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Rheological and mechanical properties of cross-linked fish gelatins

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Abstract

Gelatin was extracted from the skins of Alaska pollock (*Theragra chalcogramma*) and Alaska pink salmon (*Oncorhynchus gorbuscha*). Amino acid analysis and gel electrophoresis were used to determine their amino acid composition and molecular weight profiles, respectively. Dynamic rheology was also used to characterize the fish gelatins' gelation and melting behavior as well as their cross-linking behavior upon adding genipin and glutaraldehyde. Pollock and salmon gelatin had lower gelation and melting temperatures than that of a commercial porcine gelatin. Both fish gelatins that contained genipin showed faster cross-linking rates for samples with higher pH values. However, salmon samples exhibited greater dependence on pH. Also, pollock gelatin cross-linked faster with glutaraldehyde than with genipin. After five days of cross-linking, all porcine samples had much greater gel strengths than pollock samples. In addition, porcine samples containing genipin had gel strengths several times greater than those containing glutaraldehyde.
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Keywords: Fish gelatin; Cross-link; Rheology

1. Introduction

Gelatin has been examined as a material for various pharmaceutical and biomedical applications, including microspheres for drug delivery [1–3], bioadhesives for wound treatment [4,5], and scaffolds for tissue engineering [6]. Gelatin has relatively low antigenicity and is also relatively inexpensive. Most studies on gelatin have focused on mammalian gelatins, such as bovine and porcine, because they have superior mechanical properties to other gelatins. However, gelatin degrades readily under physiological conditions and better mechanical properties are needed for potential use as a biomedical material.

The mechanical properties of gelatin can be improved through chemical cross-linking. Once cross-linked, the sample becomes much more stable in aqueous environments. Various cross-linkers had been used to cross-link gelatin, including glutaraldehyde [1,3–5,7,8], genipin [3,4,9–12], carbodiimides [4,7,12,13], and transglutaminase [6,14–18]. Glutaraldehyde is one of the most widely used cross-linkers since it reacts rapidly with amine groups in gelatin and is also relatively inexpensive. However, there have been concerns about glutaraldehyde's toxicity.

Recently, genipin has attracted interest as an alternate cross-linker to glutaraldehyde because of its lower toxicity [3,4,9,11,12]. Like glutaraldehyde, genipin also reacts with amine groups in gelatin. Genipin is isolated from the fruits of *Gardenia jasminoides Ellis*, found in the Far East, and has been used in herbal medicine and as a food dye to create a deep blue color. Previous *in vitro* [3,4] and *in vivo* [3] studies indicated that gelatin samples cross-linked with genipin had lower toxicity than samples containing other cross-linkers. In an *in vivo* study [3], rats were implanted with gelatin microspheres cross-linked with glutaraldehyde and genipin. During the course of the experiment, the implanted genipin

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microspheres caused much less inflammation of surrounding tissue than the glutaraldehyde microspheres.

To date, studies with genipin have focused solely on porcine gelatin. However, a potential source of gelatin that has been underutilized includes Alaska pollock (*Theragra chalcogramma*) and Alaska pink salmon (*Oncorhynchus gorbuscha*). These two fishes comprised approximately 73% of the Alaskan marine finfish catch in 2000 [19]. It has been estimated that over a million tons of fish by-products are generated each year from the fishing industry in Alaska [19]. Some of these by-products are converted into fish meal and oil, but approximately 60% are dumped back into the ocean [19]. The by-products include substantial quantities of fish skin, which is a good source of gelatin. Cold water fish gelatins, such as those extracted from pollock and salmon, have very low gelation temperatures compared to mammalian and warm water fish gelatins. This is due to the cold water fishes having lower concentrations of proline and hydroxyproline than the other species. Consequently, cold water fish gelatins behave as a viscous liquid at room temperature, which severely limits their potential use in many applications.

In this study, we extracted gelatin from Alaska pollock and Alaska pink salmon skin. We then cross-linked the fish gelatins with genipin and glutaraldehyde and monitored their dynamic rheological properties during the cross-linking process. We varied the gelatin formulations by changing the cross-linker concentration and sample pH. We also compared genipin cross-linking of fish and commercial porcine gelatins. In addition, we measured the gel strength of the cross-linked samples.

