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Physical and chemical properties of gelatin from the skin of cultured Amur sturgeon (*Acipenser schrenckii*)

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Summary

To expand the usefulness of cultured Amur sturgeon, *Acipenser schrenckii*, its skin was used to explore the production of gelatin. After acetic acid pre-treatment (0.05 M for 3 h), gelatin was extracted at temperatures of 50 or 70°C for 1 or 6 h. Gelatin yield ranged from 9.42 to 12.47% (wet weight basis) ($P < 0.05$). With increasing extraction time and temperature, the content of imino acids (proline + hydroxyproline), gel strength and L*-value (lightness) decreased, while the a*-value (redness) and b*-value (yellowness) of gelatin gel increased ($P < 0.05$). Electrophoretic analysis revealed that α-chains and *β*-chains were predominant components in all extracted gelatins. Higher molecular weight proteins (*γ*-chain) were also observed. Gelling and melting temperatures of gelatin were 13.6–14.6°C and 20.3–22.6°C, respectively. Circular dichroism (CD) spectra and Fourier transform infrared (FTIR) spectroscopy revealed the triple helix loss in gelatin (A_{1235} (AIII)/$A_{1451} < 1$). Extraction conditions caused secondary structure changes in the gelatin. More likely due to the differences in the culture water temperature, gelatin exhibited gelling and melting temperatures intermediate between those of cold- and warm-water fish gelatins. The obtained gelatin can be used in food products or in the production of bioactive compounds.

Introduction

Utilization of all parts of cultured sturgeon would help diversify products and enhance profitability of the sturgeon industry. Amur sturgeon (*Acipenser schrenckii*) is an important aquaculture species in China. To maximize usefulness, the sturgeon skin was used to produce gelatin. Gelatin is a water-soluble protein obtained through breaking the triple-helical collagen structure. When collagen is heated above its denaturation temperature, hydrogen bonds that stabilize the adjacent polypeptide chains are destroyed. Individual α-chains and *β*-chains are produced from the intact trimers (*γ*-chains) and thus the rigid triple helical collagen state is transformed into a single-stranded, random-coil state (helix-coil transition) resulting in the production of gelatin (Wu et al., 2007), which is a thermo-reversible gel. Melting starts at the temperature above the melting point below the human body temperature. This melt-in-the-mouth property makes gelatin very useful in food and pharmaceutical industries (Boran et al., 2010). Gelatin is commonly obtained from pork skins, bovine hides and bones. Outbreak of bovine spongiform encephalopathy (BSE) or mad cow disease in 1980 has restricted the use of bovine gelatin worldwide. Moreover porcine gelatin is prohibited under Islam and Judaism, while bovine gelatin is not acceptable for Hindus. On the other hand, fish skin and bones are major by-products of the fish-processing industry and create disposal problems. However, they could serve as a valuable source of gelatin (Karim and Bhat, 2009).

Properties of gelatin such as gel strength, gelling and melting temperatures are the most important properties determining its commercial quality. These properties are in turn influenced by some factors such as molecular weight distribution as well as amino acid composition, which are species-specific (Gómez-Guillén et al., 2011). Gelatin is a mixture and consists of different polypeptide chains with varying molecular weights. These include α-chain (~100 kDa), *β*-chain (~200 kDa) and *γ*-chain (~300 kDa) (Eysturskar et al., 2010). The presence of α-chains, *β*-chains and higher molecular weight proteins more likely have a positive influence on the physical properties of gelatin, such as gel strength (Gómez-Guillén et al., 2002). The longer gelatin chains can participate in self-aggregation with higher junction zones (Eysturskar et al., 2010). However, proteins with a molecular weight lower than the α-chain could be produced as a result of the degradation induced by the thermal process in gelatin (Benjakul et al., 2009). Gel properties are negatively correlated with a decrease in polypeptide chain length. The occurrence of low molecular weight fractions and a decrease of the fractions of higher molecular weight and *β*-chains as a result of increased hydrolysis of gelatin negatively affect the physical properties of gelatin (Eysturskar et al., 2010).

Although thus far gelatin has been extracted from the skin of many fish species including fresh-water species (both cold- and warm-water species) and marine fishes, information on gelatin from a fish cultured at a temperature intermediate between optimum culture temperatures for cold-water (13–15°C, wells and river water from cold areas) and warm-water fish species (24–30°C, commonly from surface waters) is limited. Sturgeon (order Acipenseriformes) are neither cold-water nor warm-water fishes. On a commercial scale, they are commonly cultured in concrete tanks (round or rectangular) using well water with an approximate constant temperature throughout the year, with this temperature being intermediate between culture temperatures of cold-water and warm-water fish species.

At present, meat and caviar (salted sturgeon roe) are the only main sturgeon products. However, utilization of all parts of the fish (so-called 'deep processing') would diversify sturgeon products and lead to sustainability of the sturgeon

industry (Wei et al., 2011). While global sturgeon production through capture fisheries is decreasing as a result of overfishing and habitat deterioration, production through aquaculture shows an increasing trend (Bronzi et al., 2011; Ruban and Khodorevskaya, 2011). Increasing aquaculture production of sturgeon can result in large amounts of processed by-products, which create disposal problems and economic loss to the sturgeon industry. Amur sturgeon (*A. schrenckii*) is endemic to the Heilongjiang (Amur) River basin and confronted with over-fishing and population depletion in its natural habitat (Wang and Chang, 2006). Therefore, in response to the declining production, large-scale sturgeon farming in China was begun around the year 2000. Amur sturgeon is an important farmed sturgeon species in China, accounting for 15% of total aquaculture sturgeon production (21 000 tonnes in 2009; Wei et al., 2011). This species adapts well to culture conditions such as traditional Chinese fish culture ponds, lakes, reservoirs, and cages and grows to 900–1200 g within 1 year on a commercial diet (Wen et al., 2008). To maximize the use, the aim of the present study was to extract gelatin from the skin and measure the physical and chemical properties and secondary structure of the cultured Amur sturgeon.

