Biobased Films Prepared from Collagen Solutions Derived from Un-tanned Hides

by

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Abstract

The U.S. hide and leather industries are facing challenges of meeting environmental imperatives; quantifying, maintaining, and improving current hides and leather product quality; developing new processes and products; and improving utilization of waste. One of our contributions to address these ongoing challenges is to develop innovative uses and novel biobased products from hides to improve prospective markets and to secure a viable future for hides and leather industries. We had previously investigated the production of nonwoven and green composites from collagen fiber networks, which were extracted from un-tanned hides and from tannery solid wastes, such as splits or trimmings. Recently, we focused on preparing biobased films from un-tanned; specifically limed hides, which have potential commercial applications in medical care and food packaging. Collagen fiber networks were obtained from hides that have been processed to remove the noncollagenous materials through the hair removal and liming steps. We also focused on understanding the effects of processing steps such as bating and crosslinking treatments on the morphology and physical properties of biobased films from un-tanned hides. Results showed that the concentration of collagen solution and the methods of crosslinking with glutaraldehyde during the film formation process have significant effects on the properties of resultant films. Higher concentrations of collagen and addition of glutaraldehyde crosslinkers after solidification of the films yielded better mechanical properties. The encouraging results of this ongoing research are instrumental to produce biobased films, which have wide applications in both the medical field due to good biocompatibility and the food packaging because of excellent mechanical properties and acceptable edibility.

Introduction

Meeting environmental imperatives and improving utilization of waste are current major challenges that the U.S. hides and leather industries are facing. We have addressed these challenges by developing innovative uses and novel biobased products from the solid fibrous wastes generated from tanneries.[1-3] We hypothesize those solid fibrous wastes from a tannery can be purified into collagen fiber networks and utilized in making useful biobased products such as green composites and biobased films, all of which have great market potential. Green composites are composites made from biobased polymers and fibers or fillers that are renewable and degradable.[1] Earlier studies were devoted to understand the effects of dehydration on the morphology and physical properties of the fiber networks derived from un-tanned hides, which will be the starting material for constructing nonwoven and green composites.[1] Five dehydration methods were investigated and observation showed solvent- and freeze-drying yielded the lowest apparent density indicating a higher degree of separation in the fibrous networks that will be favorable for further processing into useful products. Mechanical testing showed the lower apparent density led to lower tensile strength, greater elongation at break, lower Young's modulus, and higher toughness. The results from comparisons showed that samples frozen and then followed by vacuum drying offer many advantages over those from the other dehydration methods in terms of economic and open fibrous structure.

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Further study investigated the use of crosslinking with glutaraldehyde and other treatments to improve the integrity of fibrous structures that offer better stability and physical properties.[2] Crosslinking is a process to apply a chemical agent - like glutaraldehyde to bridge molecular chains, thereby providing stability and improved physical properties to collagen matrices.[3] There are various crosslinking treatments currently being used today for collagen materials. These include ultraviolet light,[4] dehydrothermal treatment,[5] chemical agents such as glutaraldehyde,[6] and enzymatic crosslinking by microbial transglutaminase.[7] Two of the most common methods, i.e. transglutaminase and glutaraldehyde, were used to treat the collagen fiber networks. Results showed that glutaraldehyde treatment yielded a highly open structure, in which the fibers are well separated from each other.

We previously investigated preparations of nonwoven and green composites derived from fiber networks extracted from hides.[3] To develop the technologies for new products, pieces of limed hides were neutralized, completely dried and then ground in a Wiley Mill using a 1 mm, 2 mm or 4 mm screen size. Nonwoven sheets were prepared using paper-making technology. They were then used as reinforced components to make composites that used gelatin as the matrix. Test results showed that the fiber sizes and gelatin content had a significant effect on the properties of resultant nonwoven and composites. Finer size fibers and higher portions of gelatin yielded better tensile strength and higher stiffness. The results of this research are useful to the production of high quality fibrous products such as high efficiency air filters or green composites.

Another potential product that can be derived from un-tanned hides or tannery solid wastes is biobased films. Many reports have shown the important applications of biobased films in medical care such as wound dressing and skin repair because of the excellent biocompatibility.[8-11] Collagen is the most abundant of animal proteins, which is widely distributed in connective tissues. It was reported that in vitro, natural collagen can be formed into highly organized, three-dimensional scaffolds that are intrinsically biocompatible and provided with high tensile strength.[8] Because of these attributes, collagen has been deemed the material of choice for wound healing and tissue engineering applications.

