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Characterization of gelatin from the skin of farmed Amur sturgeon *Acipenser schrenckii*

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Abstract

Gelatin was extracted from the skin of farmed Amur sturgeon (*Acipenser schrenckii*) with a yield of 19.6% and its properties were evaluated. Its glycine and imino acids (proline and hydroxyproline) content were 336 and 138 residues/1000 residues respectively. Based on electrophoretic study, gelatin consisted of two major protein bands corresponding to α-chain and cross-linked component (β-chain). The gel strength of gelatin was 316 g, while its gelling and melting temperatures were 13 and 19.6 °C respectively as determined by temperature sweep test. Flow behavior of gelatin solutions as a function of concentration (1, 3 and 5%) and temperature (10, 30, 45 and 60 °C) indicated a clear non-Newtonian, pseudoplastic behavior at 10°C and 5% gelatin solution. Fourier transform infrared (FTIR) spectroscopic study showed major absorption bands of amide A, I, II and III at 3414.73, 1640.60, 1534.57 and 1235.01 cm^{-1} respectively.

Keywords: Gelatin, Skin, Gel strength, Rheological properties, Farmed Amur sturgeon

Introduction

Gelatin is the denatured collagen fraction having a molecular weight higher than 30 kDa (Boran et al. 2010). In aqueous solutions, gelatin is a mixture of different polypeptide chains including α-chains, β (dimers of α-chain) and γ (trimers of α-chain) components with a molar mass of around 90, 180 and 300 $\times 10^3$ g/mol respectively (Rbii et al. 2011). Gelatin is being widely used in food, drug and cosmetic industries as stabilizing, thickening and gelling agent (Boran et al. 2010; Gómez-Guillén et al. 2011; Kittiphattanabawon et al. 2010). The most important properties that makes gelatin very favorable to be used in the food industry is its low melting temperature, in which it is molten at human body temperature and higher gel strength, when compared to other common gelling agents (Boran et al.

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2010). The quality of gelatin is largely determined by its gelling strength and thermal stability. This is dependent on the amino acid composition which is species specific and molecular weight distribution as influenced by processing conditions (Gómez-Guillén et al. 2002).

Discards from fish processing are generally considered as low-value resources with negligible market value (Klompong et al. 2007). It has been reported that 30% of fish waste are in the form of bone and skin with high collagen (Gómez-Guillén et al. 2002). Through partial hydrolysis of collagen, gelatin is obtained. The market demand for gelatin is most obtained from pig and cattle skins and cattle bone. However, nowadays the importance of fish gelatin as a food additive is increasing because it does not possess any risk of contamination with cow mad disease and its potential as a kosher and halal food product for diverse ethnic groups (Gómez-Guillén et al. 2011). For a hydrocolloid used as a food additive, rheological properties are being employed as a quality index. Food formulations, product development and processing are influenced by the specific rheological properties of the food additives (Marcotte et al. 2001). Parameters such as temperature and concentration have great influence on the rheological properties of a typical hydrocolloid (Gómez-Díaz and Navaza 2003; Marcotte et al. 2001; Sopade and Kiaka 2001) There is a little information on the rheological properties of fish skin gelatin with reference to its flow behavior (Marcotte et al. 2001; Binsi et al. 2009) .

Culture of sturgeon in farms has been adopted as a promising tool to overcome the decline of wild and enhanced stocks of sturgeon as a result of over-exploitation for caviar production and serious habitat deterioration (Bronzi et al. 2011). There is growing interest to culture sturgeon species in farms in China and Amur sturgeon is currently being one of the most popular species for aquaculture in the country (Li et al. 2009). So far no gelatin has been extracted from the skin of this species and no information on its properties is available. Therefore the objective of the present work was to extract gelatin from the skin of farmed Amur sturgeon (*Acipenser schrenckii*) and to determine its physicochemical properties. The rheological properties as a function of temperature and concentration were also investigated.

Materials and methods

Chemicals

High molecular weight markers were purchased from Sigma (St. Louis, MO, USA). β-mercaptoethanol was from Biotech. Commassie brilliant blue G250 was provided from Shanghai Chemical Reagent Company Co., Ltd. (Shanghai, China). Coomassie Blue R250, bovine serum albumin, bromophenol blue, sodium dodesyl sulfate (SDS), Tris (hydroxymethyl) aminomethane, amonium persulfate, acrylamide, N,N,N´,N´,-tetramethylethylenediamine, N,N´,methylenebisacrylamide, glycerol, phosphoric acid, sodium hydroxide, ethanol, methanol, acetic acid, glycine and commercial pig gelatin were purchased from Sinopharm Chemical Reagents Co., Ltd. (Shanghai, China).