Materials and methods

Chemicals and skin sample

The protein marker was obtained from Biolab Laboratories Co. (China). Coomassie Blue R250, sodium dodecyl sulfate (SDS), Tris (hydroxymethyl) aminomethane, amonium persulfate, acrylamide, *N*,*N*,*N*′,*N*′,-tetramethylethylenediamine, *N*,*N*′,methylene-bis-acrylamide, glycerol, sodium hydroxide, methanol, acetic acid, n-butanol and glycine were purchased from Sinopharm Chemical Reagents Co., Ltd. (Shanghai, China).

Skin samples were obtained from cultured Amur sturgeon (1774.72 ± 160.47 g) purchased at a local sturgeon farm (Zhenjiang, Jiangsu, China). The fish had been cultured in concrete tanks (16 × 8 × 0.8 m) supplied with well water with an average temperature of 20°C. Five rows of scutes (bony plates on the sturgeon skin) were removed with the aid of a small knife, washed with iced water and cut with scissors into small pieces (0.5 × 0.5 cm). The skin was kept at −30°C prior to gelatin extraction and analysis (<1 month).

Extraction of gelatin

Prior to gelatin extraction the skin was soaked in 0.1 M NaOH with a skin/alkali solution ratio of 1 : 10 (w/v) to remove non-collagenous proteins and pigments. The mixture was continuously stirred using a C-MAG HS7 magnetic stirrer (IKA Werke GmbH & Co. KG, Staufen, Germany) for 6 h at 4°C; the alkali solution was changed every 90 min. The alkaline-treated skin was rinsed with cold tap water until a neutral pH or slightly basic pH of the water was reached. The alkaline-treated skin was then defatted using n-butanol with a solid/solvent ratio of 1 : 10 (w/v) for 24 h at 4°C; the solvent was changed every 6 h. The defatted skin was washed until free of any alcohol odor then soaked in 0.05 M acetic acid with a skin/solution ratio of 1 : 10 (w/v) at 4°C for 3 h to let the collagenous material swell in the skin matrix. Solutions were changed every 1 h. The acid-swollen skin was then washed in cold tap water until a neutral pH of the water was achieved. Gelatin was extracted from the swollen skins at two different temperatures (50 and 70°C) and times (1 and 6 h) with a skin/water ratio of 1 : 5 (w/v). The mixtures were continuously stirred at 200 rpm using an overhead stirrer (IKA® RW 20 D CHN, Staufen, Germany) in a temperature-controlled water bath (HH-S4; Jiangsu Zhengji Instruments Co., Ltd., Jintan, Jiangsu, China). Following extraction, skin residues were removed using two layers of cheesecloth; the filtrate was then centrifuged (K320; Sigma laboratory centrifuge, Osterode am Harz, Germany) at 8000 ***g*** for 10 min to remove insoluble material. The supernatant was collected and freeze-dried (Labconco Corp., Kansas City, MO). The dried sample was blended (BM252C; Midea, Guangdong, China) to a fine powder. Samples were then vacuum-packed and kept at −30°C prior to analysis. G50/1 denotes gelatin extracted at 50°C for 1 h, G50/6 denotes gelatin extracted at 50°C for 6 h, G70/1 denotes gelatin extracted at 70°C for 1 h and G70/6 denotes gelatin extracted at 70°C for 6 h.

Analysis

Extraction yield

Extraction yield (%) was calculated and expressed as the percentage of the freeze-dried gelatin powder weight (g) in comparison with that of fresh, wet skin (g).

Proximate analysis

Moisture, protein, fat and ash contents of the gelatin powder were determined according to the method of AOAC (2000) with the method numbers 950.46, 928.08, 960.39 and 920.153.

Determination of amino acid composition

Gelatin was hydrolyzed in 6 M HCL at 110°C for 22 h. Amino acid profile of the hydrolysate was analyzed using an Agilent 1100 Series high performance liquid chromatography system (Agilent Technologies, Inc., Santa Clara, CA).

Colour measurement

The colour of Amur sturgeon gelatin gels (6.67% w/v) was measured with an UltraScan colorimeter (HunterLab, Reston, VA) at room temperature. Values of L*, a* and b* indicating lightness, redness/greenness and yellowness/blueness, respectively, were recorded.

Electrophoretic analysis

For electrophoretic analysis, gelatin (2 mg ml^{-1}) was dissolved in 5% SDS and the mixtures incubated at 85°C for 1 h, then centrifuged (Sorvall Legend Micro 17 Centrifuge, Thermo Scientific, Germany) at 5000 ***g*** for 5 min at room temperature to remove undissolved debris. Supernatant was mixed with Laemmli sample buffer (Bio-Rad Laboratories, Inc., Hercules, CA) at a 1 : 1 (v/v) ratio and heated for 3 min in boiling water. Samples (10 μg protein) were loaded onto polyacrylamide gel made of 7.5% separating gel and 4% stacking gel and subjected to electrophoresis at a constant voltage of 20 mA/gel using a Mini-Protean® Tetra Cell unit (Bio-Rad Laboratories, Inc., Hercules, CA). After electrophoresis the gel was stained with 0.10% Coomassie blue R-250 in 50%

methanol and 6.7% acetic acid and destained with 7.5% (v/v) methanol and 5% (v/v) acetic acid. A broad molecular weight standard was used to estimate the molecular weight of the gelatin. The gel was photographed using the GelDoc-It TS™ Imaging System (UVP, Upland, CA).

