

# EFFECTS OF BATING, PICKLING AND CROSSLINKING TREATMENTS ON THE CHARACTERISTICS OF FIBROUS NETWORKS FROM UN-TANNED HIDES

by

CHENG-KUNG LIU\*, NICHOLAS P. LATONA, MARYANN M. TAYLOR, AND RENÉE J. LATONA  
*U.S. Department of Agriculture\*\**, *Agricultural Research Service*  
*Eastern Regional Research Center*  
 600 EAST MERMAID LANE  
 WYNDMOOR, PA 19038-8598

## ABSTRACT

The U.S. hides and leather industries are facing many challenges today, such as overcoming relatively high U.S. energy and labor costs; 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 efforts to address these new challenges is to develop new uses and novel biobased products from hides to improve prospective markets and to secure a viable future for the hides and leather industries. We hypothesize collagen fiber networks derived from un-tanned hides can be utilized to prepare high performance green composites and air filters, of which both have a great market potential. This study focused on understanding the effects of processing steps such as bating, pickling and crosslinking treatments on the morphology and physical properties of the fiber networks derived from un-tanned hides, which will be the starting material for constructing air filters and green composites. Results showed that glutaraldehyde treatment yielded a highly open structure, in which the fibers are well separated from each other. This could be attributed to the action of acids during the pickling step.

## RESUMEN

Las industrias de las pieles y los cueros de Estados Unidos se enfrentan a muchos retos hoy en día, tales como los abrumantes altos costos relativos de energía y mano de obra en los Estados Unidos; satisfacer los imperativos medioambientales; cuantificar, mantener y mejorar la calidad de las pieles y los actuales productos de cuero, desarrollar nuevos procesos y productos, y mejorar la utilización de los residuos. Uno de nuestros esfuerzos para hacer frente a estos nuevos desafíos es el desarrollo de nuevos usos y nuevos productos de base biológica de las pieles para mejorar los mercados de futuros y para asegurar un futuro viable para la industria de pieles y cueros. Se plantea la hipótesis que redes de fibra de colágeno derivadas de pieles no curtidas se pueden utilizar para preparar compuestos ecológicos y filtros de aire de alto rendimiento, de los cuales ambos tienen un gran potencial de mercado. Este estudio se enfoca en la comprensión de los efectos de las etapas de procesamiento tales como rendido, pickelado y tratamientos de reticulación sobre la morfología y las propiedades físicas de las redes de fibra derivadas de pieles no curtidas, que serán el material de partida para la construcción de filtros de aire y compuestos ecológicos. Los resultados mostraron que el tratamiento con glutaraldehído produjo una estructura muy abierta, en la que las fibras están bien separadas unas de otras. Esto podría atribuirse a la acción de los ácidos durante la etapa de pickelado.

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

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## INTRODUCTION

Globalization of production and markets for raw animal hides and finished leather products has resulted in new challenges to the U.S. hide and leather industries. These challenges include overcoming relatively high U.S. energy and labor costs; 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 efforts to address these new challenges is to develop new uses and novel biobased products from hides to improve prospective markets and to secure a viable future for the hides and leather industries. To improve prospective markets and to secure a viable future for the hides and leather industries, it is important to develop new uses and novel biobased products from hides. We hypothesize collagen fiber networks derived from un-tanned hides can be utilized to prepare high performance green composites and air filters, of which both have a great market potential. Earlier studies were devoted to understand the effects of a key processing step--dehydration on the morphology and physical properties of the resultant fiber networks, which will be the starting material for constructing air filters and green composites.<sup>1</sup> 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. Environmental Scanning Electron Microscope (ESEM) observations also confirmed the fibers were more separated from solvent- and freeze-drying than those from the other dehydration methods. 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 information.