2. Experimental

2.1. Gelatin extraction

A procedure for extracting gelatin from fish skin was derived from Montero and Gomez-Guillen [20] and Gudmundsson and Hafsteinsson [21]. Fresh Alaska pollock (*T. chalcogramma*) skins were obtained from an automated commercial processing plant in Kodiak, Alaska, where skinless fillets were being produced. These were immediately frozen at −30 °C in a blast freezer. Alaska pink salmon (*O. gorbuscha*) was obtained from a commercial processing plant and frozen at −30 °C. The skins were manually removed from the fish. To make gelatin, the skin was thawed and first washed with cold (2−5 °C) water (1:6 w/v) in a kettle with a paddle mixer (Groen/Dover Ind.). The washing process took 10 min. After each wash, the skin was rinsed with running tap water. Each wash and rinse step was repeated three times. Subsequently, the skin was washed with cold (2−5 °C) 0.8 N NaCl (1:6 w/v) in the kettle following the same procedure as the water wash. Excess water was then removed by draining the cleaned skin in a sieve for 5 min. The washed skin was added to cold (2−5 °C) 0.2 N NaOH (1:6 w/v) and stirred for 40 min. After this, the skin was drained and rinsed with running tap water. Each stir and rinse step was repeated three times. The skin was further washed with 0.2 N H_2SO_4 and 0.7 wt.% citric acid (both at 1:6 w/v) following the same procedure as the NaOH wash. After all washings, the skin was extracted overnight with distilled water at 45 °C without any stirring. The sample was subsequently filtered in a Buchner funnel using Whatman no. 4 filter paper. The clear filtrate was then dried in an oven for 16 h at 45 °C.

2.2. Amino acid analysis

Amino acid analysis was performed at the University of California—Davis Molecular Structure Facility. Alaska pollock, Alaska pink salmon, and porcine (Kraft 250A) gelatins were hydrolyzed in 6 N HCl/0.1% phenol solution for 24 h at 110 °C. The sample in aminoethyl cysteinyl dilution buffer was then analyzed using a Beckman 6300 amino acid analyzer.

2.3. Gel electrophoresis

SDS-PAGE was performed according to the package insert from Invitrogen Life Technologies, using 3−8% NuPAGE® Tris-Acetate gel with Tris-Acetate SDS running buffer. Samples at 67 mg/mL were dissolved in deionized water at 40 °C for 30 min and centrifuged for 5 min at 14,000 rpm in an Eppendorf 5415 centrifuge. The supernatant was used for the SDS-PAGE after it was diluted with deionized water to 400 μg/mL. Samples were denatured and reduced according to Invitrogen, using NuPAGE® LDS Sample Buffer and dithiothreitol by heating in a 70 °C water bath for 10 min. After this, 2 μg of gelatin was loaded in each lane. The reference marker was HiMark™ Unstained Protein Standard (Invitrogen). Electrophoresis was performed with an XCell SureLock electrophoresis unit (Invitrogen) at 200 V. The gel was stained with a ready-to-use Coomassie® G-250 stain from Invitrogen and protein bands were analyzed using ChemiImager™ 440 software.

2.4. Gelatin sample preparation

The gelatin solutions were prepared by first adding gelatin to deionized water or phosphate buffer solution. Different phosphate buffer concentrations were used to vary sample pH. The sample was placed in a 40 °C water bath and mixed with a stir bar for 30 min. The solution was then centrifuged for 5 min at 14,000 rpm using an Eppendorf 5415 centrifuge. After this point, sample preparation became different depending on whether genipin (Wako) or glutaraldehyde (Sigma−Aldrich) cross-linker was added to the sample. For the genipin samples, the supernatant from the centrifugation step was reheated by placing it back into the 40 °C water bath for 10 min. Genipin was added to the solution and the sample was mixed with a stir bar for an additional 20 min at 40 °C. The genipin concentrations in the gelatin samples were set at 0.5, 1, and 1.5% (w/w). For the glutaraldehyde samples, glutaraldehyde was added to the supernatant from the centrifugation step and mixed with a stir bar for 5 min at room temperature (23 °C). The amount of glutaraldehyde added to a gelatin sample was calculated so that equal moles of cross-linker reactive groups were maintained in both the glutaraldehyde and genipin

samples. Since both genipin and glutaraldehyde are difunctional cross-linkers, gelatin samples containing 0.5, 1, and 1.5% (w/w) genipin had the same moles of cross-linker reactive groups as samples containing 0.22, 0.44, and 0.66% (w/w) glutaraldehyde, respectively. The chemical structures of genipin and glutaraldehyde are shown in Fig. 1.

The pH of each gelatin sample was determined from the supernatant collected after the centrifugation step. All samples contained 5% (w/w) gelatin and the pH was measured with a Mettler Toledo MP 220 pH meter. Pollock gelatin was dissolved in deionized water, 0.01 M phosphate buffer solution, and 0.025 M phosphate buffer solution to produce sample pH's of 2.9, 3.4, and 3.7, respectively. Meanwhile, salmon gelatin was dissolved in deionized water, 0.025 M phosphate buffer solution, and 0.05 M phosphate buffer solution to produce sample pH's of 3.2, 3.8, and 4.2, respectively. The phosphate buffer solutions were made by using requisite concentrations of KH_2PO_4 (Sigma–Aldrich) and Na_2HPO_4 (Sigma–Aldrich).

2.5. Rheology

A TA Instruments AR2000 was used to characterize the rheological behavior of the gelatin samples. The sample was placed on a Peltier plate and a 60 mm stainless steel parallel plate was lowered onto the sample. The sample thickness was held constant at 1 mm. To limit evaporation, deionized water was placed in the solvent trap on the upper parallel plate. In addition, a thin layer of silicone oil (for melting and boiling point apparatus, Sigma–Aldrich) was spread over the outer edge of the sample. Dynamic rheological tests were used to characterize the gelation, melting, and cross-linking behavior of the gelatin samples. The two main parameters determined in a dynamic rheological test are the elastic modulus, G', and the viscous modulus, G''. The elastic modulus is a measure of the solid-like response of the material, whereas the viscous modulus is a measure of the liquid-like response of the material. All dynamic measurements were obtained at a frequency of 1 rad/s and a strain of 2%. In addition, all experiments were performed within the linear viscoelastic region.

