Treatment of Hides with Tara-modified Protein Products*

by

M.M. Taylor**, M.B. Medina, J. Lee, L.P. Bumanlag, N.P. Latona, E.M. Brown, and C.-K. Liu
United States Department of Agriculture, Agricultural Research Service
Eastern Regional Research Center
600 E. Mermaid Lane, Wyndmoor, PA, 19038

Abstract

In prior research, we demonstrated that gelatin could be modified with quebracho to produce products whose physicochemical properties would enable them to be used effectively as fillers in leather processing, and that leather resulting from this treatment had improved subjective properties with little effect on mechanical properties. In an extension of the study, the tannin, tara was examined for its potential in gelatin modification. The advantage for using tara is that it gives an almost colorless product, which would be desirable in production of light colored leather, as well as imparting light fastness to the leather. The conditions for optimal tara modification of gelatin were determined and the products characterized. In this present study, these tara-modified gelatins were evaluated as fillers in the treatment of wet blue and wet white. In addition, the rate of uptake of the product was also examined using an analysis developed at ERRC for the measurement of polyphenols in foods. It was found that the treated leathers, when evaluated for their subjective properties (handle, fullness, break and color), demonstrated improved properties. There were no significant differences in treated and control samples of wet blue and wet white, with respect to the mechanical properties (tensile strength, elongation, Young's Modulus, toughness index and tear strength). SEM examination of fiber structure showed differences in treated and untreated samples. Thus, another sustainable, economical resource, the polyphenolic tara, in conjunction with gelatin, has further shown its potential for use in leather production.

Introduction

The use of polyphenols in protein modification with emphasis on the effect that the polyphenols could have on physicochemical properties of modified gelatin,[1-6] has been discussed extensively in current literature. Polyphenols, extracted from plant materials, have been employed in vegetable tanning, primarily for the production of heavy leathers used in saddles, belts and shoe soles.[7,8] With respect to gelatin modification by polyphenols, it has also been reported that some vegetable tannins could be applied to gelatin to give products with interesting physical properties[9] and similarly, our investigations using the vegetable tannin quebracho led to successful application in the modification of gelatin.[10] We used the ensuing products as fillers to treat blue stock, yielding leather with improved properties.[11] In addition we applied a method, developed at ERRC[12,13] to analyze for phenolics in food, to the analysis of quebracho, and, after a slight modification, we were successful in measuring the uptake of the gelatin/quebracho product by the leather.[11] We recently described processes for modification of gelatin and whey protein concentrate (WPC) with the vegetable tannin tara and with the polyphenolic gallic acid.[14] The advantages for using tara are that it is a truly sustainable resource, and gives an almost colorless product, which is desirable in production of light colored leathers, as well as imparting light fastness to the leather[15-18] We found that these gelatin/tara products had appropriate properties so that they could be used as fillers.

*Presented in part at the 108th annual American Leather Chemists Association Meeting, June 7-10, 2012, Grandover Resort and Conference Center, Greensboro, NC

**Corresponding author e-mail: maryann.taylor@ars.usda.gov

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture (USDA). USDA is an equal opportunity provider and employer.

Manuscript received August 8, 2013, 2013, accepted for publication October 16, 2013

In the aforementioned study,[14] we determined that gelatin could be treated with tara and gallic acid at pH 9.5-10.0, 45°C for 4 h and resulting products showed changes in physicochemical properties. In this continuing study we treated wet blue and wet white with the gelatin/tara product and, after retanning, coloring and fatliquoring (RCF) assessed the effect that these treatments had on subjective and mechanical properties. Tara uptake was monitored,[12,13] as was the quebracho in previous study.[11] We furthered examined the product for fluorescent properties and, if present, to determine if this technique could be applied to monitoring product uptake. The resulting leather was also examined by scanning electron microscopy (SEM) to determine the effect of treatment on fiber structure.

EXPERIMENTAL

Materials

Commercial Type B gelatin from bovine skin, characterized in this laboratory as 175 grams Bloom, was obtained from Fisher (Fairlawn, NJ). Gallic acid and Fast Blue BB (FBBB) (4-benzoylamino-2,5-diethoxybenzenediazonium chloride hemi-[zinc chloride]) salt were obtained from Sigma (St. Louis, MO). Tara was obtained from Hermann Oak Leather Company (St. Louis, MO). Chrome-tanned stock (shoe upper) and chrome-free stock (upholstery weight) were purchased from local tanneries; area pieces were sampled from this stock. All other chemicals were analytical grade and used as received.