To make quality fibrous products, it is imperative to understand the effects of processing steps such as bating, pickling and crosslinking treatments on the physical characteristics of these fibrous products. This study further investigated the use of crosslinking treatments to improve the integrity of fibrous structures that offer better stability and mechanical properties. Crosslinking is a process to apply a chemical agent--crosslinker to bridge molecular chains, thereby providing stability and improved physical properties to collagen matrices.<sup>2</sup> There are various crosslinking treatments currently being used today for collagen materials. These include ultraviolet light,<sup>3</sup> dehydrothermal treatment,<sup>4</sup> chemical agents such as glutaraldehyde,<sup>5</sup> and enzymatic crosslinking by transglutaminase.<sup>6</sup> In this study, we used two of the most common methods, i.e. transglutaminase and glutaraldehyde to treat the collagen fiber networks with the purpose of stabilizing the structure and improving the physical properties.

## EXPERIMENTAL

### Materials and Procedures

Bovine hides were processed through the bating step and then washed. The hides were then split to approximately 4.8 mm using a Fortuna SAS splitter (Stuttgart, Germany) in order to remove the excess flesh. The bated hides were separated into two groups; one group to be treated with a microbial transglutaminase (mTgase) and the other group to be treated with a modified glutaraldehyde (Glut), which is a mixture of an aliphatic polyaldehyde and high-molecular hydroxide hydrocarbons pretannage. The bated hides to be treated with mTgase were neutralized in 1% acetic acid with 150% float till the pH was between 6 and 7. One set was taken out as the control and the remaining pieces were crosslinked with 5% mTgase (approximately 100 g units/g of product) (Activa TG-TI, Ajinomoto USA, Inc., Paramus, NJ). The other set of bated hides were processed normally to the pickling step, in which they were separated into 2 batches; just pickle as a control and then treated with a 5% modified glutaraldehyde (Sellatan CF new, TFL USA/Canada, Inc., The Woodlands, TX). The pH was then adjusted to 3.8 to 4 in order to fix the pretannage. Rectangular samples, 10- x 1-cm, were cut out parallel to the backbone as described in ASTM D2813.

The hydrothermal stability of hide samples was determined on a Multi-Cell Differential Scanning Calorimeter (DSC) (model CSC-4100) from Calorimetry Sciences Corporation, Linton, UT. Hide samples were prepared for DSC experiments by soaking in distilled water overnight and then blotting on filter paper, except for the pickled sample, which was hydrated in the pickle liquor (pH 2.0). Moist, blotted samples (100 - 250 mg) were weighed into ampoules, along with 250  $\mu$ L distilled water or pickle liquor for the pickled samples. The cells were sealed and placed in the calorimeter. The calorimeter was programmed to record heat flow as mcal/ $^{\circ}$ C while the temperature was increased from 10 $^{\circ}$ C to 180 $^{\circ}$ C at 1.0 $^{\circ}$ C/min with an equilibration period of 600 s at the start. The temperature at the peak of the calorimetry trace,  $T_p$ , was considered to be an apparent shrinkage temperature. The remaining samples were then either frozen then vacuum dried or lyophilized. The samples to be frozen then vacuum dried were placed in a refrigerator freezer overnight and then placed into a vacuum oven (Thermo-Scientific Model 3608-5, Dubuque, IA) set at room temperature ( $\sim$ 21 $^{\circ}$ C) and 96.6% vacuum (3.4 kPa absolute pressure) for 24 h. The samples to be lyophilized were placed individually in test tubes and then distilled water was added so the water level was approximately 2.5 cm above the sample. The test tubes were covered with filter paper, secured with a rubber band and then partially immersed in dry ice and acetone to freeze the water surrounding the strips. Once the water in all of the tubes was completely frozen, the test tubes were placed in a flask and then connected to a freeze drier (Labconco, Model number

7740021, Kansas City, MO) until the samples were dry. Dehydrothermal crosslinking was also carried out on bated samples, 10- x 1 cm, which were placed into a vacuum oven set at ~142°C for 24 h.