Beside medical applications, recently, there is a great interest in using biobased films for food packaging.[12-17] Food packaging films were obtained by crosslinking a collagen blend with glutaraldehyde. A network structure was formed due to crosslinking of collagen molecules by glutaraldehyde. Fourier transform infrared spectroscopy (FT-IR), SEM and TGA analyses were used to characterize the resulting films.[17] Strong hydrogen bonding was detected among the composite film molecules. The surface morphology of the film appears more homogeneous after crosslinking.[17]

Collagen/cellulose films were also reported to be made by the blend solutions of collagen and cellulose, which were dissolved in 1-allyl-3-methylimidazolium chloride. The results showed that there were strong interactions and good compatibility between collagen and cellulose in the film and the polymers have their strongest interactions at the mixing ratio of 1:1. The blend films possess better mechanical and water absorption properties than those made of collagen or cellulose only.[18]

This study presents our recent findings to convert limed and delimed-bated hides into collagen films. The most important step for preparing a collagen film or fiber is its dissolution.[19-21] Many solvent systems have been reported. But, we used an older method by Nishihara which was very effective in digesting hides to make collagen solutions, and it was less detrimental to the environment.[21] The dissolution system used a common protease which is pepsin at a suitable pH as described later. The variables we studied which affected film formation were the concentration of collagen solution and the point of application of glutaraldehyde crosslinker during the casting of films. Crosslinkers were previously identified which improved the properties of composites. For example, films prepared from gelatins, which had been modified with microbial transglutaminase, showed improved tensile strength and toughness. They were less soluble in water than films from gelatin alone (due to build of higher molecular weight), and they had improved hydrophilic properties.[7] This report presents the preparation of collagen films, their resultant mechanical properties, and their morphology by examination with SEM.

Experimental

Materials and Procedures

Hide Fiber Networks Preparation

Fleshed fresh steer hides were purchased from JBS (Souderton, PA) and sided at our facility. The fleshed hides were sided and soaked for 1 h in 150% float with 0.15% Borron TS (TFL, The Woodlands, TX) and 0.1% Proxel (Chemtan Co. Inc., Exeter, NH) with a drum speed of 6 rpm. The float was drained and the hide was washed for 5 min; 2% sodium sulfide, 2% lime, and 1% soda ash were added in addition to 100% float. The drum was run for total of 4 h with a run time of 10 min per hour. After 4 h the float was drained, 100% float was added and washed for 5 min and then drained. A 200% float was then added to the drum with 2% lime and 1% sodium sulfide and run for 20 h at 6 rpm for 3 min per hour. After 20 h the float was drained, 100% float was added and the hide was washed for 5 min, then drained again. This step was repeated for a total of two times. Pieces were cut out of the limed hide (12” x 12”), and were either stored in a refrigerator or delimed, bated and then stored in a refrigerator. For the delimed and bated hides, after the liming step the hides were refloated to 125% with 3% ammonium sulfate, 0.15% Rohapon 6000

TABLE I
Summary of Dehairing to Delime and Bate Processes.

Process	% Float	Product	Time (min)	RPM
Soak	150	0.15% Boron TS, 0.1% Proxel	60	6
Wash	150		5	6
	100	2% sodium sulfide, 2% lime, and 1% soda ash	240	6 at 10 min/hour
Drain and Washed	100	water	5	6
Relime	200	2% lime and 1% sodium sulfide	1200	6 at 3 min/hour
Drain and Washed	100	water	5	6
Drain and Washed	100	water	5	6
Delime and Bate	125	3% ammonium sulfate, 0.15% Rohapon 6000 and 0.10% Boron TS	90	6
Drain and Washed	100	water	5	6
Drain				

(TFL, The Woodlands, TX), and 0.10% Borron TS, and run for 90 min at a drum speed of 6 rpm. The float was drained, the hide was washed in a 100% float, and drained again. These steps are summarized in Table 1.

Pieces from the limed only, and delimed-bated treatments were cut into approximately 1.5” to 2” squares and allowed to air dry fully in a fume hood. They were then ground in a Wiley Mill (Thomas Scientific, Swedesboro, NJ) and sieved through a 4 mm circular screen. The collagen fibers were then stored in sealed plastic bags until the preparation of their solutions.

