Physicochemical Properties of Gelatin from Jellyfish *Rhopielma hispidium*

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Abstract

Objective of this study was to elucidate physicochemical characteristics of gelatin extracted from jellyfish (*Rhopielma hispidium*). The proximate composition, amino acids, electrophoresis, gel strength, gelling/melting points, dynamic viscoelastic properties, and viscosity of jellyfish gelatin were investigated. Jellyfish gelatin contained 12.2% moisture, 1.5% lipid, 2.1% ash and 84.8% protein. Glycine, hydroxyproline, proline and alanine were predominant amino acids in jellyfish gelatin. The gelatin showed gel strength of 31.2 kPa, gelling point at 18.0 °C and melting point at 22.3 °C. The gelatin composed of α1-chain, α2-chain, β-chain and γ-chain. During cooling and heating process, jellyfish gelatin showed lower elastic modulus (G') and loss modulus (G'″) values than mammalian gelatins. Jellyfish gelatin did not show superior rheological properties than mammalian gelatins like other fish gelatins; however, it may be used in various food and cosmetic products not requiring high gel strength.

**Keywords:** Gelatin, Jellyfish, *Rhopielma hispidium*, Physicochemical property, Gel strength
Introduction

Gelatin is a denatured protein derived from collagen by thermo-hydrolysis, and has a rheological property of thermo-reversible transformation between sol and gel (Stainsby, 1987; Cho et al., 2004). Gelatin, one of the most important gelling agents, has widely been applied in the food, pharmaceutical, cosmetic, and photographic industries (Cho et al., 2004; Cho et al., 2005).

Most of commercial gelatin is produced from by-products of mammalians, such as porcine and bovine. However, frequent occurrences of bovine spongiform encephalopathy (BSE) and foot/mouth diseases have been problems for human health, and sources from mammalians for gelatin production have been limited (Cho et al., 2005). For replacement of bovine and porcine gelatin products, fish gelatins have been widely investigated. In addition, religious constraints (Kosher and Halal foods) and attention of consumers on health issues have also resulted in a high demand of fish gelatin (Kittiphattanabawon et al., 2010). Thus far, numerous studies have reported production and physicochemical properties of gelatins from various fish sources, such as harp seal *Phoca groendlandica* (Arnesen et al., 2002), yellowfin tuna *Thunnus albacares* (Cho et al., 2005), shark cartilage (Cho et al., 2004), baltic cod *Gadus*
*morhua* (Kołodziejska et al., 2004), and black tilapia *Oreochromis mossambicus* (Jamilah and Harvinder, 2002).

Although physicochemical properties of gelatins from various fish sources, information of jelly fish as a source of gelatin production is limited. In Korea, a tremendous increase of jellyfish is becoming a big problem. Therefore, utilization of jellyfish as alternative of mammalian sources for gelatin production is needed. In the present study, the physicochemical properties of gelatin from jellyfish (*Rhopilema hispidum*) were investigated through analysis of proximate composition, amino acids, electrophoresis, gel strength, gelling point, melting point, dynamic viscoelastic properties, and viscosity.

**Material and Methods**

**Preparation of gelatin**

The jellyfish *Rhopilema hispidum* were provided by Korea Jellyfish Inc. (Busan, Korea). The sample was kept at -20°C until gelatin extraction. The extraction of gelatin was performed according to the method of Cho et al. (2005). The jellyfish was washed with tap water for 12 h to remove the foreign matters and then chopped. The sample was treated with 5
volumes (v/w) of 2% NaOH in a shaking incubator (200 rpm) at 10°C for 8 h to remove non-collagen protein and to swell the tissues. After the pretreatment, the sample was neutralized with 6 N HCl and then washed with distilled water (DW). The extraction of gelatin from the sample was performed in 6 volumes (v/w) of DW at 60°C for 5 h over agitation. The extraction solution were filtered using filter paper (No. 5A, Advantec, Japan), concentrated at 60°C, and dried at 50°C for 24 h in a hot-air dryer (WFO-601SD, EYELA, Japan). The yield of the gelatin sample was 38.5%.

**Measurement of proximate components and pH**

Moisture content (oven-drying procedure), crude protein (N×6.25), lipid (ether extraction) and ash content were estimated by the AOAC official method (Horwitz, 2000). pH was measured by melting 0.1 mg gelatin in 10 mL distilled water at 60°C with pH meter (Accumet model 15, Fisher Scientific Co., USA). The analyses were replicated three times.

**Analysis of amino acid**

The gelatin (30 mg) extracted was dissolved in 10 mL of 6 N HCl and then heated at
110°C for 24 h in a dry bath (11-718-2, Fisher Scientific Co., USA). The hydrolysates were filtered with a glass filter and vacuum-concentrated at 60°C. The concentrates were made up 10 mL with citrate phosphate (pH 2.2) and then analyzed with the automatic amino acid analyzer (S-433H, Sycam, Germany).