### Mechanical Property Evaluations

Apparent density was measured after each crosslinking condition in the dried state, which is defined as the weight per unit volume of a material including voids inherent in the material as tested. The final weight of the dehydrated samples was measured with an Adventure Pro scale (Ohaus Corp., Pine Brook, NJ). A standard ruler and caliper were used to measure the length, width and thickness. Mechanical property measurements included tensile strength, elongation, Young's modulus and fracture energy. Tensile strength is the stress in tension that is required to fracture the leather. Fracture energy is defined as the energy needed to fracture leather samples. This physical quantity is sometimes mentioned as "toughness". These properties were measured with a grip separation of 5 cm and a 5 cm/min strain rate (crosshead speed). An Insight 5 test frame and Testworks-4 data acquisition software (MTS Systems Corp., Minneapolis, MN) were used throughout this work. Each test was conducted on a minimum of five samples to obtain an average value.

### Acoustic Emission (AE)

To achieve a better understanding of the stress-strain behavior for a fibrous material in this study, AE measurements were simultaneously performed with the tensile stress-strain tests for the samples previously described. Hides are a fibrous material and as the hides are squeezed, torn or stretched by an external force, it is accompanied by a rapid movement, relocation, or breaking of structural elements such as fibrils, fibers and/or fiber bundles. As a result, sound waves are produced that can be detected by an acoustic transducer and converted into electronic signals. When the transducer detects a signal over a certain threshold (40 dB), an AE event is recorded, and this AE event is then translated by an AE analyzer as a "hit." A small piezoelectric transducer was clipped against the sample and this transducer resonates at 150 kHz (Model R15, Physical Acoustics Corp., Princeton Junction, NJ). AE signals emanating from the samples were processed into an 18 bit analog to digital PCI board with a 40 x 106 samples/sec data acquisition rate and analyzed using the software AE WIN (Physical Acoustics Corp, Princeton Jct, NJ ). This AE system has been used for studying the deformation and fracture mechanisms of fabrics, leather and green composites, in which the typical test samples were dumbbell or rectangular in shape with a thickness less than 3 mm.

### Microscopic Observations

A field-emission environmental scanning electron microscope (ESEM) was used to compare the structural difference between fibrous materials prepared with various dehydration methods. The ESEM is advantageous over conventional

scanning electron microscopy (SEM) because a relatively high vacuum in the specimen chamber is not needed, which prevents atmospheric interference with primary or secondary electrons; an ESEM may be operated with a poor vacuum (up to 10 Torr of vapor pressure, or one seventy-sixth of an atmosphere) in the specimen chamber. The ESEM was operated at low vacuum (0.3 Torr) with the voltage set at 15 kV, spot size 5.0 and working distance of approximately 10 mm. Samples were uncoated, thus preserving the original characteristics of the samples.

## RESULTS AND DISCUSSION

Figure 1 shows the various treatments greatly affected the morphology of the fibrous materials as shown in the ESEM cross-sectional micrographs for the freeze/vacuum dried fibrous networks obtained from (a) bating, (b) mTgase, (c) pickling, and (d) Glut treatments. The samples dried after bating (Figure 1a) show a very compact structure, in which there is no individual fibers to be observed. It appears that some impurities, glue-like materials, were left in the hides after bating, thus cementing the fibers together. On the other hand, Figure 1b, samples treated with mTgase show some degree of fiber separation. Interestingly, the pickling treatment yielded a very evident fibrous network structure (Figure 1c), where fibers were well separated from each other. This is sharply different from samples after either bating (a) or mTgase treated (b). The most evident fibrous structure is seen in Figure 1d, which is from the Glut-treated samples. This behavior may be ascribable to the dissolution action of impurities by acids that occurred in pickling and later in the Glut-treatment.

