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The characteristics of gelatin extracted from sturgeon (*Acipenser baeri*) skin using various pretreatments

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ABSTRACT

The physicochemical characteristics of gelatin obtained by different pretreatments of sturgeon (*Acipenser baeri*) skin with alkaline and/or acidic solutions have been studied. Visual appearance, pH, gel strength, viscosity and amino acid profile of the gelatins were evaluated. Pretreatment with alkaline solutions of $Ca(OH)_2$ and/or acetic acid (HAC) provided gelatin with a favourable colour. Pretreatment with alkali removed noncollagenous proteins effectively, whilst acid induced some loss of collagenous proteins. Gel strength and viscosity of gelatin pretreated with HAC or alkali followed by HAC were as high as gelatin extracted in the presence of protease inhibitors. Amino acid composition had no significant effect on the gelatin characteristics. The total acid concentration for the highest gel strength was inversely proportional to ionisation strength, and the preferred pH for extracting gelatin with the optimum gel strength was approximately 5.0. The results showed that any available protons, regardless of the type or concentration of the acid, inhibit protease activity, which significantly affects the gelatin characteristics.

1. Introduction

Gelatin is a fibrous protein that is extracted from collagen and is an important functional biopolymer that has a very broad application in many industrial fields, such as food, materials, pharmacy and photography. It is especially useful in the food and pharmaceutical industries for its unique chemical and physical characteristics (Rahman, Al-Saidi, & Guizani, 2008). Cattle bones, cattle hides and pork skins are the traditional materials used in gelatin production (Bowes, Elliott, & Moss, 1955; Cho, Gu, & Kim, 2005; Sobral & Habitante, 2001). In recent years, fish skin had been reported as a new alternative source of producing gelatin (Gudmundsson & Hafsteinsson, 1997; Jamilah & Harvinder, 2002). Although the properties of gelatin from fish skin are different from those from mammals and avian species, fish skin gelatin has the advantages of utilising highly abundant fish offal, and avoiding bovine spongiform encephalopathy concerns (Sadowska, Kolodziejska, & Niecikowska, 2003).

Sturgeon is famous in Western countries for its caviare (Ali & Reza, 2006; Reza & Mehdi, 2006). This fish species has been widely bred in China, especially in Hangzhou for exporting caviare. The ensuing increase of caviare means that sturgeon production will rise sharply in the future. However, the lack of utilisation of all fish parts will constrain the development of sturgeon aquaculture. It has been estimated that skin represents 20% of the waste from fish processing. Sturgeon skin is abundant in collagen, which is a useful source of gelatin. Therefore, gelatin extraction from sturgeon skin could definitely increase the value of the byproducts and solve some waste disposal problems.

The quality of gelatin for a particular application largely depends on its physicochemical properties, which are greatly influenced by both the species and tissue from which it is extracted and the method of extraction (Gilsenan & Ross-Murphy, 2000). Gelatin extraction from fish skin is generally achieved by pretreatment with acid or alkali to give the desired properties (Arnesen & Gildberg, 2006; Cho, Jahncke, Chin, & Eun, 2006; Giméneza, Turnayb, Lizarbeb, Montero, & Gómez-Guillén, 2005). The purpose of this study was to determine the effect of pretreatment on gelatin extraction from sturgeon skin and the physicochemical characteristics of the gelatin obtained to provide a possible approach for utilisation of sturgeon skin.

2. Materials and methods

2.1. Materials

Sturgeon used in this research was a cultured freshwater fish obtained from a local fish farm in Hangzhou, China. Upon arrival at the factory, the fish were killed and skinned manually. The skin was stored at −18 °C until use. All reagents used were analytical grade.

