

Functional properties of type A gelatin from jellyfish (*Lobonema smithii*)

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Abstract

Gelatin is typically derived from denatured collagen via acid, alkaline and enzyme hydrolysis. Type A and Type B gelatin commonly used in food industry are done by acid and alkaline process, respectively. In this study, desalted dried jellyfish (*Lobonema smithii*) was used to produce type A gelatin by hydrochloric acid hydrolysis at pH 1. The temperature of water extraction was 45, 60 or 75°C with extraction times of 6 or 12 h. The highest gel strength (118 g) of type A jellyfish gelatin occurred with an extraction at 45°C for 6 h. At the extraction temperature of 75°C for 6 or 12 h the gelatin gel did not form. The highest percentage (37.72%) of emulsion stability index (ESI) was observed from the gelatin produced at 75°C for 6 h. However, the same conditions did not give the highest value of foam expansion (FE). The reductions of foam stability (FS) were found in all samples after extending the storage time at room temperature for 60 min. Type A jellyfish gelatin can be produced that has gel forming, emulsifying and foaming properties. Type A gelatin produced from jellyfish can be used as an alternative source of gelatin for food application.

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Introduction

Gelatin is obtained by partial denaturation of the fibrous protein collagen by heating at acid or alkaline pH's (Haug and Draget, 2011; Lassoued *et al.*, 2014). Gelatin is commercially processed and used for food, cosmetics, pharmaceuticals, medicine, photographic adhesives, packaging and coating applications (Haug and Draget, 2011; Jellouli *et al.*, 2011). In food applications, gelatin can be functioned as a foaming agent, emulsifier, biodegradable film-forming material, colloid stabilizer and microencapsulating agent (Gómez-Guillén *et al.*, 2011). Gelatins derived from acid-treated and alkali-treated processes are known as type A and type B, respectively. Type B gelatins have lower isoelectric points (pI 4.8-5.2) compared to type A gelatins (pI 8-9) (Eysturskard *et al.*, 2009). Generally, the raw material used for producing gelatins comes from mammalian sources. Commercially 95% of gelatin is derived from pig skin and bovine hide and the other 5% is from porcine and bovine bones (Tabarestani *et al.*, 2010). In the present, the researchers were reported on gelatin from marine sources.

One major advantage of marine gelatin sources used is not associated with the risk of outbreaks

of BSE (Bovine Spongiform Encephalopathy). These gelatins are accepted by Islam, Judaism and Hinduism. Both Islam and Judaism do not consume any pork-related product, while Hinduism does not consume cow-related products (Nagarajan *et al.*, 2012). Several studies have been focused on the extraction and characterization of gelatins from marine species. The reports included fish skin (Cheow *et al.*, 2007; Tabarestani *et al.*, 2010; Ahmad and Benjakul, 2011; Sukkwai *et al.*, 2011), fish cartilage (Cho *et al.*, 2004), fish bones (Khiari *et al.*, 2013) and squid skin (Nagarajan *et al.*, 2012). The Thai jellyfish variety, Lodchong (*Lobonema smithii*), is known in English as the "white jellyfish" (Omori and Nakano, 2001). It is the important species in Asian fishery, especially Thai fishery export. The jellyfish (*Lobonema smithii*) are edible and have high collagenous protein (Klaiwong, 2009) so it can be a potential source of gelatin

The scope of the present paper was to investigate the extraction process of type A gelatin from white jellyfish (*Lobonema smithii*) by the use of hydrochloric acid. The physicochemical characteristics and functional properties of jellyfish gelatin were determined and compared to those of commercial halal bovine gelatin.

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Materials and Methods

Preparation of umbrella of dried desalted jellyfish

Salted jellyfish (*Lobonema smithii*) was obtained from Mahachai Sea Food Co., Ltd., Samutsakhon, Thailand. The samples were stored in sealed polyethylene (PE) bag and kept at 10°C. The salted jellyfish were cleaned with tap water until the salt of the wash water content measured by a salinometer (Atago®, Japan) reading was zero. The samples were dried in a tray dryer (Dwyer, TDII, Thailand) at 50°C for 24 h, ground and stored in sealed PE bag until used.