One of the important properties in a fibrous structure associated with the resultant fibrous products is the degree of fiber separation, which commonly is determined by measuring its apparent density. This data provides insight into the effect of processing and crosslinking treatments on the fibrous structure. The lower the apparent density the better fiber separation is achieved and the more desirable the fibrous structure will be. Figure 2 demonstrates that the Glut-treated samples yielded relatively lower apparent densities. As shown in Figure 1d, the fiber bundles are well separated from each other, and therefore resulted in a relatively lower apparent density. Figure 2 however, shows that the effect of treatments does not appear to be significant among bated, mTgase-treated, and pickled samples. This could be due to they were all dried in the same way (frozen first and then vacuum dried), and drying has a much more profound effect than the treatment does in this case. This is in contrast to our previous study with the different drying methods, which showed there was a more pronounced difference in the resultant apparent density.

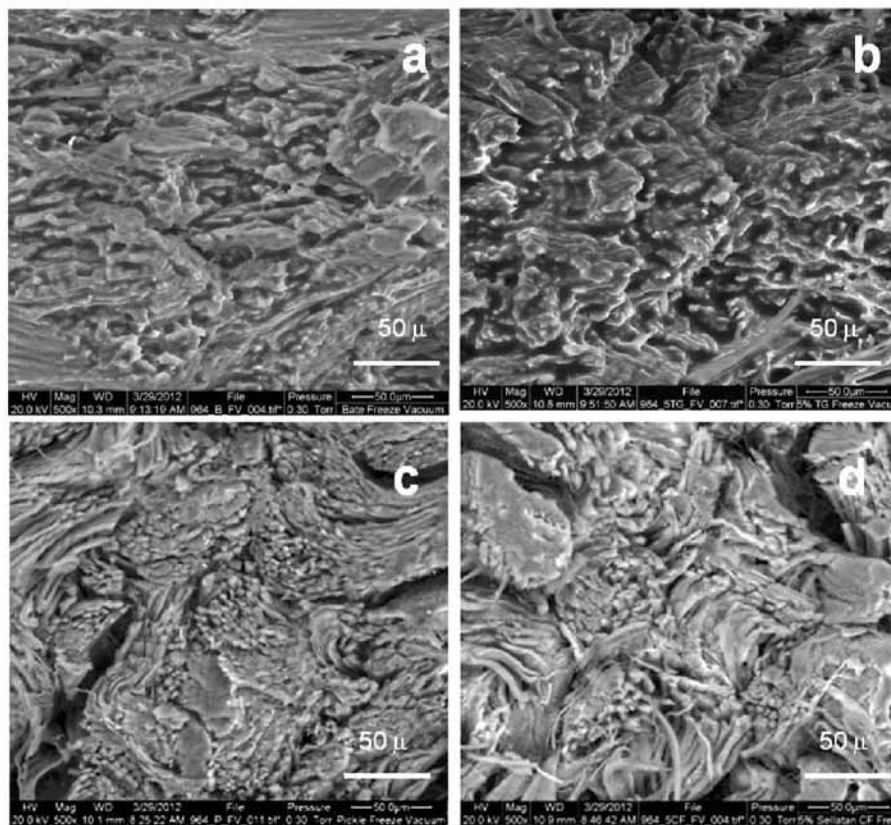


Figure 1. Micrograph of cross-section of fibrous samples after (a) bating, (b) mTgase, (c) pickling and (d) Glut treatments.

The apparent shrinkage temperature ( $T_s$ ) was measured using the DSC. As shown in Figure 3, the shrinkage temperature did not change significantly with bating and the treatment of mTgase;<sup>7</sup> however,  $T_s$  dropped when the samples were pickled and then increased with the Glut-treatment. The drop in  $T_s$  after pickling is probably due to the effect of acid and the hide opening up, whereas the increase after Glut treatment is attributable to the effects of crosslinking. This behavior has been reported in which an acid swollen collagen in the absence of salt at a pH 1.5 can shrink at temperatures below 40°C, while at the same pH the addition of salt can raise the shrinkage temperature to almost 70°C.<sup>8</sup> This behavior was observed in which the shrinkage temperature of the pickled hide using distilled water was 52°C and the shrinkage temperature using the pickle liquor was 59°C. To further prove this we soaked the pickled hide in an 8% salt solution at pH 1.9 over night and the shrinkage temperature was determined to be 69°C. The addition of salt increased the shrinkage temperature, however for our end application of air filters and green composites the salt residue would most likely have to be removed before additional processing if it is not crosslinked using Glut or other wet white tanning materials.