Collagen Dissolution Process

The process we used to extract soluble collagen from limed only, and delimed-bated hides into collagen solutions was modified from that reported by Nishihara.[21] Ground (4 mm) collagen fibers (~ 2 g) were added to 30 times their weight of water in a beaker (600 mL) to rehydrate them on a stir plate. The pH of this mixture was then lowered to 2 -2.5 by using 2N HCl. A 50 to 1 weight ratio of the sample (2 g) to pepsin (0.04 g) was added to the rehydrated collagen mixture. Two to three drops of sodium azide were next added to this mixture in order to prevent future mold growth. Several such samples were stirred for 48 to 72 h so as to solubilize the collagen fiber networks. The progress of solubilizing collagen was monitored visually as evidenced by an increase in the viscosity of its solutions. Then, 33 times the weight of the samples of 0.005N HCl (66 g) were added to the beakers, and the mixture was stirred continuously for 24 h. Additional 0.005N HCl was added so as to decrease the viscosity, and render it easier to stir. After 24 h of stirring, the collagen solutions were filtered using a fine plastic mesh screen, then neutralized using 5N or 2N NaOH to a pH of 6-7. The mixture was centrifuged, and the collagen concentrate (precipitate) was collected, washed with water, and the mixture was centrifuged again. This collagen concentrate (precipitate) was then separated from the supernatant (wash-water), and stored in a glass jar in a refrigerator until needed for film formation studies.

Instead of using the dried ground hides for making collagen solutions, we used instead freshly limed and delimed-bated hides. The cut pieces from these processed hides took a long time to dissolve or be digested with pepsin. So, they were ground first in order to facilitate their faster dissolution. Limed only and delimed-bated hides were cut into 0.5” x 0.5” squares and then ground by using a Hamilton Beach Co. (Racine, WI) Model 222 meat grinder. The delimed-bated pieces were too soft to be ground thoroughly, unlike their precursor limed hides, which were readily ground in the meat grinder, and most of their ground pieces digested with pepsin. Consequently, freshly ground limed hides were the starting material for our studies, and by extension, tanneries could utilize their waste lime trimmings as a starting material to make biobased films. Ground limed stock (2 to 10 g), and 10 times the weight in the amount of water (20 to 100 mL) were added to a beaker (600 to 2000 mL) and this mixture was stirred using a stir plate.

A collagen solution was thus prepared from this ground limed hide by using the modified pepsin dissolution method which was described above, and which was initiated by lowering the pH of this mixture to 2.0 -2.5.

Crosslinking

During the process of collagen dissolution, and specifically after the addition step of 0.005 N HCl, this mixture was stirred for 24 h and then filtered. To each beaker was then added 0.5, 1.0, and 1.5% glutaraldehyde based on the initial weight of the ground limed hide. One beaker without additional glutaraldehyde was used as the control. The solutions were stirred overnight, and then were neutralized to a pH 7 using 2N sodium hydroxide. Each of the concentrated collagen solutions was then centrifuged and its concentrate was saved. A small portion of the collagen concentrate thus crosslinked with glutaraldehyde was set aside to determine its hydrothermal stability as described below.

Casting of Collagen Films

Films were made by taking the collagen concentrate (100%) and making different reduced concentrations (10 to 80%) in water, followed by pouring approximately 20 g into a 100-mm petri dish. The solutions were allowed to air dry fully in a fume hood for 24 to 48 h. The resulting films were then put in a conditioning room at 23°C and 50% RH before testing as described in the mechanical property evaluation section. Other films were also made by taking a 50% concentrated collagen solution, pouring approximately 20 g in a petri dish and then adding 0.5, 1.0, and 1.5% glutaraldehyde. The petri dishes were then swirled for 1 min to mix and disperse the glutaraldehyde in solution. Collagen thus crosslinked with glutaraldehyde showed that its films shrunk by pulling away visually from the sides of the petri dish. In another experiment, a solution of 50% collagen was added to four petri dishes as described above. Their fully dried films were then immersed in 0.5, 1.0, and 1.5% glutaraldehyde aqueous solutions for 30 min. The control (no glutaraldehyde) was dipped in water only for 30 min. After this 30 min exposure, the residual glutaraldehyde solutions were decanted, and the crosslinked collagen films were allowed to fully air dry in the fume hood. The films were cut and tested using the conditions described in the mechanical property evaluations section.

Mechanical Property Evaluations

Mechanical property measurements on these biobased films were tensile strength, elongation, Young's modulus and toughness. Tensile strength is the stress in tension that is required to fracture the film. Toughness is calculated from the energy needed to fracture the biobased films. The films were cut to 0.5 cm wide using a razor blade and ruler, and tested using a 2.5 cm gage length. All samples were tested on a MTS electro-mechanical frame which was operated at a crosshead speed of 5 cm/min. The tests were done at approximately 23°C and 50% RH. An Insight-5 test frame and Testworks-4 data acquisition software (MTS Systems Corp., Minneapolis, MN) were used throughout this work. Each test on the MTS used a minimum of five biobased films to obtain a representative value.

