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Extraction of type A and type B gelatin from jellyfish *(Lobonema smithii)*

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Abstract

Type A (acid treated process) and type B (alkaline treated process) gelatin were extracted from the umbrella of desalted jellyfish *(Lobonema smithii)* by sulfuric acid and sodium hydroxide, respectively, at different temperatures (60 and 75°C) and times (6 and 12 h). The condition of H_2SO_4 and NaOH used were adjusted to pH 2 and pH 14, respectively. The degree of hydrolysis of type B jellyfish gelatin (77.13%) was greater than that of type A gelatin (65.30%) at 75°C for 12 h. The highest content of soluble protein (203.12 mg/ml) of type A jellyfish gelatin was lower than that of type B jellyfish gelatin (350.02 mg/ml) treated at 75°C for 12 h. The type A of jellyfish gelatin exhibited gel formation only at the condition of 75°C for 6 and 12 h, but type B jellyfish gelatin had no gel formation at all conditions used. The hue color of type A and type B jellyfish gelatin had values in the range of 47.28-83.64 and 82.89-88.58, respectively. In summary only type A jellyfish gelatin exhibiting gel formation can be produced by sulfuric acid hydrolysis (pH 2) at a temperature of 75°C for 12 h.

Introduction

Gelatin is a fibrous protein produced by thermal denaturation or partial degradation of collagen from animal skin and bone (Benjakul *et al*., 2009; Tabarestani *et al*., 2010). Gelatin has been widely used in the food, pharmaceutical industries and other technical applications (Kaewruang *et al*., 2013). Generally, sources of gelatin are derived from the skin and bone of pig and cow which are not acceptable for the Halal and Kosher food markets. Additionally, the occurrence of Foot and Mouth Disease (FMD) or Bovine Spongiform Encephalopathy (BSE) have caused anxiety about of bovine gelatin (Kittiphattanabawon *et al*., 2010). Therefore, seafood products have become a choice of gelatin source. Skin gelatins from several fish species, e.g., brownbanded bamboo shark *(Chiloscyllium punctatum)* and blacktip shark *(Carcharhinus limbatus)* (Kittiphattanabawon *et al*., 2010), unicorn leatherjacket *(Aluterus monoceros)* (Kaewruang *et al*., 2013) and seabass *(Lates calcarifer)* (Sinthusamran *et al*., 2014) have been investigated for their qualities.

Instead of using the skin of marine animals for gelatin production, the whole body as raw material from species like jellyfish has become an interesting alternative source due to the collagen content (Klaiwong *et al*., 2014). Jellyfish are marine invertebrate animals in the phylum Cnidaria. The processed jellyfish (dried) was first produced in China and recognized as one of Asian's favorite foods (Hsieh *et al*., 2001). Jellyfish are low-fat and cholesterol-free marine food and collagen is the main protein in jellyfish (Hsieh *et al*., 2001). Research related to jellyfish products such as dried protein concentrate (Saehor, 2011), collagen properties from sand jellyfish *(Rhopilema hispidum)* and white jellyfish *(Lobonema smithii)* (Klaiwong *et al*., 2014) and hydroxyl radical scavenging activity of jellyfish *(Rhopilema esculentum)* hydrolysate (Zhuang *et al*., 2009) had been investigated. Nevertheless, no information regarding gelatin extraction from white jellyfish *(Lobonema smithii)* under varying conditions has been reported. Therefore, this study aimed to determine the extraction of gelatin type A and type B from jellyfish *(Lobonema smithii)*. The qualities of dried jellyfish gelatin were also investigated.