Fish samples

Farmed Amur sturgeon, *Acipenser schrenckii* (782.5 ± 86.80 g) were obtained from a local sturgeon farm (Zhenjiang, Jiangsu province, China) and transported alive to the laboratory, where the fish were stunned by a blow on the head, bled, and skinned. Skins were washed with cold water to remove the adhering slime and tissues, thereafter cut into small pieces of approximately 0.5 × 0.5 cm with a scissor and washed again. Prepared skins were vacuum-packed and frozen at -30 °C for about one month until used.

Extraction of fish skin gelatin

Gelatin was extracted from the skin of farmed Amur sturgeon skin according to Ahmad and Benjakul (2011). Briefly, farmed Amur sturgeon skin was treated with 0.1 M NAOH for 6 h at 4 °C to remove non collagenous proteins and pigments. Alkaline solution was changed every 2 h. The alkaline-treated skins were rinsed with tap water until neutral pH of the washing water was obtained. Skins were then soaked in 0.2 M phosphoric acid at 4 °C for 24 h (acid solution was changed every 12 h). The skins were then drained and rinsed with tap water until neutral pH was achieved. Skin to solution ratio was kept at 1: 10 (w/v) for alkaline or acid treatments. Skins were mixed with distilled water at the ratio of 1:5 (w/v) and gelatin extracted at 50 °C for 6 hour in a water bath (DKZ, Shanghai Yiheng Technology Co., Ltd, Shanghai, China) with gentle stirring. The mixture was filtered and the obtained filtrate was freeze-dried. Extraction yield (%) was calculated based on the weight of freeze dried gelatin

powder (g) in comparison with that of wet fresh skin (g). The protein content of gelatin was measured by the method of Bradford (Bradford 1976) using bovine serum albumin as a standard.

Determination of gel strength

Gelatin gels were prepared according to Gómez-Guillén et al. (2002). Gelatin powder was dissolved in distilled water (at 6.67%) and stirred magnetically (IKA® C-MAG HS 7, Staufen, Germany) at 60 °C for 30 min. Gelatin solutions were kept at 7 °C for 17 h for gel maturation. Dimensions of the samples were 3.5 cm diameter and 1.5 cm height. The gel strength of the samples was determined on TA.XT-Plus texture analyzer (Stable Micro System, Surrey, UK) using standard probe. The maximum force (in g) was recorded when the penetration distance of 4 mm at a plunger speed rate of 0.5 mm/s was obtained.

Determination of amino acid composition

Amur sturgeon skin gelatin samples were hydrolyzed in 6 M HCL at 110 °C for 22 h, then amino acid profile of the hydrolysates were analyzed using HPLC (ODS HYPERSIL, Agilent 1100, USA) at a flow rate of 0.5 mL/min. The amino acids were quantified by comparing with standard amino acid profiles with the aid of ChemStation for LC 3D software (Agilent Technologies, Palo Alto, CA 94306, USA).

Eelectrophoretic study

For electrophoretic study gelatin powder (5mg/ml) was dissolved in distilled water at 60 °C and then centrifuged (BIOFUGE PRIMO R, Thermo Scientific, Germany) at 5000 g for 5 min at room temperature. Supernatant was mixed with sample buffer at a 1:1 ratio and heated for 6 min in boiling water. Samples (15 µg ptotein) were loaded onto polyacrylamide gel made of 7% separating gel and 5% stacking gel and subjected to electrophoresis at a constant voltage of 130 V using Mini Protein II unit (Bio-Rad Laboratories, Inc., Hercules, CA). The gel was stained with Coomassie blue R-250 (15% methanol, 5% acetic acid, 0.05% Coomassie blue) and destained with a mixture of methanol, acetic acid and distilled water (1:1:8 ratio). High molecular weight standards were used to estimate the molecular weight of proteins.

