Genipin-Modified Gelatin: Preparation, Characterization, and Application as a Filler for Leather

M.M. Taylor[1], K. Ding[2], and E.M. Brown[1*]

[1]USDA, ARS, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038
[2]College of Chemistry & Environmental Protection Engineering, Southwest University for Nationalities, Chengdu 610041, P.R. China

Abstract: Genipin, an iridoid compound extracted from gardenia fruits (*Gardenia jasminoides* Ellis) is a natural crosslinking agent. Because of its low cytotoxicity, genipin is increasingly used to replace both glutaraldehyde and formaldehyde as a crosslinker for proteins. Researchers have in recent years successfully demonstrated the effective use of genipin in the modification of gelatin, particularly for its use in biomedical products. We have recently reported the appropriate conditions (i.e., temperature, pH and concentration) necessary for reaction of genipin with powdered and intact hides. In this present study, we applied those reported conditions to gelatin. We investigated the crosslinking capability of genipin with gelatin to identify the parameters necessary to obtain products that could be used as fillers in leather processing. The crosslinked products should preferably possess physical properties that are compatible with the temperatures used during leather processing (28-50°C). We next applied the appropriate products to blue stock before we retanned, colored and fatliquored the hides. The properties and quality of the resultant leather were then evaluated. Using epi-fluorescent microscopy, we verified that the genipin-crosslinked gelatin was uniformly distributed through the blue stock and was not removed during washing; furthermore, Scanning Electron Microscopy (SEM) showed that the fibers appeared to be coated with the product. The physical properties of the genipin/gelatin products, as well as mechanical properties and subjective evaluation of the treated, final crust leather, will be presented along with representative epi-fluorescent microscopy and SEM images. Based on these results, we propose that genipin-modified gelatin has the potential to provide an environmentally safe alternative to the more conventional post tanning processes.
Key words: genipin, gelatin, fillers, leather

1. Introduction:

Genipin is a naturally occurring iridoid compound extracted from gardenia fruits (*Gardenia jasminoides* Ellis). Because of its low cytotoxicity, it is replacing both glutaraldehyde and formaldehyde as a crosslinking reagent. Researchers, in recent years, have successfully demonstrated the effective use of genipin in the modification of gelatin, particularly for its use in biomedical products.[1-5] Nickerson et al. have reported on the physical properties of genipin-crosslinked gelatin-maltodextrin hydrogels[6-7] as well as on the possible mechanisms and optimal reaction conditions for fixation between genipin and gelatin[8].

Prior research from our laboratory has demonstrated that glutaraldehyde-modified gelatin could be used as a filler in leather processing.[9] Glutaraldehyde treatments, although effective, require the handling of a toxic reagent. We decided to examine genipin-modified gelatin products and determine if these would also be applicable as fillers. In recent publications, we have reported the appropriate conditions (i.e. temperature, pH, and concentration) necessary for reaction of genipin with hide powder and intact hides.[10-12] In this present study, we applied those reported

*Corresponding Author: Phone: 215-233-6481. Email: ellie.brown@ars.usda.gov
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conditions, except for genipin concentration which had been significantly lowered when applied to gelatin, as reported by Nickerson.[8] We investigated the crosslinking capability of genipin with gelatin and the parameters necessary to obtain products that could be used as fillers, products with physical properties which would be compatible at temperatures used during further leather processing (28-50°C). We next applied the appropriate products to blue stock, retanned, colored and fatliquored (RCF) the hides, and evaluated the resultant crust leather. The physical properties of the genipin/gelatin products as well as mechanical properties and subjective evaluation of the treated, crust leather will be reported. Epi-Fluorescent imaging and scanning electron microscopy (SEM) studies were also performed and representative images will be shown. Finally, the effect of genipin/gelatin treatments on hydrothermal stability of leather will also be reported.

2. Experimental

2.1 *Materials:* Genipin (MW = 226.23, 98% by HPLC) was purchased from Challenge Bioproducts Co. Ltd., Taiwan, ROC, and used without further preparation. Commercial Type B gelatin from bovine skin, characterized in this laboratory as 175 grams Bloom, was obtained from Sigma, St Louis, MO. Trutan PA-65 and PRP-77 were obtained from the former Pilar River Plate Corp. (Newark, NJ); Havana Dye (Derma Havana R Powder) and Sandozol Green 5BT powder were obtained from Clariant Corporation (Charlotte, NC); Altasol-CAM and Eureka 400R were obtained from Atlas Refinery, Inc. (Newark, NJ). Chrome-tanned blue stock (upholstery weight) was purchased from a local tannery. All other chemicals were analytical grade and used as received.

