Characterization and Thermal Properties of Polygenipin-crosslinked Hide Powders

by

Jie Liu,[b] Eleanor M. Brown,[a] Cheng-Kung Liu,[a†] and Keyong Tang[b]

[a]*Eastern Regional Research Center, United States Department of Agriculture***

Wyndmoor, PA 19038, USA

[b]*School of Materials Science and Engineering, Zhengzhou University*

Zhengzhou, Henan 450001, China

Abstract

Genipin is a naturally occurring iridoid compound, it is widely used as an ideal biological protein crosslinking agent due to its low toxicity compared to glutaraldehyde and formaldehyde. Under alkaline condition, genipin could undergo ring-opening polymerization via nucleophilic attack of hydroxyl ions followed by an aldol condensation. Because of the fact that polygenipin could create long-range intermolecular crosslinking between protein chains, preliminary investigations have been carried out to study effect of polygenipin crosslinking on color and thermal stability of hide powder by using colorimetry, differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). The results show that the peak denaturation temperature (T_p) for hide powders obtained from DSC increased to a maximum and then decreased with increasing of the molecular weight of polygenipin. Degree of crosslinking was evaluated, and the results suggest that thermal stability of hide powder is influenced not only by degree of crosslinking, but also by the type of crosslinking. Thermogravimetric analysis also confirms that long-range intermolecular crosslink bridges formed between collagen molecules results in more thermally stable hide powders. This study suggests that polygenipin can be potentially useful in producing eco-friendly leather.

Introduction

Tanning is an ancient art that has been described as one of the earliest industrial activities in human history. There are many tanning techniques have been developed, optimized and successfully applied in leather production, such as the chromium, aluminum, formaldehyde, titanium, resin, and vegetable tanning. Chromium tanning, which was invented by Augustus Schultz, in 1884, is the most widely used method in tanneries due to the unique properties that it imparts to the leather.[1] However, chromium tanning generates huge amounts of solid and liquid chromium-containing wastes.[2] These wastes have posed potentially serious threats to both the environment and public health. In many countries, there are strict restrictions on the use of chromium-tanned leathers for certain purposes. [3] The environmental pressure and increasingly strict requirements for leather have boosted emerging green technologies which aimed to produce chromium free eco-friendly leather (eco-leather), in order to minimize the effects of manufacturing process, products and wastes on the environment.[4]

Over the past several decades, considerable efforts have gone into obtaining eco-friendly tanning agents. Study shows considerable evidence that today's environmentally conscious consumers strongly respond to green products and brands.[5] The utilization of eco-friendly auxiliaries or chemicals in tanning system has attracted researcher's attention in both economic and environmental points of view. Various tanning agents were investigated in the production of eco-leather. Among the mineral tanning agents, aluminum, zirconium, iron, and titanium compounds have been frequently used as alternatives to chromium. For instance, Mutlu *et al.* developed new titanium tanning agent from the metal industry wastes, for leather processing, results showed that this mineral tanning agent could be used as an alternative tanning material.[6] Iron-THPS (Tetrakis(hydroxymethyl)phosphonium sulfate) combination tanning system has been developed by Fathima *et al.* as a potential alternative to chromium salt.[7] It was reported that the physical properties of the leathers obtained are on par with or even better than the conventional chrome-tanned and iron-tanned leathers. Sundarrajan *et al.* improved the use of zirconium salt in tanning for obtaining leathers with improved hydrothermal stability. Apart from the mineral tanning agents, other reagents used in tanning include vegetable and synthetic

†Corresponding author. Tel (215) 836-6924; e-mail address: ChengKung.Liu@ars.usda.gov.

J. Liu (liujie@zzu.edu.cn) was a visiting scientist at ERRC, USDA 2016-2017; his permanent address is Zhengzhou University.

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tannins.[8] Vegetable tanning has a long history for leather production, and some researchers continue to improve it.[9] Besides, aldehyde and oil tanning also contribute to current leather production. However, no individual tanning agent as yet can substitute for chromium completely.