Application of Tara/Gelatin Product to Wet Blue and Wet White Stock (Area Samples)

Gelatin/tara products, using 10% w/v gelatin (175 Bloom) and 4% tara at pH 9.5-10.0, 45°C for 4h, were prepared as described in a previous publication[14] and were applied to wet blue and wet white stock as follows. Samples of the wet blue and wet white hides were selected for area and epi-fluorescent study. For wet blue hides, four pieces, two controls and two tests, approximately 1 foot square, were each cut sequentially from the butt, belly or neck area, and two pieces were added per drum. For wet white hides, six pieces, three tests and three controls, approximately 1 foot square, were also cut sequentially from the butt, belly or neck area, and three pieces were added per drum. The wet blue and wet white samples (tests and controls, ~650 g/drum) were placed in small Dose drums (Model PFI 300-34, Dose Maschinenbau GmbH, Lichtenau, Germany), washed (400% float based on hide weight) by drumming for 30 min at 50°C, drained and refloated in sodium bicarbonate (~1% on hide weight in 400% float). The samples were drummed at ambient temperature (25-28°C) until the pH stabilized (6.5-7.0). The floats were drained, the control samples set aside. To the test samples, the tannin product (10% gelatin, based on hide weight, modified with 4% tara, based on gelatin weight in 300 or 400% float) was added. The samples were then drummed for 1 h at ambient temperature and then for 4 h at 45°C. The floats were drained and the samples were washed twice for 10 min at 45°C (400% float), drained, patted dry, and stored at 4°C. The tests and controls were retanned, colored and fatliquored (RCF) using either a shoe upper formula (wet blue) or upholstery formula (wet white) as described in prior publications[19-21]. When completed, the wet blue samples were vacuum dried (at 60°C for 6 min) and then hung to dry at ambient temperature and humidity, conditioned and staked. The wet white samples were toggle stretched at ambient temperature and humidity, conditioned, staked and milled. The samples were subjectively evaluated. No finishing operations were done to the hide pieces and they were kept in a shelf in the conditioning room, at 20°C and 65% relative humidity for at least 3 days.

Analyses

Physical Properties, Mechanical Properties and Molecular Weight Distribution

Gel strength, melting point, and viscosity of the tara and gallic acid-treated proteinaceous solutions were determined as described in a previous publication[22]. Mechanical properties (tensile strength, elongation, Young's Modulus, toughness index, tear strength, and thickness) were determined as described in a previous paper[23]. Protein molecular weights were estimated as described previously[24]. In summary, SDS-PAGE (polyacrylamide gel electrophoresis in sodium dodecyl sulfate) was run using precast 4-15 percent gradient gels. A broad range (BRS) calibration standard (Bio-Rad, Hercules, CA), which contains a mixture of nine proteins ranging in size from 6,500 to 200,000 Daltons, was used. Samples of lyophilized protein were dissolved in sample buffer (10 mM Tris-HCl at pH 8.0 containing 1 mM EDTA, 2.5% SDS, 5% β-mercaptoethanol and 0.01% bromophenol blue) and were then heated at 40°C for 4 h. Separation was achieved using a Phast-Gel System (Pharmacia Biotech Inc., Piscataway, NJ). Gels were stained with Coomassie Blue (Pharmacia).

Phenolics Assay

Wet blue and wet white hides were treated with gelatin/tara product (10% gelatin based on hide weight and 4% tannin based on gelatin weight). To monitor the uptake of the gelatin/tara product by the wet blue or wet white, 10 mL aliquots of the floats were removed from the drums every hour from 0 to 5 h. The solutions were then subjected to modified phenolic assay[12-13] as follows. Gallic acid standard or the reaction solutions (1 mL) were transferred to borosilicate test tubes. A control blank was prepared and it contained all reagents but the standard (gallic acid), instead deionized water was added. Diazonium salt (FBBB) (0.1 mL of 0.1% solution) was added and mixed for 1 min. Sodium hydroxide (0.1mL of 5% solution) was added and the reaction was run at room temperature, vortexed for 30 sec every 30 min, for either 90 or 120 min. Each sample (2 x 200 uL) was transferred to microtiter plates and the absorbance was read at 420 nm. The

concentration of gelatin/tara product remaining in the reaction solution was estimated from the standard curve for gallic acid.