A dynamic rheological test with a temperature ramp was used to determine the gelation and melting temperatures of the gelatin samples. For gelation, the sample was initially maintained at a temperature of 40 °C for 10 min to allow for equilibration. The temperature was then slowly decreased at a rate of 0.5 °C/min to 3 °C. The gelation temperature was taken to be the temperature at which the elastic modulus began to dramatically increase in value. The test for determining melting temperature immediately followed the gelation test. After the sample reached 3 °C, the temperature was raised back to 40 °C at a rate of 0.5 °C/min. Melting occurred when the elastic modulus began to decrease in value.

A dynamic rheological test at constant temperature (25 or 40 °C) was used to characterize the cross-linking behavior of the gelatin. The sample was sandwiched between the parallel plates at the cross-linking temperature and allowed to equilibrate for 10 min. After equilibration, the evolving elastic and viscous moduli during cross-linking was monitored for 6 h.

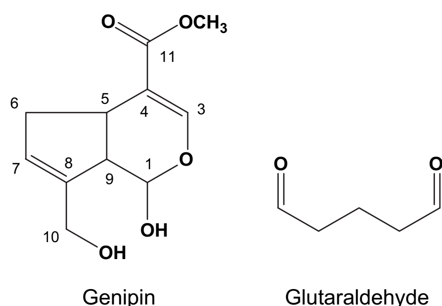

Fig. 1. Chemical structure of genipin and glutaraldehyde.

2.6. Gel strength

An Instron 5500R universal testing machine was used to measure the gel strength of the cross-linked gelatin samples. Gel strength was determined as the maximum load measured after a cylindrical plunger was plunged 4 mm into the sample. The plunger was flat-faced and had a diameter of 13 mm. Load cells of 10 and 100 N were used and the plunge rate was 4 mm/min. All samples contained 5% (w/w) gelatin and had been cross-linked for five days at room temperature (23 °C). The five-day period was chosen because the pollock samples containing glutaraldehyde had gel strength values that began to level off at this point.

3. Results and discussion

3.1. Molecular weight profile of gelatins

Pollock and salmon gelatins had slightly different molecular weight profiles compared to porcine gelatin. This is shown in Fig. 2, where we present the molecular weight profiles of the different gelatins determined from gel electrophoresis. All three gelatins contained α_1, α_2, and β-chains, the main components of gelatin produced from denatured collagen. β-Chains are comprised of two α chains. From Fig. 2, the fish gelatins had slightly lower molecular weight chains. In addition, the fish gelatins contained low molecular weight species that were not present in the porcine gelatin. This was especially evident in the salmon gelatin sample.

3.2. Gelation and melting temperatures

We first examined the gelation of pollock, salmon, and porcine gelatins as we cooled the samples from 40 to 3 °C. Porcine gelatin had a much higher gelation temperature than pollock and salmon gelatin. This is shown in Fig. 3, where we plot the elastic modulus of the gelatin samples as a function of temperature. Porcine gelatin exhibited a large increase in modulus at approximately 24 °C, whereas pollock and salmon gelatins showed large increases in modulus at 7 and 5 °C, respectively. This large modulus increase resulted from changes in the gelatin molecule's conformation during the cooling process. A previous study [22] had shown that the increase

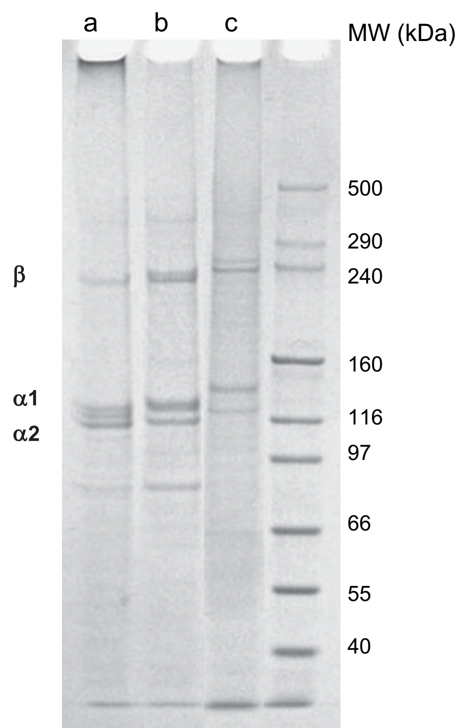

Fig. 2. Gel electrophoresis results for (a) pollock, (b) salmon, and (c) porcine gelatin samples.

in elastic modulus of fish gelatins during gelation corresponded with an increase in the concentration of triple helical structures. The triple helix structure is the basic unit of collagen, from which gelatin is derived. Thus, gelatin molecules partly revert back to the collagen structure during gelation. In addition, previous studies [22–24] had shown that gelatin samples with larger elastic modulus values contained higher concentrations of helical structures. From Fig. 3, porcine gelatin had the largest elastic modulus after gelation, suggesting that porcine gelatin had a higher concentration of helical structures than pollock and salmon gelatins. We should note that the gelatin samples had not reached equilibrium and their modulus values depended on their thermal history.