Measurement of gel strength

Gel strength of gelatin was measured according to the method of Gómez-Guillén et al. (2002) using a TA.XT-Plus texture analyzer (Stable Micro System, Surrey, UK). Gelatin powder (6.67%) was dissolved in distilled water (60°C) for 30 min and then chilled at 10°C for 17 h for gel maturation. Sample dimensions were 2.5 cm ø and 2 cm high. A probe (P/0.5R) with the speed of 0.5 mm s^{-1} was used. The maximum force (in ***g***) was recorded when a penetration distance of 4 mm was obtained.

Viscoelastic properties

Viscoelastic properties of gelatin were measured using a AR1000 rheometer (TA Instruments Ltd. Surrey, UK) according to the method of Giménez et al. (2005). Gelatin (6.67%) was dissolved in distilled water (40°C) for 30 min. The gelatin solution was cooled from 35 to 6°C and back to 35°C at a temperature gradient of 1°C min^{-1} using a frequency of 1 Hz and an applied oscillating stress of 3.0 Pa. A 4 cm parallel plate (gap 500 μm) was used as the measuring geometry. The elastic modulus (G′), viscous modulus (G″) (in Pa) and phase angle (delta) were measured as a function of temperature. Gelling and melting temperatures were taken as those where the phase angle was at its transition point, tan δ becomes 1 and δ becomes 45°.

Circular dichroism (CD) spectra

Circular dichroism was carried out according to the method of Wierenga et al. (2003) using a Mos-450 CD spectropolarimeter (Biologic, Claix, France). Gelatin samples (0.125 mg ml^{-1}) were dissolved in 10 mM sodium phosphate buffer (pH 7.0); the CD spectra were measured at room temperature, with a scan speed of 100 nm min^{-1} from 190 to 250 nm. All spectra were corrected for the corresponding protein-free sample.

Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared spectroscopy of gelatin was performed using FTIR spectrophotometer (NICOLET NEXUS 470; Thermo Electron Corp., MA). Gelatin powder was mixed with kBr and placed on the crystal cell of the FTIR spectrophotometer. Measurement was performed at 4000–500 cm^{-1} at room temperature and automatic signals collected in 32 scans at a resolution of 4 cm^{-1}.

Statistical analysis

Experiments were run in triplicate. Data were analyzed by 2×2 factorial design using the general linear method (GLM) and means were compared by Duncan's multiple range test using an SPSS package (SPSS 16.0 for windows; SPSS Inc, 246 Chicago, IL). Data are expressed as mean ± standard deviation (SD) and the probability value of $P < 0.05$ was considered significant.

Results and discussion

Gelatin yield

After the processing of cultured Amur sturgeon, 56.46% of fish weight was by-products; skin represented 7.40% of the by-products (data not shown). Yields of 9.42, 11.91, 12.12 and 12.47% (wet weight basis) were obtained for G50/1, G50/6, G70/1 and G70/6, respectively. However, the yields of G50/6, G70/1 and G70/6 were not different ($P > 0.05$). The degree of conversion of collagen into gelatin depends on the raw material pretreatment conditions, the extracting parameters such as time, temperature and pH, and the properties and the preservation method of the initial raw material (Giménez et al., 2005; Kittiphattanabawon et al., 2010). The extraction yields of gelatin from the skin of other fish species were 8.3% for sole (*Solea vulgaris*), 7.4% for megrim (*Lepidorhombus boscii*) (Gómez-Guillén et al., 2002) and 20.1% for farmed giant catfish (*Pangasianodon gigas*) (Jongjareonrak et al., 2010). The variations in gelatin recovery from these fish species is attributed to the differences in intrinsic characteristics of their skin and collagen molecules, the content of collagen, the amount of soluble components in the skins, incomplete collagen hydrolysis, and the loss of collagen during washing (Uriarte-Montoya et al., 2011).

Proximate composition. Proximate composition of G50/6 was measured. The gelatin had protein, moisture, fat and ash contents of 89.56, 9.74, 0.26 and 0.30%, respectively (data not shown). A high amount of protein in the gelatin indicates an efficient removal of fat and water from the skin material (Benjakul et al., 2009). The gelatin moisture content was below the limit for edible gelatin (15%) (Nagarajan et al., 2012). The ash content was below 0.5%, indicating the negligible presence of inorganic matter. The ash content was

Table 1
Effect of extraction temperature and time on amino acid composition (residues/1000 residues) of gelatin from the skin of cultured Amur sturgeon