Differential Scanning Calorimetry

The hydrothermal stability of film samples was determined on a Multi-Cell Differential Scanning Calorimeter (DSC) (model CSC-4100) from Calorimetry Sciences Corporation, Lindon, UT. Collagen concentrate (~ 250 mg) was weighed, put in the machine's cell, and the cell's assembly placed in the calorimeter. The calorimeter was programmed to record heat flow (mcal/°C units) while the temperature was increased from 20°C to 130°C at a rate of 1.0°C/min. The samples were equilibrated for a period of 600 s before the start of the test. The peak temperature in the output graph, T_s, was considered to be the apparent shrinkage temperature.

Microscopic Observations

A scanning electron microscope (SEM) was used to compare the structural differences among the different cast films of collagen. The films were freeze fractured, and then glued to specimen holders using Duco cement. They were sputter-coated once for 30 seconds with a thin layer of gold using an EMS Q150R sputter coater (Quorum Technologies, Ltd., Laughton, East Sussex, England). Images were collected using a FEI Quanta 200F scanning electron microscope (Hillsboro, OR), and operated in the secondary electron imaging mode.

RESULTS AND DISCUSSION

The thickness of the biobased film was a key property. Figure 1 shows the concentration of the collagen solution has a direct effect on the resultant thickness of the film. The higher the concentration of collagen that was in solution, the thicker the resultant film became.

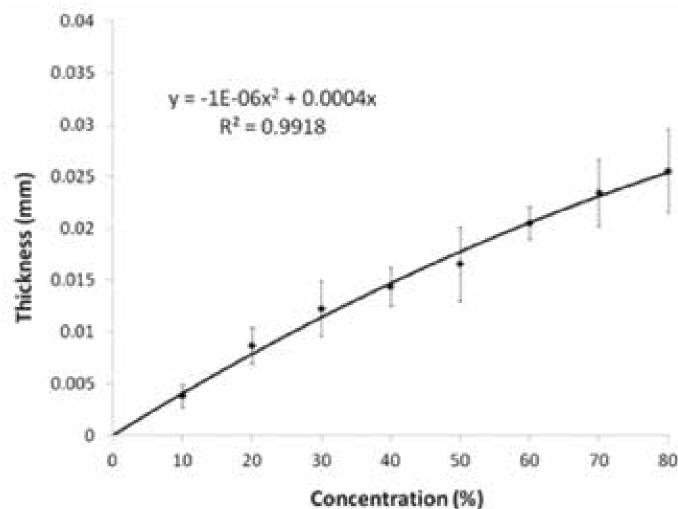

Figure 1. Thickness as a function of collagen concentration.

A film needs some degree of tensile strength and elongation for use in a load bearing application. Figure 2 shows that both tensile strength and elongation increased with collagen concentration. They peak at around 50% concentration, and then slowly move downward with its further increase in collagen concentration.

Figure 3 shows that the concentration of collagen on its films had similar effects on Young's modulus and toughness. Both properties improved with increasing collagen concentration up to 50%, and then leveled off (Figure 3a) or gently decreased (Figure 3b). This general trend is probably ascribable to a denser structure of film that was cast with a higher collagen concentration, thereby improving its mechanical properties. One thus concludes that those films which were cast from a 50% collagen solution reached a peak saturation point of performance, and the other films cast from still more concentrated solutions (or thicker films) did not benefit from added improvement in mechanical properties.

The starting material in this investigation was limed hides. But, it is reasonable to expect that the additional treatments of deliming and bating will affect the properties of its biobased films. Bating is a further step to purify the hide before tanning in the leathermaking process by digesting undesired proteins. We found it interesting to discern the effect of bating on the resultant film properties. So, Figure 4, shows that both tensile strength and Young's modulus increased with limed and bated collagen concentration. But the bating process demonstrated a significant decrease in both mechanical properties.

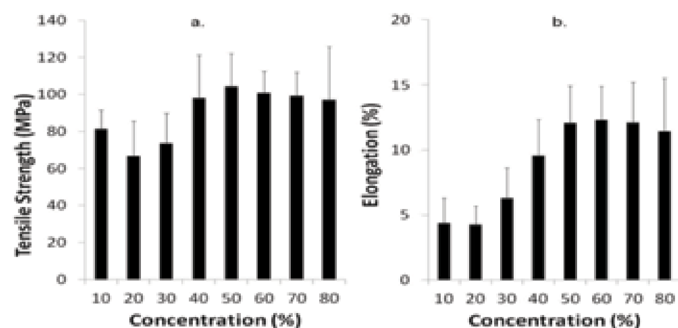

Figure 2. (a) Tensile strength and (b) elongation as a function of collagen concentration.