Tanning of leather may be understood as the crosslinking of collagen to form a stable three-dimensional matrix. Genipin, which is a naturally occurring iridoid compound, has been widely used as an ideal biological crosslinking agent due to its low toxicity compared to glutaraldehyde and formaldehyde.[10] It was reported that genipin is about 5000~10000 times less cytotoxic than glutaraldehyde.[11] Genipin is extracted from gardenia fruits according to modern microbiological process.[12] Vegetable tannin, which is another important class of tanning agent from natural resources, can be obtained from different parts of plants including woods, barks, fruits, and leaves. Though genipin and vegetable tannins share similarities in their origins, their chemical structure and crosslinking mechanisms are quite different. Genipin crosslinking was expected to cause the formation of amide and tertiary amine bonds between the crosslinking agents and the amino-containing molecules.[13] By now, genipin has been used to crosslink different kinds of proteins and polysaccharides, such as gelatin, collagen, chitosan, silk fibroin, casein and carrageenan.[12-15] To exploit the potential of genipin as eco-friendly tanning agent, Ding *et al.* studied the effect of genipin crosslinking on thermal stability of bovine hide powder. The results indicated that the hide powder which had been treated with genipin at pH ~7.0 exhibited significant improvement in thermal stability.[16] Their further research suggested that it is possible to develop a novel chrome-free tanning process using citrate-masked aluminum sulfate and genipin in a combination tannage.[17]

Genipin is a crystalline, water-soluble powder possessing bi-functional characteristics. It has been recognized that pristine genipin is unstable in solution and undergoes self-polymerization. According to the study by Mi and coworkers, the ring-opening polymerization of genipin was initiated by extracting protons from the hydroxyl groups at C-1 of deoxyloganin aglycone, followed by opening the dihydropyran ring to conduct an aldol condensation.[18] At a higher pH, a higher degree of self-polymerization of genipin is likely to occur than at lower pH. Earlier research conducted by Ge et al. has shown that the polymerized genipin (polygenipin) has a molecular weight of 300-42000 and had approximately 1-185 monomer units.[19] The effect of degree of polymerization of polygenipin on physical properties of gelatin and collagen hydrogels has been systematically studied by other researchers. The results indicated that polygenipin with different degrees of polymerization could create short-range and long-range intermolecular crosslinking between protein chains.[20] However, the crosslinking effects of polygenipin on physical properties of hide and leather have not been described previously.

The purpose of the present work was to evaluate the potential effectiveness of polygenipin crosslinking agent for improving thermal properties of hide powders. In this study, polygenipin was prepared at different environmental pH, and the effect of polygenipin crosslinking on color and thermal properties of hide powder were studied. Revealing the thermal properties, especially the thermal denaturation behavior of hide and leather, is of paramount importance for understanding and improving the properties of leather. We anticipate that our findings will provide a new route to fabrication of eco-leather.

Experimental

Materials

Bovine hide powder (HP) was prepared from fleshed bovine hides that had been pre-processed and limed as described in an earlier report.[21] Pieces of limed hides were neutralized to approximately pH 7.0 using 0.5 M acetic acid. The hides were cut into approximately 2.5 to 5.5 cm^2 pieces and air dried. After being air-dried completely, the limed hide pieces were ground in a Wiley Laboratory Mill (Model 4, Thomas Scientific, USA) using a 1-mm screen. The hide powders were collected and stored in sealed plastic bags before use.

Genipin (98%) was purchased from Challenge Bioproducts Co. Ltd. Taiwan. Sodium chloride, hydrochloric acid and other reagents were from Sigma-Aldrich. All chemicals were of the highest grade available and used as received unless indicated otherwise.

Preparation of Polygenipin

The polygenipin was obtained by ring-opening polymerization of genipin in alkaline condition. 10 mL of fresh prepared genipin solution at the concentration of 10 mg/mL were adjusted to pH = 7.0, 8.0, 9.0, 10.0, and 11.0 using 0.1 M NaOH solution. After occasional stirring at 25°C for 20 h, the solutions were adjusted to neutral by 0.01 M HCl solution. Then the polygenipin solutions were diluted to 20 mL with PBS buffer (50 mM, pH 7.0). These solutions should be prepared just before crosslinking of hide powder. The UV-vis spectra of polygenipin solutions were recorded using a Cary 50 spectrophotometer (Agilent Technologies, USA) after suitable dilution with the PBS buffer.

Preparation of the Polygenipin Crosslinked Hide Powders

Hide powder (1.0 g) was weighed into a 30-mL glass threaded vial with cap and soaked in 10 mL PBS (pH ~ 7.0) for 20 h at 25°C. The polygenipin solution (20 mL, 5 mg/mL) immediately prepared as described above was then poured into the vial. The vial was placed in a thermostated shaking bath at 35°C for 30 h. After cooling to room temperature, the mixture was filtered, washed copiously with distilled water to remove any unreacted crosslinking agents and chemicals. The crosslinked hide powder was air-dried at room temperature and stored in a desiccator over

silica gel until analysis. The obtained polygenipin crosslinked hide powders were named PGH-7, PGH-8, PGH-9, PGH-10, and PGH-11 when the used polygenipin was prepared at pH 7.0, 8.0, 9.0, 10.0, and 11.0, respectively. In order to investigate the effect of crosslinking time on the properties of hide powder, we prepared polygenipin crosslinked hide powder samples at pH 10.0 with different crosslinking times in the range 8-102 h.