Amino acids	G50/1	G50/6	G70/1	G70/6
Alanine	120.28[a]	108.10[b]	118.83a	117.39a
Arginine	54.20a	46.51[b]	53.82[a]	50.99[a]
Aspartic acid/asparagine	45.49[a]	48.84[a]	51.79[a]	50.59[a]
Cysteine	0.59[a]	0.56[a]	0.95[a]	0.47[a]
Glutamic acid/glutamine	79.62[b]	79.99[b]	85.51[a]	82.97[a]
Glycine	358.70[b]	352.10[b]	351.86[b]	366.58[a]
Histidine	5.85[a]	5.57[a]	5.66[a]	5.95[a]
Hydroxyproline	49.74[a]	50.69[a]	35.09[b]	39.11[b]
Isoleucine	12.44[a]	12.42[a]	11.19[a]	12.75[a]
Leucine	18.58[a]	18.48[a]	20.67[a]	18.94[a]
Lysine	26.54[a]	26.73[a]	26.36[a]	24.49[a]
Methionine	12.24[a]	12.04[a]	12.22[a]	12.52[a]
Phenylalanine	14.20[a]	14.10[a]	14.42[a]	14.34[a]
Proline	107.70[b]	135.70[a]	124.19[a]	107.69[b]
Serine	48.39[a]	47.98[a]	38.57[a]	49.55[a]
Threonine	23.96[a]	18.56[a]	24.25[a]	20.18[a]
Tyrosine	3.67[a]	3.59[a]	4.22[a]	3.70[a]
Valine	18.30[a]	17.99[a]	19.16[a]	18.68[a]
Imino acids	157.44[b]	186.39[a]	159.28[b]	146.80[b]
Hydrophobic amino acids*	662.44[a]	670.93[a]	672.54[a]	668.90[a]

Mean (n = 2).
*Calculated as the sum of residues glycine, valine, alanine, leucine, isoleucine, proline, phenylalanine and methionine/1000 residues.
G50/1 denotes gelatin extracted at 50°C for 1 h; G50/6 at 50°C for 6 h; G70/1 at 70°C for 1 h; G70/6 at 70°C for 6 h.
Different lowercase letters in same row = significant differences ($P < 0.05$).

also lower than the limit given for edible gelatin (2%) (Nagarajan et al., 2012).

Amino acid composition. Amino acid composition of Amur sturgeon gelatin is shown in Table 1. The dominant amino acid in the gelatin was glycine (351.86–366.58 residues/1000 residues), suggesting that gelatin was obtained from its mother collagen. Glycine represents about one-third of the total residues in collagen and occurs as every third residue (Gómez-Guillén et al., 2002). Gelatin extracted at a higher temperature for a longer time (G70/6) had a higher glycine content. The high content of glycine in G70/6 was more likely due to the presence of free glycine as a result of hydrolysis of gelatin at higher extraction temperature (Nagarajan et al., 2012). Imino acids (proline 107.69–135.70 residues/1000 residues and hydroxyproline, 35.09–50.69 residues/1000 residues) were second in abundance. Glycine, alanine, proline and hydroxyproline were abundant amino acids in gelatin from shark skin (Kittiphattanabawon et al., 2010). The amino acid composition of gelatin is characterized by a repeating sequence of Gly-X-Y triplets, where X is often proline and Y is often hydroxyproline. Imino acids have a role in the stabilization of triple-helical structure in renatured gelatin (Haug and Draget, 2009). Hydroxyproline plays a singular role in the stabilization of the triple-stranded collagen helix due to its hydrogen-bonding ability through its hydroxyl group (Gómez-Guillén et al., 2011). Imino acid content of Amur sturgeon gelatin was lower than that of warm-water fish gelatin (198 residues/1000 residues) or mammalian gelatin (223 residues/1000 residues) (Karim and Bhat, 2009). Gildsen and Ross-Morphy (2000) stated that thermal stability of gelatin correlates to the imino acid contents of gelatin. Imino acid content, especially that of hydroxyproline, depends on the temperature of fish habitat and affects the thermal stability of collagens and their derivatives. Therefore lower content of imino acids in Amur sturgeon gelatin could be due to the lower culture water temperature (20°C) compared to that of warm-water fishes (commonly 24–30°C). A high content of alanine (108.10–120.28 residues/1000 residues) was present in gelatins. Alanine plays a role in viscoelastic properties of gelatin (Gómez-Guillén et al., 2002). Glutamic acid/glutamine and aspartic acid/aspargine were also found in high levels. In general, gelatins extracted at 70°C had higher acidic amino acid (glutamine and aspargine) contents than gelatins extracted at 50°C. With an increasing extraction temperature, deamidation may occur and might thus form glutamic acid and aspartic acid (Nagarajan et al., 2012). On the other hand, G70/6 showed the lowest level of lysine, a basic amino acid. Tyrosine (3.59–4.22 residues/1000 residues) and histidine (5.57–5.95 residues/1000 residues) were present in gelatin at low levels. Negligible amounts of cysteine (<1 residues/1000 residues) were found in gelatins. This could indicate the presence of small quantities of stroma protein such as elastin, which is highly insoluble and unusually stable in salt (Giménez et al., 2005). The content of hydrophobic amino acids (glycine, valine, alanine, leucine, isoleucine, proline, phenylalanine and methionine) in G50/1, G50/6, G70/1 and G70/6 were 662.44, 670.93, 672.54 and 668.90 residues/1000 residues, respectively. Tilapia and horse mackerel gelatins with higher gel strength than the non-gelling cod gelatin contained higher amounts of hydrophobic amino acids (Badii and Howell, 2006). In the present study, no relationship was observed between the total hydrophobic amino acids and gel strength.

Colour of gelatin gels

The colour of cultured Amur sturgeon gelatin extracted at different temperatures and times expressed as L*, a* and b* is shown in Table 2. When extraction times and temperatures increased, L*-value (lightness) decreased, while a* (redness) and b*-value (yellowness) increased ($P < 0.05$). Nagarajan et al. (2012) showed a decrease in lightness and increase in redness and yellowness of splendid squid (*Loligo formosana*) gelatin with increasing extraction temperatures. The higher redness and yellowness in gelatin extracted at higher temperatures were presumed to be due to the co-extraction of pigments into gelatin. Moreover, increased hydrolysis of gelatin as a result of increasing extraction temperature could produce more free amino groups, which in turn could undergo a browning reaction along with carbonyl compounds in the skin. The colour of gelatin can be influenced by the fish species and raw material (Koli et al., 2011).