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2.2. Methods

2.2.1. Pretreatment

Sturgeon skins were allowed to thaw below 10 °C and the adherent tissues from the skins were scraped manually with a scalpel. Fat on the skins was removed by vigorous stirring in chilled water. After being cleaned in distilled water, the skins were cut into small pieces (0.5 × 0.5 cm) with a scalpel. Cleaned skins (20 g) were pretreated following the procedures described by Zhou and Regenstein (2005) with varying concentrations of alkaline and/or acidic solution (1:6 w/v) for 60 min at 0–4 °C. Pretreatment 1: cleaned skins were treated with NaOH or $Ca(OH)_2$ at [OH^-] concentration 0.01, 0.05, 0.1, 0.2 and 0.5 mol/L. Pretreatment 2: cleaned skins were treated with acetic acid at total acid concentration 0.075 mol/L. Pretreatment 3: cleaned skins were first treated with $Ca(OH)_2$ at concentration of 0.025 mol/L for 60 min, followed by treatment with acids (acetic, citric and sulphuric acid) at total acid concentration of 0.01, 0.025, 0.05, 0.075, 0.1, 0.15 and 0.2 mol/L for 60 min. After pretreatment, the skins were rinsed and drained with tap water for gelatin extraction. The pretreatment solutions (5 mL) were collected and concentrated to 2 mL for analysis.

2.2.2. Extraction of gelatin

The pretreated skins were extracted with distilled water (1:3 w/v) at 50 °C for 18 h. The extracted solution was filtered through double layer gauze and Whatman filter paper (No. 4). The control samples were directly extracted with distilled water in the absence or presence of a mixture of protease inhibitors (to study the effect of endogenous proteases on collagen during preparation), which consisted of 5 mM EDTA disodium salt, 0.2 mM phenylmethane sulfonyl fluoride (PMSF; Sigma–Aldrich Inc., St. Louis, MO, USA) and 2 μM pepstatin (Sigma–Aldrich Inc., St. Louis, MO, USA). The extraction solutions were diluted with distilled water to a protein concentration of 50 mg/mL for analysis of gel strength, viscosity properties, pH and colour.

2.2.3. Analyses

2.2.3.1. Gelatin yield. Protein concentration was determined by the Lowry method (Zhou & Regenstein, 2006), and the extraction yield was calculated by the following equation:

$$\text{Yield } (\%) = 100 \times (\text{protein concentration in g/mL}) \times (\text{volume of extract in mL})/20\ \text{g}$$

2.2.3.2. Gel strength. The gel strength of the gelatin solutions was determined using a Texture Analyser (QTS-25, CNS Farnell, England) using a 12.5 mm diameter plunger pressed 10 mm into the gelatin gels at a speed of 30 mm/min and a trigger of 5 g.

2.2.3.3. Viscosity properties. The viscosity of the gelatin solutions was determined at room temperature according to the procedures described by Montero, Jiménez-Colmenero, and Borderías (1991).

2.2.3.4. pH determination. The pH of the gelatin solutions was determined using a pH metre (PB-10, Sartorius, Germany) at 25 °C.

2.2.3.5. Colour measurement. The colour of the gelatin solutions was determined by measuring the lightness, redness and yellowness values (L^*, a^* and b^*) (Jamilah & Harvinder, 2002) using a lab Miniscan portable colorimeter (CIE-LAB, Xingguang Inc., Beijing, China).

2.2.3.6. Amino acid analysis. Gelatin solutions were hydrolysed in vacuum in 6 M HCl (1:1 v/v) at 110 °C for 22 h in the presence of 1% phenol (v/v), and the hydrolysates were analysed using an amino acid analyser (Hitachi 835-50, Hitachi Inc., Japan).

2.2.3.7. SDS–PAGE. SDS–PAGE was performed as described by Jamilah and Harvinder (2002) using 75% resolving gel and 40% stacking gel. Gelatin solutions (5 mg/mL) and molecular weight standard were diluted 1:2 with sample buffer and then loaded onto the gel (Bio-Rad Laboratories, CA, USA). After electrophoresis, the gel was stained with Coomassie Brilliant blue R-250 and then scan with gel image system (Tanon 1600, Tianlong Inc., Tianjin, China) for content distribution of protein.