Color Parameters

Color parameters of hide powders were measured using a portable digital colorimeter (JZ-300, Shenzhen Kingwell Instrument Co., Ltd, China). Hide powders were pressed against a white plate (white standard) to form pellets, and the CIELAB color scale was used to measure color: L* defines lightness, which has values from 0 (black) to 100 (white). a* denotes the red/green value, from -60 (green) to 60 (red); b* denotes the yellow/blue value, from -60 (blue) to 60 (yellow). [22] Total color difference value (ΔE*) was calculated according to the following equation:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL*, Δa*, and Δb* are the differences between the corresponding color parameters of the samples and that of white standard (L* = 93.58, a* = 1.54, and b* = -0.50). Values were expressed as the means of eleven measurements on different areas of each sample.

Degree of Crosslinking (DC)

The DC of genipin crosslinked hide powders was determined with the ninhydrin assay.[23] The assay determines the amount of free amino groups remaining in the sample before and after crosslinking. Weighed hide powder samples (~5.0 mg) to be assayed were soaked in 1.0 mL de-ionized water for 72 h at 25°C, and subsequently refluxed at 100°C with 1.0 mL ninhydrin reagent (2% solution, Sigma-Aldrich Chemical Co., USA) for 20 min. After the solution was cooled down to room temperature and diluted with 50% (v/v) isopropanol, the optical absorbance of the solution was measured at 570nm with a Cary 50 UV-vis spectrophotometer (Agilent Technologies, USA). The amount of free amino groups in the sample is proportional to the absorbance of the solution after heating with ninhydrin reagent. Concentration of free amino groups in each sample was calculated by referring to a standard curve of glycine concentration vs. absorbance. The degree of crosslinking was calculated according to:

$$DC(\%) = \frac{M_{HP} - M_{PGH}}{M_{HP}} \times 100$$

where, M_{HP} and M_{PGH} are the amounts of free amino groups in raw hide powder and polygenipin crosslinked hide powder, respectively. Results reported here were averaged on three independent runs.

Differential Scanning Calorimetry (DSC)

DSC analysis of hide powder was performed using MCDSC (multi-cell DSC) from TA Instrument to observe the thermal denaturation behavior under nitrogen flow. Hide powder samples were pretreated by soaking in distilled water for 2 h before characterization to avoid the influence of hydration level on temperature of transition. The sample was heated to a temperature range from 0°C to 100°C at the rate of 1°C/min with an equilibration period of 10 min at the start. The peak temperature of the main endotherm was taken as phase transition temperature or denaturation temperature (T_p).

Thermogravimetric Analysis (TGA)

TGA experiments were carried out on a TA Q500-1708 thermogravimetric analyzer with a sample mass of approximately 6 ~ 8 mg, and the mass was recorded as a function of temperature. The samples were heated from room temperature to 600°C at a heating rate of 10°C/min. The experiments were performed under a nitrogen flow of 40 mL/min in the balance and 60 mL/min in the sample.

Results and Discussion

Characterization of Polygenipin

So far as we know, genipin molecule is unstable and undergoes ring-opening self-polymerization in alkaline environment. The OH^- in the alkaline aqueous solution can attack the genipin molecules and open the genipin ring to form aldehyde groups. Subsequently, the ring-opened genipin molecules can polymerize to form polymers or oligomers via an aldol condensation.[18] The chemical structures of genipin and polymeric genipin are shown in Figure 1.

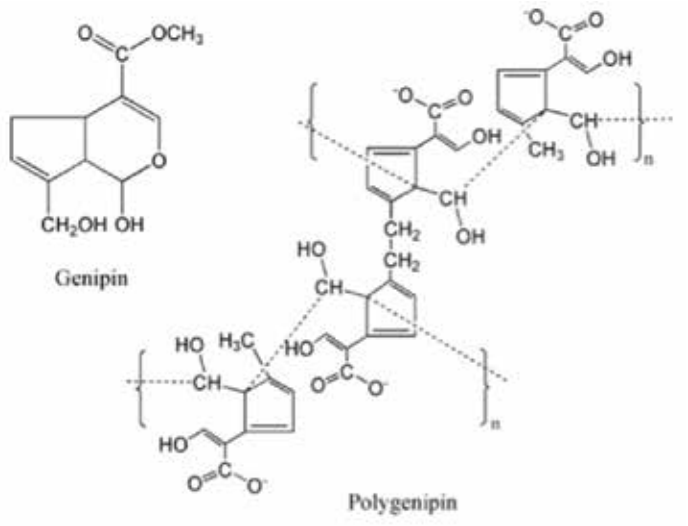

Figure 1. Chemical structures of genipin and polygenipin.