Protein patterns of gelatin

Protein patterns of gelatin from the skin of Amur sturgeon extracted for different times at various temperatures are shown in Fig. 1. All gelatins had α-chain and β-chain (dimer of α-chain), the major protein constituents. Apart from α-chain and β-chain, the higher molecular weight proteins (γ-chain) were also observed in all gelatins. Proteins or peptides with a molecular weight lower than the α-chain were also observed in gelatins, while they were not present in commercial pig gelatin. No obvious changes in protein bands were observed among gelatins. The presence of α-chains,

Table 2
Effect of extraction temperature and time on gel strength, colour, gelling and melting temperatures of gelatin from skin of cultured Amur sturgeon

Characteristic	G50/1	G50/6	G70/1	G70/6
Gel strength (g)	115.69 ± 3.66^{ab}	121.43 ± 3.54^{a}	94.77 ± 1.89^{c}	64.19 ± 1.52^{d}
Colour				
L*	26.69 ± 0.69^{a}	26.63 ± 0.25^{ab}	25.89 ± 0.52^{b}	24.80 ± 0.9^{c}
a*	0.28 ± 0.00^{b}	0.46 ± 0.07^{a}	0.43 ± 0.02^{a}	0.34 ± 0.09^{ab}
b*	−4.14 ± 0.44^{c}	−4.38 ± 0.31^{c}	−2.34 ± 0.39^{b}	−0.65 ± 0.13^{a}
Gelling and melting temperatures				
Gelling temperature (ºC)	14 ± 0.6^{a}	14.6 ± 1.2^{a}	14.4 ± 0.4^{a}	13.6 ± 0.8^{a}
Melting temperature (ºC)	22 ± 1.2^{a}	22.6 ± 1.0^{a}	21.6 ± 1.0^{a}	20.3 ± 0.8^{b}

Mean ± SD (n = 3).
Different lowercase superscripts in same row = significant differences ($P < 0.05$).
G50/1 denotes gelatin extracted at 50°C for 1 h; G50/6 at 50°C for 6 h; G70/1 at 70°C for 1 h; G70/6 at 70°C for 6 h.

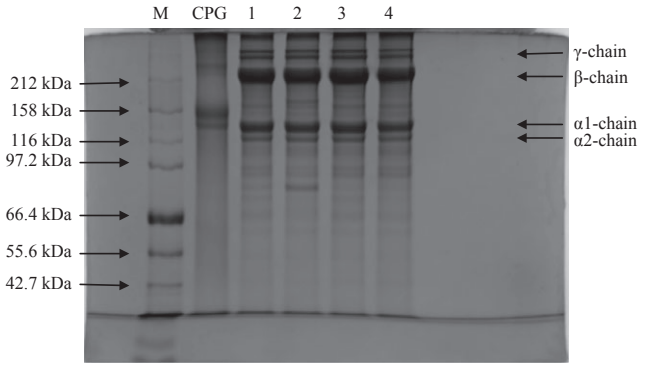

Fig. 1. SDS-PAGE pattern of cultured Amur sturgeon skin gelatin. M: molecular weight protein markers; CPG, commercial pig gelatin; 1: gelatin extracted at 50ºC for 1 h; 2: at 50°C for 6 h; 3: at 70°C for 1 h; 4: at 70°C for 6 h

β-chains and higher molecular weight proteins more likely have a positive influence on physical properties of gelatin, such as gel strength (Gómez-Guillén et al., 2002).

Gel strength

The gel strength of gelatin from the skin of cultured Amur sturgeon extracted at different temperatures and times is presented in Table 2. Gel strength is one of the most important functional properties of gelatin. During thermal treatment, collagen is transformed to soluble gelatin, in which the typical helical structure of collagen is changed to random coils. Gelatin has the ability to dissolve in warm water to form a thermo-reversible gel. When gelatin solution is cooled, single helices start to form from the random coils (coil to helix transition). The formation of three-dimensional networks is responsible for the strength and rigidity of the gelatin gels (Karim and Bhat, 2009). The gel strength of G50/1, G50/6, G70/1 and G70/6 were about 115, 121, 94 and 64 ***g***, respectively ($P < 0.05$). Among all samples, G70/6 exhibited the lowest gel strength. The longer α-chains and β-chains present in gelatins at higher amounts extracted at lower temperatures could undergo aggregation and form stronger gel networks more efficiently than α-chains and β-chains at the lower amount in gelatins extracted at higher temperatures