Subjective Evaluation RCF Leather

Each treated and untreated sample of wet blue or wet white hide was evaluated (one evaluator) with respect to handle, fullness, grain (break) and color. A rating value from 1 to 5 was assigned for each parameter, with 1 being the worst and 5 being the best. From these ratings, an overall evaluation was determined and this value (from 1 to 5) was reported.

Optical Microscopy (with Epi-fluorescent Attachment)

Gelled samples of unmodified 10% w/v gelatin and gelatin modified with 4% tara were prepared and then checked for fluorescence using an epi-fluorescent microscope. Control and treated samples of wet blue or wet white were sectioned, using a razor (from grain to flesh) and mounted onto a glass slide. They were examined using an Eclipse E600 Polarizing Microscope (Nikon Instruments Company, Melville, NY), at 4X magnification, operating in optical mode. The instrument was equipped with a X-Cite™ 120 Fluorescence Illuminator System which was fitted with a metal halide lamp (EXFO Photonic Solutions, Inc., Mississauga, ON, Canada), with two filter cubes or optical blocks, containing epi-fluorescence interference and absorption filter combinations including an excitation filter, dichromatic beamsplitter (often referred to as a mirror), and a barrier (or emission) filter (515-555 nm or 600-660 nm), and with a digital camera (DS-Fi1)[25].

Scanning Electron Microscopy (SEM)

Wet blue and wet white samples, after treatment and after RCF, along with their respective control samples were cut into small strips (6.5 cm x 1 cm), placed in a test tube to which nano pure water was added (to cover strip) and freeze-dried. Two pieces (1.5 mm thick) were cut from each of the dry samples and were mounted onto the surfaces of carbon adhesive tabs with the help of Duco cement. After drying for 1 h, silver paint was applied to the exposed surface area around the samples. The samples were sputter-coated with a thin layer of gold using a Scancoat Six Sputter coater. Samples were viewed using a Quanta 200 FEG Environmental Scanning Electron microscope, FEI Company (Hillsboro, OR) in high vacuum-secondary electron imaging mode at an accelerating voltage of 10 kV (spot size 3.0, pressure 0.3 torr). Digital images were collected at 50, 250, 500, and 1000x magnifications.

RESULTS AND DISCUSSION

In our studies we wish to demonstrate the efficacy of polyphenol modification of gelatin and produce products that could be used in leather processing. We therefore established that a product made from a modification of gelatin with quebracho, yielded a crust leather with improved subjective properties.[10,11] In a continuing study, we examined whether tara could be equally as efficient in gelatin modification.[14] We prepared gelatin/tara products and identified those products whose properties made them possible candidates as fillers for leather. We found that when 10% gelatin was reacted with 4% tara at pH 9.5-10.0 and at 45°C, for 4 h, the physicochemical properties of the products compared to an unmodified control sample were superior (gel strength, 316 g vs 241 g, melting point, 45.4°C vs 35.0°C, and viscosity@60°C, 5.57 cP vs 4.58 cP). These are characteristics of products that are amenable to being used as fillers. In this continuing study, we apply these compounds to wet blue and wet white hides, study the uptake of product in floats, examine the leather using epi-fluorescence microscopy and SEM, evaluate the leather for subjective properties and analyze for mechanical properties.

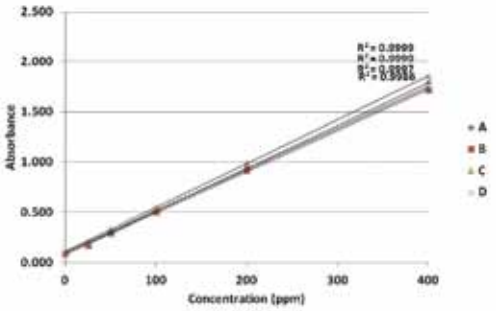

Figure 1. Phenolics assay showing standard curves (n=4) using gallic acid standard.

Phenolic Assay for Tara

The assay as described in a prior publication[11] to determine uptake of quebracho, was applied to the vegetable tannin tara to determine the uptake of a gelatin/tara product by wet blue and wet white. In the previous study, we used quebracho as the standard instead of the reported gallic acid. In this present study, we returned to using gallic acid as the standard. The standard curves resulting from four studies can be seen in Figure 1. The average R^2 value was found to be 0.9995 with a standard deviation (SD) of 0.0006.

