% TNBS solution and 2 mL of phosphate buffer (pH 8.2) and incubated at 50°C for 30 min in the dark using a temperature-controlled water bath (WNB 45, Memmert, Germany). The reaction was terminated by adding 2 mL of 0.1 M sodium sulphite and cooling at room temperature for 15 min. The absorbance was measured at 420 nm using a spectrophotometer (SP 830 plus, Metertech, Taiwan). The percentage of DH was calculated according to the following equation (2):

$$DH (\%) = [(L - L_b) / (L_{max} - L_b)] \times 100 \quad (2)$$

where L = The α -amino groups content of hydrolysate sample, L_b = The α -amino groups content in the original substrate (blank), and L_{max} = The total α -amino groups the sample hydrolysed by 6 N HCl at 100°C for 24 h.

2.3.5 Determination of gel strength

The gelatine gel was prepared slightly modified according to the standard method [26]. The dried gelatine sample was dissolved in distilled water at 60°C and continuously stirred until gelatine was solubilised completely to obtain the final concentration of 6.67% (w/v). The gelatine sample (6.67%) was poured in a container with the size of 2.5 cm in height and 3 cm in diameter and then refrigerator at 4°C for 18 h before analysis. The gel strength was determined using a texture analyser (TA-XT2i, Stable Micro Systems, UK) equipped with a 1.27 cm diameter flat-faced cylindrical Teflon plunger (P/0.5R). The parameter of pre-test and post-test speeds were set at 1.5 and 1.0 mm/s, respectively. The maximum force was expressed to be defined as the gel strength (g). The gel strength of jellyfish gelatine was compared to commercial fish and bovine gelatine (McGarrett, JR F&B Co., Ltd, Thailand).

2.3.6 Colour measurement

The colour of jellyfish gelatine gel prepared at 6.67% (w/v) was evaluated by CIE L* a* b* system and measured using a colourimeter (ColorQUEST 45/0, HunterLab, USA). The results of the CIE L*a*b* colour parameters were displayed as L* (lightness 0-100), a* (redness (+)/greenness (-)), and b* (yellowness (+)/blueness (-)). The colour of jellyfish gelatine was compared to commercial fish and bovine gelatine (McGarrett, JR F&B Co., Ltd, Thailand). The hue angle of the sample was calculated according to the following equation (3):

$$\text{Hue angle} = \tan^{-1} (b^* / a^*) \quad (3)$$

2.4 Statistical analysis

All experiments were performed in triplicate, except for the gel strength and colour determination performed in quintuplicate. The data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test for statistical significance ($p < 0.05$) using the statistical package for social science (SPSS) 22.0.

3 Results and discussions

3.1 Quality of desalted jellyfish by-products

The desalted jellyfish by-products had protein, ash, fat, and moisture content of $8.68 \pm 0.19\%$, $1.04 \pm 0.23\%$, $0.98 \pm 0.08\%$, and $89.10 \pm 0.10\%$, respectively. These results were slightly different from other studies, which reported that the desalted jellyfish (*Lobonema smithii*) (washed 2 cycles) had protein, ash, fat, and moisture content of 4.79-5.55%, 1.19-1.39%, 0.91-0.95%, and 91.86-93.36%, respectively [19, 22]. Differences in proximate results might be due to differences in the seasonal catch or varying salting process [22]. Despite no determination of collagen in this study, previous reports showed that most of the jellyfish proteins were collagen protein [16, 27, 28].