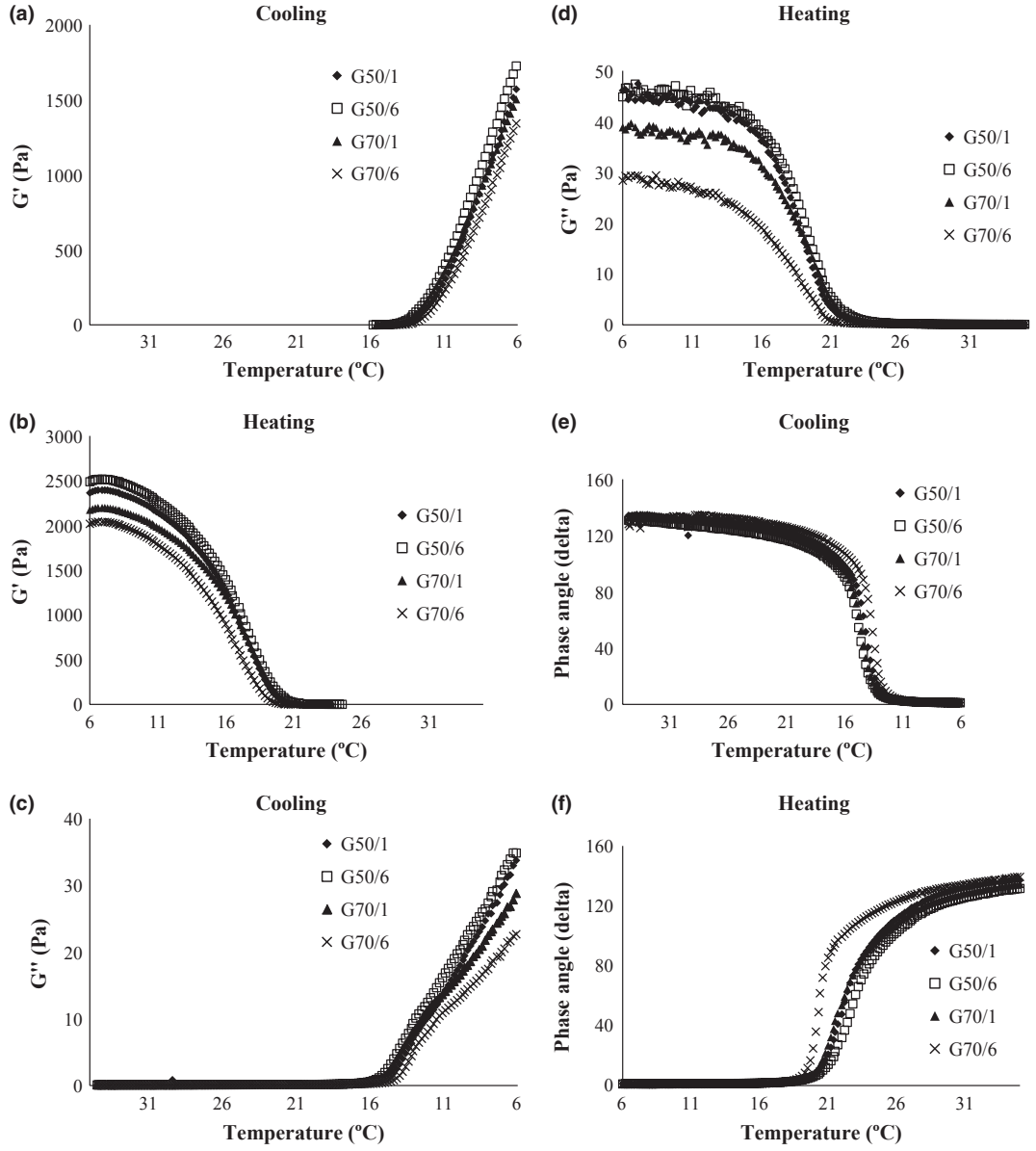

Fig. 2. Changes in elastic modulus (G′), viscous modulus (G″) (in Pa) and phase angle (delta) of 6.67% (w/v) gelatin solution during cooling from 35°C to 6°C (a, c, e) and heating from 6°C to 35°C (b, d, f)

(Nagarajan et al., 2012). Therefore in G70/6, peptide chains possibly had less ability to form a strong network. Furthermore, the gelatin molecules might not align themselves orderly and as a result the ordered network could not be developed. Gel strength of gelatin could also be influenced by amino acid composition (Gómez-Guillén et al., 2011). The imino acid contents of G70/6 with lower gel strength were lower than other gelatins with higher gel strength (Table 1). Commercial gelatins (pigskin and bovine gelatins) have gel strength ranges from 50 to 300 g and gelatins with gel strengths below 100 g are considered as low-bloom gelatin (Haug and Draget, 2009). G70/1 and G70/6 with gel strengths lower than 100 g could be considered as low-bloom gelatins.