### Mechanical Properties

Figure 4 displays the resultant mechanical properties of samples with various treatments. Measurements show mTgase-treated

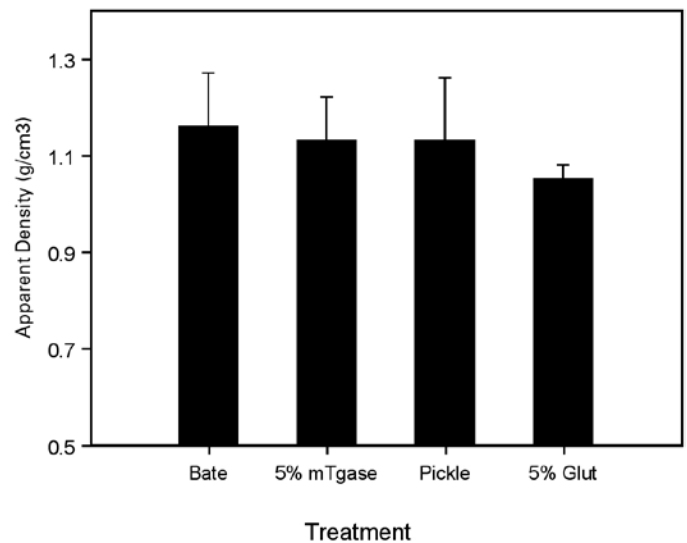


Figure 2. Apparent density vs. treatments.

samples yield the highest tensile strength. Glut-treated samples, on the other hand, show the lowest tensile strength. There does not appear to be much difference in the tensile strength between the bated and mTgase-treated samples. But the tensile strength does decrease for the pickled and Glut-treated samples. This is probably due to the relatively low apparent density of these two samples resulting from the acid swelling.

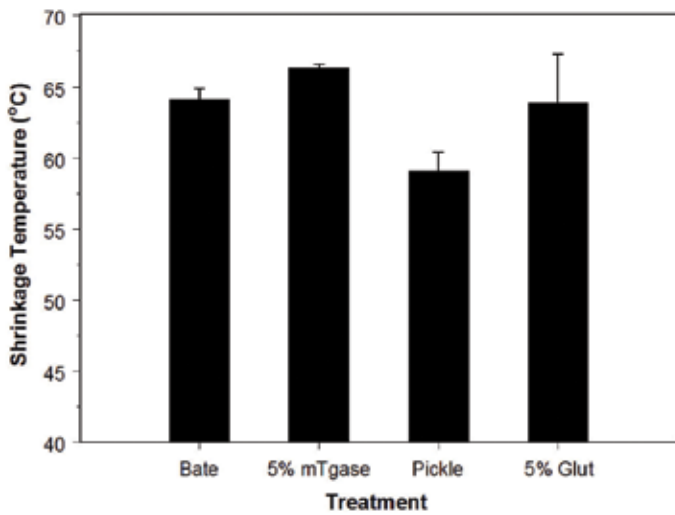


Figure 3. Apparent shrinkage temperature vs. treatment.