After cooling the gelatin samples down to 3 °C, we incrementally raised the temperature back to 40 °C. Porcine gelatin melted at a higher temperature than pollock and salmon gelatins. The melting behavior of the gelatins is shown in Fig. 4, where we plot the elastic modulus of the gelatins as a function of temperature. Each gelatin sample had an elastic modulus that decreased to a low value at a specific temperature, after which the modulus remained relatively constant. The porcine gelatin reached this temperature at approximately 32 °C, whereas the pollock and salmon gelatins reached this temperature at approximately 14 and 12 °C, respectively. The decrease in elastic modulus resulted from a loss of network structure. Previous studies [22,24,25] showed that the concentration of helical structures decreased in value during melting. Each gelatin sample eventually transformed into a viscous liquid at higher temperatures, with the sample having a viscous modulus (G″) greater than its elastic modulus (G′) (data not shown).

The difference in thermostability between porcine, pollock, and salmon gelatins can be attributed to each gelatin having different proline and hydroxyproline concentrations. Results from amino acid analysis of the different gelatins are shown in Table 1. Porcine gelatin contains 22.9 mol% proline and hydroxyproline, whereas pollock and salmon gelatin contain 15.4 and 16.4 mol%, respectively. A higher proline and hydroxyproline concentration had been shown to result in higher gelation and melting temperatures [22,26]. This is consistent with the results shown in Figs. 3 and 4.

3.3. Gelatin cross-linking

We initially examined the effects of genipin concentration on the cross-linking behavior of pollock gelatin. An increase in genipin concentration resulted in large increases in cross-linking rate. This is shown in Fig. 5, where we plot the elastic modulus of the pollock samples with different genipin

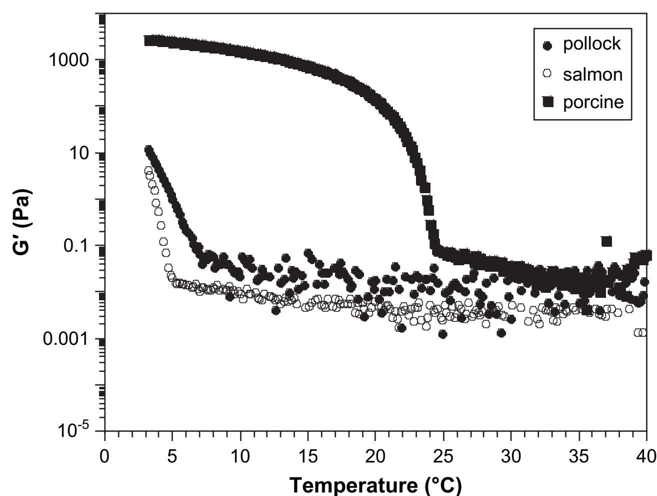

Fig. 3. Elastic modulus of 5% (w/w) pollock, salmon, and porcine gelatin samples during gelation. The temperature was decreased at 0.5 °C/min and the frequency of oscillation was 1 rad/s.

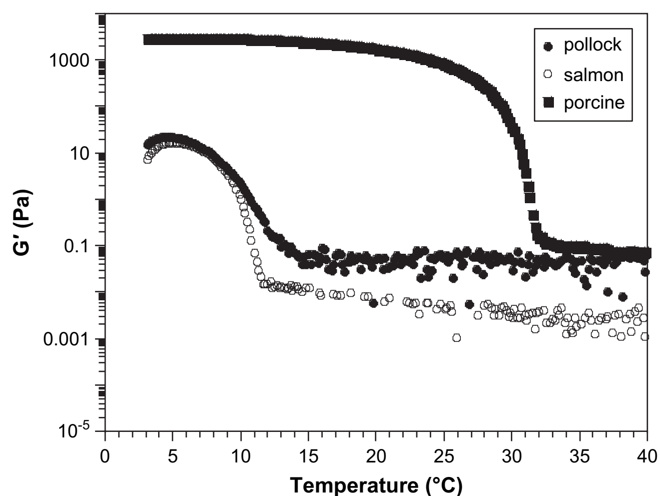

Fig. 4. Elastic modulus of 5% (w/w) pollock, salmon, and porcine gelatin samples during melting. The temperature was increased at 0.5 °C/min and the frequency of oscillation was 1 rad/s.