Viscoelastic properties

Dynamic viscoelastic studies were performed on a AR1000 rheometer (TA Instruments Ltd. Surrey, England) as described by Gómez-Guillén et al. (2002). Dry gelatin powder was dissolved in distilled water at a concentration of 6.67% at 40 °C and the mixture was stirred magnetically (IKA® C-MAG HS 7, Staufen, Germany) until solubilization was obtained. Gelatin solution cooled from 40 °C to 5 °C, kept at 5 °C for 10 min and back to 40 °C at a scan rate of 1 °C/min, at a frequency of 1 Hz and oscillating applied stress of 3.0 Pa. A 4 cm parallel plate (gap 500 µm) was used as the measuring geometry. The phase angle (rad) was presented as a function of temperature. The gelling and melting temperatures of gelatin are determined as the crossover temperature point ($G' = G''$), where the phase angle (δ) is 45° ($\tan \delta = 1$) (Eysturskarð et al. 2009).

Flow behavior of gelatin

Flow properties of gelatin were measured on a AR1000 rheometer (TA Instruments Ltd., Surrey, England). Gelatin powder was dissolved in distilled water at 40 °C and stirred magnetically until complete solubilization. Samples were placed into the sample cup and then equilibrated for 2 min at the required temperature and subjected to linearly increasing shear rate from 0.1 to 100 s^{-1} as a function of gelatin concentration (1, 3 and 5%) and temperature (10, 30, 45 and 60°C). The measuring geometry used was 6 cm cone and plate (2°) with truncation of 59 µm. The flow behavior index (n) and consistency coefficient (k) values were calculated by applying the power law equation: $\tau = k\gamma^n$ where τ is the shear stress (Pa), γ is the shear rate (s^{-1}), k is the consistency index (Pa s^n), and n is the flow behavior index.

Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared spectroscopy of gelatin powder was performed using FT-IR spectrophotometer (NICOLET NEXUS 470, Thermo Electron Corporation, USA). Briefly, samples consisted of 2 mg of gelatin powder was mixed with 100 mg potassium bromide (Muyonga et al. 2004) and placed on the crystal cell and of the FTIR spectrophotometer. Measurement was performed at 4000-500 cm^{-1} at room temperature and automatic signals were collected in 32 scans at a resolution of 4 cm^{-1}.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan multiple range test by SPSS (SPSS Inc., Chicago, IL) 16. The significance of results was at 5%.

Results and discussion

Yield and protein content of gelatin

Yield and protein content of gelatin are shown in Table 1. In this study phosphoric acid was used for acid pretreatment. Phosphoric acid with high ionic strength and lower pH can cause more repulsive force among collagen molecules , which facilitated the swelling process. This led to highly loosen structure in collagen. With the highly loosen structure of collagen, warm water can penetrate better into the skin matrix. As a result the extraction efficiency can be improved (Ahmad and Benjakul 2011).

The average yield of gelatin from the skin of farmed Amur sturgeon was 19.59% which was close to the yield reported for shark (Kittiphattanabawon et al. 2010) and farmed Giant catfish (Sai-Ut et al. 2010). The yield of 7-16% was reported by Zhou and Regenestein (2005) for the skin of Alaska pollock. The obtained yield for bigeye snapper (Binsi et al. 2009) and marine species studied by Gómez-Guillén et al. (2002) was in the range of 4-8%. Protein is the main constituent of gelatin. The amount of protein in gelatin is between 85 and 92 percent and the reminder is composed of minerals and moisture that may still remain after freeze drying (Schrieber and Gareis 2007). The protein content of Amur sturgeon skin gelatin was determined as 90.4%. The contents of protein of gelatin were 88.5% for Nile tilapia (Zeng et al. 2010), 93.1% for shark (Esmaeili Kharyeki 2011), 94.6% for bigeye snapper (Binsi et al. 2009), 90.43 and 89.81% for rohu and common carp respectively (Ninan et al. 2011).