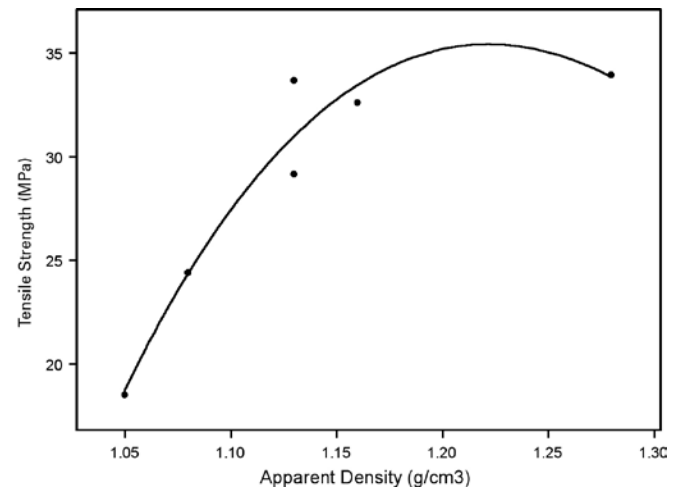


Figure 5. Apparent density vs. tensile strength.

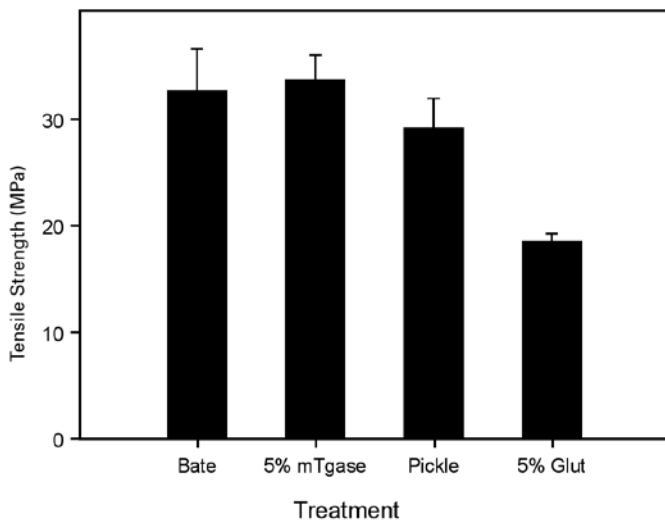


Figure 4. Tensile strength vs. treatment.

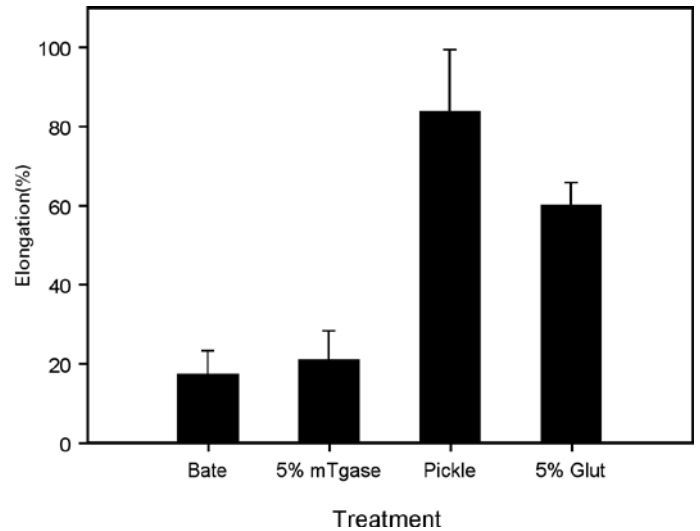


Figure 6. Elongation vs. treatment.

A close relationship is clearly demonstrated in Figure 5 that the denser structure produces the greater tensile strength. The higher apparent density indicates increased concentration of load-bearing collagen fibers, thereby yielding greater tensile strength.

Figure 6 showed that samples with a more evident fibrous structure such as those shown in Figure 1c and Figure 1d have greater elongations. This is in contrast to the samples in Figures 1a and 1b in which the fibers are much more fused together preventing them from stretching and realigning when the samples were strained.