Table 1
Amino acid composition in gelatin (mol%)

Amino acid	Alaska pollock	Alaska pink salmon	Porcine
Aspartic acid	5.21	5.12	4.66
Hydroxyproline	5.30	5.56	9.78
Threonine	2.68	2.55	1.87
Serine	5.85	4.73	3.39
Glutamic acid	7.17	7.25	7.44
Proline	10.1	10.8	13.1
Gylcine	35.7	35.5	31.7
Alanine	10.9	12.5	11.1
Valine	1.67	1.41	2.18
Cystine	0.14	0.08	0.09
Methionine	1.13	1.00	0.29
Isoleucine	1.07	0.97	1.05
Leucine	2.10	1.83	2.50
Tyrosine	0.24	0.13	0.37
Phenylalanine	1.20	1.27	1.37
Homocystine	0.16	0.12	0.03
Hydroxylysine	0.61	0.76	0.70
Lysine	2.78	2.47	2.80
Histidine	0.80	0.87	0.50
Arginine	5.18	5.06	5.08

concentrations as a function of time. We cross-linked pollock gelatin at 40 °C because the pollock samples did not show any increase in elastic modulus after 6 h at 25 °C (data not shown). From Fig. 5, the pollock sample containing 0.5% (w/w) genipin did not show an increase in elastic modulus until after a few hours. Meanwhile, samples containing higher genipin concentrations showed a rapid rise in modulus within the first hour. After 6 h, the sample containing 1.5% (w/w) genipin had a modulus value more than one order of magnitude greater than that of the sample containing 0.5% (w/w) genipin.

We then held the genipin concentration constant at 0.5% (w/w) and increased the pollock gelatin concentration. The samples with higher gelatin concentrations exhibited faster cross-linking rates. The results are shown in Fig. 6, where we plot the elastic modulus of the pollock samples with various gelatin concentrations as a function of time. There are more amine groups at higher gelatin concentrations, leading to faster cross-linking and a more rapid increase in modulus.

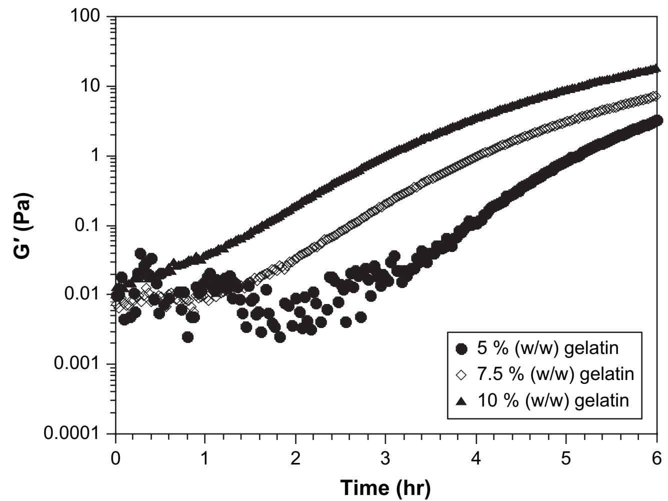

Fig. 6. Elastic modulus of pollock samples containing 0.5% (w/w) genipin with various gelatin concentrations as a function of time. The temperature was 40 °C and the frequency of oscillation was 1 rad/s.

We next examined the effects of solution pH on the cross-linking rate of pollock gelatin. We again kept the genipin concentration constant at 0.5% (w/w) and increased the sample pH. An increase in pH dramatically increased the pollock gelatin cross-linking rate, as shown in Fig. 7. The pollock sample with a pH of 2.9 did not have an appreciable elastic modulus until a few hours into the reaction. However, the samples with higher pH showed a rise in modulus after only 1 h.

Salmon gelatin samples with higher pH also cross-linked faster than samples with lower pH. In this case, salmon samples exhibited a greater dependence on pH than pollock samples. This is shown in Fig. 8, where we plot the elastic modulus of the salmon samples with different pH's as a function of time. We cross-linked the salmon samples at 40 °C and each sample

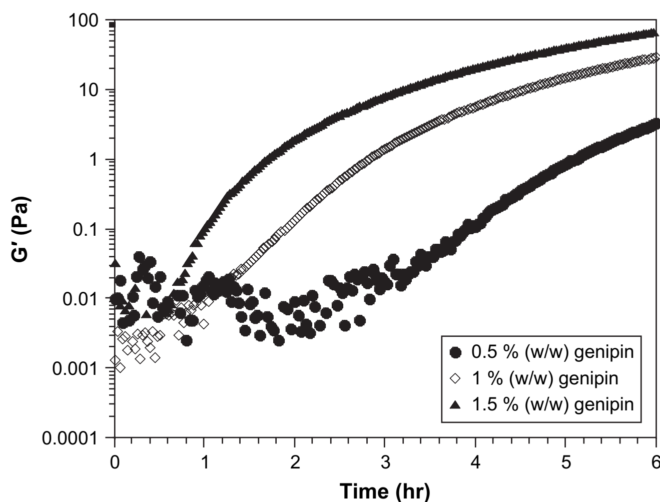

Fig. 5. Elastic modulus of 5% (w/w) pollock gelatin samples with various genipin concentrations as a function of time. The temperature was 40 °C and the frequency of oscillation was 1 rad/s.