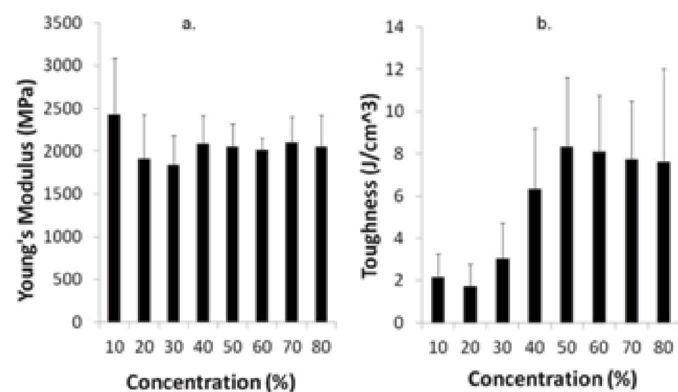

Figure 3. (a) Young's modulus and (b) toughness as a function of collagen solution.

Crosslinking of Films

Crosslinking is a chemical process which uses an agent (crosslinker) to bridge molecular chains, thereby providing hydrothermal stability and improved physical properties to collagen matrices.[2] In this study, we used a very common collagen crosslinker, glutaraldehyde, to crosslink or post treat collagen films for the purpose of stabilizing their structure (decrease disorder in molecular chains) and improve their physical properties. The apparent shrinkage temperature (T_s) was measured using the DSC. Typical DSC curves are displayed in Figure 5 for the collagen concentrate (control solid curve), and for the test sample that was crosslinked with 1.5% glutaraldehyde (dotted curve). Both samples showed a

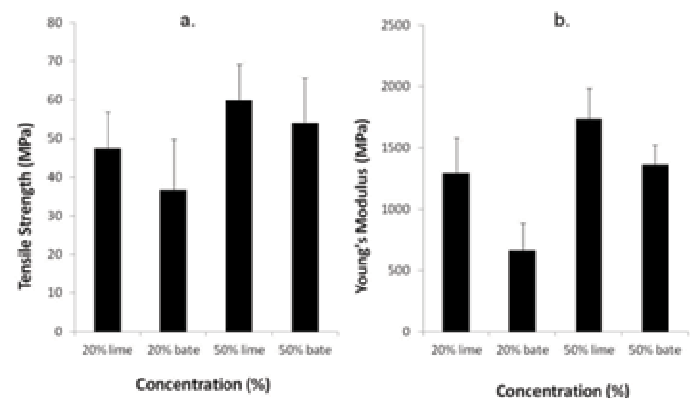

Figure 4. (a) Tensile strength and (b) Young's modulus vs. limed and bated collagen concentration.

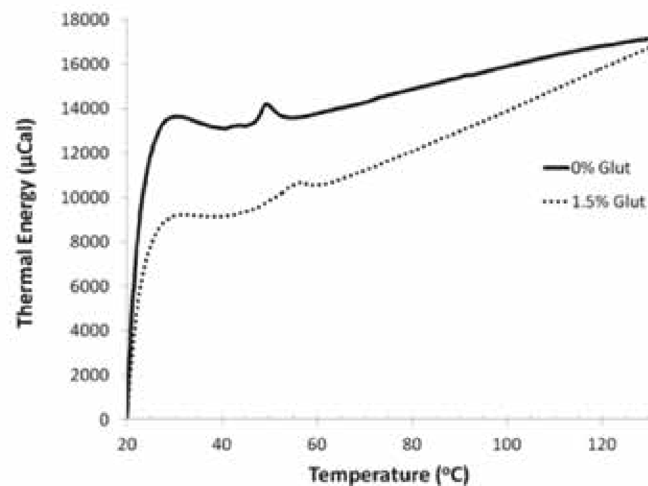

Figure 5. DSC curves of crosslinked and non-crosslinked collagen concentrates.

characteristic peak in DSC which is their shrink temperature. But the peak in the dotted curve (crosslinked sample) was shifted to the right. This meant a higher apparent shrink temperature due to the stabilization, or the endowed order of more collagen molecular chains in the film. Figure 6 shows that shrink temperature increased with increasing glutaradehyde concentration.