**SDS-polyacrylamide gel electrophoresis (SDS-PAGE)**

SDS-PAGE was performed according to the method of Laemmli (1970). For polyacrylamide gel, 5% stacking gel and 6% separating gel were used. Gelatin solution (10 mg/mL) and tracking dye mixtures containing 0.5 M Tris-HCl buffer (pH 6.8), 5% 2-mercaptoethanol, 20% glycerol, and 0.1% bromophenol blue were heated at 100°C for 5 min. After heating, the solution was injected on the gel and then electrophoresis was performed at 15 mA/gel with Mini-Protean 3 (Bio-Rad Laboratories, Hercules, CA, USA). The gel was separated, dyed in 0.25% (w/v) comassie brilliant blue R250 and then decolorized. Calf skin collagen (Sigma-Aldrich Inc, St Louis, MO) was used as the marker protein.

**Measurement of gel strength**
Gel strength was measured according to the method of Cho et al. (2005) using rheometry (Compac-100, Sun Scientific Co., Ltd., Japan). Gelatin was dissolved with DW (6.67%, w/v) at 60°C for 30 min until completely dispersed and then kept at 7°C for 17 h. After cool maturation, the gel strength was measured with the following conditions; plunger, 12.7 mm diameter; penetration depth, 4 mm; penetration speed, 2 cm/min.

**Measurement of gelling and melting points**

Gelling and melting points were measured according to the method of Gudmundsson (2002) and Cho et al. (2005). The gelling point was evaluated from the intersection point where the elastic modulus (G', Pa) and the loss modulus (G", Pa) during the cooling process. The melting point was done during the heating process in a same manner as for the gelling point.

**Measurement of dynamic viscoelastic properties**

Dynamic viscoelastic properties were measured by a rheometer (Rheostress 1 RS30, HAAKE Co., Ltd., Karlsruhe, Germany). The concentration of gelatin was made into 6.67% with water bath at 60°C. The measurement was performed at frequency at 1 Hz, the temperature
at 0.5°C/min; oscillating applied stress 3 Pa and gap at 4.2 mm. The ranges of temperature were divided into two ones: a range of cooling down from 40°C to 5°C and a range of heating up from 5°C to 40°C, for measurement of elastic modulus (G', Pa) and loss modulus (G'', Pa).

**Measurement of viscosity**

Viscosity was measured according to the method of Kittiphattanabawon et al. (2005) with a slight modification. Gelatin (0.04%, 100 mL) was added into 0.1 M acetic acid at 60°C. Spindle No. 40 of Brookfield Synchorolectic viscometer (Model DV II+, Brookfield Eng Labs Inc., Stoughton, MA, USA) was used to measure viscosity at 60 rpm. The temperature for the measurement ranged from 15°C to 50°C, at 15°C/min. At this time, the experiment was carried out after the solution was maintained at each temperature for 10 min.

**Measurement of denaturation temperature**

Denaturation temperature was measured by the method of Kimura et al. (1988) with a slight modification. Denaturation temperature was expressed by measuring viscosity of gelatin (0.03%, 5 mL) at intervals of 5°C at 20-50°C with Ostwald-Fenske viscometer (Canon
Instrument Co., State College, Pa., USA). During the measurement, the temperature of gelatin was maintained for 10 min by ranges of temperature before the experiment was conducted. The denaturation temperature (Td) was evaluated at the half position of the measured value.

Results and Discussion

Proximate composition

The proximate composition and pH value of jellyfish gelatin were shown in Table 1. Jellyfish gelatin contained 12.2% moisture, 84.8% crude protein, 1.5% crude lipid, and 2.1% crude ash. The ash content of gelatin plays a role in a very important factor in the quality of gelatin. According to the US standards of food (Gelatin, FCC, 1994), the maximum ash content of gelatin is 3%. The jellyfish gelatin contained ash less than the regulation level. The pH value of jellyfish gelatin was 7.32.

Amino acid composition

Jellyfish gelatin contained high levels of glycine (Gly, 18.90%), proline (Pro, 8.15%),
and hydroxyproline (Hyp, 13.93%) (Table 2). This is originated from a repeated structure (Gly-Pro-Hyp) of collagen, the precursor of gelatin. Ledward (1986) reported that gelatin has a repeated structure of Gly-X-Y. When Pro and Hyp were located in the X and Y positions, the gelatin structure was stable. The total content of Gly-Pro-Hyp is one of the important factors that express a thermal stability of gelatin (Burjandze, 2000). Imino acids are involved in formation of hydrogen bond (Ledward, 1986). Generally, mammalian gelatins contain more imino acids (Pro and Hyp) than fish gelatins (Gilsenan and Ross-Murphy, 2000; Haug et al., 2004). In the present study, jellyfish gelatin showed lower imino acid content than mammalian gelatins reported in previous studies. The contents of alanine and lysine in jellyfish gelatin were 6.9% and 2.5%, respectively. Gómez-Guillén et al. (2002) reported that the low content of alanine causes a poor function of gelation, and lysine also stabilizes gelatin structure by forming cross-linking structures between chains.