3.2 Effect of pre-treatment with different HCl concentrations on yield, salt content, and DH

3.2.1 Yield

After pre-treatment with 0.1, 0.2, and 0.3 M HCl, the yields of jellyfish gelatine were 2.97%, 5.60%, and 6.34%, respectively, calculated on a dry weight basis (Table 1). The gelatine yield significantly increased with increased HCl concentration from 0.1 to 0.2 M but not with 0.3 M HCl. However, the jellyfish gelatine yields of this study showed lower values from the other works with different types and concentrations of acid used and extraction temperature and time. The highest yield of type A jellyfish gelatine was 39.47% (on a dry weight basis) when pre-treated with a sulfuric acid solution (pH 2) and extracted at 75°C for 12 h [15]. The tilapia skin gelatine pre-treated with 0.1 M NaOH and extracted at 55°C for 6 h showed the highest yield of 21.10% on a dry weight basis [29]. The highest yield of blacktip shark and the brown-banded bamboo shark was 21.17-24.76% and 19.06-22.81% (based on wet weight), respectively when pre-treated with a 0.1 M NaOH, 1 M HCl, and 0.2 M acetic acid and extracted at 45, 60, and 75°C for 6 and 12 h [9]. Thus, compared to the yields from other studies, the shortened duration of HCl pre-treatment for only 2 h and hot water extraction for 3 h was insufficient for the jellyfish gelatine process due to the leftover jellyfish by-products greater than 90% remained.

Table 1. Yield, salt content, and degree of hydrolysis of jellyfish gelatine at different HCl concentrations pre-treatment.

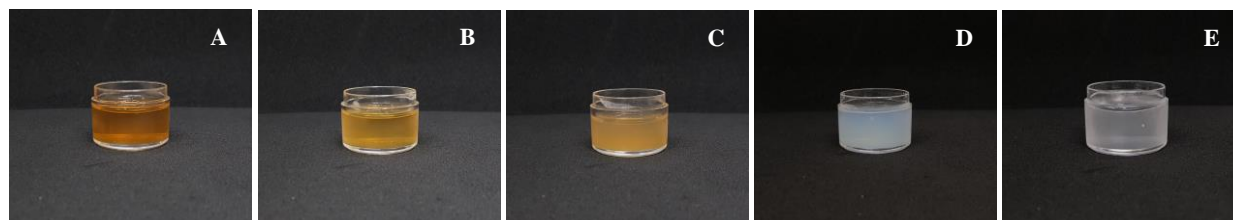
Sample	Yield* (%)	Conductivity* (us/cm)	NaCl content* (%)	DH* (%)
0.1 M HCl	2.97±0.97 ^b	1,029.67±4.72 ^a	0.047±0.00 ^a	29.74±0.6 ^c
0.2 M HCl	5.60±1.01 ^a	996.67±5.50 ^b	0.046±0.00 ^b	33.63±0.45 ^b
0.3 M HCl	6.32±1.08 ^a	947.33±2.08 ^c	0.043±0.00 ^c	36.47±0.68 ^a

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

Table 2. Gel strength and colour of jellyfish gelatine at different HCl concentrations pre-treatment and commercial gelatines (bovine and fish gelatine).

Sample	Gel strength* (g)	Colour*			
		L*	a*	b*	h*
0.1 M HCl	223.61±3.72 ^c	4.43±0.61 ^{cd}	5.68±0.44 ^a	8.64±0.32 ^a	33.05±2.46 ^a
0.2 M HCl	214.47±4.12 ^c	5.70±0.93 ^{bd}	5.71±0.53 ^a	9.01±0.66 ^a	32.43±3.81 ^a
0.3 M HCl	118.89±0.48 ^d	7.24±1.18 ^b	5.94±0.02 ^a	9.19±0.85 ^a	32.98±2.31 ^a
Bovine	358.62±3.42 ^a	16.76±2.39 ^a	0.71±0.46 ^c	-9.66±1.08 ^b	3.98±2.27 ^b
Fish	306.11±3.79 ^b	3.67±1.10 ^d	4.92±0.41 ^b	-9.90±0.37 ^b	26.45±2.69 ^c

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

**Fig. 2.** Gelatine gel (A) jellyfish gelatine gel pre-treated at 0.1 M HCl, (B) jellyfish gelatine gel pre-treated at 0.2 M HCl, (C) jellyfish gelatine gel pre-treated at 0.3 M HCl, (D) commercial bovine gelatine gel, and (E) commercial fish gelatine gel.

