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Chemical Modification of Gelatin by a Natural Phenolic Cross-linker, Tannic Acid

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Chemical modification of gelatin by a natural phenolic compound tannic acid (TA) at pH 8 was studied, and the properties of the modified gelatin materials were examined. The cross-linking effect was predominant when the TA content was lower, resulting in the formation of a partially insoluble cross-link network. The cross-linking structure was stable even under boiling, and the protein matrix became rigid, whereas the mechanical properties were enhanced. An effective cross-linking effect on gelatin matrix was achieved when the amount of TA was around 3 wt %. Further increase in the TA content enhanced the grafting and branching reactions between gelatin and TA in conjunction with the hydrogen bonding between gelatin and TA molecules. These effects produced an increase in molecular mobility of gelatin matrix, and the materials displayed a behavior similar to that of plasticized protein materials.

KEYWORDS: Gelatin; tannic acid; natural phenolic cross-linking; mechanical properties

INTRODUCTION

Protein-based natural polymer materials have a long history in food packaging and coating formulations. Gelatin, a water-soluble protein produced by the hydrolysis of animal collagen, has been widely used as a biomaterial in pharmaceutical, food, and medical applications because of a range of favorable properties including barrier to gases such as oxygen and carbon dioxide (*1*−*7*). The protein can be obtained/derived from inexpensive sources of waste or byproduct from manufacturing processes including tanning, pharmaceutical, and food production and can be regarded as a renewable and biodegradable material (*8*). However, gelatin exhibits poor mechanical properties, especially when exposed to wet and/or humid conditions, and this high water sensitivity compromises its broader application. To enhance its mechanical strength and water resistance in natural protein-based materials, structure modifications are normally required. Chemical cross-linking of proteins is an effective way in which to introduce stable covalent bonds between protein segments that consequently improve both the mechanical and water resistance properties of the protein matrix (*9*). For gelatin, an aldehyde cross-linker, such as glutaraldehyde, has been widely used because of its high cross-linking efficiency (*10*, *11*). However, during biodegradation glutaraldehyde manifests toxicity (*12*), and thus alternative cross-linkers such as carbodiimides, epoxy, and genipin have been studied (*13*−*15*).

The development of additives derived from natural products for protein-based materials has attracted a great deal of scientific and industrial interest. Natural phenolic compounds (*16*) as antioxidant reagents have been widely used as modifiers in food processing and leather fabrication. In polymer resin-based bonders or coatings, tannin is often used as a natural additive to replace phenolic compounds. Several natural phenolic compounds derived from plants have been reported to be interactive or reactive with proteins with resultant improvements in gel or film properties of gelatin-based materials (*17*−*23*). In this paper, we report on our recent work in which we have used tannic acid (TA) as a natural phenolic cross-linker to modify gelatin and improve its mechanical performance. The chemical structure of TA has been described in the literature (*24*−*26*). It is a hydrolyzable tannin with multiple phenolic functionalities due to its high molecular weight. Possible chemical reaction pathways between gelatin and natural phenolic compounds have been postulated (*22*). The amino functional groups of amino acid units in gelatin (such as lysine, arginine, and histidine) would react with phenolic reactive sites of TA under alkaline conditions to form covalent C−N bonds and generate cross-linked networks (*27*). Other reactive groups in gelatin (e.g., the hydroxyl group in serine units) may also promote such reactions via a similar mechanism. On the other hand, the phenolic hydroxyl and carboxyl groups of TA could associate with gelatin chains via hydrogen bonding. The extent of these reactions and interactions in gelatin/TA systems plays an important role in determining consequent material properties.

To investigate this, changes in molecular weight of the gelatin after reaction with a small amount of TA were examined while the materials were still soluble. High-resolution solid state NMR was used to examine the chemical and phase structures and the

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correlation of these structures with the swelling properties and mechanical behaviors of the modified gelatin materials. The hydrophilic−hydrophobic properties of the material surface were also studied. Results were compared with gelatin systems cross-linked with an aldehyde cross-linker (glyoxal).

MATERIALS AND METHODS

Samples and Preparation Conditions. All raw materials used in this study, including gelatin (type B obtained from bovine skin), tannic acid (TA), and glyoxal (Gx, 40%) were obtained from Sigma-Aldrich without further purification or treatment.

Gelatin and TA were dissolved in distilled water (10 wt %) separately, and the pH of each solution was adjusted to 8.0 by NaOH (46%) solution. Gelatin solution was heated to 45 °C to ensure complete dissolution. The TA solution was then slowly added to the gelatin solution at 58 ± 1 °C with stirring at predetermined TA/gelatin ratios and allowed to react for predetermined periods of time. During reaction, the pH of these solutions was monitored using a pH-meter and held constant at a pH of 8.0 by the dropwise addition of NaOH solution (1 M). Oxygen was bubbled into the systems throughout the reaction. For the systems with a Gx additive, a Gx solution (40%) was added to the gelatin solution at predetermined Gx/gelatin ratios but without oxygen bubbling.

Gelatin films (thickness of around 0.3 mm) were prepared via solution casting at room temperature and dried for 2 weeks after the reaction. Compression-molded samples were obtained by hot pressing the cast samples (at 120 °C for 5 min under a pressure of 12 ton). All samples were conditioned at a relative humidity of 52% at 22 °C for 7 days before testing and characterization. The moisture content for all samples after conditioning was determined by weight loss after drying at 105 °C for 6 h, and the value was between 17 and 19%.