Viscoelastic properties

Changes in the elastic modulus (G′) and viscous modulus (G″) (in Pa) of cultured Amur sturgeon gelatin solutions (6.67% w/v) as a function of temperature (1°C min^{-1}) during cooling (from 35 to 6°C) and heating (from 6 to 35°C) are shown in Fig. 2. During cooling, a rapid increase in G′ was observed in all gelatins (Fig. 2a) which is due to the rapid formation of junction zones and a strong reinforcement of the gel network (Binsi et al., 2009). Gelatin extracted at the lower temperature (50°C) had better gelling ability, as indicated by a higher increase in G′ and G″ during cooling, but those extracted at the higher temperature (70°C) formed gel with lower G′ and G″ (Fig. 2a,b). Gelling temperatures for G50/1, G50/6, G70/1 and G70/6 were 14, 14.6, 14.4 and 13.6°C, respectively (Table 2) as indicated by the sharp decrease in phase angle (δ) (Fig. 2e). G70/6 showed the lowest gelling temperature and consequently the phase angle decrease occurred at a lower temperature compared to other gelatins. Moreover, lower elastic and viscous modulus at 6°C (cooling ramp) was observed for this gelatin, which could be due to the lower amounts of α-chains, β-chains and higher molecular weight proteins, resulting in less ability of renaturation of the fully collagen native form (Giménez et al., 2005). During the heating ramp, an increase in G′ was observed when compared to that of the cooling ramp, attributed to the quick cold maturation at a low temperature before the heating ramp began (Gómez-Guillén et al., 2002). The main differences in the properties of gelatin from mammalian and fish are that fish gelatin has a lower gelling and melting temperature. Porcine and bovine gelatins have gelling and melting temperatures in the ranges of 20–25 and 28–31°C, respectively (Karim and Bhat, 2009). Melting temperatures at which the phase angle began to increase rapidly (Fig. 2f) for G50/1, G50/6, G70/1 and G70/6 were 22, 22.6, 21.6 and 20.3°C, respectively (Table 2). G70/6 had a lower melting temperature than other gelatins ($P < 0.05$). However, the melting temperatures of G50/1, G50/6 and G70/1 were not different ($P > 0.05$). Thermal stability of gelatin is influenced by imino acid contents of gelatin and molecular weight distribution (Gómez-Guillén et al., 2002). Cold-water fish gelatins exhibit lower gelling and melting temperatures, compared to those of mammalian gelatins due to their lower imino acid contents (Gudmundsson, 2002). Gelling and melting temperatures of cultured Amur sturgeon gelatin were higher than those of cold-water fish gelatins (4–8 and 14–16°C, respectively) but were lower than those of warm-water fish gelatins (21–22 and 28–29°C, respectively) (Haug and Draget, 2009). As mentioned earlier, the temperature in which Amur sturgeon was cultured is intermediate between optimum growth temperatures for cold-water fishes and warm-water fishes. The difference in culture water temperature might be the possible reason why gelling and melting temperatures of Amur sturgeon gelatin was intermediate between those of cold- and warm-water fish gelatins.

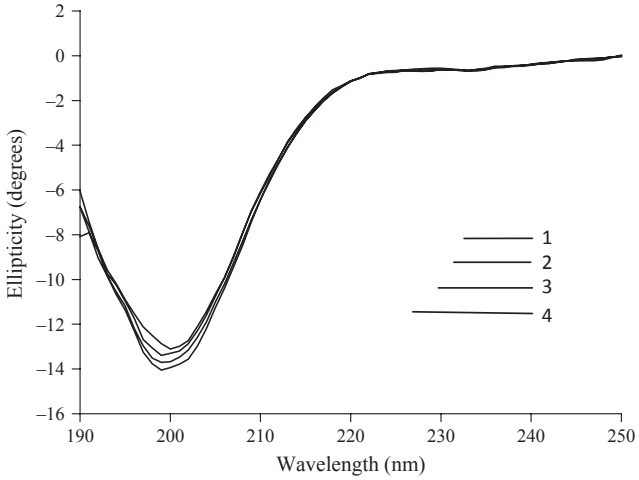

Fig. 3. Circular dichroism (CD) spectra of cultured Amur sturgeon skin gelatin. 1: gelatin extracted at 50°C for 1 h; 2: at 70°C for 6 h; 3: at 50°C for 6 h; 4: at 70°C for 1 h

Circular dichroism (CD) spectra

Circular dichroism spectra of protein solutions give information about the secondary structure of protein. In order to investigate the effect of extraction conditions on secondary structure of gelatin, circular dichroism study was carried out. Triple helical conformation of collagen reveals a positive peak at 220–230 nm and a negative peak at around 200 nm (Aewsiri et al., 2011). The triple helical structure of collagen can transform into the random coil configuration when it is heated above the denaturation temperature (Zhang et al., 2006). After complete denaturation of collagen, the positive peak at 220–230 nm, characteristic of the triple-helix, disappears completely and only a negative peak at 200 nm of gelatin remains (Aewsiri et al., 2011). The negative peak at 200 nm corresponds to the random conformation (Wu et al., 2007). CD spectra of Amur sturgeon gelatin revealed a maximum negative peak at 199–200 nm but lacked a positive peak at around 220–230 nm, suggesting that the triple helical configuration of collagen collapsed (Fig. 3). This result is in agreement with Zhang et al. (2006) and Aewsiri et al. (2011). Secondary structure of gelatin extracted at 50°C for 1 h consisted of 19.7% α-helix, 21.8% β-sheet, 20.2% turn and 43% random coil. An increasing extraction time and temperature led to a more random structure (46.7–58.3%), whereas α-helix, β-sheet and turn contents decreased (Table 3). Results indicated that extraction conditions caused the changes in the secondary structure of gelatin.

Table 3
Secondary structure content (%) of gelatin from skin of cultured Amur sturgeon using circular dichroism spectra

Sample codes	α-helix	β-sheet	Turn	Random coil
G50/1	19.7^{a}	21.8^{a}	20.2^{a}	43.0^{d}
G50/6	19.8^{a}	19.3^{b}	19.2^{b}	46.7^{c}
G70/1	17.3^{b}	16.3^{c}	16.1^{c}	54.9^{b}
G70/6	15.1^{c}	15.1^{d}	15.7^{d}	58.3^{a}

Different lowercase superscripts in the same column = significant differences ($P < 0.05$).

Table 4
FTIR characteristics of different gelatins from skin of cultured Amur sturgeon

Sample codes	$\Delta\nu$ ($\nu_I - \nu_{II}$) (cm^{-1})	A_{1235} (AIII)/A_{1451}
G50/1	79.16[d]	0.85[a]
G50/6	107.65[c]	0.85[a]
G70/1	113.98[b]	0.85[a]
G70/6	123.48[a]	0.85[a]

Different lowercase superscripts in the same column = significant differences ($P < 0.05$).