Materials and Methods

Preparation of dried jellyfish

The umbrella of minced salted jellyfish *(Lobonema smithii)* was obtained from Mahachai Seafoods Co., Ltd., Samut Sakhon, Thailand

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and kept in sealed polyethylene bags at 10±2°C in the Department of Agro-Industrial, Food and Environmental Technology lab, King Mongkut's University of Technology North Bangkok, Thailand. The minced salted jellyfish was washed with tap water for several times until the salt content was below 1°Brix, was determined with a salinometer (Atago®, S/Mill-E, Japan). The samples were dried by a hot air oven (Bluem, G 01350, USA) at 50°C for 24 h. The dried jellyfish having the moisture content of 9.61 ± 0.06% were ground and stored in polyethylene bags and packed in a desiccator at room temperature until used.

Extraction of gelatin from the dried jellyfish

Gelatin was extracted by modifying the method of Ahmed and Benjakul (2011). The dried jellyfish were soaked in sulfuric acid solution pH 2 for acid-treated (type A) and sodium hydroxide solution pH 14 for alkaline-treated (type B). The ratio of dried sample: solution used was 1: 15 (w/v). The mixture was stirred continuously for 24 h at 4°C using a speed of 150 rpm in a shaking incubator (WiseCube, WIS-20R, Korea). At the end of reaction, pH of type A gelatin was adjusted with sodium hydroxide solution (1 N) and type B gelatin used sulfuric acid solution (1 N) to pH 7. The mixture was kept in a temperature controlled water bath (Memmert, Schwabach, Germany) for 6 and 12 h at different temperature (at 60 and 75°C). The extract was filtered twice using two layers of filter cloth. The filtrate was collected and dried in a hot air oven (Bluem,G 01350, USA) at 50°C for 24 h. The dried powders of gelatin (type A and type B) were obtained, kept in sealed polyethylene bags and stored in the desiccator at room temperature.

Determination of soluble protein concentration

The soluble protein concentration of gelatin was measured by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

Determination of degree of hydrolysis (DH)

DH of gelatin was determined according to the method of Benjakul and Morrissey (1997) with slight modification. Properly diluted samples (125 µl) were mixed thoroughly with 2.0 ml of 0.2125 M phosphate buffer, pH 8.2, followed by the addition of 1.0 ml of 0.01% 2,4,6-trinitrobenzene sulfonic acid (TNBS) solution. The mixtures were then placed in a water bath at 50°C for 30 min in the dark. The reaction was terminated by adding 2.0 ml of 0.1 M sodium sulfite. The mixtures were cooled down at ambient temperature for 15 min. The absorbance was measured at 420 nm. The DH can be determined using Equation 1.

$$\% DH = [(L_t - L_0)/(L_{max} - L_0)] \times 100 \quad (1)$$

when L_t is the absorbance at 420 nm of hydrolysate at a given time, L_0 is the absorbance of control and L_{max} is the absorbance of hydrolysate at the complete hydrolysis.

Yield of gelatin

The yield of dried gelatin was calculated based on Equation 2.

$$\% Yield = [\text{weight of dried gelatin (g)}/ \text{weight of dried minced jellyfish (g)}] \times 100 \quad (2)$$

Determination of gel strength of jellyfish gelatin

Gelatin gels were prepared using the method of Fernandez-Diaz *et al.* (2001) with slight modification. Gelatin samples were dissolved in distilled water (6.67% (w/v)).

The gelatin solution was heated to 50°C until it was completely solubilized and then poured into plastic bottles with the size of 3 cm (diameter) and 2 cm (height). The gelatin solution was cooled in a refrigerator at 10°C for 16-18 h to form the gel. The gel strength was determined using a Texture Analyzer (Texture profile analyzer, TA-XT2i, UK). A load cell of 5 kN equipped with a 50 mm diameter flat faced hemispherical probe plunger was used. The maximum force (g) was recorded when the penetration distance reached 40 mm. The pre-test speed and post-test speed of the plunger was 1, 10 mm/s, respectively.

Color measurement

Color of gelatin solution (6.67%) was determined using a colorimeter (Hunter lab, Color Quest XT, USA). The values of L^*, a^*, b^* indicating lightness/ brightness (0 = black, 100 = white), redness (+a)/ greenness (-a) and yellowness (+b)/blueness (-b) were observed. Hue angle can be calculated according to Equation 3.