Characterization. The molecular weights of gelatin were determined by gel permeation chromatography (GPC) using a triple detection system that included a Wyatt Dawn F laser photometer operating at 90° (right angle laser light scattering, RALLS) coupled with an online Waters 410 differential refractometer (for measurement of refractive index, RI) and Viscotek T50A differential viscometer. The three detectors were calibrated with pullulan standards of known molecular weight, intrinsic viscosity, and narrow polydispersity. A Waters Ultrahydrogel column was used. GPC experiments were conducted with 0.1 M potassium sulfate and 5 mM sodium azide solution as the mobile phase under a flow rate of 1.0 mL/min and column temperature of 45 °C.

The equilibrium water uptake (wt %) of the gelatin films was determined by soaking film samples in distilled water (1 g of sample in 20 g of water) for 24 h at room temperature, removing the samples from the soak, and carefully removing any surface absorbed water on the swollen samples (by tissue paper) and measuring the weight difference between the swollen and dry samples. To determine film solubility, soaked (swollen) samples in water (1 g of film in 20 g of water) were mixed with 30 g of boiling distilled water and boiled for 5 min. The insoluble component (obtained after filtering or centrifuging) was dried at 105 °C for 6 h and the solubility calculated from the difference of the dry weight before swelling and that after boiling.

Tensile properties were determined at room temperature on an Instron 5566P with a crosshead speed of 50 mm/min. To minimize variation in sample moisture content arising from environmental factors between conditioning and testing, samples were sealed in PE bags immediately after conditioning and tested directly. The data for each sample were an average of the testing of 7−10 dog-bone cut specimens (effective length 30 mm, width of 6 mm).

Dynamic mechanical analysis (DMA) was carried out on a PerkinElmer PYRIS Diamond DMA in dual cantilever bending mode at a frequency of 1 Hz. The temperature range was set from −100 to 120 °C with a heating rate of 2 °C/min. The storage modulus (E'), the loss modulus (E''), and tan δ (E''/E') were recorded as a function of temperature throughout the experiment.

To determine film hydrophicity, the surface water contact angle was determined using a NRL C.A. goniometer at room temperature. Distilled water was carefully dropped onto the surface of film samples, and the contact angle between the sample surface in contact with the water drop and the tangent of the water drop was measured as a function of time during a period of 5 min. The average contact angle values were obtained from measuring three to five specimens of each sample. A high contact angle indicates hydrophobicity, whereas a low angle means hydrophilicity.

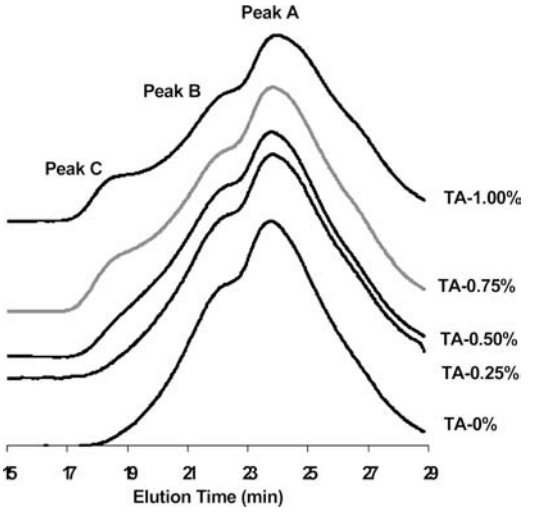

Figure 1. GPC results of gelatin after reaction with tannic acid at pH 8 and 58 °C for 3 h. Peaks A, B, and C represent the components corresponding to low, medium, and high molecular weights, respectively.

NMR experiments were conducted at room temperature using a Varian Unity plus spectrometer at resonance frequencies of 75 MHz for ^{13}C and 300 MHz for ^{1}H. High-resolution solid-state ^{13}C NMR spectra were measured under cross-polarization, magic angle spinning, and high-power dipolar decoupling (CP/MAS/DD) conditions. The 90° pulse was 6.0 μs for H-1 and C-13, whereas the spinning rate of MAS was set at a value in the range of 6−7 kHz. A contact time of 1.0 ms was used for measuring CP/MAS spectra, whereas the repetition time was 2 s for all experiments. The chemical shift of ^{13}C CP/MAS spectra was determined by taking the carbonyl carbon of solid glycine (176.3 ppm) as an external reference standard. The proton spin−lattice relaxation times in the rotating frame (^{1}H $T_{1\rho}$) were measured through the decay of ^{13}C magnetization prepared by CP after varied ^{1}H spin-locking time as reported previously (*28*, *29*).