Gelatin preparation

The preparation of gelatin consists of two steps which are acid treatment and gelatin extraction. For the first step, ground dried jellyfish were soaked with hydrochloric solution at pH 1 in the ratio of 1:15 (w/v). The mixture was stirred continuously at 4°C for 24 h using a shaker (WiseCube, WIS-20R, Korea) at the speed of 150 rpm. After the soaking treatment, the slurries were adjusted with 1 M potassium hydroxide until it had a pH of 7. For the second step of extraction, the swollen jellyfish proteins were heated at 45, 60 and 75°C for 6 and 12 h in a temperature-controlled water bath (Memmert, Schwabach, Germany). The gelatin solutions were filtered through four layers of cheese cloth, dried at 50°C for 24 h with tray dry (Binder G 01350, USA) and ground with a blender (Moulinex, Thailand). All gelatin samples were weighed and kept in a desiccator with silica gel until used. The yield of dried gelatin was calculated using Equation 1.

$$\text{Yield (\%)} = (\text{weight of dried gelatin/weight of dried jellyfish}) \times 100 \quad (1)$$

Proximate analysis

The protein, moisture, ash and fat contents of the dried jellyfish gelatin powders were determined according to the method of AOAC (2000).

Color measurement

The color of gelatin solutions (6.67% w/v) was measured by a colorimeter (Hunter Lab Inc., Reston, VA, USA) reported by the CIE system. L(0-100), a*(+/-) and b*(+/-) parameters indicating darkness/lightness, redness/greenness and yellowness/blueness, respectively. The colorimeter was calibrated with a black and a white standard. Hue angle (h*) showed the calculated value between 0-360 by using Equation 2.

Hue angle

$$\begin{aligned} &= \arctangent(b^*/a^*) && \text{when } a^* > 0 \text{ and } b^* \geq 0 \\ &= \arctangent(b^*/a^*) + 180^\circ && \text{when } a^* < 0 \\ &= \arctangent(b^*/a^*) + 360^\circ && \text{when } a^* > 0 \text{ and } b^* < 0 \end{aligned}$$

(2)

Amino acid composition of jellyfish gelatin

The dried gelatin samples (10 mg) were hydrolyzed in 6 N HCl at 110°C for 24 h in a block heater (Model SBH 130D, Stuart Scientific, Manchester, UK). After the samples were concentrated by flushing with nitrogen gas, they were adjusted to 5 ml with distilled water and filtered through a 0.45 µm cellulose acetate filter (VertiPure™ CA Syringe Filter, Vertical Chromatography Co., Ltd. Bangkok, Thailand). Amino acid analysis was performed according to the method of Yan *et al.* (2007). Briefly, a 10 µl aliquot of each sample was analyzed by reverse phase-high performance liquid chromatography (RP-HPLC Model 1200, Agilent Technologies, Inc. Santa Clara, CA, USA) on a fused silica capillary (C18; 250 mm x 4.6 i.d., 5µm film thickness; Prevail™ column Alltech®, Deerfield, IL, USA). Mobile phase used was 5 mM heptafluorobutyric acid (HFBA) in 0.5% trifluoroacetic acid (TFA) (A) and acetonitrile : H₂O (95:5) (B). Gradient conditions of B were 0, 0, 15, 35% at 0, 3, 8 and 17 min, respectively. Flow rate used was 1.0 ml/min. This technique cannot be detected some amino acid (serine, valine, and methionine). Only the sample of jellyfish gelatin with maximum gel strength was analyzed for amino acid composition.

Determination of gel strength

The strength of gelatin gel was determined according by the method of Fernandez-Diaz *et al.* (2001) with a slight modification. Jellyfish gelatin gels were prepared by dissolving 6.67% (w/v) dried gelatin powder in distilled water at room temperature for 30 min. The solutions were heated at 60°C in a water bath for 30-60 min until gelatin powder was completely solubilized and then cooled in a refrigerator at 10°C for 16-18 h prior to analysis. The gel strength was determined by a Texture Analyzer (TA.XT2i, Stable Micro System, UK) with a load cell of 5 kN equipped with a 1.27 cm diameter flat faced cylindrical Teflon® plunger. The maximum hardness (in gram) was recorded when the penetration distance reached 4 mm. The speed of the plunger was 0.5 mm per second. The dimension of the measured sample was 3 cm in diameter and 2.5 cm in height.