2.2.3.8. Statistical analysis. All data were analysed using the analysis of variance (ANOVA) and Duncan's multiple range tests to determine the significant differences between means (SAS, v6 software).

3. Results and discussion

3.1. Removal of proteins

The pretreatment solutions were collected to determine the effect of different pretreatments on protein removal. The results showed that pretreatment induced removal of proteins from sturgeon skin, but the composition of the proteins removed were sensitive to acidic and alkaline solutions (Fig. 1). The protein bands in lane 3 ($Ca(OH)_2$) pretreatment solution) were more intense than the bands in lane 2 (NaOH pretreatment solution). This indicates that the $Ca(OH)_2$ pretreatment solution had higher amounts of protein than the NaOH pretreatment solution. The different effect between $Ca(OH)_2$ and NaOH was that NaOH caused significant swelling of sturgeon skin during pretreatment, whilst $Ca(OH)_2$ did not. This observation was noticed in a study of pollock skins (Zhou & Regenstein, 2005). Skin treated with $Ca(OH)_2$ followed by acetic acid (HAC) (1:6 w/v) at total acid concentration of 0.075 M induced the dissolution of more total proteins than without pretreatment. No significant amount of collagen was observed in alkaline pretreatment solutions, whilst α and β collagen were found in acidic pretreatment solutions (Fig. 1). These results were similar to those reported previously for Alaska pollock skin (Zhou & Regenstein, 2005). Gudmundsson and Hafsteinsson (1997) and Muyonga, Cole, and Duodu (2004) also reported that alkaline and acid treatments were critical for removing unwanted material such as noncollagenous proteins with minimum collagen loss. Undeland, Kelleher, and Hultin (2002) confirmed that preliminary

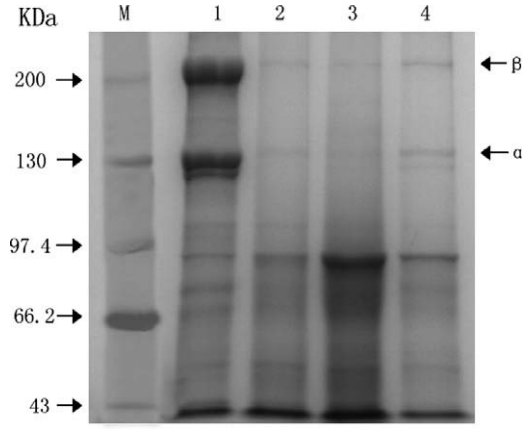

Fig. 1. SDS–PAGE profile for solutions from sturgeon skin pretreated with different acids and/or bases. M: molecular weight standards; lane 1: HAC; lane 2: NaOH; lane 3: $Ca(OH)_2$; lane 4: $Ca(OH)_2$ and HAC. The α and β refer to the α and β chains of sturgeon skin gelatin.

extraction with base could effectively remove noncollagenous proteins during the isolation of collagen from fish muscle.