RESULTS AND DISCUSSION

When a small amount of TA (< 1 wt %) was included in gelatin systems (after reaction at 58 °C for 3 h and then casting), dissolution rates became slower than those of gelatin alone, but films remained soluble in water when heated to 40−45 °C. **Figure 1** shows GPC curves of gelatin/TA solutions measured at 45 °C after the reaction. Gelatin-only solution (TA-0%, prepared by dissolving gelatin in distilled water at 45 °C without further heating) displayed two peaks (A and B) corresponding to two groups of molecular weights. After reaction with TA, a shift to shorter elution times was observed, suggesting an increase in molecular weight. As the amount of TA increased to > 0.75 wt %, a third peak (peak C) was clearly observed at a shorter elution time than peaks A and B, indicating the formation of a substance with an even higher molecular weight than either of these. The magnitude of peak C increased as the TA content increased from 0.75 to 1 wt %. The best fitting data of the TA−gelatin GPC curves are summarized in **Table 1** together with that of gelatin without TA (TA-0%-H) prepared under the same reaction conditions and heated at 58 °C for 3 h. It is noted that the lowest molecular weight was that of TA-0%-H (molecular weight ∼ 45K), and this was attributed to protein hydrolysis occurring under these reaction conditions. In contrast to this, the molecular weight of samples using 0.25−0.50 wt % of TA increased compared to that of TA-0% and TA-0%-H. Further increasing the TA content (0.75−1.00 wt %) resulted in the formation of the C component (around 8%) with a molecular weight as high as 7−11M. Such a significant

Table 1. Molecular Weight of Gelatin/TA Gels after Reaction at pH 8

	GPC peak A			GPC peak B			GPC peak C		
sample	M_w	PD[a]	%	M_w	PD	%	M_w	PD	%
TA-0%	98.6K	1.3	70.0	790.8K	1.4	30			
TA-0%-H	45.0K	1.5	100.0						
TA-0.25%	116.2K	1.1	69.4	1139K	1.7	30.6			
TA-0.50%	101.6K	1.3	64.0	1772K	2.4	36.0			
TA-0.75%	151.4K	1.2	66.9	937.9K	1.3	24.6	7628K	1.2	8.5
TA-1.00%	261.5K	1.1	66.3	1719K	1.4	25.6	10660K	1.1	8.1

[a] PD, polydispersity of molecular weight.

increase in molecular weight, particularly when compared to the substantial decrease in molecular weight observed between TA-0% (~99K) and TA-0%-H (~45K), provides direct evidence for the formation of covalent linkages between gelatin molecules through reaction with TA.

Due to the significant degradation of the gelatin-only system under the reaction conditions (TA-0%-H), the TA-0% gelatin sample was taken as the reference sample in this study. As the pH also plays an important role in modification of protein structures and material performance (*30*−*33*), the TA-0% gelatin film was cast after dissolving at 45 °C and adjusting the solution pH to 8 without further heating. To avoid hydrolysis under reaction conditions, film samples with TA > 1 wt % were obtained via reactions for 30 min at the same temperature.

Gelatin films reacted with ≤1 wt % of TA displayed similar swelling properties as gelatin, and all of them dissolved in water when heated above 45 °C. The increase in molecular weight had no significant effect on the water sensitivity of the materials. However, the solubility of gelatin/TA films decreased significantly when the content of TA increased to 3 wt %; the majority (~80%) of gelatin films was insoluble even when boiled in water for 5 min. This indicates a significant number of covalent linkages were formed between gelatin and TA, resulting in a stable cross-linked network. The equilibrium water uptake data (shown in **Figure 2**) also decreased significantly as the TA content was increased to 3 wt %. The water uptake value of the compression-molded films (120 °C for 5 min) was lower than that of cast films, suggesting that thermal cross-linking could also contribute to the observed properties of the protein matrix. However, the water uptake in these films was still substantial (500−700 wt %), indicating the cross-link density was low and materials were still water sensitive. These data are consistent with those reported previously for similar systems (*22*). In contrast, Gx (1−3 wt % to gelatin) cross-linked gelatin absorbed only 100−150 wt % of water (**Figure 2**). It is thought that this difference could be due to much longer cross-link segments formed in gelatin/TA systems than those formed in gelatin/Gx films. Because the gelatin samples with <1 wt % TA displayed minimal differences in mechanical properties as compared to the TA-0% sample, property testing was conducted only on samples with TA content >1 wt %.

The mechanical properties of gelatin/TA films (moisture content of 17−19 wt % after conditioning) including tensile strength, elongation at break, and Young's modulus are listed in **Table 2** together with results for Gx cross-linked gelatin samples. When the amount of TA was below 6 wt %, both tensile strength and modulus marginally increased from baseline levels (from 83 to 89 MPa and from 1789 to 1982 MPa, respectively) and, interestingly, so did elongation at break (from 9.8 to 13.8%). In Gx cross-linked samples, both tensile strength and modulus were significantly increased but elongation at break was reduced more than half, which is typical for cross-linked polymer materials. It would therefore seem that the TA additive plays a dual role as

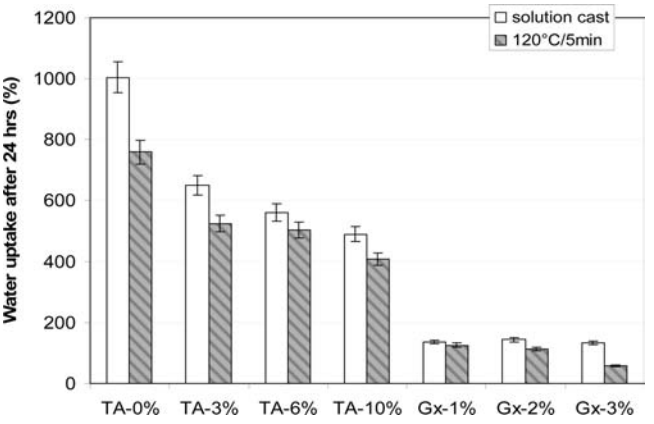

Figure 2. Equilibrium water uptake data of the gelatin films after cross-linking with TA or Gx.