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

The hue angle is the color of the object is between 0-360 degrees : purple-red to orange-red (0-45), orange-red to yellow (45-90), yellow to yellow-green (90-135), yellow-green to green (135-180), green to blue.(180-225), blue-green to blue (225-270), blue to purple (270-315) and purple to purplish-red (315-360). Hue angle values were determined by McGuire (1992).

Table 1. Extraction soluble protein, degree of hydrolysis, yield and gel strength of jellyfish gelatin extracted at different condition

Gelatin solution	Condition	Soluble protein (mg/ml)	(%DH)	Yield (%)	Gel strength (g)
Type A	60°C for 6h	26.14 ± 0.10^{c}	22.56 ± 0.70^{d}	26.45 ± 0.11^{c}	no gel
	60°C for 12h	23.81 ± 0.22^{d}	28.13 ± 0.71^{c}	24.39 ± 0.19^{d}	no gel
	75°C for 6h	60.83 ± 0.69^{b}	30.82 ± 0.69^{b}	34.65 ± 0.18^{b}	62.33 ± 0.57^{b}
	75°C for 12h	203.12 ± 1.04^{a}	65.30 ± 0.53^{a}	39.47 ± 0.04^{a}	108.36 ± 0.43^{a}
Type B	60°C for 6h	301.01± 0.77^{c}	68.68 ± 0.11^{c}	44.65 ± 0.13^{d}	no gel
	60°C for 12h	420.88 ± 0.75^{a}	74.74 ± 0.22^{b}	46.52 ± 0.15^{c}	no gel
	75°C for 6h	301.61 ± 0.69^{c}	50.63 ± 0.44^{d}	59.54 ± 0.26^{b}	no gel
	75°C for 12h	350.02 ± 0.13^{b}	77.13 ± 0.45^{a}	67.45 ± 0.12^{a}	no gel

Mean ± SD (n = 3).
Different lowercase letters within the same column indicate significant differences ($p< 0.05$).

Amino acid analysis

Amino acid content of jellyfish gelatin was measured by using method of AOAC (2005). The dried gelatin (10 g) was hydrolyzed with 6N HCl at 110°C for 48 h in block heater (Model SBH 130D, Stuart Scientific, Manchester, UK). The hydrolyzed gelatin samples were flushed with nitrogen gas, adjusted the volume with 5 ml distilled water, and filtered by using a cellulose filter of 0.4 µm (VertiPure™ CA Syringe Filter, Vertical Chromatography Co., Ltd. Bangkok, Thailand). The filtrate was analyzed by High Performance Liquid Chromatography (RP-HPLC Model 1200, Agilent Technologies, Inc. Santa Clara, CA, USA) equipped with an Evaporative Light Scattering Detector (ELSD) (Model 3300, Alltech®, Deerfield, IL, USA).

Statistical analysis

Statistical analysis was performed using the SPSS program (SPSS 19.0 for windows. SPSS Inc., Chicago. IL, USA). Data were presented as means ± standard deviation.

The difference of mean values were analyzed by one way analysis of variance (ANOVA) and analyzed using Duncan's multiple range test. Differences with $p<0.05$ were statistically significant.

Results and Discussion

Soluble protein and degree of hydrolysis of extracted gelatin

The soluble protein contents of jellyfish gelatin type A and B are shown in Table 1. The pHs used for type A and type B gelatin extraction were 2 and 14. The type A jellyfish gelatin extracted at a temperature of 75°C for 12 h and type B extracted at 60°C for 12 h had high soluble protein of 203.12 and 420.88 mg/ml respectively. At the same extraction of 75°C for 12 h, the high DHs of type A and type B jellyfish gelatin were 65.30% and 77.13%, respectively. The results showed increased of soluble protein if extraction temperatures and times increased.