3.2. Characteristics of gelatin extract

Different gelatin characteristics were observed with various pretreatments of sturgeon skins prior to extraction (Table 1). Gelatin produced from $Ca(OH)_2$ pretreatment generally had higher L^* value than those produced from NaOH pretreatment. However, concentration of alkali seemed to play a role. For instance gelatins from the 0.005 M $Ca(OH)_2$ pretreatment had similar L^* and b^* values to the gelatins from the NaOH pretreatment. Gelatin yields from skins pretreated with $Ca(OH)_2$ were in the ranged from 2.40% to 3.52%, which were a little higher than those treated with NaOH at the same hydroxide concentration. Pretreatment with $Ca(OH)_2$ followed by HAC provided higher extraction yields than pretreatment with $Ca(OH)_2$ at 0.025 M but lower than pretreatment with HAC at 0.075 M. The gelatin extracts with only alkaline pretreatments had gel strengths ranging from 30.03 to 89.03 g cm, whilst those pretreated with HAC at total acid concentration of 0.075 M or $Ca(OH)_2$ of 0.025 M followed by HAC at total acid concentration of 0.075 M improved gel strength up to 207.65 and 296.95 g cm, respectively. However, the yield was similar with $Ca(OH)_2$ pretreatment. Similar results were obtained for viscosity. HAC at total acid concentration of 0.075 M or $Ca(OH)_2$ at a (OH^-) of 0.025 M followed by HAC at total acid concentration of 0.075 M improved viscosity up to 0.333 and 2.283 Pa s, respectively. This suggested that the combination of the two pretreatments provided the optimum conditions for gelatin extraction, during which some noncollagenous proteins were further destroyed but with less loss of collagenous proteins. The pH of gelatin extracted after alkaline pretreatments ranged between 8 and 10, whilst using HAC alone the pH was 4.51, and using alkali followed by HAC the pH was 6.03. The pH values of gelatin extracted using HAC were similar to those of the controls extracted with protease inhibitors.

3.3. Amino acid composition and molecular weight distribution

The amino acid composition of gelatin from sturgeon skin pretreated using different methods is shown in Table 2. Glycine was the most abundant amino acid found in the gelatin, with a composition of about 25 mg/100 mg protein. Cysteine, histidine and tyrosine were found in low concentrations. Control samples of gelatin directly extracted with distilled water in the absence or presence of protease inhibitors provided gelatin with higher amino acid content, whilst amino acid content in the gelatin pretreated with HAC or $Ca(OH)_2$ followed by HAC was generally lower. In this study, proline and hydroxyproline for different pretreatments accounted for about 18% of the total amino acid composition, which was similar to those reported for collagens from cold water fish species (16–18%) (Gilsenan & Ross-Murphy, 2000; Gudmundsson & Hafsteinsson, 1997; Jongjareonrak, Benjakul, Visessanguan, Nagai, & Tanaka, 2005). There was no significant difference in amino acid composition amongst the different pretreatments. However, gel strength and viscosity were significantly different amongst the various pretreatments (Table 2). It has been reported (Arnesen & Gildberg, 2007) that the total glycine, proline and hydroxyproline content was an important factor affecting the rigidity of the collagen structure. But our results showed that collagen rigidity was affected more by molecular weight distribution, which was determined by the different pretreatments (Fig. 2 and Table 3). Sturgeon skin extracted without inhibitors produced a large amount of low molecular weight proteins (Fig. 2, lane 2). After pretreatment with alkali, protein species with higher molecular weight were observed in the gels of the resultant gelatine samples (Fig. 2, lanes 5–8). In addition, the molecular weight of the gelatin extracted in the gelatin, increased with increasing hydroxide concentration (lanes 6 and 7). Sturgeon skin pretreated with HAC alone (lane 3), or $Ca(OH)_2$ followed with acetic acid (lane 4), or directly with inhibitors (lane 1) increased the molecular weight of the extracted protein species significantly. This is shown by the presence of high molecular weight proteins including the α and β bands in the lanes of the gel. The high molecular weight proteins with both the α and β bands can be seen in the SDS–PAGE gel. Thus, molecular weight is a factor that affects the characteristics of collagens and degradation of these extracts was the result of acid or alkaline pretreatment, which inhibited the protease activity. High hydroxide concentration was found to inhibit protease activity and decrease protein degradation, whilst acid and a combination of alkaline and acid pretreatment inhibited protease activity and decreased the enzymatic degradation of gelatin extracts as well, as shown in Fig. 2.