Table 2. Mechanical Properties of Gelatin/TA and Gelatin/Gx Films[a]

sample	moisture (wt %)	tensile strength (MPa)	strain at break (%)	Young's modulus (MPa)
TA-0%	16.4	83	9.8	1789
TA-1%	16.8	84	11.7	1984
TA-3%	17.2	89	10.4	1920
TA-6%	18.6	86	13.8	1982
TA-10%	18.2	75	15.1	1734
Gx-1%	17.9	103	6.0	3464
Gx-3%	18.3	148	3.0	3972

[a] Error of 5−8%.

both a cross-linker (improving tensile strength and modulus) and plasticizer (improving elongation) in a similar fashion to some additives in wheat protein based natural polymer systems modified by polymer grafting and cross-linking (*34*−*36*). When the amount of TA was increased to 10 wt %, tensile strength and modulus were reduced to levels lower than those of the unmodified gelatin (i.e., TA-0%), but elongation continued to increase, indicating that the plasticization effect was predominant, the same as for many other plasticized protein materials (*37*−*40*).

Similar phenomena were also observed in DMA measurements shown in **Figure 3**. The behavior of these gelatin films was similar to that observed in normal plastic films; the E' storage modulus decreased slowly as the temperature increased toward the glass transition temperature (T_g). Significant reduction of E' occurred when T_g started with the simultaneous observation of a tan δ peak corresponding to the T_g. The onset of the E' decrease corresponding to T_g transition was taken as the starting temperature of glass transition, $T_{g\text{-start}}$. For the gelatin films cast after reaction with TA at pH 8 (**Figure 3A**), the addition of a small amount of TA resulted in an increase in E' modulus at room temperature (e.g., at 20 °C, below T_g), but the $T_{g\text{-start}}$ was effectively unchanged at 52−53 °C (as shown in **Table 3**).

As noted, the highest modulus at room temperature (20 °C) was obtained for samples containing 6 wt % of TA with further increases in the amount of TA resulting in a reduction of the modulus. In all samples modified with TA, the tan δ maximum at T_g was higher than that of TA-0%, suggesting an increase in molecular mobility at the T_g transition and consistent with a dual effect of cross-linking and plasticization derived from the TA additive. It is thought that a TA content of 6 wt % might be the optimum proportion showing both effects (highest E' at 20 °C and highest tan δ maximum for T_g). The plasticization effect became more pronounced when the films were cast after neutralization of

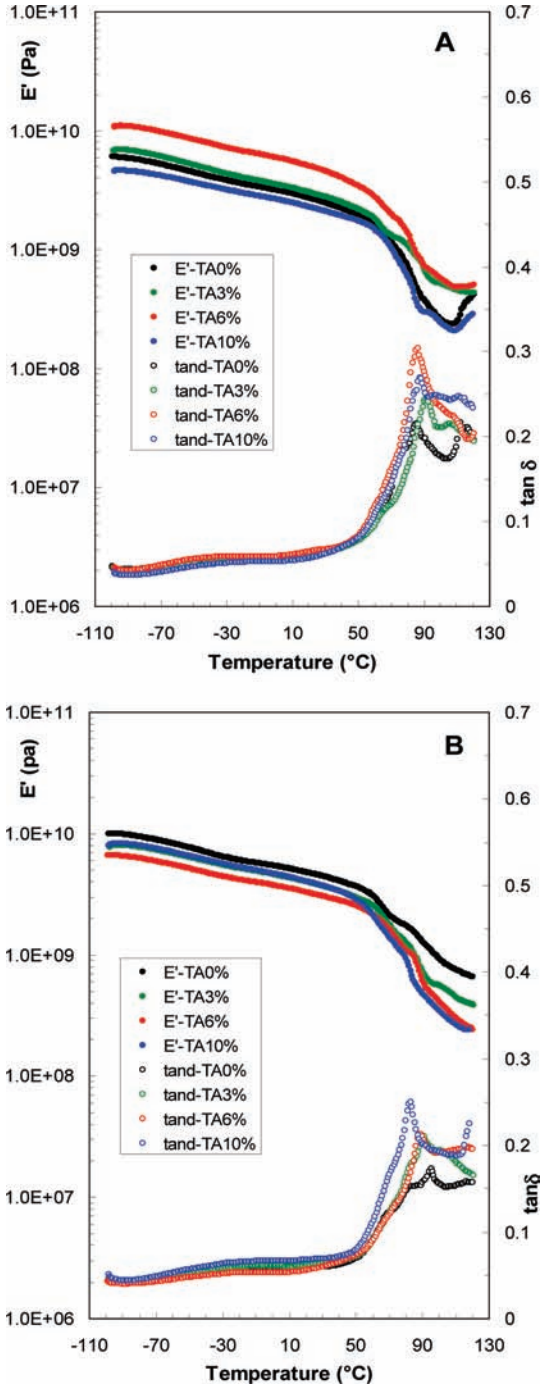

Figure 3. DMA results (E' storage modulus, tan δ) of gelatin films: (**A**) reaction and cast at pH 8; (**B**) reaction at pH 8 but cast at pH 7.

the reaction solution to pH 7 as shown in **Figure 3B**. Here the E' of the TA-0% film (cast after adjusting the pH of the gelatin solution to 8 then neutralizing to 7) was highest at temperatures below T_g with the TA-6% sample displaying the lowest E' in the same temperature range (**Table 3**). When the TA content was increased, T_g shifted to lower temperatures and tan δ maximum related to the T_g transition also increased, indicating that plasticization effects derived from the TA additives were predominant and similar to those observed in other plasticized protein materials (*37*−*40*).