In this study, the polygenipin with different polymerization degrees was prepared by adjusting pH to alkalinity. The photographs and UV-vis curves of polygenipin solutions obtained at 5 different pH values are presented in Figure 2. Figure 2(A) shows that the colors of genipin solution becoming deeper and deeper from colorless to brownish, with the increase in the pH of the solution. Several studies have shown that the polymerization of genipin would lead to such a significant color change of the solution. In the meantime, a color change of crosslinked hide powder from dark blue to dark brown was perceivable by the naked eye (Figure 2(B)). The UV-vis spectra of polygenipin are depicted in Figure 2(C). Genipin possessed a major UV absorption at 240 nm. The increase in pH values from 7.0 to 11.0 led to the intensity of the genipin absorption peak at 240 nm decreasing gradually, owing to the opening of the dihydropyran ring. It was observed that the solution colors and UV spectra of genipin at pH 7.0, 8.0, and 9.0 was only slightly changed, indicating that the ring-opening polymerization didn't occur significantly at weak alkaline solution. With the increase of pH from 9.0 to 11.0, the characteristic peak at 240 nm was lowered and red-shifted to 270 nm. Such a shift may be due to the increase in the number of un-saturated π bonds in the polygenipin. [13, 18] The relationship between the molecular weights of polygenipin and pH values has been investigated by other authors using gel permeation chromatography (GPC). Mi et al. and Ge et al. have both reported a positive correlation between the molecular weight of polygenipin and pH value.[18, 19] They found that the degree of polymerization and molecular weight of polygenipin increase with increasing pH of the solution.

Color Parameters of Polygenipin Crosslinked Hide Powders

Uniform color is one of the important properties of leather. The color change of hide powders was studied before and after polygenipin crosslinking by application of CIELAB colorimetric method. Table I shows the variance results on color parameters L*, a* and b* for the un-crosslinked and polygenipin-crosslinked hide powders. The obtained results indicated that raw hide powder had the highest L* (lightness) and b* (yellowness) values, and lowest a* (redness) and ΔE^* (difference in color) values. No significant differences ($p<0.05$) in ΔE^* values were found for PGH-7, PGH-8 and PGH-9 samples but values differ considerably from that of the raw hide powder. In the crosslinked hide powders, PGH-10 and PGH-11 had lower value of ΔE^* but higher L* and b* values, than those of other crosslinked samples, probably due to the presence of polymerized genipin within the collagen matrix, indicating a tendency to yellowness.

Degree of Crosslinking

The reduction percentage in the free amino group content for each hide powder sample was determined by the ninhydrin assay and was used as a measure of the degree of crosslinking (DC). After the crosslinking, it was noted that the values of DC lie between 22.5±2.1% and 44.4±1.5% (Table II). The results indicated that polygenipin was an effective crosslinking agent for hide powder, also has the potential for being used as tanning agent for leather production. The values of DC of the PGH-8 and PGH-9 did not change significantly as compared to that observed in PGH-7 ($p>0.05$). However, the DC increases with further increase in pH of polygenipin reaction environment, reaches a

Table I
Color parameters of the un-crosslinked and polygenipin-crosslinked hide powders.

Samples	L*	a*	b*	ΔE*
HP	61.36±0.49^{a}	19.21±4.67^{d}	7.26±2.39^{a}	37.83±1.69^{d}
PGH-7	31.01±1.49^{d}	33.05±5.98^{ab}	-11.95±2.09^{e}	71.20±2.54^{a}
PGH-8	29.87±1.26^{e}	30.16±3.12^{bc}	-10.04±0.81^{cd}	70.55±1.44^{a}
PGH-9	32.52±0.56^{c}	34.86±4.27^{a}	-11.03±1.15^{de}	70.46±2.05^{a}
PGH-10	33.75±1.32^{b}	33.44±4.10^{ab}	-9.52±3.09^{c}	68.55±1.71^{b}
PGH-11	33.80±0.29^{b}	28.45±2.55^{c}	-4.85±1.24^{b}	65.74±1.18^{c}

Values are given as mean ± standard deviation. Different letters (a, b, c...) in the same column indicate significant differences between the means obtained by Duncan test ($p< 0.05$).