Determination of emulsifying properties

The emulsion stability index (ESI) of gelatin samples was determined according to the method

of Kittiphattanabawon *et al.* (2004) with a slight modification. Soybean oil (3 ml) was mixed with 9 ml of each gelatin solution (1% gelatin). The mixture was homogenized using a homogenizer (Model T25 basic; IKA Labortechnik, Selangor, Malaysia) at 20,000 rpm for 1 min. 10 ml of the mixture was pipetted into a 15 ml centrifuge tube and centrifuged at high speed 22,540 g for 10 min at room temperature. The stability of emulsion was calculated using Equations 3.

$$\%ESI = ((W_1 - W_2) / W_1) \times 100 \quad (3)$$

where W_1 and W_2 are the weight of sample mixture and the weight of water separated, respectively.

Determination of foaming properties

The method of Shahidi *et al.* (1995) was used to determine foam expansion (FE). Gelatin solution were prepared at 1% concentration and homogenized at high speed of 17,000 rpm for 1 min. The mixture was carefully transferred into a 25 ml cylinder for volume measurement. The reading was done rapidly within 10 sec after the foam was set in the cylinder. The percentage of FE was calculated using Equations 4.

$$\%FE = (V_T / V_0) \times 100 \quad (4)$$

where V_T and V_0 are total volume after whipping and the original volume (ml) before whipping, respectively.

Statistical analysis

The data analyses were performed using a SPSS 19.0 software program (SPSS Inc., Chicago, Ill., U.S.A.). ANOVA was used to find differences between treatment and using Duncan's test with a confidence level of 95%. The values were reported as mean \pm SD for all measurements.

Results and Discussion

Proximate composition

The dried desalted jellyfish contained protein as the major component (69.85 \pm 0.79%), moisture (9.88 \pm 0.07%), fat (8.76 \pm 0.37%) and ash content (<1%), respectively. The important element of gelatin is protein and the jellyfish can use as a raw material in the production of gelatin.

Jellyfish gelatin yield

The yields of jellyfish gelatins in difference extraction are given in Fig 1. It was found the jellyfish gelatins extracted at 45, 60 and 75°C for

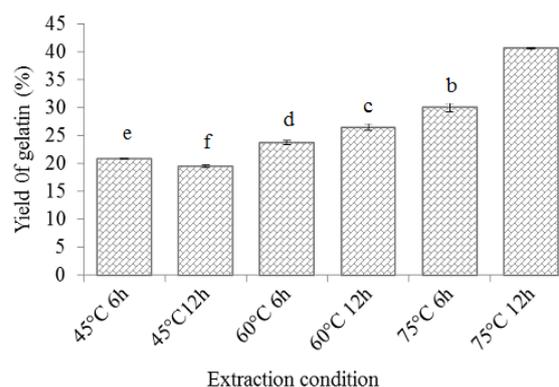


Figure 1. Gelatin yield as a function of extraction temperature (°C) and extraction time. Bars represent standard deviation (n = 3). Different letters on the bars denote significant differences ($P \leq 0.05$)

6 h were 20.85, 23.71 and 29.97%, respectively but those extracted at the same temperature for 12 h were 19.43, 26.46 and 40.54%, respectively. The increased extraction time and temperature could possibly increase opening of collagen cross-links, thereby swelling its collagen structure and make it easier to cause denaturation. The results suggested that when a higher temperature was applied, the bonding between α -chains in the native collagen were more newly established. As a consequence, the triple helix structure became amorphous and could be more readily extracted into the medium leading to a higher yield. It was noted that an extraction time longer than 6 h did increase the yield. Increased the extraction time also provided more energy to destroy the bonding, in which more free α or β -chains were released from the materials (Sinthusamran *et al.*, 2014). The cross-linked proteins stabilized by covalent bonds in the collagen network might be destroyed at the higher extraction temperature and time. The results suggested that the degree of conversion of collagen into gelatin depended on the processing parameters such as temperature, extraction time, pH, pretreatment conditions and properties of the starting raw material (Karim and Bhat, 2009).