This also suggests that the drying time (2 weeks) under alkaline conditions was critical for forming stable cross-linking gelatin/TA structures. pH played an important role in the modification of protein structures (*30*−*33*), and hydrolysis could happen under an increased pH condition occurring during the drying process.

Table 3. Key Data of DMA Testing of Gelatin/TA Samples

sample		E' at 20 °C (GPa)	$T_{g\text{-start}}$ (°C)	tan δ (°C)	tan δ max
reaction and cast at pH 8	TA-0%	2.72	52.4	85.7	0.216
	TA-3%	3.04	53.5	91.1	0.244
	TA-6%	5.06	52.1	86.4	0.303
	TA-10%	2.28	52.8	87.5	0.269
reaction at pH 8 then cast at pH 7	TA-0%	4.79	60.4	95.2	0.173
	TA-3%	4.00	60.8	90.9	0.210
	TA-6%	3.28	57.8	88.2	0.212
	TA-10%	4.04	54.2	82.9	0.250

Thus, films cast at pH 7 generally displayed a higher E' below T_g in conjunction with a higher T_g value than those cast at pH 8. The formation of different aggregation structures or hydrogen-bonded domains under different pH values could also contribute to the mechanical performance of the gelatin/TA films.

The surface hydrophilic−hydrophobic properties of the gelatin/TA films were examined by water contact angle measurement with data shown in **Figure 4**. For the films cast after reaction at pH 8 (**Figure 4A**), only the TA-1% film displayed higher contact angles than TA-0%, whereas all others showed lower values, indicating that film hydrophobicity was promoted only when a very small amount of TA was used, with higher levels enhancing the hydrophilicity of the film surface. For the Gx cross-linked gelatin samples, the contact angle data were more stable over time, although the initial data were slightly lower than that of TA-0%, suggesting more stable surface properties. The low contact angle data for the samples containing a higher amount of TA are likely due to the hydrophilic nature of the long cross-linked segments derived from TA and a low cross-link density. Compression molding at 120 °C for 5 min significantly enhanced the hydrophobicity of these film surfaces such that all contact angles were increased (**Figure 4B**). In contrast to uncompressed films, both TA-1% and TA-3% displayed higher values than the TA-0% sample, suggesting that thermal cross-linking plays a significant role in enhancing surface hydrophobicity. The potential for changes in surface morphology or polymer chain packing density arising during compression molding to modify hydrophobic/hydrophilic properties has not been examined.

High-resolution solid-state NMR is a powerful technique able to explore the intermolecular interactions, molecular motions, and phase structures of protein materials as demonstrated in our previous publications (*33*−*40*). The ^{13}C CP/MAS NMR spectra of the gelatin/TA samples are shown in **Figure 5**. The phenolic resonances of TA are in the range of 100−160 ppm (shown in **Figure 5** on the right) with hydroxyl-substituted resonances at 140 and 145 ppm, whereas the ortho- and para-substituted carbons appear at 114 and 121 ppm. We note that most TA resonances became broad and the hydroxyl-substituted resonances (~145 ppm) shifted to low field (high ppm direction) in gelatin/TA systems. This reflects strong intermolecular interactions between gelatin and TA components, which is also consistent with the plasticization effect observed in the DMA study.

The cross-linking−plasticization effect was further examined by measuring proton spin−lattice relaxation times in the rotating frame (^1H $T_{1\rho}$) as this parameter is sensitive to the molecular motions of polymer chains in the regions of tens of kilohertz. The data of gelatin/TA samples cast after reaction at pH 8 were observed via ^{13}C resonances and are listed in **Table 4**. Only a single-exponential $T_{1\rho}$ decay was obtained from all ^{13}C resonances in all samples, and the data observed from different resonances in each sample were similar. This indicates strong spin−diffusion interactions among the various possible phases