2.2 Methods:

2.2.1 Preparation of genipin-modified gelatin and blue stock treatment

Genipin-modified gelatin was prepared as shown in Figure 1 in which reaction times ranged from 0 to 24 h and genipin concentrations ranged from 0 to 0.7%. Blue stock was sampled from the butt, belly and neck areas (two pieces were cut from each area, one for test and one for control). Treatment of blue stock, (Trials A, B, and C), optimally with 0.4% genipin, pH=7.5, @ 35°C for 4 h,, is shown in Figure 2; the samples were then RCF as described in Figure 3.

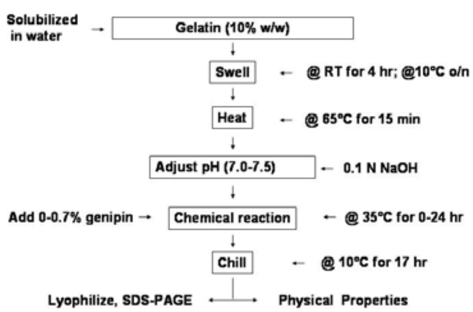


Figure 1: Preparation of Genipin-modified Gelatin*

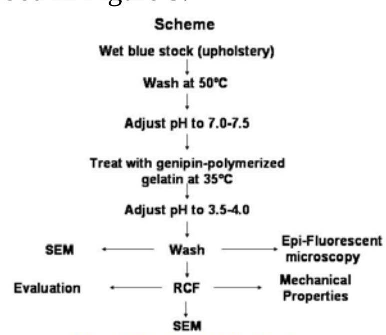


Figure 2: Treatment of Blue Stock*

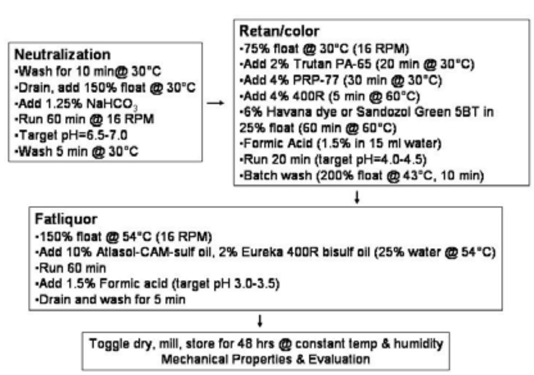


Figure 3: RCF of Blue Stock*

2.3 Analyses:

2.3.1Product characterization, extractables and hydrothermal stability (Ts):

Gel strength, melting point (MP), viscosity, and molecular weight distribution (by SDS-PAGE) of genipin modified gelatin products were determined as described in previous publications.[13-14] Percent extractables in RCF samples was determined as described in ASTM 3495-83. Hydrothermal stability of the blue stock before and after treatment and after RCF were measured.[15]

2.3.2 Microscopic Studies:

Genipin-gelatin has fluorogenic properties [16] (emission @ 610 nm) and the treated blue stock was examined for effectiveness of filler uptake using an epi-fluorescent microscope. Scanning electron microscopy (SEM) studies were carried out on blue stock before and after treatment with genipin gelatin products. Both of these techniques were described previously.[17]

2.3.3 Mechanical Properties:

The samples were stored in a conditioned room at 20°C and 65% relative humidity according to ASTM D1610-01. Mechanical property measurements were performed parallel to the backbone with a strain rate of 10 in/min and a gage length of 4 inches. The mechanical property measurements included: thickness, tear strength, tensile strength, elongation, Young's Modulus and toughness index. [17] An upgraded Instron mechanical property tester, model 1122, and Testworks 4 data acquisition software (MTS Systems Corp., Minneapolis, MN) were used throughout this work.

2.3.4 Subjective evaluation and yellowing test:

Each treated and untreated sample was evaluated with respect to handle, break, fullness and color. A value from 1 to 5 was assigned for each parameter, with 1 being the worst and 5 being the best. From these values, an overall evaluation was determined and this value was reported. Yellowing tests were performed on 3 inch (76 mm) square pieces which were heated at 120°C for 72 h. Ratings compared to a control were on a scale of 1 to 5, with 1 being the worst (highest color change) and 5 being the best (least affect on color).