Colors

Color of gelatin is an important aesthetic property depending on the application for which the gelatin is being used. The color of jellyfish gelatin and commercial bovine gelatin, was expressed in terms of L^* , a^* , b^* and h^* (hue angle). Jellyfish gelatin extracted at 45°C for 12 h and 60°C for 6 h showed the greater lightness (L^*) and yellowness (b^*) value compared to the color of bovine gelatin. However, the results of all jellyfish gelatins had the hue angle less than 90° indicating orange to yellow color which is darker than the hue value of bovine

Table 1. Amino acid content of jellyfish gelatin and other gelatins (amino acid g/100g sample)

Amino acids	Jellyfish gelatin (45°C 12 h)	Bovine gelatin	Sin croaker (Cheow <i>et al.</i> , 2007)	New Zealand hoki (Mohtar <i>et al.</i> , 2014)
Ser	0.00±0.00	0.00±0.00	2.2 ± 0.17	3.61 ± 0.11
Asp	4.44±0.21	4.22±0.03	1.81 ± 0.08	3.64 ± 0.09
Ala	0.69±0.00	0.82±0.01	20.6 ± 0.40	10.9 ± 0.30
Thr	0.64±0.00	0.81±0.01	3.94 ± 0.24	2.08 ± 0.19
Glu	8.03±0.02	8.90±0.15	3.3 ± 0.15	7.69 ± 0.27
Cys	2.88±0.05	0.00±0.00	0.41 ± 0.11	0.00±0.00
Lys	0.00±0.00	4.09±0.08	3.78 ± 0.11	2.77 ± 0.13
His	0.47±0.04	0.66±0.02	1.25 ± 0.04	0.86 ± 0.07
Arg	2.13±0.09	1.95±0.06	4.27 ± 0.13	5.29 ± 0.09
Val	0.00±0.00	0.00±0.00	3.14 ± 0.72	2.52 ± 0.24
Met	0.00±0.00	0.00±0.00	2.2 ± 0.18	1.07 ± 0.15
Tyr	0.48±0.01	0.34±0.02	2.52 ± 0.22	0.08 ± 0.03
Ile	1.23±0.07	1.12±0.04	1.19 ± 0.05	1.58 ± 0.05
Leu	1.82±0.19	2.11±0.03	2.59 ± 0.28	2.17 ± 0.17
Phe	0.56±0.03	1.25±0.00	2.2 ± 0.19	1.58 ± 0.10
Trp	0.20±0.01	0.23±0.02	2.48 ± 0.35	0.00±0.00
Gly	12.35±0.30	70.98±1.32	32.2 ± 0.6	32.8 ± 0.62
Hyp	5.62±0.11	8.62±0.37	8.09 ± 0.46	9.85 ± 0.33
Pro	4.19±0.07	11.37±0.06	1.88 ± 0.16	9.90 ± 0.11

gelatin. It can be concluded that factors such as raw material and extraction condition influence the color characteristics of extracted gelatin when compare with the commercial bovine gelatin. In general, color does not influence the functional properties; however light color is preferred because of less impact on the color attributes of the product (Shyni *et al.*, 2014).

Amino acid composition

The highest gel strength occurred with jellyfish gelatin extracted at 45°C for 12 h. The amino profile of jellyfish gelatin and the commercial bovine gelatin are shown in Table 1. The jellyfish gelatin had much higher glycine than other amino acids (12.53 residues/100 residues). However, the amino acid composition of jellyfish gelatin displayed low contents of hydroxyproline, proline and glycine, which are common characteristics, when compared to bovine gelatin. Glycine is the most dominant amino acid in gelatins (Arnesen and Gildberg, 2002). No serine, valine and methionine were found in jellyfish gelatin and bovine gelatin compared to sin croaker and New Zealand hoki gelatin. Bovine gelatin was reported to contain no cysteine that is characteristics of all bovine gelatins (Balti *et al.*, 2011; Lassoued *et al.*, 2014). It was in agreement with previously published data that the mammalian gelatins contain higher amounts of imino acid (proline and hydroxyproline) content than marine gelatin (Cheow *et al.*, 2007; Mohtar *et al.*, 2014). The results suggested that the starting raw material of gelatin differ in amino acid composition. This could result in different functionality and stability such as gel formation or foaming.