3.4. Effect of type and concentration of acids on gelatin extraction

Three acids (acetic, citric and sulphuric) with different total acid concentration (0.01, 0.025, 0.05, 0.075, 0.1, 0.15 and 0.2 M) were used to study the effects of the type of acid and acid concentration on gel strength (Fig. 3). The results showed that gel strength was low at total acid concentration of 0.01 M. Further increasing of the acid concentration for all three acids did not increase the gel strength to the same extent. The total acid concentration necessary to reach high gel strength for the three acids was in the order of

Table 1
The yield and properties of gelatin extracts (mean ± SD based on three determinations).

Pretreatment	Extraction yield (%)	Gelatin pH	Gel strength (g cm)	Viscosity (Pa s)	Colour of gelatin		
					L^*	a^*	b^*
0.01 M NaOH	2.24 ± 0.01	8.32 ± 0.40	43.42 ± 9.18	0.032 ± 0.009	51.49 ± 1.48	2.00 ± 0.49	6.31 ± 0.25
0.05 M NaOH	2.74 ± 0.14	10.02 ± 0.38	89.03 ± 1.77	0.039 ± 0.014	51.47 ± 1.73	1.96 ± 0.17	6.08 ± 1.74
0.1 M NaOH	2.83 ± 1.19	10.39 ± 0.01	84.30 ± 1.02	0.037 ± 0.008	52.70 ± 2.75	1.94 ± 0.26	4.41 ± 0.90
0.2 M NaOH	2.86 ± 0.98	10.69 ± 0.01	70.98 ± 11.70	0.035 ± 0.006	53.07 ± 4.54	1.79 ± 0.56	4.60 ± 0.64
0.5 M NaOH	2.87 ± 0.27	10.87 ± 0.04	37.43 ± 23.61	0.033 ± 0.006	50.96 ± 2.52	2.2 ± 0.44	5.93 ± 2.56
0.005 M $Ca(OH)_2$	2.40 ± 0.46	8.15 ± 0.04	32.65 ± 4.27	0.036 ± 0.004	52.55 ± 4.97	2.14 ± 0.20	4.49 ± 0.35
0.025 M $Ca(OH)_2$	3.06 ± 0.20	9.88 ± 0.51	79.63 ± 9.33	0.137 ± 0.010	53.78 ± 1.64	2.12 ± 0.11	1.87 ± 0.09
0.05 M $Ca(OH)_2$	3.28 ± 0.21	10.05 ± 0.14	83.68 ± 6.49	0.160 ± 0.009	53.75 ± 4.38	2.19 ± 0. 29	1.67 ± 0.13
0.1 M $Ca(OH)_2$	3.52 ± 0.14	10.08 ± 0.07	37.54 ± 9.49	0.029 ± 0.004	54.01 ± 3.12	2.15 ± 0.14	1.34 ± 0.22
0.25 M $Ca(OH)_2$	3.38 ± 0.40	10.10 ± 0.16	30.03 ± 3.43	0.025 ± 0.006	53.82 ± 3.37	2.11 ± 0.14	1.51 ± 0.29
0.075 M HAC	3.29 ± 1.40	4.51 ± 0.08	207.65 ± 8.21	0.333 ± 0.019	53.53 ± 6.73	2.83 ± 0.62	4.35 ± 1.87
0.025 M $Ca(OH)_2$– 0.075 M HAC	3.16 ± 2.45	6.03 ± 0.54	296.95 ± 22.90	2.283 ± 0.091	54.39 ± 5.03	3.05 ± 0.22	3.13 ± 0.18
Inhibitor	4.42 ± 1.13	5.85 ± 0.07	256.21 ± 13.59	0.858 ± 0.038	53.39 ± 3.26	2.92 ± 0.60	5.15 ± 0.99
Original	5.40 ± 0.18	7.10 ± 0.11	5.20 ± 0.30	0.190 ± 0.009	53.56 ± 1.05	2.88 ± 0.67	3.71 ± 1.41

Table 2
Amino acid composition of gelatin from sturgeon skin using different pretreatments (mg/100 mg protein).