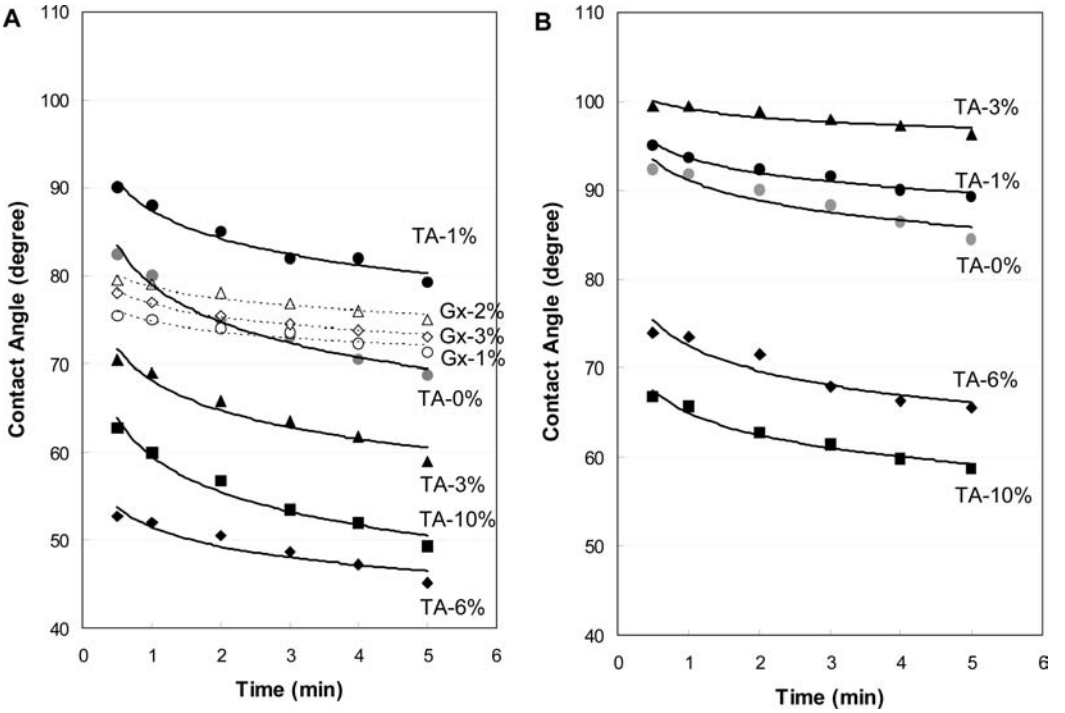

Figure 4. Contact angle data of gelatin/TA and gelatin/Gx films: (**A**) solution cast (**B**) compression molded at 120 °C/5 min after solution casting.

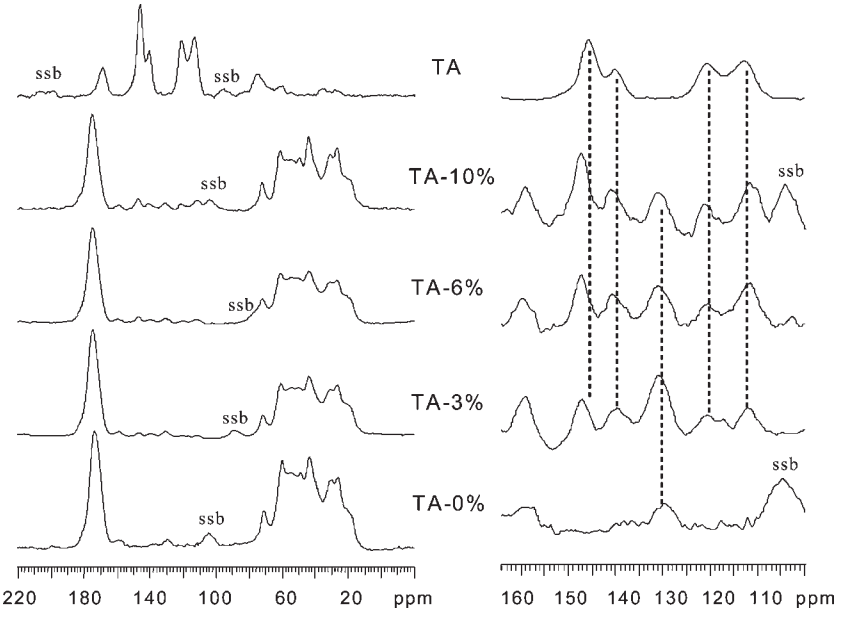

Figure 5. ^{13}C CP/MAS NMR spectra of TA and gelatin/TA films cast after reaction with enlarged spectra at a region of 100−165 ppm shown on the right.

Table 4. ^{1}H $T_{1\rho}$ (Milliseconds) of the Gelatin Component in Gelatin/TA Samples Observed via ^{13}C CP/MAS Spectra

sample	174 ppm	72 ppm	44 ppm	27 ppm
TA-0%	4.3 ± 0.2	4.2 ± 0.2	4.2 ± 0.2	4.1 ± 0.3
TA-1%	4.7 ± 0.1	4.8 ± 0.2	4.8 ± 0.3	4.6 ± 0.2
TA-3%	4.5 ± 0.1	4.5 ± 0.2	4.5 ± 0.2	4.3 ± 0.1
TA-6%	4.7 ± 0.1	4.4 ± 0.3	4.5 ± 0.2	4.4 ± 0.3
TA-10%	3.9 ± 0.1	4.2 ± 0.2	4.0 ± 0.2	4.1 ± 0.2
TA	4.0 ± 0.2 at 145 ppm		4.2 ± 0.4 at 115 ppm	

(such as cross-linked regions, plasticized phases, and others if existing) had averaged out the relaxation processes of all protons within the systems. The materials were homogeneous at the scale of the spin−diffusion path length within the $T_{1\rho}$ time, ca. 2−3 nm.

At room temperature, which is below the T_g of the gelatin/TA materials, the cross-linking effect would result in a rigid polymer network and thus cause the ^{1}H $T_{1\rho}$ data to increase. In contrast, an efficient plasticization effect would mobilize gelatin chains and reduce the $T_{1\rho}$ value. As the ^{1}H $T_{1\rho}$ values of pure TA and gelatin are identical, the spin−diffusion interaction between these two components should not vary the relaxation rates. Introducing a small amount of TA (ca. 1 wt %) into gelatin indeed produced an increase in $T_{1\rho}$ values, but when this was further increased (> 3 wt %), values slightly decreased such that $T_{1\rho}$ values for TA-10% and TA-0% were similar. Such $T_{1\rho}$ variation reflects a shift from a predominantly cross-linking effect (when the TA content was low) to chain mobility enhancement (as TA content was increased). The cross-link density was relatively low in these gelatin/TA cross-link

networks due to either low reactivity or a low concentration of reactive functional groups in gelatin. TA not involved in cross-linking may remain associated with gelatin molecules via hydrogen bonding and act as a plasticizer. At higher levels of TA, grafting or branching reactions could be also promoted, leading to formation of single covalent linkages between TA and gelatin chains, which could block normal triple-helix aggregation of gelatin. This could also contribute to producing a mobile gelatin matrix. The overall effect is a significant mobilization of the protein matrix leading to a lower $T_{1\rho}$ and corresponding changes in mechanical properties of the gelatin materials. However, further research is needed to provide more detailed evidence to clarify the matter.