3.2.2 Conductivity and salt content

The effect of electrolytes has directly influenced gelatine gel quality. For the jellyfish gelatine process, increased HCl concentration decreased conductivity and sodium chloride. The conductivity value and sodium chloride of jellyfish gelatine solutions were in the range of 947.33-1,029.67 $\mu\text{s}/\text{cm}$ and 0.047-0.043%, after pre-treatment with 0.1, 0.2, and 0.3 M HCl (Table 1). Before washing, the sample used in this study was salted jellyfish that was cured by sodium chloride (NaCl), sodium bicarbonate (NaHCO_3), and potassium alum sulfate ($\text{KAl}(\text{SO}_4)_2$). Previous studies reported that the salt content, determined by the titration method, of the salted jellyfish before and after washing for two cycles was 14% and 1%, respectively, but elements of Na, Al, Si, P, S, Cl, K, Ca, Fe, and Br were also found in washed samples [19, 22]. The salt elements remained in the protein structure of desalted jellyfish via the binding of positively charged ions (e.g., Na^+ , K^+ , Al^{3+} , Ca^{2+} , Fe^{2+}) and negatively charged ions (e.g., Cl^- , Si^- , Br^- , PO_4^{3-}) to the negatively and positively charged amino acids in the

jellyfish protein structure [22, 30]. Collagen in the salted jellyfish found 7 polar amino acids, including charged acidic amino acids (aspartic acid and glutamic acid), charged basic amino acids (arginine and lysine), and uncharged amino acids (serine, threonine, and tyrosine), and 10 nonpolar amino acids including alanine, cysteine, glycine, hydroxyproline, isoleucine, leucine, methionine, phenylalanine, proline, and valine [17]. All charged polar amino acids can be engaged in ionic bonds via electrostatic attraction, and all uncharged amino acids can be formed hydrogen bonds. In this study, during the gelatine process, it was noticed that the swollen jellyfish protein structure increased as the HCl concentration increased. As a result, in the system, the increases of H^+ ion and Cl^- ion might affect the charged and uncharged amino acids, causing the unfolded triple helix structure of collagen and increased binding water molecules to denatured jellyfish collagen [30]. Thus, the excess Cl^- ion might be reformed into NaCl, thereby reducing the salt content from the swollen collagen structure. In addition, the salt was diluted during the washing step. The effect of salt on fish gelatine showed that 1.5%

NaCl addition caused a loss in molecular order in secondary structure, affecting low gel strength [31]. Therefore, it is necessary to remove the excessive salt content before the drying step that might be interfering with the gel property.

3.2.3 DH

The DH of jellyfish gelatine solutions increased significantly with increasing HCl concentration. The results showed that DH of jellyfish gelatine was in the range of 29.74-36.47% (Table 1). The highest DH of jellyfish gelatine was pre-treated with 0.3 M HCl, which was lower than other reports that used 1 and 6 N HCl for 12 and 24 h and heated at 95°C, yielding high %DH of 73.70 and 75.40 [32]. The different chemicals affected DH. When pre-treated with a sulfuric acid solution (pH 2) and extracted at 60 and 75°C for 6 and 12 h, DH of jellyfish gelatine values were 65.30-77.13% [15]. During HCl treatment, the triple helix structure of jellyfish collagen was denatured with a higher HCl concentration by breaking down the hydrogen and covalent bonds [33], resulting in increased helix-to-coil transition and converted to soluble gelatine. Thus, the condition used in this study was relatively mild, compared to other studies [14, 15], and the shorten acid pre-treatment and water extraction. This condition might not be applicable for producing jellyfish gelatine when the desalted jellyfish was used as a raw material.