Figure 7 presents the effects of treatment methods on the stiffness of the samples. Stiffness is quantified by measuring the Young's modulus, which is the initial slope of the stress-strain curve. As expected the bated samples yielded the highest stiffness, whereas the Glut-treated samples produced the softest samples.

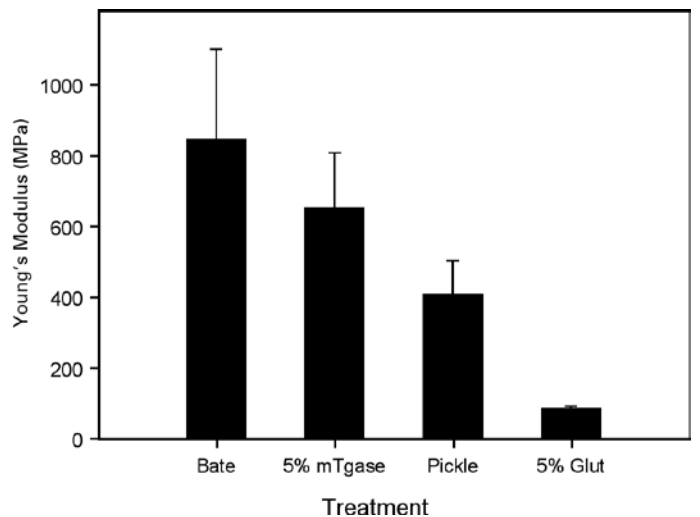


Figure 7. – Young's modulus vs. treatment.

Toughness has been described in a previous report as a quantity associated with the product of tensile strength and elongation.<sup>9</sup> The application of fibrous materials often requires good toughness, which reflects a superior balance of strength and flexibility with good deformability, thereby minimizing the stress concentration and yielding a better tearing strength. Toughness was characterized by measuring the energy needed to fracture a sample — fracture energy, which is obtained by integrating the area under the stress-strain curve. As shown in Figure 8, the pickled and Glut-treated samples yield better toughness values than those of the bated and mTgase-treated samples. This is ascribed to the higher degree of fiber separation in the fibrous structure produced from pickling and Glut treatments. A better fiber separation leads to a more even stress sharing and results in a higher energy required to fracture the samples. There does not appear to be a difference with the bated and mTgase samples. Also we see a decrease in the toughness with the addition of the Glut, which could be due to the crosslinking effect of the Glut on the collagen fibers.

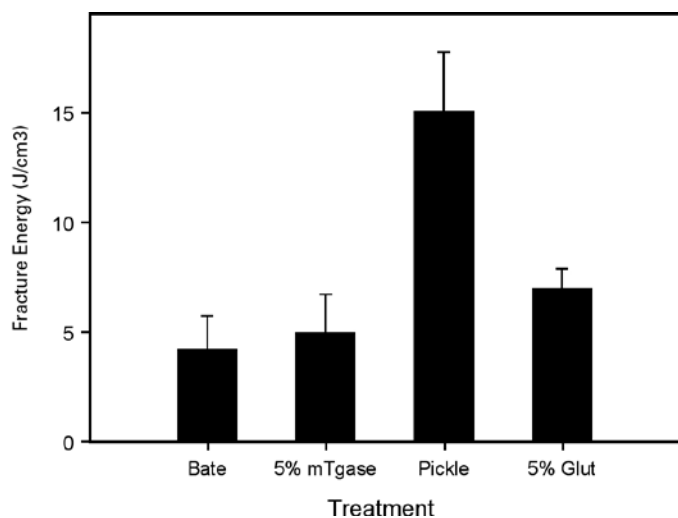


Figure 8. Fracture Energy vs. treatment.