In the initial experiments, the commercial product tara was analyzed for percent active tannin. Tara as received had a high amount of insolubles and it was determined that these should be removed before analysis. The sample was dissolved in warm water, stirred, and the sample was then centrifuged to isolate the soluble tannin. These samples were then subjected to the phenolic assay described in experimental section with gallic acid as the standard. Six samples were run and each sample was run in quadruplicate. The SD within each sample and overall for six samples are shown (Table 1). For the most part the SD within each sample is quite low and between the six samples the value is 3.4. So in summary, the commercial

TABLE I
Analysis for tannin in commercial Tara sample.

Sample[1]	% Tannin	SD[2]
1	48.9	0.9
2	46.4	0.8
3	41.9	0.4
4	43.3	5.2
5	39.2	3.9
6	44.6	2.5
Aver	44.0	2.3
SD[3]	3.4	1.9

[1]n=4, analyses run in quadruplicate
[2]SD within each sampling
[3]SD between six samples

sample used in these studies has an average of about 44.0% tannin (SD 3.4) (based on total weight), with an average of about 26.4% insolubles (SD 6.25). These values for soluble tannin and insolubles compare to those reported in the literature[17-18].

Treatment of Wet Blue

Wet blue hides were treated with the gelatin/tara product deemed to have optimal physicochemical properties necessary for application as a filler (10% gelatin (w/v), 4% tara, pH 9.5-10.0, 45°C, for 4 h). Products prepared for each trial were monitored for physical properties and molecular weight distribution (indicating the bands for gelatin have been diminished when compared to the control) and results were analogous to those reported earlier[14]. Pieces of wet blue, cut sequentially along the backbone of the hide were treated with gelatin/tara product (10% gelatin based on weight of hide with 4% tara based on weight of gelatin).

Preliminary studies indicated that gelatin fluoresces after modification with tara (as did gelatin treated with quebracho). After treatment, pieces were examined using the epi-fluorescent microscope to determine if the leather was filled (Figure 2a and b). The pieces treated with the product fluoresce more (Figure 2b) than the control samples (Figure 2a) (blue stock naturally has fluorescence at these wavelengths). The wet blue samples were retanned, colored and fatliquored and subjectively evaluated (Figure 3) and in all properties there was a significant improvement over the untreated control samples. The data shown are the average of five trials with 2 samples in each trial.

With respect to mechanical properties (Figure 4), as indicated by error bars there were no significant differences between the test samples and the controls. Again the data shown are the average from five trials, two samples per trial and five specimens per analysis.

Samples were also taken for SEM analysis (Figure 5). Differences in the fiber structure of the tests and the controls can be seen, in that the controls, after treatment and after RCF, have a more distinct fiber separation than the tests. These observations are similar to what we observed in gelatin/quebracho treated blue stock[11].

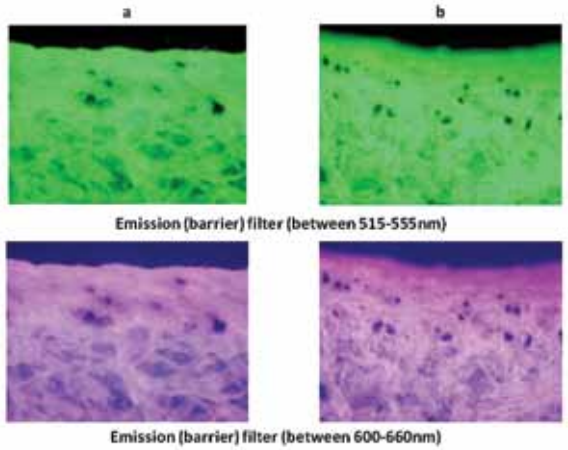

Figure 2. Epi-fluorescent micrographs of blue stock; treated with pH-adjusting agents alone (control) (a), and test sample treated with tara-modified gelatin (b); two emission (barrier) filters, between 515-555 nm and 600-660 nm were used.

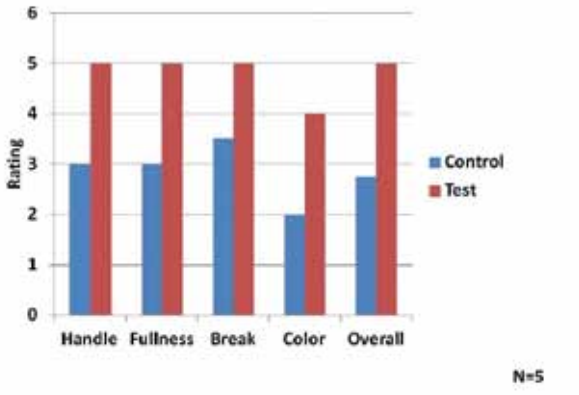

Figure 3. Subjective evaluation (handle, fullness, break, and overall) using rating scale of 1 = worst to 5 = best, of wet blue (area pieces), treated with pH-adjusting agents alone (controls) and with tara-modified gelatin (tests), then RCF; data was averaged from five trials.