Gel strength

Gel strength is an important criteria employed for grading of gelatins. It is well known that gelation of gelatin is due to a partial recovery of the triple helical structure in collagen, in which Gly-Pro-Hyp rich regions are of crucial importance for the initiation and stabilization of the triple helices. Commercial gelatin possesses gel strength in the range of 50- 300 g (Schrieber and Gareis 2007). Gelatin derived from fish typically has lower gel strength, compared with its mammalian counterpart (Gudmundsson 2002). Gel strength of Amur sturgeon skin gelatin was 316.27 g, which was higher than that reported by Ninan et al. (2011) for common carp (181.31 g) and rhou (188.63 g), Kasankala et al. (2007) for grass carp (267 g), Binsi et al. (2009) for bigeye snapper (108 g), Mohtar et al. (2010) for hoki (197 g), Zeng et al. (2010) for Nile tilapia (260 g) and Eysturskarð et al. (2009) for cod, haddock and saithe (100-200 g). However a higher gel strength (426 g) was reported for gelatin from the skin of yellowfin tuna (Cho et al. 2005). Generally gelatin with higher contents of proline and hydroxyproline exhibits higher gel strength as these two amino acids are involved in the formation of nucleation zone in renatured gelatin (Gómez-Guillén et al. 2011). Moreover the prevalence of low molecular weight fractions explained the lower gel strength of gelatins (Eysturskarð et al. 2010).

Molecular weight distribution

Protein pattern of gelatin from the skin of farmed Amur sturgeon is shown in Figure 1. Gelatin consists of a mixture of polypeptide representing collagen type I with α-chains, β chains (two covalently cross-linked α-chains) and γ-chains (three covalently cross-linked α-chains) together with higher and lower molecular weight fragments. A strong decrease in β and γ-chains and the almost disappearance of higher molecular weight fragments and the increased prevalence of degradation fragments represents the application of severe extraction condition (Gómez-Guillén et al. 2011). SDS-PAGE pattern of Amur sturgeon skin gelatin had the protein bands with MW between 116 and 200 kDa, corresponding to α-chain and β-chain. Similar pattern was reported by Binsi et al. (2009) on bigeye snapper, *Priacanthus hamur* skin gelatin. Higher molecular weight components as well as some low molecular weight fragments were also observed. The appearance of low molecular fragments is attributed to the hydrolysis of gelatin during the extraction process. Molecular weight distribution of gelatin is commonly influenced by the processing conditions (Gómez-Guillén et al. 2011). When higher temperature was used to extract gelatin from the skin of shark, high molecular weight components were disappeared (Kittiphattanabawon et al. 2010).

Table 1. Extraction yield, protein content and gel strength of gelatin extracted from the skin of farmed Amur sturgeon

	Yield (%)	Protein (%)	Gel strength (g)
Mean ± SD	19.59 ± 2.73	90.39 ± 6.84	316.27 ± 18.90

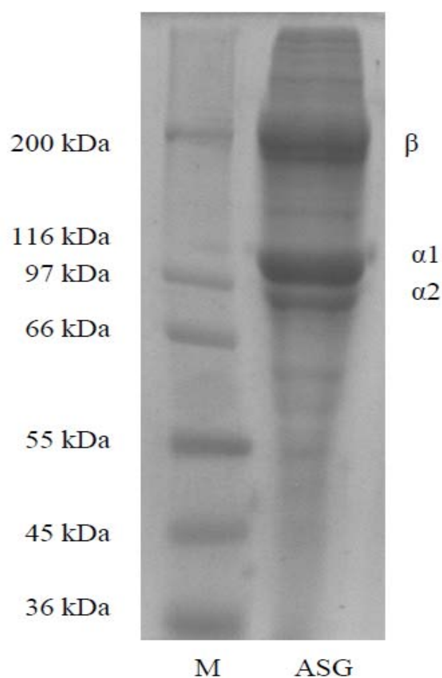

Fig. 1. SDS-PAGE pattern of gelatin extracted from the skin of farmed Amur sturgeon. M and ASG denote high molecular weight protein markers and Amur sturgeon gelatin respectively.

Amino acid composition

The amino acid composition of Amur sturgeon skin gelatin is shown in Table 2. Amino acid composition of fish gelatin is species specific and has important role in molecular properties of gelatin. Amur sturgeon skin gelatin contained high amounts of glycine (336 residues/1000 residues) which is the main amino acid in gelatin (Schrieber and Gareis 2007). The content of glycine in Amur sturgeon skin gelatin is higher than that reported by Benjakul et al. (2009) and Kittiphattanabawon et al. (2010) for bigeye snapper (246.57-259.38 residues/1000 residues) and shark species (321-322 residues/1000 residues) but lower than sole, megrim and cod gelatin in the range of 344-352 residues/1000 residues (Gómez-Guillén et al. 2002). Amur sturgeon gelatin had high content of alanine (118 residues/1000 residues). The content of imino acids (proline and hydroxyproline) of Amur sturgeon skin gelatin was 138 residues/1000 residues, lower than that reported for bigeye snapper, shark and Nile tilapia (Benjakul et al. 2009; Kittiphattanabawon et al. 2010; Zeng et al. 2010).