**Electrophoretic profiles**

Fig. 1 shows electrophoretic patterns of jellyfish gelatin and calf skin collagen (a marker protein) by SDS-PAGE. The calf skin collagen was comprised of α1-chain and α2-chain (α1 : α2 = 1 : 2), β-component (cross linked dimer of α-chains), and γ-component (cross linked...
trimer of α-chains). Giraud-Guille et al., (2000) reported that the molecular weights of α₁, α₂, β and γ chains are 93, 93, 186 and 279 kDa, respectively. It was found that jellyfish gelatin composed of α₁-chain, α₂-chain, β-chain and γ-chain and the α₁-chain was less clear than that of calf skin collagen. Lower content of high molecular weight fractions (β- and γ-chains) for bone gelatins was associated with lower viscosity, melting, and setting point and longer setting time (Muyonga et al., 2004).

**Gel strength, gelling and melting points**

In assessment of gelatin quality, physical properties such as gel strength, gelling and melting points are important. The gelation of gelatin is occurred by physical crosslinking leading to the formation of junction zones and ultimately a three-dimensional branched network (Gilsenan and Ross-Murphy, 2000). Generally, fish gelatins show lower gel strength than mammalian gelatins (Norland, 1987; Choi and Regenstein, 2000). Among fish, tropical fish such as tilapia and tuna possesses a superior gel strength than cold-water fish such as cod (Gudmundsson and Hafsteinsson, 1997; Gómez-Guillén et al., 2002; Cho et al., 2005). The gel strength (31.2 kPa) of jellyfish gelatin was lower than porcine (147.4 kPa) and bovine (107.9 kPa) gelatins (data not shown). Fig. 2 shows changes in gel strength of jellyfish gelatin as
affected by the concentration. Until the gelatin concentration reached at 5%, jellyfish gelatin did not form the gel. However, after the gelatin concentration reached at 1.5%, the gelatins from porcine and bovine formed the gel (data not shown). Jellyfish gelatin needed higher concentration than porcine and bovine gelatins to form the gel. Gel strength is a function of complex interactions determined by amino acid composition and the ratio of α-chain and the amount of β-component (Cho et al., 2004). Gómez-Guillén et al. (2002) reported gel structure of gelatin is more stable when the imino acid (Hyp and Pro) content is higher, and when the amount of aggregates of higher molecular weight is less. The total content of Gly-Pro-Hyp is one of the important factors that express a thermal stability of gelatin (Burjandze, 2000). The lower gel strength in jellyfish gelatin is probably due to its lower amount of total Gly+Hyp+Pro, which stabilized gelatin structures.

Gelling and melting points are also an important indicator on the gelatin quality. Generally, mammalian gelatins possess higher gelling and melting points than fish gelatins (Choi and Regenstein, 2000; Gilsenan and Ross-Murphy, 2000; Gudmundsson, 2002). The gelling and melting points of jellyfish gelatin were 18.0°C and 22.3°C (Table 3). Mammalian gelatins have much higher gel set temperatures than both warm- and cold-water fish gelatins (Avena-Bustillos et al., 2006). Cho et al. (2005) reported that the gelling and melting points of mammals were 23.8°C and 33.8°C in bovine gelatin; 25.6°C and 36.5°C in porcine gelatin,
respectively. The gelling and melting points of jellyfish gelatin were lower than those of mammalian gelatins. This tendency has been reported in the previous studies on the gelatins from other fish, such as tuna (Cho et al., 2005) and tilapia (Gilsenan and Ross-Murphy, 2000; Gudmundsson, 2002). The results suggest that jellyfish gelatin has useful physical properties different from mammalian gelatins.

**Dynamic viscoelastic properties**

Dynamic viscoelastic profiles were measured during both the cooling process (40-5°C) and heating process (5-40°C) at the rate of 0.5°C/min. Fig. 3 and 4 show the changes in elastic modulus (G', Pa) and the loss modulus (G'', Pa), respectively. The elastic modulus (G') and the loss modulus (G'') are an important indicator for gelling ability of gelatin. In general, when G'' value is higher than G' value, it shows the state of sol, whereas when G' value shows higher than G'' value, it shows the state of gel. Intersection point of G' and G'' values means a gelling point (Winter and Chambon, 1986). The G' values of jellyfish gelatin sharply increased when it decreased during cooling under 18°C whereas G'' values gradually increased. G' and G'' values of the gelatins at 5°C of heating process were higher than those at 5°C of cooling process. The reason for the increase is that the gelatin gel were continued to stabilize for a few minutes until
the measurement restarted. Gelatins with larger elastic modulus values, also indicated by higher gelation temperatures, contained higher concentrations of helical structures (Joly-Duhamel et al., 2002). The results show that the mammalian gelatins possess a higher melting point and more thermo-stability than jellyfish gelatin.