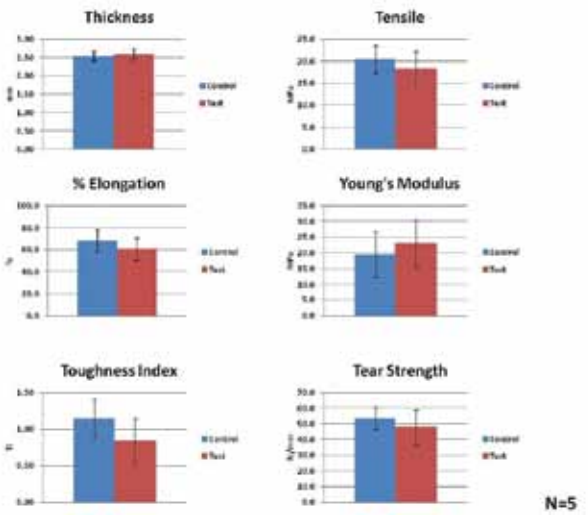

Figure 4. Mechanical properties (with error bars indicating SD) of area pieces of wet blue, treated with pH-adjusting agents alone (controls) and with tara-modified gelatin (tests) and then RCF; data was averaged from five trials, and within the trials, each mechanical property is an average of five determinations.

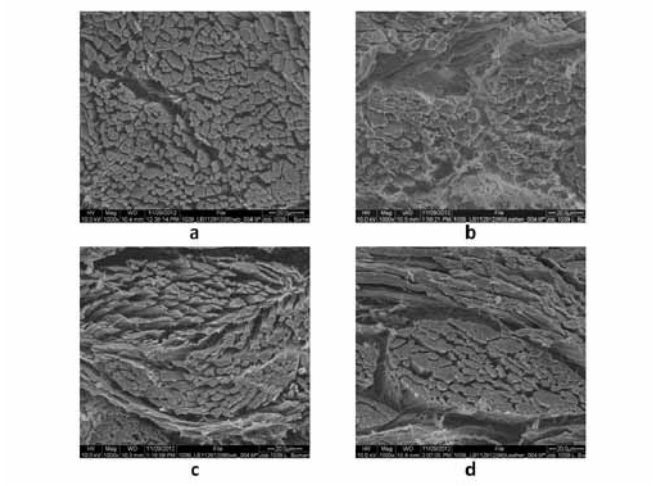

Figure 5. SEM micrographs (1000x) of wet blue; untreated control sample after pH adjustment (a) and after RCF (b) and test sample, after treatment with tara-modified gelatin (c) and after RCF (d); (— = 20μm).

Percent Uptake of Tara/Gelatin by Wet Blue

Samples of float from treatment of blue stock with gelatin/tara were taken every hour up to five hours and analyzed for phenolics using a method developed at ERRC by Medina[12-13]. After 4-5 hours, the hides have taken up approximately 45-50% of the product offered (Figure 6). These results point to a lesser amount of product uptake than was found when gelatin/quebracho was added to wet blue. This could indicate that the hide has reached saturation and a smaller offer of tara/gelatin product would be feasible.

Treatment of Wet White

Six pieces of wet white, cut sequentially along the backbone of the hide were treated with gelatin/tara product (10% gelatin based on weight of hide with 4% tara based on weight of gelatin). After treatment, pieces were examined using the epi-fluorescent microscope to determine if the leather was filled (Figure 7a and b). The wet white auto-fluoresces strongly and three neutral density filters were utilized to decrease the intensity. The images (control, Figure 7a and test, Figure 7b) show that the treated wet white (Figure 7b) had picked up the tara/gelatin filler and is evenly distributed; a highly intensive band is also seen at the grain of the treated sample.