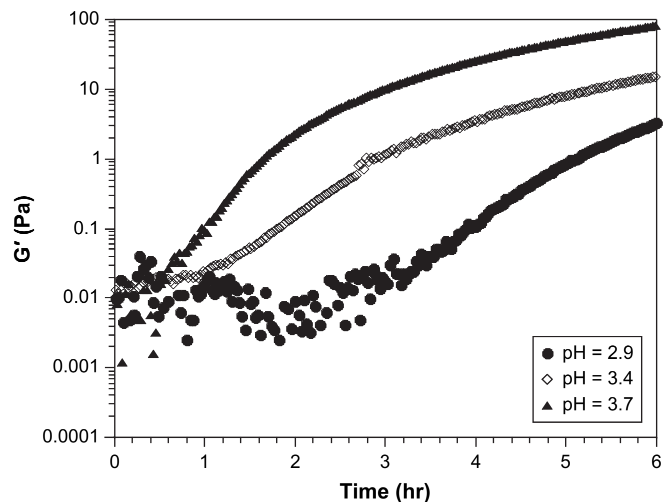

Fig. 7. Elastic modulus of 5% (w/w) pollock gelatin samples containing 0.5% (w/w) genipin with different pH's as a function of time. The temperature was 40 °C and the frequency of oscillation was 1 rad/s.

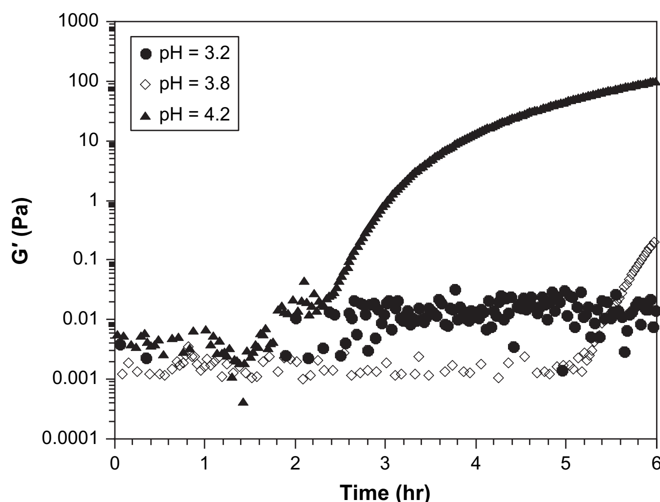

Fig. 8. Elastic modulus of 5% (w/w) salmon gelatin samples containing 0.5% (w/w) genipin with different pH's as a function of time. The temperature was 40 °C and the frequency of oscillation was 1 rad/s.

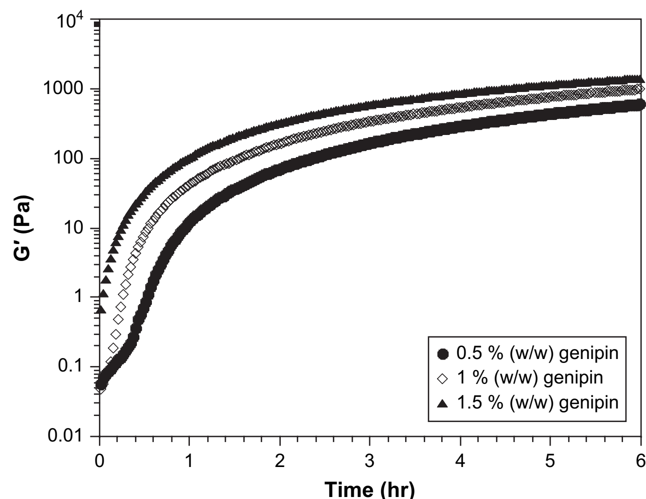

Fig. 9. Elastic modulus of 5% (w/w) porcine gelatin samples with various genipin concentrations as a function of time. The temperature was 40 °C and the frequency of oscillation was 1 rad/s.

contained 0.5% (w/w) genipin. From Fig. 8, salmon gelatin dissolved in deionized water (pH = 3.2) did not have an appreciable elastic modulus throughout the entire experiment. However, the sample with a pH of 4.2 exhibited large increases in elastic modulus. As a comparison, pollock samples with an approximately one unit difference in pH showed much less variation in cross-linking behavior.

The effects of pH on gelatin cross-linking can be explained by the protonation of free amine groups in gelatin. At a higher pH, fewer amine groups become protonated, resulting in more free amines. In the genipin samples, genipin reacts with any free amine group [27]. Since a higher pH sample has a greater concentration of free amine groups than a lower pH sample, the reaction proceeds at a faster rate in the higher pH sample. A faster cross-linking reaction leads to a greater increase in elastic modulus, consistent with the results shown in Figs. 7 and 8.

To compare fish gelatin cross-linking to porcine gelatin cross-linking, we added genipin to the porcine samples. The porcine samples displayed immediate increase in modulus values from the start of the experiment, unlike salmon and pollock samples, which exhibited some lag time. The cross-linking curves for the porcine samples are shown in Fig. 9, where we plot the elastic modulus of porcine samples with various genipin concentrations as a function of time. We cross-linked the porcine samples at 40 °C. From Fig. 9, the porcine samples had much larger elastic modulus values after 6 h than the pollock and salmon samples that were dissolved in deionized water (see Figs. 5 and 8). Also, the porcine samples did not show as large a dependence on genipin concentration as the pollock and salmon samples.