Viscosity

Fig. 5 shows changes in relative viscosity of jellyfish gelatin solution (0.04%, w/v) at different temperatures. At the lower temperatures, the gelatin molecules begin to form triple helical junction zones to occur more cross-linking and develop eventually some network structure, and the viscosity rapidly increases (Avena-Bustillos et al., 2006). At higher temperatures, the gelatin molecules behave as random coils in solution and all gelatins had low viscosities. When gelatin solution undergoes heat treatment, the hydrogen bonds of gelatin molecules were broken down and then the viscosity is decreased (Nagai et al., 1999; Nagai and Suzuki, 2000; Nagai and Suzuki, 2002). The viscosity of jellyfish gelatin solution tended to rapidly decrease until the temperature reached at 32°C, and decreased gradually at the ranges from 33°C to 50°C. According to a report of Kittiphattanabawon et al. (2005), when gelatin was extracted from the bones and skins of a bigeye snapper, the viscosity of the gelatin decreased
gradually at ranges from 35°C to 50°C. The change in viscosity of jellyfish gelatin showed a similar tendency to report of Kittiphattanabawon et al. (2005).

**Acknowledgements**

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**References**


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compared to mammalian gelatin. Food Hydrocoll 18, 203–13.


Table 1. Proximate composition and pH value of gelatin extracted from jellyfish *Rhopilema hispidium*

<table>
<thead>
<tr>
<th>Items</th>
<th>Content or value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>12.2±0.3%</td>
</tr>
<tr>
<td>Crude protein</td>
<td>84.8±0.2%</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>1.5±0.3%</td>
</tr>
<tr>
<td>Crude ash</td>
<td>2.1±0.3%</td>
</tr>
<tr>
<td>pH</td>
<td>7.32</td>
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Table 2. Amino acid composition of gelatin extracted from jellyfish *R. hispidum*

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Contents (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyproline</td>
<td>13.93</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>5.46</td>
</tr>
<tr>
<td>Threonine</td>
<td>1.99</td>
</tr>
<tr>
<td>Serine</td>
<td>2.53</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>6.09</td>
</tr>
<tr>
<td>Proline</td>
<td>8.15</td>
</tr>
<tr>
<td>Glycine</td>
<td>18.90</td>
</tr>
<tr>
<td>Alanine</td>
<td>6.88</td>
</tr>
<tr>
<td>Valine</td>
<td>1.94</td>
</tr>
<tr>
<td>Isoleucine</td>
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<tr>
<td>Leucine</td>
<td>2.22</td>
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<tr>
<td>Tyrosine</td>
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</tr>
<tr>
<td>Phenylalanine</td>
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</tr>
<tr>
<td>Lysine</td>
<td>2.49</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.62</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.62</td>
</tr>
<tr>
<td>Imino acids$^1$</td>
<td>22.08</td>
</tr>
</tbody>
</table>

$^1$Imino acids mean proline and hydroxyproline.
Table 3. Gel strength, gelling point and melting points of gelatin extracted from jellyfish *R. hispidium*

<table>
<thead>
<tr>
<th>Items</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel strength (kPa)</td>
<td>31.2±1.2</td>
</tr>
<tr>
<td>Gelling point (°C)</td>
<td>18.0</td>
</tr>
<tr>
<td>Melting point (°C)</td>
<td>22.3</td>
</tr>
</tbody>
</table>
Fig. 1. SDS-PAGE patterns of gelatin from jellyfish *Rhopilema hispidum*. Calf skin collagen was used as a maker protein. A, calfskin collagen; B, jellyfish gelatin.
Fig. 2. Changes in the gel strength of gelatin from jellyfish *R. hispidium* as affected by the concentration.
Fig. 3. Changes in elastic modulus (G', kPa) during cooling (40-5°C) and heating (5-40°C) process of gelatin solutions from jellyfish *R. hispidum*. A cooling and heating rate was 0.5°C/min, and a 6.67% (w/v) gelatin solution was used.
Fig. 4. Changes in loss modulus ($G''$, kPa) during cooling (40-5°C) and heating (5-40°C) process of gelatin solutions from jellyfish *R. hispidium*. A cooling and heating rate was 0.5°C/min, and a 6.67% (w/v) gelatin solution was used.
Fig. 5. Changes in relative viscosity of gelatin solution (0.04%, w/v) from jellyfish *R. hispidium* at different temperatures.