Yield of gelatin

The highest yield of type A and type B jellyfish gelatin were 39.47% and 67.45% (on dry weight basis) at the extraction condition of 75°C for 12 h. The degree of conversion of collagen into gelatin depends on the processing parameters (temperature, extraction time and pH) as well as the pretreatment conditions, the properties and the preservation method of the starting raw material (Karim and Bhat 2009). The increased temperature provided more energy to disrupt bonding stabilizing the collagen structures and peptide bonds of α-chains. As a result, a large amount of gelatin could be extracted as the temperature was elevated (Nagarajan *et al.*, 2013).

Gel strength

Jellyfish gelatin type A had the highest gel strength (108.36 g) when the extraction condition of 75°C for 12 h was used (Table 1). All the type B gelatins did not form gels. The results suggested that the α-chains or β-chains of collagen might undergo conformation changes, when extracted with high temperatures and times. Gel strength of gelatin was decreased by the effect of heat to destroy gelatin structure (Cho *et al.*,

Table 2. Colors of jellyfish gelatin solution extracted at different condition

Gelatin solution	Condition	L^*	a^*	b^*	hue angle
Type A	60°C for 6h	42.13 ± 0.13^{d}	-3.17 ± 0.06^{c}	3.45 ± 0.38^{d}	47.28 ± 2.68^{d}
	60°C for 12h	43.02 ± 0.34^{c}	-3.08 ± 0.03^{c}	4.73 ± 0.34^{c}	56.69 ± 2.00^{c}
	75°C for 6h	52.49 ± 0.31^{a}	-2.82 ± 0.12^{b}	8.03 ± 0.29^{b}	70.62 ± 1.39^{b}
	75°C for 12h	46.39 ± 0.09^{b}	-1.34 ± 0.06^{a}	12.14 ± 0.18^{a}	83.64 ± 0.35^{a}
Type B	60°C for 6h	24.55 ± 0.07^{b}	-0.63 ± 0.01^{d}	5.08 ± 0.16^{d}	82.89 ± 0.18^{b}
	60°C for 12h	27.81 ± 0.15^{a}	-0.59 ± 0.02^{c}	8.57 ± 0.18^{b}	86.00 ± 0.23^{ab}
	75°C for 6h	24.10 ± 0.03^{c}	-0.18 ± 0.02^{a}	8.07 ± 0.03^{c}	85.37 ± 5.67^{ab}
	75°C for 12h	22.45 ± 0.13^{d}	-0.23 ± 0.03^{b}	9.59 ± 0.04^{a}	88.58 ± 0.16^{a}

Mean ± SD (n = 3).
Different lowercase letters within the same column indicate significant differences ($p< 0.05$).

2005). The larger bundles or strands might not form a fine and ordered gel structure, three-dimensional (junction zone), which reduces the strength of the gel network (Karim and Bhat 2009). The gel network stabilization of the triple-stranded collagen helix may be due to its hydrogen bonding ability through its hydroxyl group (Sinthusamran *et al.*, 2014). The differences in gel strength obtained from different raw materials had been previously reported, including splendid squid *(Loligo formosana)* (85-132 g) (Nagarajan *et al*., 2012), rainbow trout *(Oncorhynchus mykiss)* (459 g) (Tabarestani *et al*., 2010), brownbanded bamboo shark *(Chiloscyllium punctatum) a*nd blacktip shark *(Carcharhinus limbatus)* (206-214 g) (Kittiphattanabawon *et al.*, 2010).

Color

Differences in the color of gelatin solutions were observed. Type A extracted at 75°C for 6 h showed the higher L^* value (lightness) (52.49) than other conditions used ($p<0.05$), while the higher a^* value (redness) was contradicted with the data in Table 1. The a^* (redness) values in Table 1 were all in negative values and b^* value (yellowness) was found in the gelatin type A extracted at 75°C for 12 h. The colors of two gelatins were clearly demonstrated in hue value. The hue angle is the color of the object is between 0-360 degrees. The hue angle values of type A and type B jellyfish gelatin were 83.64 and 88.58, respectively, indicating that the samples were in the green-yellow color area. The changes in color of gelatin with increasing temperatures and times of extraction obtained. The yellowness color might be due to a non-enzymatic browning reaction taken place at the higher temperature and extraction time increased (Ajandouz and Puigserver, 1999). The result indicated that extraction temperature had a direct impact on color of gelatin and extraction time did not.