Figure 7 shows the further effect of glutaradehyde concentration on the thickness of the resultant films. Figure 7a pertained to the application of glutaradehyde to the collagen concentrate, Figure 7b pertained to the application of glutaradehyde during film formation, and Figure 7c belonged to the application of glutaradehyde after the film was fully cast or developed. It appeared that the thickness of the resultant films was affected more when glutaraldehyde was added to the system during film formation (Figure 7b). This is probably due to thickening of the collagen solution by glutaradehyde which resulted in a thicker film as also shown for the samples treated with 0.5% glutaradehyde or greater. An additional increase in % glutaradehyde did not cause an additional increase in film thickness.

Our observations also indicated that the timing of glutaraldehyde addition had a significant effect on the properties of the films. It appeared that the ideal time to add glutaradehyde was after the collagen film has been cast and had solidified or developed fully. In this case, the addition of glutaraldehyde did not interrupt the process of film formation such that the resultant physical properties were not affected greatly as seen in Figure 8c. By contrast, the resultant tensile strength decreased significantly when glutaradehyde was

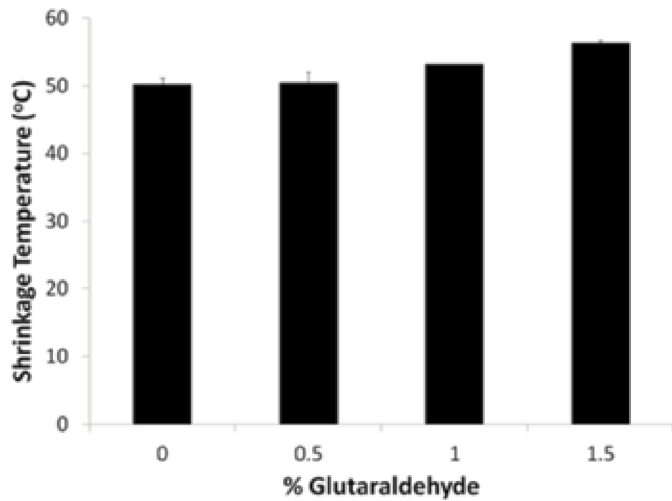

Figure 6. Apparent shrinkage temperature vs. % glutaraldehyde.

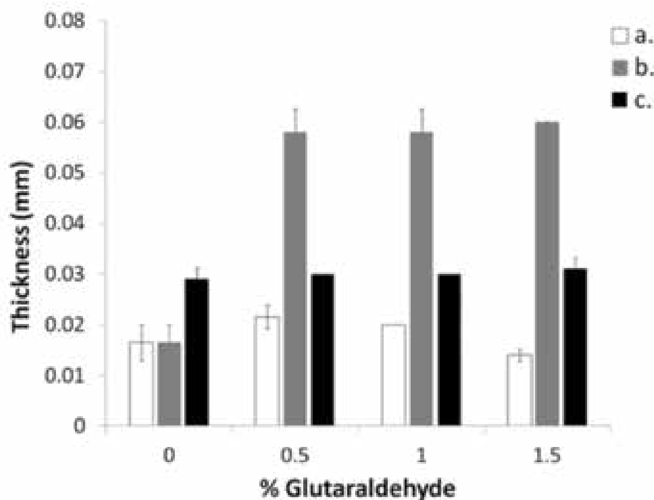

Figure 7. Thickness as a function of glutaradehyde concentration: (a) crosslinked in collagen concentrate, (b) crosslinked during the film formation; and (c) crosslinked after film casting.

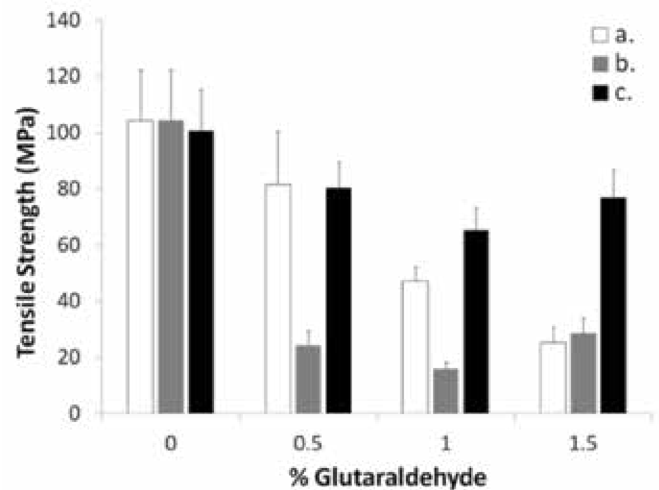

Figure 8. Tensile strength as a function of glutaradehyde concentration: (a) crosslinked in collagen concentrate, (b) crosslinked during the film formation; and (c) crosslinked after film casting