The wet white samples were retanned, colored and fatliquored and subsequently evaluated. A 400% float was used initially and no improvement in properties was observed (Figure 8a). We subsequently decreased the float to 300% and saw a significant effect (Figure 8b) on subjective properties. Handle and fullness improved the most along with slightly better results on break and overall appearance. Mechanical

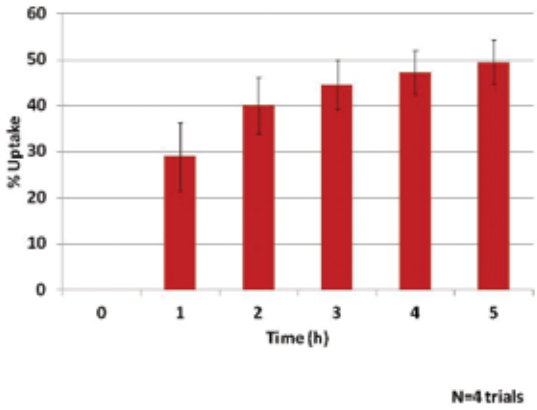

Figure 6. Percent uptake (using phenolics assay) of gelatin/tara product by blue stock (n=4), with error bars indicating SD).

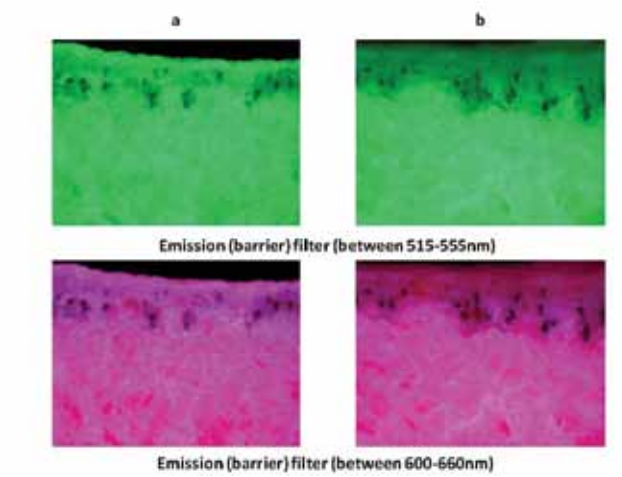

Figure 7. Epi-fluorescent micrographs of wet white stock; treated with pH-adjusting agents alone (control) (a), and test sample treated with tara-modified gelatin (b); two emission (barrier) filters, between 515-555 nm and 600-660 nm were used.

properties were determined. Samples treated with 300 and 400% floats were calculated separately and then the data was combined (Figure 9). There appears to be no significant difference found (as indicated by the error bars) between the test samples and the control samples.

Samples were taken after treatment and again after RCF for SEM imaging (Figure 10) and pictures of selected images showed a difference in that the control samples had a regular structure whereas the structure of the treated hides was not defined. Similar lack of separation was seen when blue stock was treated with either the gelatin/quebracho or gelatin/tara products.

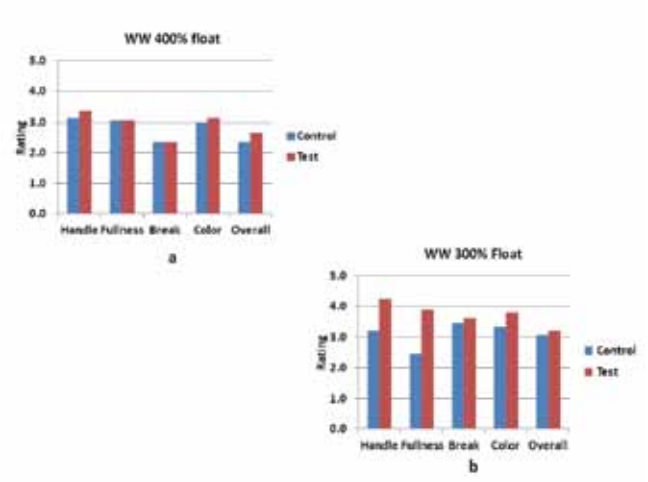

Figure 8. Subjective evaluation (handle, fullness, break, and overall) using rating scale of 1 = worst to 5 = best, of wet white (area pieces), treated with pH-adjusting agents (controls) and with tara-modified gelatin (tests); (a) using a 400% float, two trials and (b) using a 300% float, four trials.