After examining genipin as a cross-linker for gelatin, we next used glutaraldehyde and compared its cross-linking effectiveness to genipin. We added glutaraldehyde to the pollock samples and first characterized the reaction at 25 °C. Pollock samples containing glutaraldehyde showed much faster cross-linking rates than pollock samples containing genipin. The glutaraldehyde cross-linking results are shown in Fig. 10, where we plot the elastic modulus of pollock samples containing different glutaraldehyde concentrations as a function of time. We had previously determined that pollock samples containing genipin did not show an increase in elastic modulus during 6 h of cross-linking at 25 °C. Even when we increased the temperature to 40 °C (see Fig. 5), the genipin samples cross-linked at a slower rate than the glutaraldehyde samples. These results indicated that glutaraldehyde could effectively cross-link pollock gelatin much faster than genipin at room temperature. Previous studies involving porcine gelatin [3,4] and chitosan [28] had also shown that glutaraldehyde cross-linked each sample faster than genipin.

When we increased the temperature from 25 to 40 °C, the pollock samples containing glutaraldehyde exhibited even faster cross-linking rates. This is shown in Fig. 11, where we plot the elastic modulus of pollock gelatin with various glutaraldehyde concentrations as a function of time. The cross-linking

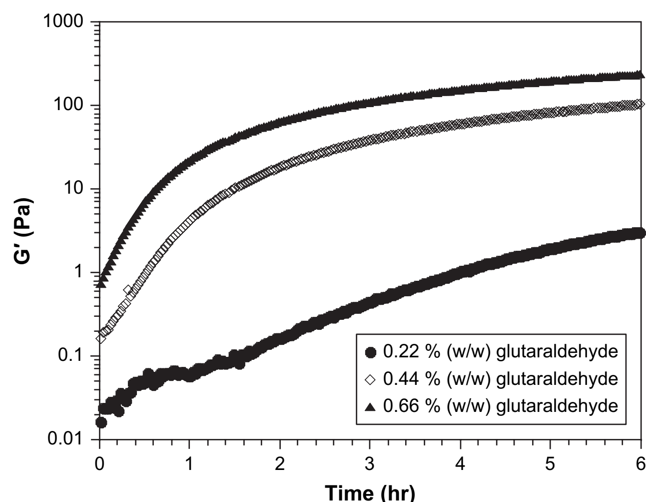

Fig. 10. Elastic modulus of 5% (w/w) pollock gelatin samples with various glutaraldehyde concentrations as a function of time. The temperature was 25 °C and the frequency of oscillation was 1 rad/s.

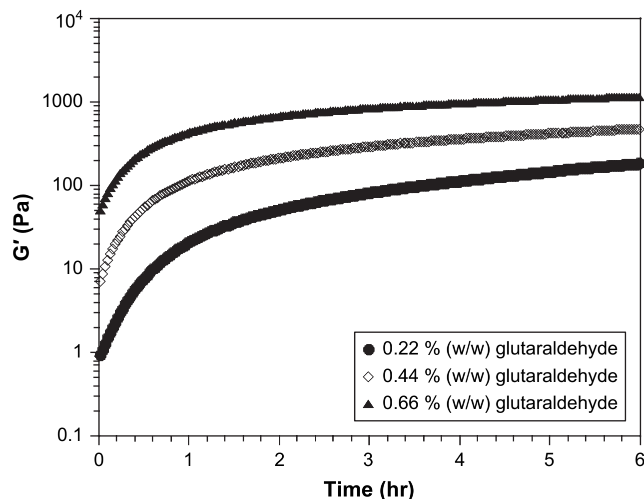

Fig. 11. Elastic modulus of 5% (w/w) pollock gelatin samples with various glutaraldehyde concentrations as a function of time. The temperature was 40 °C and the frequency of oscillation was 1 rad/s.

rates were so fast that the samples had already developed a sizeable modulus even before the start of the measurements. Also, the modulus curves had already begun to level off within 2 h of reaction time.

Genipin and glutaraldehyde react with gelatin via different reaction mechanisms, which may in part explain differences in the cross-linking rates. Genipin reacts with amino acids in gelatin that contain amine side groups, such as lysine and arginine [27]. This reaction involves two different mechanisms [10,28,29]. One mechanism is a nucleophilic attack by an amine group (on C3, see Fig. 1) that eventually leads to the formation of a heterocyclic amine. The second mechanism involves a nucleophilic substitution reaction that results in the replacement of the ester group on genipin with a secondary amide linkage. This second reaction is thought to be slower than the first one [10]. Meanwhile, glutaraldehyde reacts with amine groups in gelatin to produce imine linkages. Mi et al. [28] also postulated that the slower genipin reaction, when compared to glutaraldehyde, could be due to the greater steric hindrance of the cross-linking sites located on genipin.