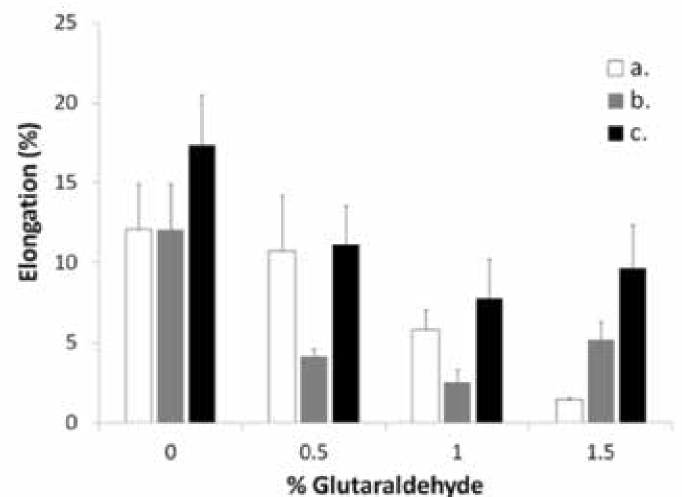

Figure 9. Elongation as a function of glutaradehyde concentration: (a) crosslinked in collagen concentrate, (b) crosslinked during the film formation; and (c) crosslinked after film casting.

added to the collagen solution at the onset (Figure 8a) or during its film casting (Figure 8b), meaning during the liquid state of both. A similar behavioral trend was also observed for elongation (Figure 9), Young's modulus (Figure 10), and toughness (Figure 11) .

Figure 12 displays the top view SEM micrographs of films prepared according to: (a) without glutaradehyde cross linking (b) wetted with water (c) glutaradehyde added to collagen solution- (d) film post treated with glutaradehyde. The micrographs did not show drastic differences in surface morphology, except the post treatment of the films (Figure 12d) appeared somewhat smoother after crosslinking. By contrast, the SEM cross-sectional views (Figure 13) showed very different pictures of internal structure among these four types of films. It appeared that the crosslinked samples (Figure 13c and 13d) had a denser structure than those of the non-crosslinked films (Figure 13a and Figure 13b).

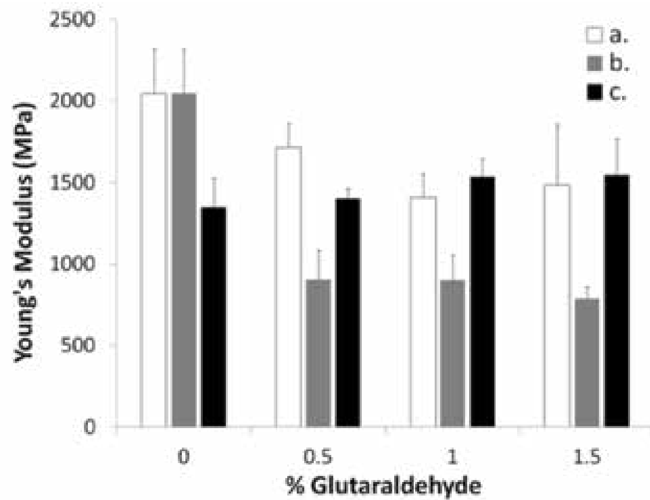

Figure 10. Young's modulus as a function of glutaradehyde concentration: (a) crosslinked in collagen concentrate, (b) crosslinked during the film formation; and (c) crosslinked after film casting.

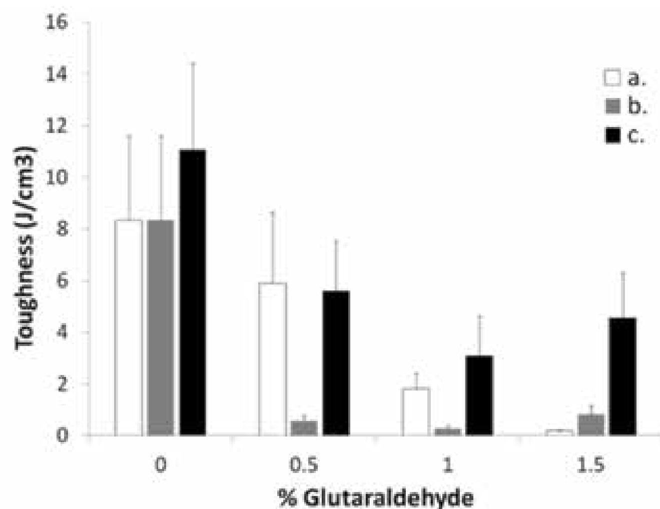

Figure 11. Toughness as a function of glutaradehyde concentration: (a) crosslinked in collagen concentrate, (b) crosslinked during the film formation; and (c) crosslinked after film casting.