maximum of 44.4±**1.5**% (PGH-10) and then decreases to 31.2±1.8% (PGH-11). The difference in the type of crosslinks formed within the hide powder was responsible for this observation: genipin and low-molecular-weight polygenipin could mainly form intramolecular crosslinks (within a collagen molecule) and short-range intermolecular crosslinks between adjacent collagen molecules, whereas polygenipin with higher molecular weight tends to create long-range intermolecular/interfibrilar crosslinking between protein chains.[17, 19, 20] Consequently, low molecular weight crosslinking agents may easily penetrate into the collagen fiber bundles and react with surface collagen molecules, the physical barrier effect caused by crosslinked surface layer may leads to a decrease in genipin/low-molecular weight polygenipin concentration in the core of collagen bundles. For higher-molecular-weight polygenipin, on the contrary, it may take a longer time for the formation of crosslinked barrier-layer surface on the collagen fiber, and thus lead to a rise in the degree of crosslinking of the hide powder. With further increase in the molecular weight of polygenipin, the polymerization reaction will dramatically reduce the aldehyde groups in the polymeric genipin molecules, resulting in the decrease of crosslinking between crosslinking agent and collagen. Additionally, the diffusion process within hide powder is controlled by the size of polygenipin macromolecules as described by the Stokes-Einstein relation, it was difficult for high-molecular-weight polygenipin to enter into the collagen bundle network and the degree of crosslinking decreased accordingly.

Previous studies performed by Mu et al. demonstrated that the degree of crosslinking of the genipin-crosslinked collagen hydrogels decreased with the increase of the polymerization degree of genipin.[20] Different sample preparation methods may be responsible for the difference in effect of molecular weight of polygenipin on degree of crosslinking. Mu et al. mixed bovine tendon collagen

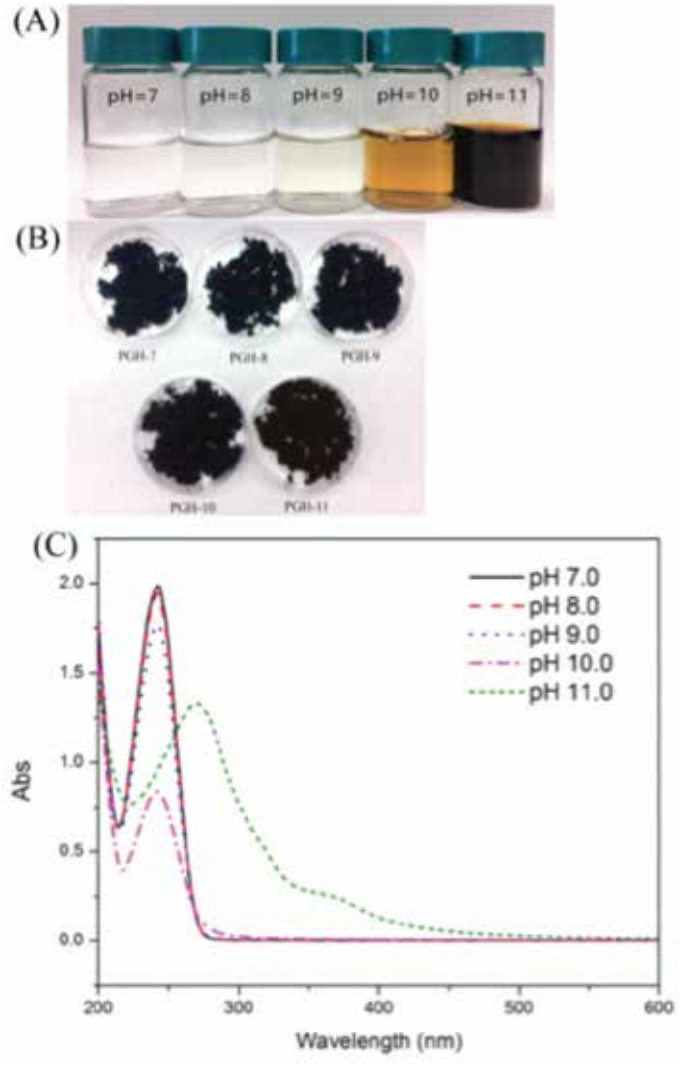

Figure 2. Photographs of (A) polygenipin solutions (~10 mg/mL) and (B) polygenipin crosslinked hide powders; (C) UV-vis spectra of polygenipin solutions.

Table II
Degree of crosslinking of polygenipin crosslinked hide powders.

Samples	PGH-7	PGH-8	PGH-9	PGH-10	PGH-11
DC (%)	22.5±2.1^{c}	23.8±1.9^{c}	25.2±2.6^{c}	44.4±1.5^{a}	31.2±1.8^{b}

Values are given as mean ± standard deviation. Different letters (a, b, c) in the same row indicate significant differences between the means obtained by Duncan test ($p< 0.05$).