3.3 Gel strength of gelatine gel

Gelatine gel is a biopolymer network that is reversibly crosslinked and primarily stabilised by hydrogen-bonded junction zones [34]. Gel strength affects the texture quality in food products [14]. The gel strength of 6.67% jellyfish gelatine gel pre-treated at different HCl concentrations is shown in Table 2. The jellyfish gelatine decreased gel strength values from 223.61, 214.47, and 118.89 g when increased HCl concentration from 0.1, 0.2, and 0.3 M HCl. The gel strength of jellyfish gelatine was pre-treated with 0.1 M HCl, which was significantly different from those with 0.2 and 0.3 M HCl, while gel strength of jellyfish gelatine was pre-treated at 0.2 and 0.3 M HCl did not show significantly different. The gel strength values of jellyfish were lower than those of bovine gelatine (358.62 g) and fish gelatine (306.11 g). Previous studies reported that jellyfish gelatine's gel strength was inferior to bovine and fish gelatine due to differences in collagen type, amino acid composition, the alpha and beta chain ratio in the gelatine, and the gelatine peptides of the alpha chain [20]. However, the gel strength of jellyfish gelatine gel, in this study, was higher than the gel strength values of Cuttlefish (*Sepia Officinalis*) skins (198 g) [35] and squid (*Loligo formosana*) skins (156 g) [4]. Compared to the same jellyfish sample, the jellyfish gel strength reported in this study was higher than the previous work that showed the gel strength of 18-118 g [14, 15]. Recently, the highest gel strength of jellyfish (*Lobonema smithii*) gelatine was 323.74 g [20] which applied a higher extraction time of

12 h. As a result, the concentration of HCl pre-treatment, pre-treatment time, and water extraction time directly affected the gel strength. Therefore, in this study, applying a mild concentration of HCl (0.1 M) for 2 h and shortening hot water extraction time for only 3 h might not be suitable for the jellyfish gelatine process. Despite low gel strength, jellyfish gelatine might be used in frozen and refrigerated food products and used for crystal growth modification.

3.4 Colour of gelatine gel

The colour of jellyfish gelatine gel (6.67%) pre-treated at different HCl concentrations, commercial bovine gelatine gel, and commercial fish gelatine gel are presented in Table 2. The colour of gelatine gel was expressed as lightness (L*), redness (a*), yellowness (b*), and hue angle (h*). The lightness, redness, and yellowness value of jellyfish gelatine gel increased significantly with increasing HCl concentration. The change of redness and yellowness value related to hue angle (h*) of gelatine gel. The colour of jellyfish gelatine showed light to dark brown (tata. 2) with no differences in hue values that were 33.05±2.46, 32.43±3.81, and 32.98±2.31 after pre-treatment with 0.1, 0.2, and 0.3 M HCl, respectively. The colour of commercial bovine gelatine gel and commercial fish gelatine gel showed a transparent gel (Fig. 2) which the hue value was 3.98±2.27 and 26.45±2.69.

The intense brown of jellyfish gelatine gel could be due to a non-enzymatic browning reaction (Maillard reaction), in which reducing sugars, possibly ribose and deoxyribose that typically found in RNA and DNA [36], react with a free amino acid at the appropriate conditions of temperature during jellyfish gelatine drying [37]. Indeed, the drying condition for 3 days at 60°C might be too long, and no bleaching step was performed during jellyfish gelatine extraction. Then, the effect of the bleaching agent on jellyfish gelatine could be further studied.

4 Conclusion

Jellyfish by-products can be a source of gelatine production which reduced food waste and save time. The stepwise pre-treatment, including sodium hydroxide, hydrochloric acid, and hot water extraction, directly impacts gelatine qualities. The jellyfish treated with 0.1 M HCl showed the highest gel strength of 223.61 g but showed the lowest gelatine yield of 2.97%. The colour of jellyfish gelatine gel was light to intense brown colour compared to clear gelatine gel from fish and bovine gelatine. Therefore, improved jellyfish gelatine properties, including yield, colour, and gel strength, are still needed for further study.

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