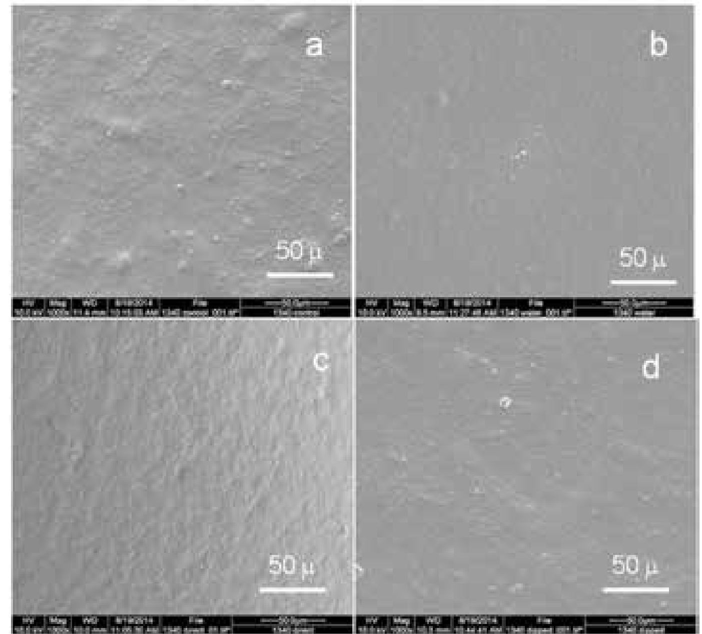

Figure 12. Micrographs show the surface structure of films (a) without glutaradehyde crosslinking (b) wetted with water (c) glutaradehyde was added in collagen solution- (d) film treated with glutaradehyde.

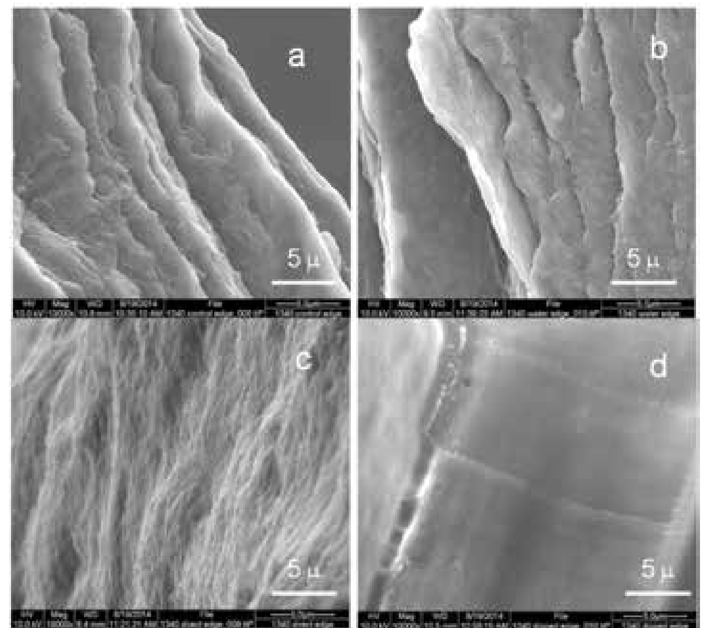

Figure 13. Micrographs show the cross-section of films (a) without glutaradehyde crosslinking, (b) wetted with water, (c) glutaradehyde was added in collagen solution, and (d) film treated with glutaradehyde.

CONCLUSIONS

Hides are the most valuable byproduct of the meat packing industry. The U.S. is the world's 3rd largest hide producer of approximately 35 million cattle hides annually. Due to fierce competition in global markets, the American leather and hides industry's long term viability will depend on the industry adopting more environmentally friendly processes and implementing new technology for producing novel products from raw hides and recycled tannery waste. One of our contributions to address these industrial challenges was to develop these novel biobased collagen film products from hides and from their tannery waste. This study focused on preparations of biobased films from un-tanned hides, including limed hides and delimed-bated hides, which correspond with their actual tannery waste of limed splits and their trimmings. The aqueous concentration of collagen extracted from the hides and the timing of the addition of glutaraldehyde during the preparation of films had significant positive effects on their mechanical properties. The encouraging results of this ongoing research are instrumental to produce commercially viable biobased films. These films could be used in the field of medicine because of their good compatibility with living tissue, and in packaging of food due to their excellent mechanical properties, and accepted edibility.

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