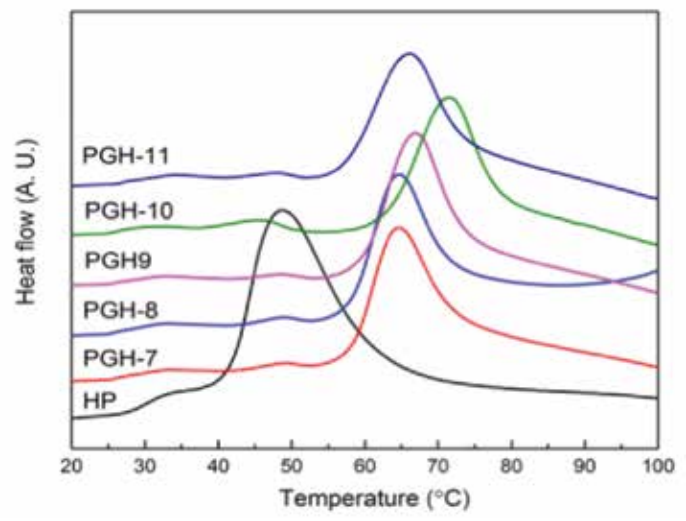

Figure 3. The DSC thermograms of raw hide powder and polygenipin crosslinked hide powders obtained at different pH. (crosslinking time = 30h)

solution with polygenipin to obtain a homogeneous slurry, then the hydrogel was shape-fixed after standing for several days at 30°C. It can be assumed that the concentration of polygenipin in collagen solution was uniform. In their experiment, the aldehyde groups in the polygenipin molecules will be the major factor in determining the degree of crosslinking.

Differential Scanning Calorimetry

Thermal denaturation of collagen is a time-dependent irreversible transformation of the native triple helical structure into uncoiled structures.[24,25] Figure 3 shows the DSC curves of the thermal denaturation of un-crosslinked hide powder and crosslinked samples from PGH-7 to PGH-11 in excess water at a heating rate of 1°C/min. For each curve, a major endothermic peak and some minor peaks at temperatures lower than the major peak were observed. Recent works showed that the major peak was due to the irreversible helix-coil transition of collagen, while the minor peak before denaturation was ascribed to the breaking of inter-hydrogen bonds among collagen molecules, or the transition of gelatin-like structures produced by denaturation.[26,27] As shown in Figure 3, the denaturation peak temperature (T_p) of hide powder became higher after crosslinking, indicating that polygenipin can effectively improve the thermal stability of collagen. The increased thermal stability of collagen in crosslinked fibers can be explained by a "polymer-in-a-box" mechanism.[28] According to this mechanism, the rate of collagen unfolding is depressed by the proximity of the neighboring collagen molecules in the fiber. These molecules can be described as the walls of a box to confine collagen molecules within the fiber lattice, and such confinement effect reduces the configurational entropy of random-coil domain. Here, when polygenipin was used as crosslinking agent, it stabilized the collagen molecules in a fiber lattice by reducing the separation of the molecules, i.e. by reducing the lateral dimensions of the box, and thereby increases the value of T_p. Miles et al. mentioned that crosslinking causes dehydration of the collagen fibers and it is the reduced hydration that causes the increased thermal stability.[28] Though the hide powders were fully saturated with water before the DSC analysis in this study, crosslinking affects the denaturation temperature of collagen fibers because it reduces the extent to which fibers can swell in a given environment.

Meanwhile, Figure 3 shows that the T_p significantly increases from 48.7°C (HP) to 71.5°C (PGH-10), and then decreases to 66.2°C for PGH-11. The difference in the peak temperatures between various crosslinked hide powders can be ascribed mainly to the different degree of crosslinking. According to Table I, there was no significant difference in degree of crosslinking between PGH-7, PGH-8 and PGH-9, and polygenipin with low molecular weights mainly creates short-range crosslinking between collagen molecules.[19] Consequently, the values of T_p are quite similar from PGH-7 to PGH-9. In contrast, a pH of 10.0 for genipin polymerization results in hide