The stability of triple helical structure in renatured gelatin is proportional to the total contents of imino acids (proline and hydroxyproline). Hydroxyproline via its hydrogen bonding property through its –OH group has a major role in the stabilization of triple helical structure of collagen (Gómez-Guillén et al. 2011). In fact gelatin with high amounts of hydroxyproline offers better viscoelastic properties (Gómez-Guillén et al. 2002). Amur sturgeon gelatin contained 50 residues/1000 residues of serine. It has been reported that hydroxyl side chains of serine has important role in the generation of hydrogen bonds and helical structure during gel strengthening (Kittiphattanabawon et al. 2010). Low contents of cysteine (0.3 residues/1000 residues) and tyrosine (3.6 residues/1000 residues) were observed in Amur sturgeon gelatin. This is the general characteristic for all gelatins (Binsi et al. 2009).

Table 2. Amino acid composition (residues/1000 residues) of gelatin extracted from the skin of farmed Amur sturgeon

Amino acids*	Average
Alanine	118
Arginine	53
Asparagine	47
Cysteine	0.29
Glutamine	77
Glycine	336
Hydroxyproline	45
Histidine	53
Isoleucine	12
Leucine	17
Lysine	25
Methionine	11
Phenylalanine	15
Proline	93
Serine	50
Threonine	26
Tyrosine	3.6
Valine	17
Imino acids	138

*n=2

Viscoelastic properties

Gelling and melting points of gelatin solution (6.67% w/v) were determined. Information on viscoelastic behavior and the sol-gel transition of polymeric solutions can be obtained by the combination of elastic (G') and viscosity (G") modulus. In aqueous solution, gelatin exists as flexible single random coils. Upon cooling the solution, the single helices start to form from the random coils. With further cooling of the sol, the formation of triple helix starts. Gelling point is defined as a point that the viscosity begins to increase sharply with decreasing temperature. During cooling ramp, a large amount of helices are formed and network formation occurred. This process is dominated by intermolecular interactions (Zandi et al. 2007). Gelling temperature of Amur sturgeon skin gelatin was at 13 °C, while pig skin gelatin formed gel at 21 °C (Figure 2A). Gelling temperatures of gelatin from cold-water and warm-water fish species are around 4-12 °C and 18-19 °C (Gómez-Guillén et al. 2011), respectively. Fish gelatin posseses lower gelling temperature, when compared with mammalian counterparts (Boran et al. 2010; Gudmundsson 2002). The onset of melting of Amur sturgeon skin gelatin was at about 18.4 °C and molt at 19.6 °C (Figure 2B). The gel loses most of its elasticity (G') when heated above the melting point and a viscous polymer solution is formed (Zandi et al. 2007). Melting point of Amur sturgeon skin gelatin is higher than those of bigeye snapper (Binsi et al. 2009), flounder (Fernández-Díaz et al. 2003), cod and hake (Gómez-Guillén et al. 2002) but was lower than those of yellowfin tuna (Cho et al. 2005), sole and megrim (Gómez-Guillén et al. 2002) and tilapia (Gudmundsson 2002). It has been shown that the thermal stability of gelatin is proportional to the number and stability of proline-rich regions in collagen and gelatin molecules. Cold-water fish species are reported to possess lower content of imino acids, compared with warm-blooded animals. Amur sturgeon skin gelatin contained lower amounts of imino acids, compared with megrim and sole and began to melt at lower temperature. In spite of lower amount of imino acids observed in gelatin from the skin of Amur sturgeon in comparison with that of hake (Gómez-Guillén et al. 2002), it showed relatively higher melting temperature. In fact, viscoelastic properties of gelatin in addition to amino acid composition can also be influenced by protein pattern and particularly the percentage of α-chains, and β- and γ-components (Gómez-Guillén et al. 2002) which offer good ability of renaturation in gelatin to the native collagen. SDS-PAGE pattern of Amur sturgeon skin gelatin (Figure 1) revealed that α-chain and β- components were the major constituents along with higher molecular weight components (γ-component) which in turn explain the better thermal stability in this gelatin.