Fourier transform infrared (FTIR) spectra

FTIR spectra of cultured Amur sturgeon skin gelatin extracted at different times and temperatures is depicted in Fig. 4. FTIR spectra of gelatin displayed major peaks in the amide region. The amide-A band originates from N–H stretching frequency, amide-I peak arises from C=O stretching, amide-II peak is represented by N–H bending and C–N stretching vibrations, while the amide-III peak is a complex system mainly associated to CH_2 residual groups from glycine and proline (Uriarte-Montoya et al., 2011). In the FTIR spectrum of the cultured Amur sturgeon gelatin (Fig. 4), the four characteristic absorption bands can be observed. A free N–H stretching vibration occurs in the range 3400–3440 cm^{-1}, however when the NH group of shorter peptide fragments are involved in hydrogen bond, its position is shifted to a lower frequency, usually 3300 cm^{-1} (Ghica et al., 2009). Amide-A band of gelatin extracted at 50°C for 1 h appeared at 3417 cm^{-1}, but their amide-A band was shifted to lower frequencies (3319–3344 cm^{-1}) in gelatins extracted at the higher temperature (70°C) for a longer time (6 h). Triple helical structure integrity is determined by A1235 (AIII)/A1451 ratio, which has to be higher or equal to one (Albu et al., 2009). In this study, A1235(AIII)/A1451 ratio was lower than one (0.85) for all gelatins (Table 4). During acid pre-treatment, acid provides the power for destabilization of the triple helical structure of collagen through disrupting acid labile cross-links at the telopeptide region and amide bonds of the triple helix as well as non-covalent intra- and inter-molecular bonds. With the loosened structure of swollen collagen, warm water could penetrate into the skin matrix (Ahmad and Benjakul, 2011). During warm water extraction, hydrogen bonds stabilizing the triple helix of skin collagen were destroyed and helix-to-coil transition occurred. This resulted in the conversion of collagen to soluble gelatin (Benjakul et al., 2009). This is in agreement with the lack of a positive peak at 220 nm, characteristic of the triple-helix, in circular dichroism spectra (Fig. 3). The difference between amide I and II bands, $\Delta\nu$ ($\nu_I - \nu_{II}$) gives information on the presence of a denaturation process in which values higher than 100 cm^{-1} show enhanced hydrolysis (Ghica et al., 2009). Values lower than 100 cm^{-1} suggests that α-helix chain was preserved (Albu et al., 2009). In collagen-tannic acid matrices, $\Delta\nu$ ($\nu_I - \nu_{II}$) values ranging between 83 and 92 cm^{-1} were obtained, indicating that cross-linking does not affect the integrity of the triple helical structure of collagen (Ghica et al., 2009). $\Delta\nu$ ($\nu_I - \nu_{II}$) values for Amur sturgeon gelatin ranged from 79 to 123 cm^{-1} (Table 4), which increased with increasing extraction time and temperature, indicating higher denaturation of α-helix chain at severe extracting conditions. $\Delta\nu$ ($\nu_I - \nu_{II}$) value lower than 100 cm^{-1} implies that α-chains in G50/1 extracted at a lower temperature can participate in self-aggregation with a higher junction zone to form a stronger gel network in renatured gelatin. Thus, extraction for a longer time at a higher temperature can cause greater loss of the molecular order as a result of thermal uncoupling of intermolecular cross-links and disruption of intra molecular bondings.

Conclusion

Cultured Amur sturgeon skin can serve as a valuable raw material for gelatin production when the optimal conditions for gelatin production are implemented. Higher extraction time and temperature led to a darker gelatin and decreased the content of imino acids and gel strength. Extraction conditions caused changes in the secondary structure of gelatin.

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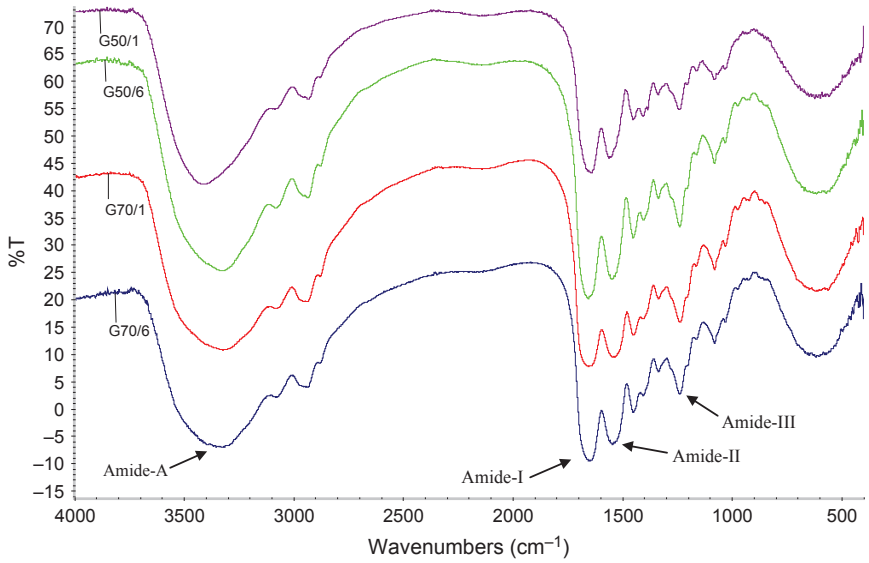

Fig. 4. FTIR spectra of cultured Amur sturgeon skin gelatin

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