In summary, this work has demonstrated that cross-linking reactions between gelatin and TA at pH 8 resulted in a significant molecular weight increase initially and then the formation of partially insoluble materials. At a low TA content, the cross-linking effect was predominant and the cross-linked structure was stable even under boiling. Both the rigidity of the protein matrix increased and the mechanical properties of the gelatin/TA films were enhanced. At higher TA content, grafting and branching reactions between gelatin and TA were enhanced as well as the amount of TA molecules not involved in cross-linking. The overall effect produced an enhancement of molecular motions of gelatin matrix that displayed similar behavior to plasticized protein materials. The optimum amount of TA to achieve an effective cross-linking effect on gelatin matrix was approximately 3 wt %.

LITERATURE CITED

(1) Veis, A. *The Macromolecules Chemistry of Gelatin*; Academic Press: New York, 1964.

(2) Hastings, G. W.; Ducheyne, P. *Macromolecular Biomaterials*; CRC Press: Boca Raton, FL, 1984.

(3) Esposito, E.; Cortesi, R.; Nastruzzi, C. Gelatin microspheres: influence of preparation parameters and thermal treatment on chemico-physical and biopharmaceutical properties. *Biomaterials* **1995**, *20*, 2009–2020.

(4) Arvanitoyannis, I.; Psomiadou, E.; Nakayama, A.; Biba, S.; Yamamoto, N. Edible films made from gelatin, soluble starch and polyols. *Food Chem.* **1997**, *60*, 593–604.

(5) Arvanitoyannis, I.; Nakayama, A.; Aiba, S. Edible films made from hydroxypropyl starch and gelatin and plasticized by polyols and water. *Carbohydr. Polym.* **1998**, *36*, 105–119.

(6) Sobral, P. J. A.; Menegalli, F. C.; Hubinger, M. D.; Roques, M. A. Mechanical, water vapor barrier and thermal properties of gelatin based ediable films. *Food Hydrocolloids* **2001**, *15*, 423–432.

(7) Yakimets, I.; Wellner, N.; Smith, A. C.; Wilson, R. H.; Farhat, I.; Mitchell, J. Mechanical properties with respect to water content of gelatin films in glassy state. *Polymer* **2005**, *46*, 12577–12585.

(8) Chiellini, E.; Cinelli, P.; Corti, A.; Kenawy, E. R. Composite films based on waste gelatin: thermal-mechnical properties and biodegradation testing. *Polym. Degrad. Stab.* **2001**, *73*, 549–555.

(9) Gerrard, J. A. Protein−protein crosslinking in food: methods, consequences, applications. *Trends Food Sci. Technol.* **2002**, *13*, 389–397.

(10) Bigi, A.; Cojazzi, G.; Panzavolta, S.; Rubini, K.; Roveri, N. Mechanical and thermal properties of gelatin films at different degrees of glutaraldehyde crosslinking. *Biomaterials* **2001**, *22*, 763–768.

(11) Martucci, J. F.; Ruseckaite, R. A.; Vazquez, A. Creep of glutaraldehyde-crosslinked gelatin films. *Mater. Sci. Eng., A* **2006**, *435*−*436*, 681–686.

(12) Olde, D.; Dijkstra, P. J.; van Luyn, M. J. A.; van Wachem, P. B.; Nieuwenhuis, P.; Feijen, J. Glutaraldehyde as a crosslinking agent for collagen-based biomaterials. *J. Mater. Sci. Mater. Med.* **1995**, *6*, 460–472.

(13) Kuijpers, A. J.; Engbers, G. H. M.; De Smedt, S. C.; Meyvis, T. K. L.; Demeester, J.; Krijgsveld, J.; Zaat, S. A. J.; Dankert, J. Characterization of the network structure of carbodiimide cross-linked gelatin gels. *Macromolecules* **1999**, *32*, 3325–3333.

(14) Sung, H. W.; Huang, D. M.; Chang, W. H.; Huang, R. N.; Hsu, J. C. Evaluation of gelatin hydrogel crosslinked with various crosslinking agents as bioadhesives: in vitro study. *J. Biomed. Mater. Res.* **1999**, *46*, 520–530.

(15) van Wachem, P. B.; Zeeman, R.; Dijkstra, P. J.; Feijen, J.; Hendricks, M.; Cahalan, P. T.; van Luyn, M. J. A. Characterization and biocompatibility of epoxy-crosslinked dermal sheep collagens. *J. Biomed. Mater. Res.* **1999**, *47*, 270–277.

(16) Spanos, G.; Wrolsted, R. E. Phenolics of apple, pear, and white grape juices and their changes with processing and storage − a review. *J. Agric. Food Chem.* **1992**, *40*, 1478–1487.

(17) Matheis, G.; Whitaker, J. R. Modification of proteins by polyphenol oxidase and peroxidase and their products. *J. Food Biochem.* **1984**, *8*, 137–162.