Flow behavior

Flow behavior parameters (flow behavior index, consistency coefficient, regression coefficient and viscosity) of Amur sturgeon skin gelatin are shown in Table 3. The value of flow behavior index (n) (except for 5% at 10 °C) was not affected by temperature and was between 0.82 and 1, indicating slightly non-Newtonian and shear thinning nature of Amur sturgeon gelatin. With the increase in gelatin concentration, flow behavior index did not change significantly (except in 5% gelatin). Changes of n value as a function of concentration and temperature have been noted in previous studies (Sopade and Kaika 2001; Gómez-Díaz and Navaza 2003; Binsi et al. 2009). Flow behavior index of Amur sturgeon skin gelatin (except at 10 °C and 5% concentration) was higher than starch, pectin, xantan, carrageenan (Marcotte et al. 2001), sago starch (Sopade and Kaika 2001) and Qodume shirazi seed gum (koocheki et al. 2009) and was similar to carboxymethyl cellulose (Gómez-Díaz and Navaza 2003) and chitosan (Sathivel et al. 2007), indicating a more Newtonian nature at higher temperature or lower concentrations. However at 10 °C and 5% concentration, the gelatin n value (0.31) was similar to that of sago starch (Sopade and Kaika 2001). The low n value ensures a great distance of flow from the Newtonian behavior and high viscosity. This behavior was similar to that found in other shear-thinning hydrocolloids, where the decreases are obtained as shear rate is increased. Hydrocolloids with low n value and higher viscosity show better mouth feel (Marcotte et al. 2001). Marcotte et al. (2001) indicated a clear Newtonian behavior for commercial fish gelatin; however bigeye snapper skin gelatin showed non-Newtonian nature at all concentrations and temperatures (Binsi et al. 2009).

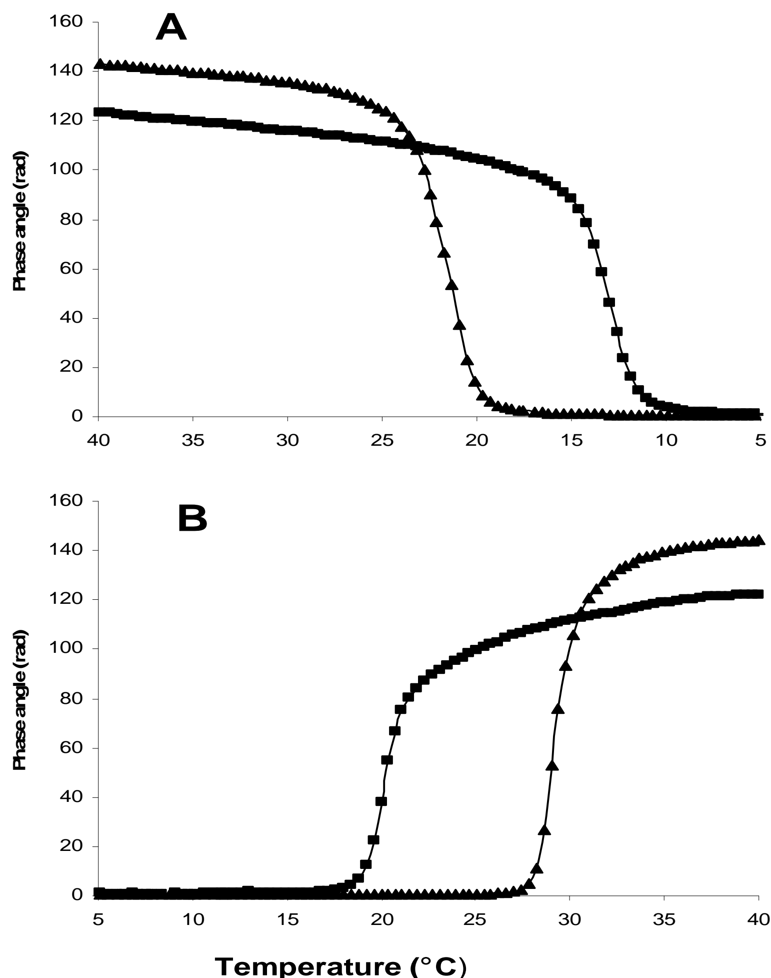

Fig. 2. Changes in the phase angle (rad) of Amur sturgeon skin gelatin (■) and pig skin gelatin (▲) are presented during cooling from 40 to 5 °C (A) and subsequent heating from 5 to 40 °C (B) of 6.67% (w/v) gelatin solution.