Amino acid	NaOH	$Ca(OH)_2$	HAC	$Ca(OH)_2$–HAC	Inhibitor	Original
Aspartic acid	6.5393	6.4455	6.4864	6.4791	6.7008	6.5055
Threonine	2.1730	2.0109	1.5410	1.6120	1.9620	1.9910
Serine	2.7378	2.2503	1.2056	1.3735	2.1509	2.2949
Glutamic acid	10.3869	10.4866	10.4753	10.4662	10.7926	10.6384
Proline	12.4455	12.7357	12.7835	13.0795	12.6663	12.5817
Glycine	24.8798	25.1875	25.4079	25.9942	25.0628	25.3305
Alanine	9.5765	10.1613	10.1306	9.9562	9.7131	9.8202
Cysteine	0.4605	0.4537	0.4815	0.5036	0.4303	0.4147
Valine	2.0604	1.9870	2.0625	2.0224	2.1208	2.0010
Methionine	2.0240	1.9731	1.9905	2.0643	1.9829	1.8799
Isoleucine	1.9788	1.9048	2.0352	2.0023	1.9839	1.9444
Leucine	2.7616	2.6574	2.7123	2.6685	2.8917	2.7796
Tyrosine	0.3272	0.3002	0.2575	0.1851	0.2798	0.1226
Phenylalanine	2.4701	2.5284	4.5302	2.5992	2.5795	2.6672
Lysine	3.7695	3.8448	3.3977	3.9159	3.9759	3.9290
Histidine	0.7352	0.7489	0.7278	0.7009	0.7528	0.7622
Arginine	8.9999	9.0632	9.0206	9.1699	9.0192	9.0732
Hypro	5.6738	5.2607	4.7540	5.2074	4.9345	5.2641

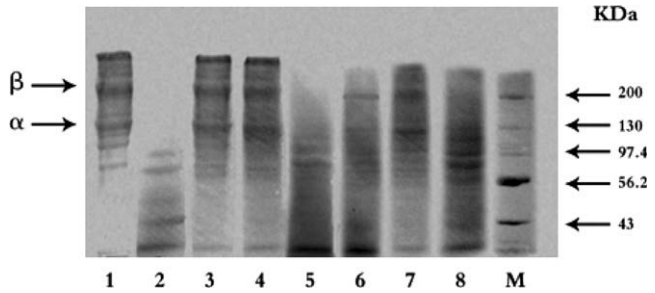

Fig. 2. SDS–PAGE profiles of gelatin extracts from sturgeon skin using different pretreatments. Lane 1: inhibitor; lane 2: original (no inhibitor); lane 3: HAC; lane 4: 0.025 M $Ca(OH)_2$ and 0.075 M HAC; lane 5: 0.005 M $Ca(OH)_2$; lane 6: 0.025 M $Ca(OH)_2$; lane 7: 0.05 M NaOH; lane 8: 0.01 M NaOH; M: molecular weight standards. The α and β refer to the α and β chains of sturgeon skin gelatin.

Table 3
Content analysis of α and β collagen for SDS–PAGE gel (%).

Lane	Inhibitor	HAC	0.025 M $Ca(OH)_2$ and 0.075 M HAC	0.025 M $Ca(OH)_2$	0.05 M NaOH
α	24.86	24.71	26.35	11.20	13.46
β	17.34	17.26	19.65	7.02	16.54

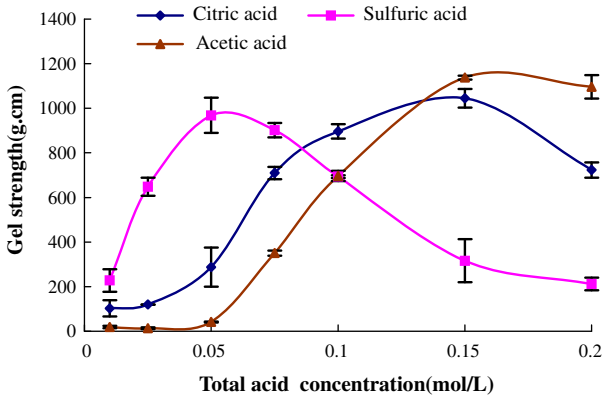

Fig. 3. Effect of various concentrations of acids on gel strength.