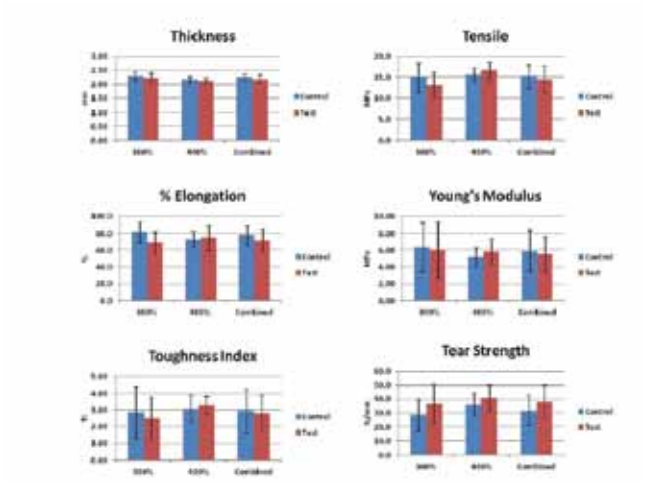

Figure 9. Mechanical properties (with error bars indicating SD) of area pieces of wet white, treated with pH-adjusting agents (controls) and with tara-modified gelatin (tests), and then RCF; shown are 400% (n=2) and 300% (n=3) trials, and combined data; each mechanical property is an average of five determinations.

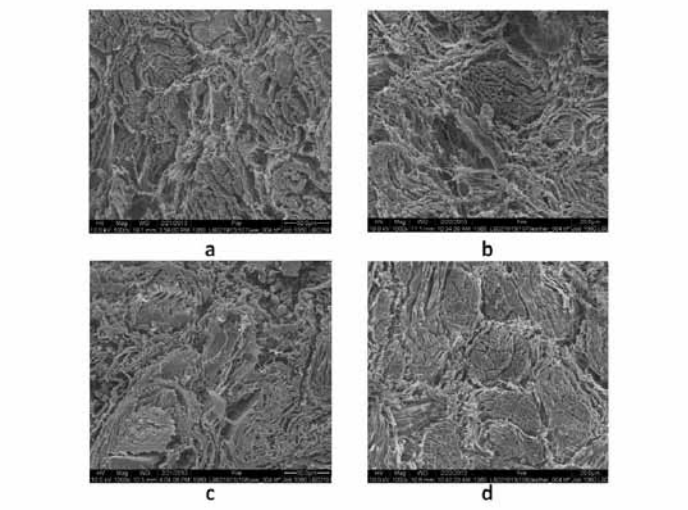

Figure 10. SEM micrographs (1000x) of wet white; untreated control sample after pH adjustment (a) and after RCF (b) and test sample, after treatment with tara-modified gelatin (c) and after RCF (d); (— = 20μm).

Percent Uptake of Tara/Gelatin by Wet White

With respect to determining the uptake of product in wet white, it appeared that percent uptake was decreasing as time of reaction increased (the reaction is appearing to reverse). Experiments were carried out to determine if substances from the chemical makeup of wet white hides were interfering in the analysis. Indeed it was found that there were moieties that were absorbing in the same range and these moieties increased as the treatment proceeded. Even if the analyses were corrected for these interferences from wet white, the percent uptake still could not be calculated. It is possible that there may be compounds in the hides that were interfering with the FBBB reaction. At the present time this analysis cannot be used for uptake of product in wet white and perhaps another analysis, for example a protein assay, needs to be explored.

CONCLUSIONS

Gelatin/tara product can be used effectively as a filler in treatment of wet blue and wet white. A phenolic assay was developed for the analysis of tara and was utilized in determining active tannin in commercial samples. It was further applied to determine percent uptake of product when treating wet blue (ca. 45-50% uptake after 4 h), but when this technique was applied to wet white, there appeared to be interferences in the reaction. The gelatin/tara product fluoresces and this was a useful tool in determining distribution of product. In both wet blue and wet white, there were improvements in the subjective properties and at the same time there were no statistical differences observed in mechanical properties. SEM studies show that the fiber structure in treated wet blue and wet white hides is not as organized and more compressed than the open and more defined structure we see in the control samples.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the assistance of Renee Latona, Guoping Bao and Joseph Uknalis.