Table 3. Power law model parameters and viscosity of Amur sturgeon skin gelatin solutions as a function of concentration and temperature

Sample/temperature	Viscosity* (Pa s)	Flow behavior index (n)	Consistency coefficient (Pa s^n)	Regression coefficient (R^2)
1% gelatin				
10°C	0.0068 ± 0.0010[c]	0.82 ± 0.08[d]	0.0156 ± 0.0040	0.97
30°C	0.0016 ± 0.0000[c]	0.97 ± 0.02[ab]	0.0018 ± 0.0003	0.99
45°C	0.0012 ± 0.0000[c]	0.98 ± 0.03[ab]	0.0013 ± 0.0002	0.99
60°C	0.0010 ± 0.0002[c]	0.99 ± 0.01[ab]	0.0009 ± 0.0002	0.99
3% gelatin				
10°C	1.014 ± 0.1757[b]	0.88 ± 0.04[cd]	1.9091 ± 0.6915	0.99
30°C	0.0040 ± 0.0004[c]	0.99 ± 0.01[ab]	0.0042 ± 0.0006	0.99
45°C	0.0027 ± 0.0003[c]	0.95 ± 0.07[abc]	0.0035 ± 0.0006	0.99
60°C	0.0022 ± 0.0002[c]	0.97 ± 0.06[ab]	0.0026 ± 0.0005	0.99
5% gelatin				
10°C	6.74 ± 0.054[a]	0.31 ± 0.03[e]	155.2 ± 17.73[a]**	0.99
30°C	0.0093 ± 0.0009[c]	1.00 ± 0.00[a]	0.0092 ± 0.0009	0.99
45°C	0.0063 ± 0.0007[c]	0.98 ± 0.03[ab]	0.0065 ± 0.0006	0.99
60°C	0.0045 ± 0.0002[c]	0.92 ± 0.05[bc]	0.0067 ± 0.0018	0.99

* At 100 s^{-1} shear rate for all samples.
**Statistically different ($P < 0.05$).

The consistency coefficient (k) values in this study were between 0.0009 and 155.2 Pa. A non significant decrease in k values was observed when temperature increased, indicating the decrease in viscosity as the temperature increased. On the other hand, upon increasing gelatin concentration from 1 to 5%, k increased (Table 3). Previous studies (Marcotte et al. 2001; Sopade and kiaka 2001; Gómez-Díaz and Navaza 2003) have noted the major effects of temperature on the consistency coefficient of different hydrocolloids. Consistency coefficient is a strong function of concentration and temperature, while flow index (n) does not show such strong dependency (Maecotte et al. 2001). K value of Amur sturgeon gelatin was lower, compared with other hydrocolloids such as starch, pectin, xantan and carrageenan (Marcotte et al. 2001). However at 10 °C for 5% gelatin solution, the value was much higher (Table 3). The increase of k value is attributed to water holding capacity of the hydrocolloid (Gómez-Díaz and Navaza 2003).

Viscosity of gelatin at different concentrations and temperatures is shown in Table 3. Viscosity was influenced by concentration. At 5% and 10 °C, gelatin solution exhibited the highest viscosity. The increase in temperature is accompanied by the decrease in viscosity (Gómez-Díaz and Navaza 2003). At higher temperature, the thermal energy of the molecules in the solution increases and intermolecular distances increased. Therefore interactions between molecules become weaker (Koocheki et al. 2009). In this study, although not significant, with the increasing temperature, viscosity slightly decreased. This result was in agreement with Marcotte et al. (2001) who noted the decrease in viscosity as a function of temperature for commercial gelatin.

FTIR Spectra

Fourier transform infrared (FTIR) spectroscopy is an important and well established technique to study secondary structure of proteins and polypeptides. Nine characteristic FTIR absorption band, namely A,B and I-VII can be observed from a typical IR spectra, of which amide I band (1700-1600 cm^{-1}) is the most sensitive and widely used in studies of protein secondary structure. Amide I band is mainly due to C=O stretching vibration (about 80%) of the amide group coupled with in-plane NH bending (less than 20%) (Kong and Yu 2007). Amide II (1575-1480 cm^{-1}) derives mainly from in-plane NH bending and CN stretching vibration and shows less protein conformational sensitivity compared with amide I, while other amide vibrational bands have less practical use in protein conformational studies (Kong and Yu 2007). Amide I and II bands of Amur sturgeon skin gelatin appeared at the wavenumber of 1640.60 and 1534.57 cm^{-1} (Figure 3). Amide I bands of gelatin from bigeye snapper skin was found at around 1630 cm^{-1} (Benjakul et al. 2009).