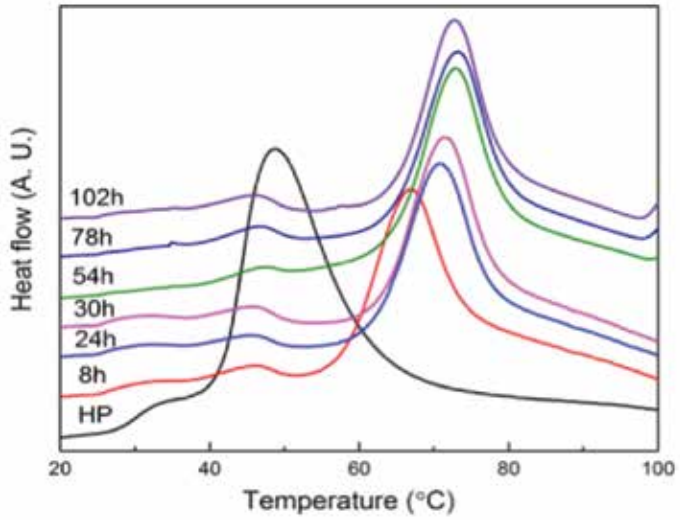

Figure 4. The DSC thermograms of raw hide powder and polygenipin crosslinked hide powders obtained at different crosslinking time. (pH = 10.0)

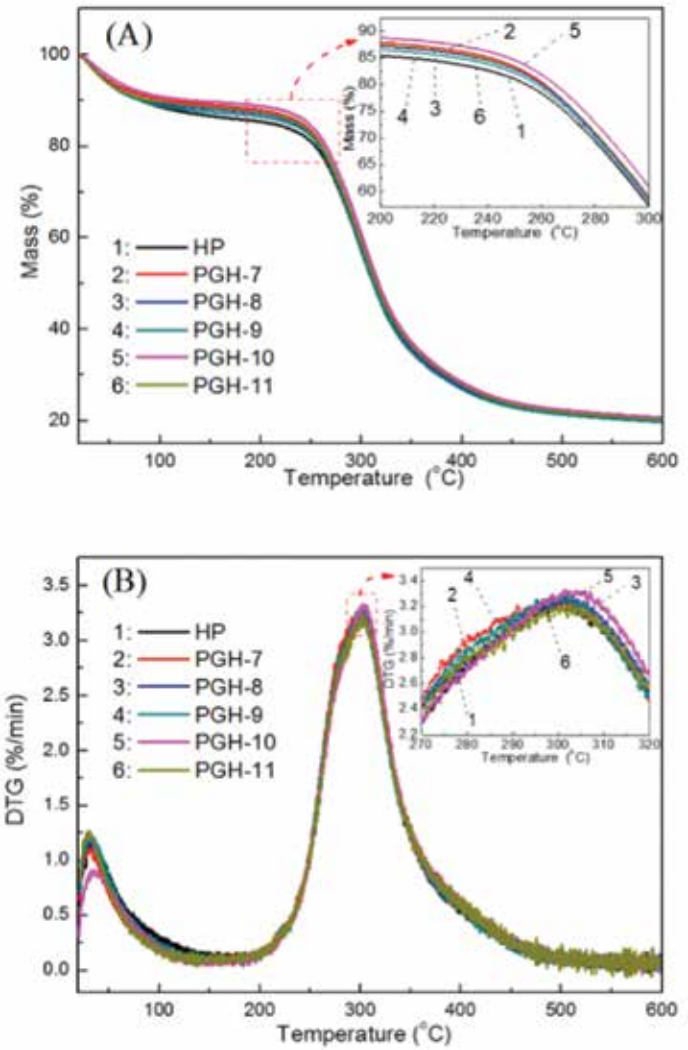

Figure 5. (A) TG and (B) DTG curves of the polygenipin-crosslinked hide powders and un-crosslinked hide powder at a heating rate of 5 °C/min.

powder with the highest degree of crosslinking. As expected, PGH-10 has the highest T_p. It is noteworthy that the T_p of PGH-11 is lower than that of PGH-9, though the degree of crosslinking of the former is significantly higher than the latter. This observation indicates that short-range and long-range crosslinking may have different effects on thermal stability of the hide powders. Long-range crosslinking leads to flexible collagen matrices due to the long chain of polygenipin, the hide powders crosslinked with high-molecular-weight polygenipin may have enhanced stability and elasticity by maintaining large distances between crosslinking sites. By contrast, genipin and low-molecular-weight polygenipin crosslinked collagen matrice may have more rigid networks owing to the closely-spaced crosslinking sites, which reduced the flexibility of the fixed collagen molecules, and therefore exhibit enhanced thermal stability even with a lower degree of crosslinking.