Amino acid composition

The total amino acid content of jellyfish gelatin type A was 43.30 g/100 g sample which was higher than that of jellyfish gelatin type B (35.40 g/100g sample; Table 3). The gelatin type A and B contains glycine, proline and hydroxyproline of 11.22, 3.83, 4.63 and 8.66, 3.65,0.00 g/100g sample, respectively. Generally, glycine occurs every third position in the α-chain was derived from its collagen (Benjakul *et al.*, 2009; Balti *et al*., 2011). The loss of hydrolysis might be occurred during extensive alkaline hydrolysis and extraction. Giménez *et al.* (2009) and Gómez-Guillén *et al.* (2002) reported lower hydroxyproline content in gelatin extracted at 70°C and 80°C. The decomposition of hydroxyproline may correlate with gel formation which is not found for jellyfish gelatin type B. Hydroxyproline is formed from proline by a post-translational modification of enzymatic hydroxylation reaction and is used almost exclusively in structural proteins including collagen. Non-hydroxylated collagen is commonly termed pro-collagen. A difference in amino acid contents were reported for gelatin from splendid squid skin (184 residues/1000 residues) (Nagarajan *et al*., 2012), salmon skin (166 residues/1000 residues) (Arnesen and Gildberg 2007) and bovine gelatin (219 residues/1000 residues) (Jellouli *et al*., 2011). The amino acid content also influences the functional properties of gelatin. Kittiphattanabawon *et al.* (2010) reported that gelatin with higher content of amino acids had higher bloom strength than gelatin

Table 3. Amino acid composition of type A and type B jellyfish gelatin

Amino acids	Number of amino acid g/100g sample	
	type A jellyfish gelatin (75°C 12 h)	type B jellyfish gelatin (75°C 12 h)
Hydroxyproline	4.63 ± 0.06	0
Serine	0	0
Threonine	0.63 ± 0.01	0.52 ± 0.01
Methionine	0.53 ± 0.02	0.78 ± 0.03
Glutamine	6.09 ± 0.10	6.73 ± 0.16
Asparagine	3.54 ± 0.03	3.98 ± 0.18
Cysteine	0.26 0.02	0
Lysine	2.15 ± 0.03	2.17 ± 0.12
Arginine	3.81 ± 0.03	0.30 ± 0.01
Histidine	0.47 ± 0.02	2.07 ± 0.20
Glycine	11.22 ± 0.19	8.66 ± 0.25
Proline	3.83 ± 0.09	3.65 ± 0.17
Alanine	0.68 ± 0.01	0.60 ± 0.01
Valine	1.61 ± 0.05	1.81 ± 0
Leucine	1.63 ± 0.09	1.60 ± 0.02
Isoleucine	1.04 ± 0.01	0.90 ± 0.09
Phenylalanine	0.44 ± 0.03	0.66 ± 0.01
Tyrosine	0.39 ± 0	0.61 ± 0.01
Tryptophan	0.25 ± 0	0.29 ± 0.01
Total	43.30 ± 0.40	35.40 ±0.80

containing lower levels of amino acids. Thus jellyfish gelatin type B should not be used in foods in which gel forming is required.

Conclusion

Type A and B gelatin extracted from jellyfish by sulfuric acid or sodium hydroxide had different characteristics and gelling properties. The type A jellyfish gelatin exhibiting good gel formation can be produced with an extraction temperature of 75°C for 12 h. For all of the extraction conditions used, jellyfish gelatin type B had no gel formation. Future study of jellyfish gelatin functionalities is needed for proper used in food application.

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