Pollock gelatin showed superior cross-linking to salmon gelatin when both were dissolved in deionized water. Consequently, we focused on measuring gel strengths of the cross-linked pollock samples. We also determined gel strengths of porcine samples as a comparison. After five days of cross-linking at room temperature, all porcine samples had much greater gel strengths than pollock samples. This is shown in Fig. 12, where we plot gel strength of the cross-linked samples as a function of cross-linker concentration. Also, porcine samples containing genipin had gel strengths several times larger than those containing glutaraldehyde. In contrast, pollock samples showed the opposite behavior, with glutaraldehyde samples having greater gel strengths than genipin samples. We could explain this by noting that the pollock samples were not fully cross-linked, since their gel strengths still increased in value even after two weeks of cross-linking (data not shown). In fact, pollock samples containing genipin eventually had greater

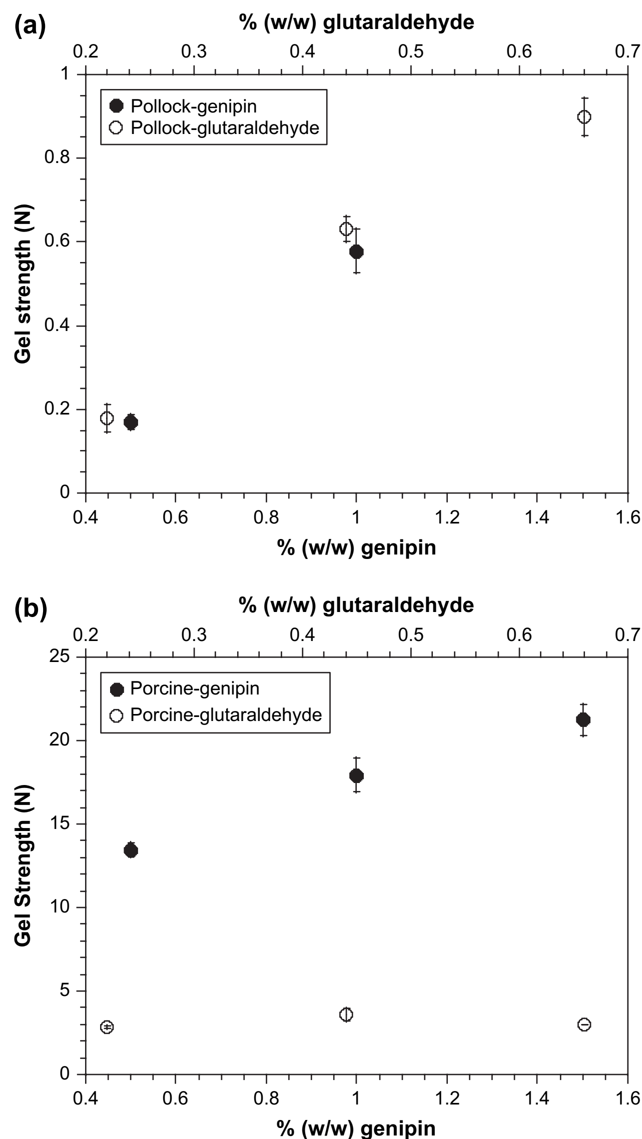

Fig. 12. Gel strengths of (a) 5% (w/w) pollock and (b) 5% (w/w) porcine gelatin samples cross-linked with genipin and glutaraldehyde. The samples were cross-linked for five days at room temperature (23 °C). Results for the pollock sample containing 1.5% (w/w) genipin were not shown because some genipin had precipitated out of solution during the cross-linking process.

gel strengths than those containing glutaraldehyde, much like the porcine samples. We decided to limit cross-linking to five days because the glutaraldehyde samples had gel strengths that began to level off at this point. The difference in gel strengths of genipin and glutaraldehyde samples might be explained by the stability of the different cross-links. A previous study [30] had shown that porcine pericardia tissue cross-linked with genipin produced a more stable structure than that cross-linked with glutaraldehyde. The authors postulated that the lower stability of the glutaraldehyde sample might be due to the reversibility of the glutaraldehyde reaction. This might explain the gel strength data, since a reversible glutaraldehyde reaction results in a lower number of intermolecular cross-links. This leads to a lower cross-link density and lower gel strength.

4. Conclusions

We compared the gelation and melting behavior of gelatin extracted from the skins of Alaska pollock and Alaska pink salmon to that of a commercial porcine gelatin. Both pollock and salmon gelatins gelled and melted at lower temperatures than porcine gelatin. This was consistent with the lower proline and hydroxyproline concentrations found in the fish gelatins.

We then cross-linked the gelatins using genipin and glutaraldehyde. For samples containing genipin, both pollock and salmon gelatins displayed faster cross-linking rates at higher pH. However, salmon gelatin samples showed greater dependence on pH. We found that pollock samples containing glutaraldehyde cross-linked much faster than those containing genipin. We also measured gel strengths of pollock and porcine samples after five days of cross-linking with genipin and glutaraldehyde. All porcine samples had much greater gel strengths than pollock samples. In addition, porcine samples containing genipin had gel strengths several times greater than those containing glutaraldehyde.

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