3 Results and Discussion

3.1 Product preparation and characterization

Recent research[1-8, 10-12] has defined some of the parameters for genipin reaction with gelatin and collagen, such as optimal pH and temperature. Our ultimate goal, the preparation of a filler for leather, necessitated we prepare a product with physical properties, in particular its viscosity, which would reflect a not-too-high-degree of polymerization, and thus would allow the product to be easily handled at temperatures in which leather was processed (e.g., 28 to 50°C). Therefore, starting with the reported conditions of pH 7.5 and temperature of 35°C, our first objective was to react 175 Bloom gelatin with 0.5% genipin, and determine the effect of time (0-24 h) on physical properties, e.g. gel strength, viscosity and melting point.[17] Next, while keeping the pH, temperature, and time constant (4 h), we examined the effect of genipin concentration (0-0.7%) on these physical properties. From these studies we determined that a feasible product could be made by using 0.4% genipin on a 10 % w/w gelatin solution at pH 7.0-7.5 and running the reaction for 4 h.[17] The physical properties of this product are shown in Table 1 and show that the gel strength, melting point and viscosity have increased over that of an untreated control sample. The molecular weight distribution (Figure 4) indicates that the bands representative of gelatin, from 14.4 KDa to 116.2 KDa, have diminished, and, at the same time, the band that does not enter the gel has increased in intensity.

3.2 Product application, RCF and mechanical properties

Blue stock was treated with this product (Figure 2) and the initial studies were aimed toward examining whether the filler was distributed evenly throughout the hide and not removed during washing. Genipin protein products have fluorogenic properties[16] and we used this characteristic to examine the treated blue stock using the epi-fluorescent microscope. Representative images of

Table I
Physical Properties of genipin-modified gelatin[a]

	Average[b]	StdDev
Gel Strength[c] (g)	502.2	54.2
MP[d] (°C)	42.3	0.5
Viscosity[e] (cP)	17.2	2.3

[a]10%gelatin, 0.4%genipin, pH=7.5, temp=35°C, time=4h; [b] n=3
Control Sample: [c]gel strength=411.6 g; [d]MP=37.3°C; [e]viscosity=5.7 cP

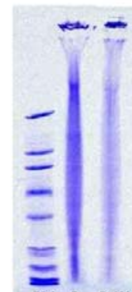


Figure 4. SDS-PAGE of genipin-modified gelatin

treated and untreated blue stock are shown in Figure 5 (emissions @ 519 and 610 nm). These studies confirmed that indeed the hides were filled. We next applied the product to butt, belly and neck areas of hide. During these studies we took samples before and after treatment for SEM studies and as seen in Figure 6, the treated sample's fibers appear to be more separated and the fibers appear to be coated when one compares this image to the untreated control sample.

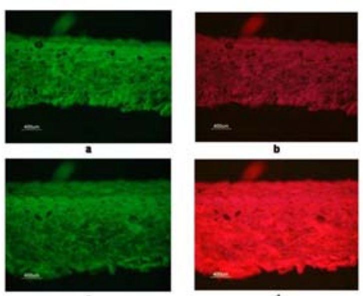
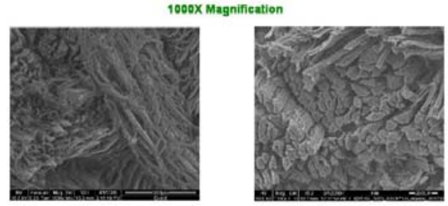


Figure 5: Epi-Fluorescent Micrographs of Untreated (a & b) and Genipin-Gelatin Treated (c & d) Blue Stock

1000X Magnification



Blue stock to which no filler was added, just pH adjusting agents

Blue stock to which genipin-polymerized gelatin was added as filler

Figure 6: SEM of Untreated and Genipin-Gelatin Treated Blue Stock

The hides were subsequently RCF (Figure 3) and the resulting products were analyzed for mechanical and subjective properties. A summation of mechanical properties is shown in Figure 7 and one can see that when the data from each area for each mechanical property (thickness, tensile strength, percent elongation, Young's Modulus, toughness index (TI) and the calculation of the tear strength) are averaged, the data from three experiments show no significant difference between the test samples and the control samples (Figure 7). Taken as a whole, the mechanical properties of the genipin/gelatin-filled leather samples are not significantly different from those of the control samples, indicating that the addition of the filler does not adversely affect these properties.