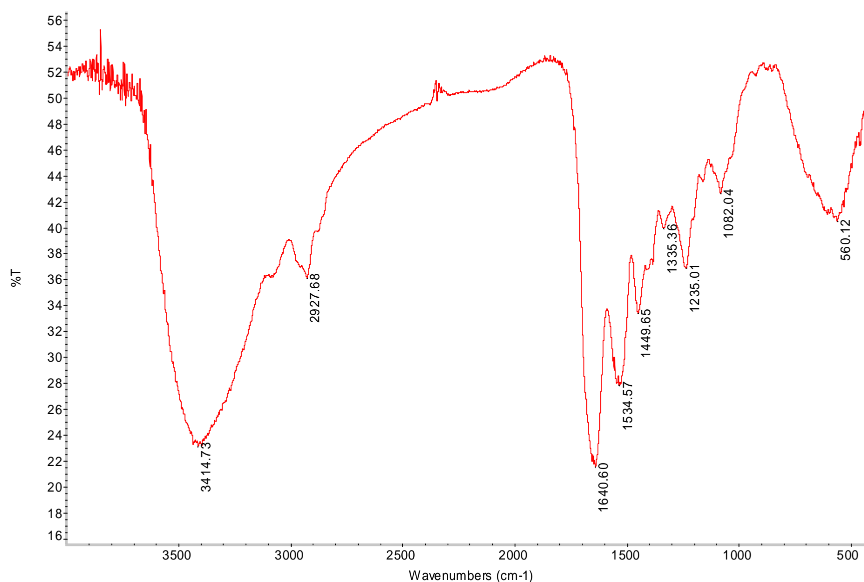

Fig. 3. Fourier transform infrared (FTIR) spectroscopy of gelatin from the skin of farmed Amur sturgeon

Amide I band of Amur sturgeon skin gelatin appeared at lower wavenumber, compared with that of unicorn leatherjacket and shark skin gelatin. Higher frequencies of amide I bands is attributed to greater loss of molecular order of triple helix due to uncoupling of intermolecular cross-links and disruption of intra molecular bonding when gelatin was extracted at higher temperature or longer time (Kittiphattanabawon et al. 2010; Ahmad and Benjakul 2011). In addition, amide III band of Amur sturgeon gelatin was detected at 1235 cm^{-1} which was associated with loss of triple-helix state of the molecules and transformation of α-helical to random coil structure due to denaturation of collagen to gelatin (Muyounga et al. 2004). Amide III band of gelatin at 1237 cm^{-1} was reported by Benjakul et al. (2009) in bigeye snapper. Amide A band derives from the stretching vibration of N-H group (Kong and Yu 2007). In this study, N-H stretching band appeared at 3414.73 cm^{-1}. N-H stretching bands at around 3288.79 were observed for bigeye snapper gelatin (Benjakul et al. 2009). N-H stretching vibration of amide A occurs normally at wavenumber of 3440-3400 cm^{-1} (Muyonga et al. 2004). When N-H group of shorter peptides are involved in hydrogen bonding, the position of the band in amide A region shifts to lower frequencies. Amide A band of unicorn leatherjacket skin gelatin treated with phosphoric acid and extracted for 8 hour, shifted to lower wavenumber, compared with gelatin extracted for 4 hour, indicating the involvement of N-H group of shorter peptide fragments in hydrogen bonding (Ahmad and Benjakul 2011).

Conclusions

Gelatin extracted from the skin of farmed Amur sturgeon showed higher yield when compared with that of some fish species. Gelatin had α- and β-chains as major protein components. Gel strength of Amur sturgeon gelatin is comparable to that of mammalian gelatin. Similar with the other fish gelatins, farmed Amur sturgeon skin gelatin had lower gelling and melting temperature than mammalian gelatin. Flow behavior index of gelatin was higher than other hydrocolloids indicating more Newtonian nature at higher temperatures or lower concentrations. However it exhibited a clear non-Newtonian with shear-thinning behavior and high viscosity at 5% and 10°C. Extraction of gelatin helps in better utilization of farmed Amur sturgeon waste.

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