Gel strength

Gel strength is an important function of gelatin and is determined by amino acid composition and the ratio of α -chain and the amount of β -component

(Balti *et al.*, 2011). The gel strength of jellyfish gelatin extracted with different temperature and time is shown in Table 2. The values of gel strength decreased as the extraction temperature and time increased ($p < 0.05$), except for jellyfish gelatin extracted at 45°C for 12 h. All values of gel strength of jellyfish gelatins were lower than that of bovine gelatin. No gel formations occurred with jellyfish gelatins prepared with an extraction temperature of 75°C for 6 or 12 h. Muyonga *et al.* (2004) and Cho *et al.* (2005) reported that higher temperatures of gelatin extraction exhibited a lower gel strength. A weak gelatin gel was associated with the formation of small fragments (Gómez-Guillén *et al.*, 2002). Kittiphattanabawon (2010) reported no gel formation of gelatin when the extraction temperature was above 60°C. Different gel strengths reported for gelatin from different marine species are also shown in Table 2. Among these marine gelatins, the gel strength of jellyfish gelatin is similar to salmon and rohu. The result was in agreement with Gómez-Guillén *et al.* (2002) that gelatin extracted at higher temperatures showed a lower gel strength. Factors associated with gelatin quality are pH, molecular weight distribution, and proline and hydroxyproline content of raw material as well as extraction conditions that could result in less organized triple helical structure (Shyni *et al.*, 2014; Lassoued *et al.*, 2014). Typically, the gel matrix is formed during gelation by the crosslinking of peptide chains. Denaturation is the change of a native protein conformation to another, more unfolded conformation, in which functional groups (such as sulfhydryl groups or hydrophobic groups) become exposed. Subsequently, these exposed groups can interact with each other to form aggregates (Wang and Damodaran, 1991). When the protein concentration is high enough, aggregation leads to formation of a gel. However, the shorter

Table 2. Gel strength of extracted jellyfish gelatin gels, bovine gelatin gels and different marine gelatin gels. Different letters on the bars denote significant differences ($P \leq 0.05$)

Extraction condition and the starting raw material of gelatin	Gel strength (g)	Reference
45°C 6h	47±1.47 ^c	
45°C 12h	118±8.07 ^b	
60°C 6h	56±1.92 ^c	
60°C 12h	18±0.67 ^d	
commercial	354±30.55 ^a	
cod	71	Arnesen and Gildberg, 2007
bigeye snapper	106	Jongjareonrak et al., 2006
Atlantic salmon	108	Arnesen and Gildberg, 2007
rohu	124	Shyni et al., 2014
splendid squid	132	Nagarajan et al., 2012
sturgeon	141	Nikoo et al., 2014
skipjack tuna	177	Shyni et al., 2014
shortfin scad	177	Cheow et al., 2007
cuttlefish	181	Balti et al., 2011
dog shark	206	Shyni et al., 2014
red snapper	219	Jongjareonrak et al., 2006
New Zealand hoki	231	Mohtar et al., 2014
seabass	369	Sinthusamran et al., 2014

chain lengths of gelatins cannot form a strong gel due to the lower inter-junction zones (Intarasirisawat et al., 2007). For commercial product, the strength of gelatin gels are separated as high-Bloom (200-300 g), medium-Bloom (100-200 g) and low Bloom (50-100 g) (Haug and Draget, 2011).

Emulsifying properties

Gelatin can be used as a foaming, stabilizing and emulsifying agent in food, pharmaceutical and technical applications due to its surface-active properties (Karim and Bhat, 2009). The oil in water (O/W) emulsion in this study was formed from a mixture of soybean oil, water with jellyfish gelatin used as an emulsifier. The function of jellyfish gelatin as a stabilizer and emulsifier was measured as the emulsion stability index (ESI). Results showed the jellyfish gelatin extracted at 75°C for 6 h had a significantly higher ESI than any jellyfish gelatins and bovine gelatin ($p < 0.05$) (Figure 2). The hydrophobic portion of jellyfish peptide may interact with the hydrophobic part of soybean oil, thereby stabilizing the emulsion. The shorter chains of the peptide might migrate to the oil-water interface faster, which would stabilize smaller particle size emulsions (Kaewrung et al., 2013). A number of factors that influence the stability of emulsions such as interfacial tension between the two phases, characteristics of the absorbed film in the interface, magnitude of the