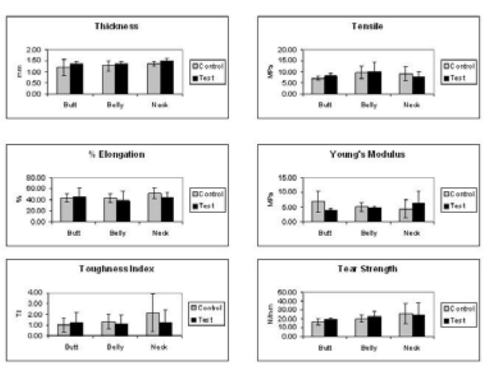


Figure 7: Mechanical Properties of Leather*

3.3 Product evaluation and hydrothermal stability (Ts)

All samples were evaluated with respect to handle, break (grain), fullness and color. The samples were rated on a scale of 1 to 5, with 1 being the worst and 5 being the best. From these ratings an overall rating was given and these results are summarized in Figure 8. Of the 15 pairs analyzed (5 from each trial) 8 pairs (53 %) of tests were better than the control, 4 pairs (27%) were equal to control, and 3 pairs (20%) in which controls exceeded the tests. In general, these evaluations showed that the test samples fared better than the controls, particularly in the neck area.

The yellowing test was performed on all samples. The samples from Trials A and B were treated with a medium brown dye, and, the test samples did more poorly than the controls because the addition of proteins after tanning may have an adverse effect on this test, particularly if a lighter dye is used.[18] The average evaluations of Trials A and B are shown in Figure 8. In Trial C, samples were treated with a dark green dye, and after testing no differences between the tests and the controls in these samples could be seen; thus no evaluation was performed.

Finally, the hydrothermal stability or shrinkage temperature of genipin/gelatin-treated blue stock, untreated control samples and their respective retanned, colored and fatliquored samples was determined, and as seen in Table II the shrinkage temperature of the treated samples increased almost four degrees to 100°C, and sustained a three minute boil. There may be enough unreacted genipin remaining in the prepared polymer which could possibly be reacting with the collagen to increase the hydrothermal stability, an attribute of genipin and combination genipin tannages, described by Ding et al. in recent publications.[10-12] However, after retanning, coloring and fatliquoring, the shrinkage temperature of the samples dropped, a phenomenon described earlier by Kronick et al.[19]

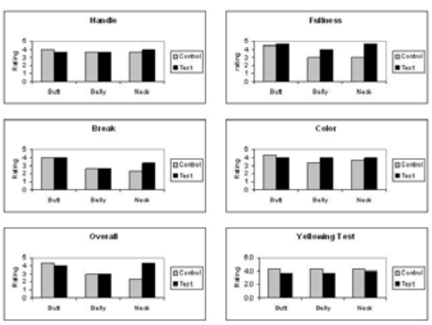


Figure 8: Subjective Evaluation and Yellowing Test of Leather

Table II
Shrink Temperature (Ts)
Genipin/Gelatin Treated Blue Stock*

Sample[a]	Ts Blue Stock[b]	Ts RCF[c]
Control (pH adj)	95.5°C	81.5° C
Test	100°C[d]	83° C

[a]Washed 2x's, [b]N=4; [c]N=2; [d]Sustained a 3 minute boil

4 Conclusions

This study has shown that genipin-modified gelatin has the potential to be used as a filling agent for leather. Optimum parameters for producing a product that would be amenable (melting point and viscosity) to working in the post tanning processing of leather were determined. The combination of genipin and gelatin gave a product with fluorogenic properties and we were able to monitor filling effectiveness using epi-fluorescent microscopy. RCF-treated blue stock was analyzed for mechanical and physical properties and the mechanical properties were not significantly different from those of the control pieces and, with respect to subjective evaluation, the treated products fared better than the controls. The hydrothermal stability of the blue stock and RCF samples was also determined and there was a significant improvement in the shrink temperature in the genipin/gelatin-treated blue stock samples. In the RCF samples, there was a drop in hydrothermal stability in both the control and test samples, but the test samples' shrinkage temperature still remained higher than the controls. SEM images showed that the fibers of the filled blue stock samples appear to be separated and the structure is more open. These studies show that genipin-modified gelatin has the potential to provide an environmentally safe alternative to the more conventional post tanning processes.

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