REFERENCES

1. Strauss, G., and Gibson, S.M.; Plant phenolics as cross-linkers of gelatin gels and gelatin-based coacervates for use as food ingredients. *Food Hydrocolloids* **18**, 81-89, 2004.
2. Cao, N., Fu, Y., and He, J.; Mechanical properties of gelatin films cross-linked, respectively, by ferulic acid and tannin acid. *Food Hydrocolloids **21**, 575-58***4*, 2007.
3. Balange, A. and Benjakul, S.; Enhancement of gel strength of bigeye snapper (*Priacanthus tayenus*) surimi using oxidised phenolic compounds. *Food Chemistry **113**, 61-70*, 2009.
4. Balange, A. and Benjakul, S.; Effect of oxidised phenolic compounds on the gel property of mackerel (*Rastrelliger kanagurta*) surimi. *LWT - Food Science and Technology* **42**, 1059-1064, 2009.
5. Balange, A. K. and Benjakul, S.; Effect of oxidised tannic acid on the gel properties of mackerel (*Rastrelliger kanagurta*) mince and surimi prepared by different washing processes. *Food Hydrocolloids* **23**, 1693-1701, 2009.
6. Kosaraju, S. L., Puvanenthiran, A., and Lillford, P.; Naturally crosslinked gelatin gels with modified material properties. *Food Research International* **43**, 2385-2389, 2010.
7. Haslam E.; Vegetable tannage - Where do the tannins go? *J. Soc. Leather Techn. Chem.* **81**, 45-51, 1997.
8. Slabbert, N. P.; The basics of practical tanning systems reconciled with vegetable tanning theories. *JALCA* **94**, 1-7, 1999.
9. Peña, C., de la Caba, K., Eceiza, A., Ruseckaite, R., and Mondragon, I.; Enhancing water repellence and mechanical properties of gelatin films by tannin addition. *Bioresource Technology* **101,** 6836-6842. 2010.
10. Taylor, M.M., Lee, J., Bumanlag, L.P., Latona, R., Brown, E.M., and Liu, C.-K.; Preparation and characterization of polyphenol-modified gelatin products. *JALCA* **107**, 51-59, 2012.
11. Taylor, M.M., Medina, M., Lee, J., Bumanlag, L.P., Brown, E.M., and Liu, C.-K.; Treatment of wet blue with quebracho-modified gelatin biopolymer products. *JALCA* **107**, 416-421, 2012.
12. Medina, M. B.; Determination of the total phenolics in juices and superfruits by a novel chemical method. *Journal of Functional Foods* **3**, 79-87, 2011.
13. Medina, M. B.; Simple and rapid method for the analysis of phenolic compounds in beverages and grains. *Journal of Agricultural and Food Chemistry* **59**, 1565-1571, 2011.
14. Taylor, M.M., Lee, J., Bumanlag, L.P., Latona, R.J., Brown, E.M., and Liu, C-.K.; Preparation and evaluation of tara-modified proteins. *JALCA* **108**, 16-22, 2013.
15. http://www.otto-dille.de/english/tara.html
16. http://taninos.tripod.com/hidrolisables.htm
17. Castell, J., Fabregat, C., Sorolla, S., Olle, L., Bacardit, A., and Solano; D.; Optimizing a sustainable and innovative wet-white process with tara tannins. *JALCA* **106**, 278-286, 2011.
18. Castell, J., Sorolla, S., Jorba, M., Aribau, J., Bacartit, A., and Ollé, L.; Tara *(Caesalpinia spinosa)*: The sustainable source of tannins for innovative tanning processes. *JALCA* **108**, 221-230, 2013.
19. Taylor, M.M., Marmer, W.N., and Brown, E.M.; Effect of fillers from enzymatically modified proteins on mechanical properties of leather. *JALCA* **103**, 128-137, 2008.
20. Taylor, M.M., Lee, J. Bumanlag, L.P., Hernàndez Balada, E., Cooke, P.H., and Brown. E.M.; Treatment of low-quality hides with fillers produced from sustainable resources. Effect on properties of leather. *JALCA* **104**, 324-334, 2009.
21. Taylor, M.M., Lee, J., Bumanlag, L.P., Hernàndez-Balada, E., and Brown, E.M.; Treatments to enhance properties of chrome-free (wet white) leather. *JALCA* **106**, 35-41, 2011.
22. Taylor, M.M., Cabeza, L.F., Marmer, W.N., and Brown, E.M.; Enzymatic modification of hydrolysis products from collagen using a microbial transglutaminase. I. Physical Properties. *JALCA* **96,** 319-332, 2001.
23. Liu, C.-K., and McClintick, M.D.; Measurements of the initial strain energy of leather. *JALCA* **92**, 157-171, 1997.
24. Taylor, M.M., Marmer, W N., and Brown, E M.; Molecular weight distribution and functional properties of enzymatically modified commercial and experimental gelatins. *JALCA* **99,** 129-140, 2004.
25. http://www.microscopyu.com/articles/fluorescence/filtercubes/filterindex.html