The time dependence of thermal stability of polygenipin crosslinked hide powders was also examined. The DSC thermograms for the raw hide powder and polygenipin crosslinked hide powders obtained at pH 10.0 and different crosslinking time are displayed in Figure 4. With increasing crosslinking time, the main endothermic peak moved to higher temperature in the polygenipin-crosslinked hide powder. It could be found that the T_p reached 64.0°C after 8 h of incubation in polygenipin solution. Further extension of the crosslinking time leads to a noticeable increase in the T_p of hide powders. After 30 h of crosslinking, the T_p reached 73.0°C. Although increasing the crosslinking time to more than 30 h did somewhat increase the T_p of the hide powders, it was observed the value of T_p changes only slightly by 102 h, showing that the equilibrium of the crosslinking reaction was reached gradually at the crosslinking time under investigation.

Thermogravimetric Analysis

The mass evolutions with temperature of hide powders crosslinked with polygenipin prepared at different pH values, together with the original un-crosslinked hide powder are shown in Figure 5. All samples display similar behavior with three main stages of mass loss in the temperature range of 22°C - 600°C. The temperature range for the first step of mass loss is 22°C - 230°C, which corresponds to the loss of low molecular mass compounds, mainly free and bound water. The second stage gave a high degradation rate at about 230°C - 400°C, is mainly related to the thermal degradation of hide powder and polygenipin. The higher temperature stage which exceeds 400°C can be attributed to the decomposition of more thermally stable structure. The behaviors described were similar to those observed in the leather samples.[29-31]

The onset temperature of the main degradation (T_o), the residue at 600°C, the maximum rate of mass loss (R_{max}) and the temperature corresponding to maximum rate of mass loss (T_m) of hide powders are summarized in Table III. The data show that the thermal stability of hide powder is slightly improved by polygenipin crosslinking. Comparison of T_o indicates that un-crosslinked hide powder started to degrade at lower temperature than those of hide powders crosslinked by polygenipin, and the value of T_o gradually increases from PGH-7 to PGH-11, which means that the polymerization of genipin is conducive to improving thermal stability of hide powder. This observation can also be explained by the long-range crosslinking between collagen chains. The presumed reaction mechanism of amino-group-containing protein with genipin is through nucleophilic attack by primary amine groups on the olefinic carbon atom at C-3 of deoxyloganin aglycon on genipin and was followed by opening the dihydropyran ring to form a heterocyclic amine.[18, 32] The intermediate compounds could further associate

Table III

The onset temperature of the main degradation (T_o), the maximum rate of mass loss (R_{max}), the temperature corresponding to maximum rate of mass loss (T_m) and the residue at 600°C of the investigated hide powders.

Samples	T_o (°C)	R_{max} (%/min)	T_m (°C)	Residue at 600°C (%)
HP	253.6	3.19	300.2	20.0
PGH-7	254.4	3.23	300.4	20.6
PGH-8	254.5	3.25	301.8	20.1
PGH-9	254.9	3.28	301.8	19.7
PGH-10	257.2	3.31	304.1	20.6
PGH-11	254.5	3.18	302.1	20.2

to form networks with short chains of crosslinking bridges. However, the polygenipin with different molecular weights may create different kinds of crosslink bridges, which can be used to produce gelatin or collagen hydrogels with different mechanical and textural properties altered by adjusting the molecular weight of polygenipin.[19, 20] Under basic conditions, the ring-opened dihydropyran ring of genipin molecule can form new aldehyde groups, which can form C=N crosslinking by Schiff's base formation with ε-amino groups of collagen. Compared to the short-range crosslinking effects of genipin, the long-range crosslinking tends to improve the thermal stability of polymer networks, which can be attributed to an enhanced thermal stability of polymerized genipin macromers or oligomers compared with genipin monomer. Moreover, it has been reported that the polymerized genipin is stable even under acidic conditions. In this study, with the increase of the molecular weight of polygenipin, more long-range intermolecular crosslink bridges may be formed between collagen molecules, therefore leading to more thermally stable hide powders.

Conclusions

Crosslinked hide powders were obtained by using polygenipin as crosslinking agents. The results indicated that the color of hide powder changes from dark-blue to dark brown with the increase of molecular weight of polygenipin. Measurement of degree of crosslinking showed that when the molecular weight of polygenipin increases to a certain extent, the highest extent of crosslinked hide powder was obtained. The peak temperatures for the denaturation process associated with the phase change were measured by DSC for the hide powder before and after crosslinking, revealing that the highest denaturation temperature was 73.0°C compared with 48.7°C in the un-crosslinked hide powder. With the increase of crosslinking time, a rapid increase of T_p was observed after crosslinking for 8 h, followed by a gradual increase toward an equilibrium value at about 30 h. Thermogravimetric analysis demonstrated that long-range intermolecular crosslink bridges formed between collagen molecules results in more thermally stable hide powders. Results of current study suggest that polygenipin can improve the thermal properties of hide powder, thereby opening new routes for production of chrome-free eco-friendly leather.

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