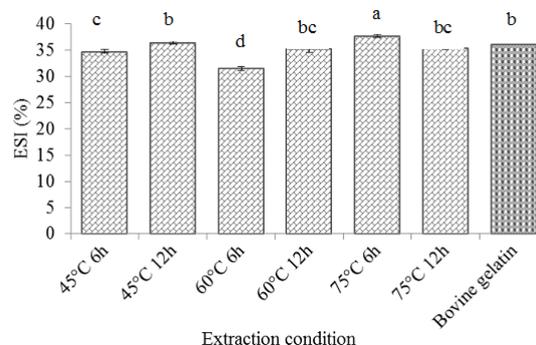


Figure 2. Emulsion stability index as a function of extraction temperature (°C) and extraction time (h). Bars represent standard deviation ($n = 3$). Different letters on the bars denote significant differences ($P \leq 0.05$)

electrical charge on the globules, size and surface per volume ratio of the globules, weight per volume ratio of dispersed and dispersion phases and viscosity of the dispersion phase (Morr, 1981).

Foaming properties

Gelatin can form and stabilize foams and is widely used in confectionary product such as marshmallows (Lassoued et al., 2014). Foam formation is generally controlled by transportation, penetration and reorganization of protein molecules at the air-water interface (Koli et al., 2012). In this study, the whipping process incorporates air into the gelatin solution to form bubbles. The integrity

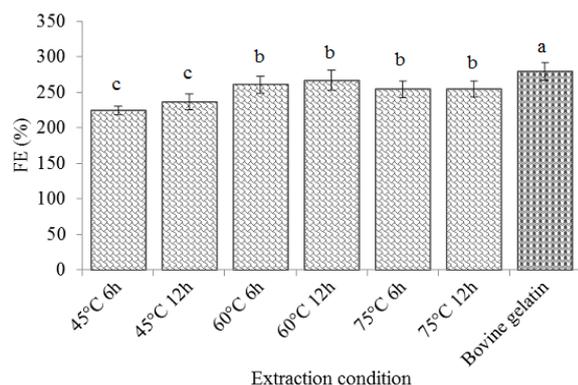


Figure 3. Foam expansion as a function of extraction temperature ($^{\circ}\text{C}$) and extraction time (h). Bars represent standard deviation ($n = 3$). Different letters on the bars denote significant differences ($P \leq 0.05$)

of foam is measured by foam expansion (FE). The %FE of all gelatin samples generally increased with increasing time for extraction (Figure 3). All jellyfish gelatins had slightly lower % FE compared to bovine gelatin. This might be due to the migration of shorter peptides to the air-water interface (Kaewrung *et al.*, 2013). However, the size of gelatin foam was not measured in this study. The hydrophobic regions facilitate the adsorption at the interface, a process that is followed by partial unfolding surface denaturation (Lomakina and Miková, 2006). Intermolecular protein-protein interaction enhances the cohesive nature of the film, thereby imparting stability and elasticity to the membrane surrounded the foam. This interaction appears to be dependent on the presence of a high ratio of nonpolar/polar side chains in the protein (Johnson and Zabik, 1981). The results suggested that jellyfish gelatin could be functioned as a foaming agent. Similar findings were reported by Lueyot and Thumthanaruk (2014) that the white jellyfish (*Lobonema smithii*) protein hydrolysate (WJPH) with highest degree of hydrolysis (60.62%) exhibits a good foaming and emulsifying properties.

Conclusions

Jellyfish could be a promising source for producing gelatin. All jellyfish gelatins from different extraction temperatures and times showed the lower values of gel strength than that of commercial bovine gelatin. To obtain gelatin with a high gel strength and high yield, the recommended extraction conditions were 45°C for 12 h. Beside gel properties, foams were formed using gelatin produced at all extraction conditions used in this study. The ability to form weak gels or emulsification and foaming may find new applications for jellyfish gelatin type A used in food industry.

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