(18) Asquith, T. N.; Butler, L. G. Interactions of condensed tannins with selected proteins. *Phytochemistry* **1986**, *25*, 1591–1593.

(19) Wu, J.; Chiu, S.-C.; Pearce, E. M.; Kwei, T. K. Effect of phenolic compounds on gelatin behavior of gelatin gels. *J. Polym. Sci: Part A: Polym Chem.* **2001**, *39*, 224–231.

(20) Chatterjee, S.; Chowdhury, R.; Bhattacharya, P. A mathematical analysis of the effect of pH and protein concentration on adsorption of proteins on immobilised tannin. *Biochem. Eng. J.* **2000**, *5*, 77–82.

(21) Rawel, H. M.; Kroll, J.; Hohl, U. C. Model studies on reactions of plant phenols with whey proteins. *Nahrung/Food* **2001**, *2*, 72–81.

(22) Strauss, G.; Gibson, S. M. Plant phenolics as cross-linkers of gelatin gels and gelatin-based coacervates for use as food ingredients. *Food Hydrocolloids* **2004**, *18*, 81–89.

(23) Xing, F.; Cheng, G.; Yi, K.; Ma, L. Nanoencapsulation of capsaicin by complex coacervation of gelatin, acacia and tannins. *J. Appl. Polym. Sci.* **2005**, *96*, 2225–2229.

(24) Cao, N.; Fu, Y.; He, J. Mechnical properties of gelatin films crosslinked, respectively, by ferulic acid and tannin acid. *Food Hydrocolloids* **2007**, *21*, 575–584.

(25) Roux, D. G. Recent advances in the chemistry and chemical utilization of the natural condensed tannins. *Phytochemistry* **1972**, *11*, 1219–1230.

(26) Isenburg, J. C.; Karamchandani, N. V.; Simionescu, D. T.; Vyavahare, N. R. Structureal requirements for stabilization of vascular elastin by polyphenolic tannins. *Biomaterials* **2006**, *27*, 3645–3651.

(27) Zhang, X.; Do, M. D.; Casey, P.; Sulistio, A.; Qiao, G. G.; Lundin, L.; Lillford, P.; Kosaraju, S. Chemical cross-linking gelatin with natural phenolic compounds as studied by high-resolution NMR spectroscopy. *Biomacromolecules* **2010**, *11*, 1125–1132.

(28) Alexlson, D. E.; Russell, K. Characterization of polymers by means of ^{13}C NMR spectroscopy. *Prog. Polym. Sci.* **1985**, *11*, 221–282.

(29) McBrierty, V.; Packer, K. *Nuclear Magnetic Resonance in Solid Polymers*; Cambridge University Press: Cambridge, U.K., 1993.

(30) Kato, A.; Tanaka, A.; Lee, Y.; Matsudomi, N.; Kobayashi, K. Effects of deamidation with chymotrypsin at pH 10 on the functional properties of proteins. *J. Agric. Food Chem.* **1987**, *35*, 285–288.

(31) Gennadios, A; Brandenburg, A. H.; Weller, C. L.; Testin, R. F. Effect of pH on properties of wheat gluten and soy protein isolate films. *J. Agric. Food Chem.* **1993**, *41*, 1835–1839.

(32) Kayserilioglu, B. S.; Stevels, W. M.; Mulder, W. J.; Akkas, N. Mechanical and biochemical characterization of wheat gluten films as a function of pH and co-solvent. *Starch* **2001**, *53*, 381–386.

(33) Zhang, X.; Hoobin, P.; Burgar, I.; Do, M. The pH effect on the mechanical performance and phase mobility of thermally processed wheat gluten-based natural polymer materials. *Biomacromolecules* **2006**, *7*, 3466–3473.

(34) Zhang, X.; Hoobin, P.; Burgar, I.; Do, M. Chemical modification of wheat protein-based natural polymers: cross-linking effect on mechanical properties and phase structures. *J. Agric. Food Chem.* **2006**, *54*, 9858–9865.

(35) Zhang, X.; Do, M.; Bilyk, A. Chemical modification of wheat protein-based natural polymers: formation of polymer networks with alkoxysilanes to modify molecular motions and enhance the material performance. *Biomacromolecules* **2007**, *8*, 1881–1889.

(36) Zhang, X.; Do, M. Plasticization and crosslinking effects of acetone-formaldehyde and tannin resins on wheat protein-based natural polymers. *Carbohydr. Res.* **2009**, *344*, 1180–1189.

(37) Zhang, X.; Burgar, I.; Do, M.; Lourbakos, E. Intermolecular interactions and phase structures of plasticized wheat proteins materials. *Biomacromolecules* **2005**, *6*, 1661–1671.

(38) Zhang, X.; Do, M.; Hoobin, P.; Burgar, I. The phase composition and molecular motions of plasticized wheat gluten-based biodegradable polymer materials studied by solid-state NMR spectroscopy. *Polymer* **2006**, *47*, 5888.

(39) Kurniawan, L.; Qiao, G. G.; Zhang, X. Chemical modification of wheat protein-based natural polymers: grafting and crosslinking reactions with poly(ethylene oxide) diglycidyl ether and ethyl diamine. *Biomacromolecules* **2007**, *8*, 2909–2915.

(40) Kurniawan, L.; Qiao, G. G.; Zhang, X. Formation of wheat-protein-based biomaterials through polymer grafting and crosslinking reactions to introduce new functional properties. *Macromol. Biosci.* **2009**, *9*, 93–101.

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