**Acoustic Emission Studies**

Acoustic emission (AE) tests were performed simultaneously with the tensile tests because the AE results may reveal some structural information that other methods cannot offer. A chronological course of the test is displayed in Figure 9, which demonstrates the hits rate vs. time profiles during tensile testing of the samples. As demonstrated in Figure 9, the bated and mTgase samples yield only narrow peaks, whereas pickled and Glut-treated samples produce a peak followed by a creep in the hits rate (hits/sec) vs. time (sec) curve. The creep in the hits rate curve is probably ascribable to the softness of the structure that does not fracture but only elongates. A well separated fibrous structure produces more acoustic activities as demonstrated in Figure 9c-d, in which the pickled- and Glut-treated samples generate more AE hits compared to the first two treatments. This is due to the more pronounced

fibrous structure produced by either the pickled- or Glut-dried treatments. Figure 10 shows cross-section micrographs with the corresponding hits rate curves of the fibrous samples after (a) pickling and (b) dehydrothermal treatments. The high heat in the dehydrothermal treatment caused the fibers to be fused together and make the sample very brittle, as shown in Figure 10b, resulting in a very small and narrow AE peak. The pickled sample on the other hand produces more of an open structure and yielded a much broader AE peak.

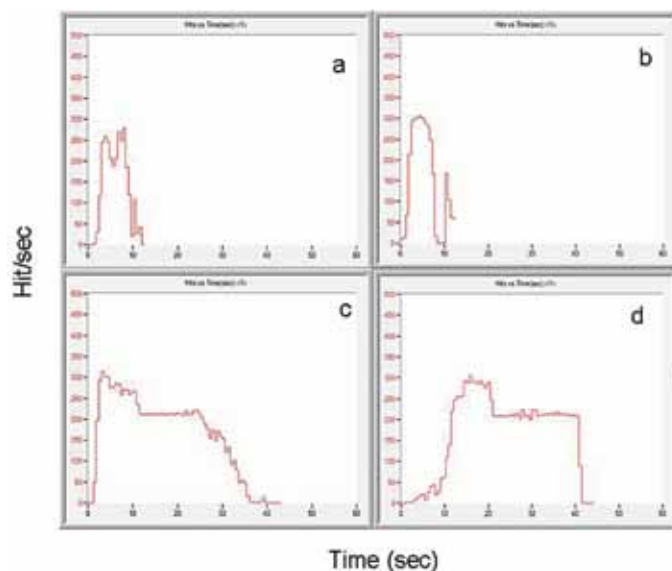


Figure 9. Hits rate vs. time curves for samples after (a) bating, (b) mTgase, (c) pickling and (d) Glut treatments.

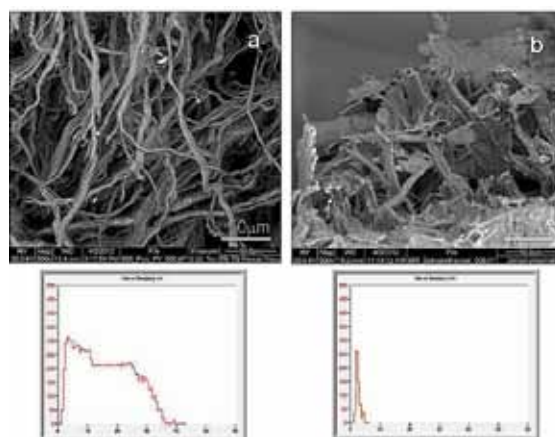


Figure 10. Micrograph of cross-section with corresponding hits rate curves of fibrous samples after (a) pickling, (b) dehydrothermal treatments.

**CONCLUSIONS**

This study was devoted to understand the effects of bating, pickling and crosslinking treatments on the morphology and physical properties of the fiber networks derived from un-tanned hides, which will be the starting material for

constructing our proposed air filters and green composites. Results showed that glutaraldehyde treatment yielded a highly open structure, in which the fibers are well separated from each other. This could be attributed to the action of acids during the pickling step prior to the glutaraldehyde treatment. In addition, the environmental scanning electron microscope images and acoustic emission results demonstrate the significant effect of the pickling step on the opening the collagen fiber structure in un-tanned hides.

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