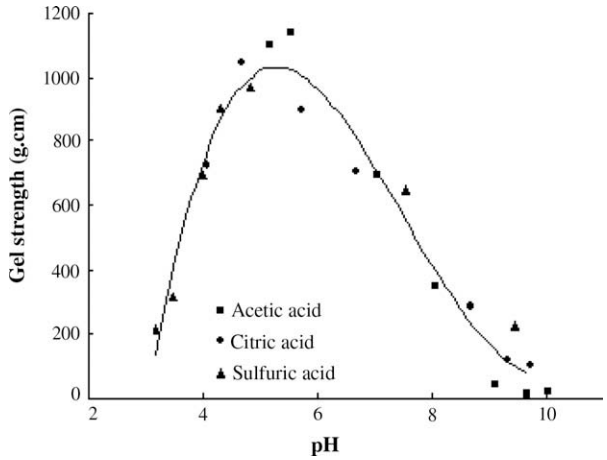

Fig. 4. Relationship between final pH and gel strength.

HAC > citric acid > sulphuric acid, which is the opposite order as ionisation strength with HAC < citric acid < sulphuric acid. Gel strength was the highest when the skin was pretreated with total acid concentration of 0.15 M using HAC. Pretreatment with total acid concentration of 0.05 M using sulphuric acid or 0.15 M using citric acid also gave peak values of gel strength. Any deflection from these total acid concentration values decreased the gel strength significantly. These results were different from those reported by Zhou and Regenstein (2005), which stated that gel strength reached a peak at total acid concentration of 0.05 M for these three acids. This discrepancy may be due to the variations in skin from different species.

The effect of the final pH of the gelatin extracts on gel strength is shown in Fig. 4. The results indicated that the extracts had similar gel strengths at comparable final pH values, and were not dependent on the type or concentration of acid that was used for the pretreatments. The results also showed that high gel strength was obtained at pH values near 5.0. Therefore, the type of acid and total acid concentration had an effect on gel strength, but they were not the main factors. The most important factor was concentration of hydrogen ion. The total acid concentration required for the three acids to reach the maximum gel strength was different because they have unique ionisation constants (Montero et al., 1991). Thus, although in some cases the total acid concentration of each acid was the same, the ionised (H^+) available in solution was different, which resulted in a different final pH during gelatin extraction, and led to differences in gel strength. The more intense the ionisation was, the faster the gel strength reached a high level (Fig. 3).

4. Conclusions

Sturgeon skins pretreated with alkali and/or acid induced removal of protein with different molecular weight distributions. Proteins with low molecular weight predominated in basic pretreatment solutions, but α and β collagens were found in acidic pretreatment solutions. Pretreatment with a combination of alkali and acid decreased the loss of collagen, but it increased gel strength and viscosity significantly.

Generally, glycine was the most abundant amino acid, and cysteine, histidine and tyrosine content was low in all extractions pretreated using the various methods. The total amino acid content was highest in extracts without any pretreatment, followed by alkali, acid and a combination of alkali and acid pretreatment. The presence of amino acids such as proline and hydroxyproline was relatively lower in pretreatments with acid and a combination of

alkali and acid; and protein molecular weights focused around 130 and 200 kDa with these two pretreatments, which induced high gel strength and viscosity.

The gelatin characteristics were highly sensitive to the final pH of the extract, therefore pH was the major factor for efficient extraction. It was determined that the type of acid and the concentration of acid did not matter, rather, a final pH around 5.0 was optimum for gelatin extraction.

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