# Treatment of Wet Blue with Fillers Produced from Quebracho-modified Gelatin*

*by*

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## Abstract

Gelatin modified with quebracho to produce high molecular weight, high viscosity products was investigated as a filler in leather processing. The uptake of quebracho/gelatin product by the wet blue was on the average about 55% of the 10% gelatin/quebracho product offered; the reaction appeared to be complete after about 4 h. The uptake of quebracho alone by the wet blue was run as a control; after 2 h almost 100% of the quebracho was taken up by the hide. As a second control, the effect of eliminating vegetable tannins (quebracho and mimosa) from the retan, color, and fatliquor (RCF), on properties of the crust was tested. Epi-fluorescent imaging and scanning electron microscopy (SEM) results indicated distinctive differences between the two control samples and the gelatin/quebracho treated hide. The gelatin/quebracho treated samples had superior subjective properties when compared to untreated controls; differences in mechanical properties were dependent on whether vegetable tannin was present or absent in RCF. Thus a filler produced from a common vegetable tannin (quebracho) and a waste product from the leather industry (gelatin) can add economic value to leather by improving its quality.

## Resumen

Gelatina modificada con quebracho para producir un producto de alto peso molecular y alta viscosidad, se investigó como un agente rellenante en el procesamiento del cuero. La absorción del producto elaborado de quebracho/gelatina por el "wet-blue", fue en promedio aproximadamente el 55% del 10% ofrecido; la reacción parecía haber terminado luego de aproximadamente 4 horas. La absorción de quebracho individualmente ofrecido al wet-blue se efectuó cómo control; luego de 2 horas la gran mayoría del quebracho fue absorbida por el cuero. Cómo un control secundario, el efecto de la eliminación de los taninos vegetales (quebracho y mimosa) del recurtido, teñido y engrase (RCF), sobre las propiedades de la resultante crosta, fue comprobada. Imágenes obtenidas por epi-florecencia y microscopía electrónica por barrido (SEM) resultantes, indicaron diferencias en características entre los dos controles y el cuero tratado por gelatina/quebracho. Las muestras obtenidas por el uso de gelatina/quebracho demostraron propiedades superiores subjetivas en comparación a los controles sin los tratamientos; diferencias en las propiedades mecánicas dependieron si los taninos estuvieron presentes en RCF. Entonces un rellenante producido de un tanino vegetal común y un desperdicio de la industria del cuero (gelatina) pueden añadir valor económico al cuero por aumento en su calidad.

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## INTRODUCTION

Vegetable tanning, using polyphenols extracted from plant materials, has been employed primarily for the production of heavy leathers used in saddles, belts and shoe soles.[1,2] Polyphenols involved in vegetable tannage have recently been investigated for their ability to crosslink gelatin.[3-8] Polyphenols are known to react under oxidizing conditions with side chain amino groups of peptides, leading to formation of cross-links in proteins.[3] It has also been reported that some vegetable tannins themselves could be applied to gelatin to give products with interesting physical properties.[9] In a recent study,[10] we prepared a gelatin/quebracho product whose properties made it amenable as a filler for leather. Samples of treated blue stock along with controls were retanned, colored and fatliquored (RCF) and were subjectively evaluated. In every subjective property evaluated, there was an improvement in the treated samples. Mechanical properties indicated that there were no significant differences between tests and controls.

In this present study, we will attempt to optimize on treatment conditions by determining the percent uptake and rate of the reaction using a modification of a phenolic assay developed at ERRC.[11-12] We further examined the effects that treatment of blue stock with quebracho alone, as well as the elimination of vegetable tannins (mimosa and quebracho) from the RCF, would have on properties. Scanning electron microscopy (SEM) and epi-fluorescent imaging were employed to assess properties of different treatments (quebracho treated blue stock, control without quebracho, and gelatin/quebracho product). Studies on the rate of the reaction, subjective and mechanical properties of the RCF leather, as well as epi-fluorescent and SEM images, will be presented.

## EXPERIMENTAL

### Materials

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

### *Application of Gelatin/Quebracho Product to Wet Blue Stock (Area Samples)*

Gelatin/quebracho products, using 10% w/v gelatin (175 Bloom) and 2% quebracho at pH 9.0, 45°C for 4h, were prepared as described in a previous publication[10] and were applied to wet blue stock as follows (Figure 1). Samples of the hide were selected for area and epi-fluorescent study (four pieces, two controls and two tests, approximately 1 foot square) each cut sequentially from the butt, belly or neck area, two pieces /drum, ~325g each. The samples were placed in two 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 45°C, drained and refloated in sodium bicarbonate (2% on hide weight in 400% float or 2600 mL). The samples were drummed (in Dose drums) at ambient temperature (25-28°C) until the pH stabilized (6.5-7.0). The floats were drained, one set of control samples was set aside, another set of control samples were treated with quebracho alone (0.2% or 1.3 g, based on the amount that was added in the test runs). To the test samples, the tannin product (10% gelatin, 65 g, based on hide weight, 650 g, modified with 2% quebracho, 1.3 g, based on gelatin weight) was added in a 400% float (2600 mL). 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 in a 400% float (2600 mL), drained, patted dry, and stored at 4°C. The tests and controls were RCF using a shoe upper formula for wet blue as described in prior publications.[13,14] In selected trials, vegetable tannins (mimosa and quebracho) were eliminated in the retan step. When completed, the RCF samples were vacuum dried (at 60°C for 6 min) and then hung to dry at ambient temperature and humidity. The samples were wetback and covered with plastic for 2 h; the plastic was removed, the samples were staked, subjectively evaluated, and then were placed on a shelf in the conditioning room at 20°C and 65% relative humidity for at least 3 days before mechanical properties were performed. No finishing operations were done to the hide pieces.

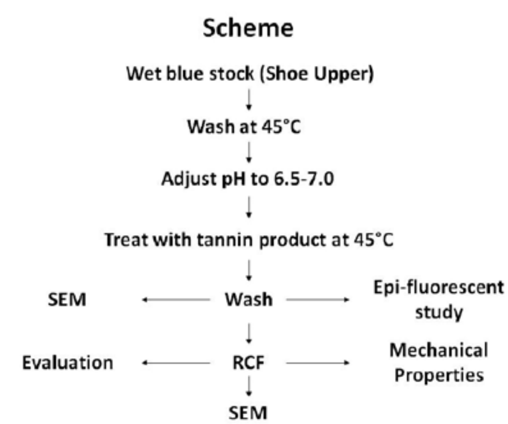


Figure 1. Flow diagram for quebracho and gelatin/quebracho treatment of blue stock and protocol for sampling and evaluation of RCF crust.

### Analyses

#### *Phenolics Assay*

Wet blue was treated with either quebracho alone or with gelatin/quebracho product (10% gelatin based on hide weight and 2% tannin based on gelatin weight). To monitor the uptake of the gelatin/quebracho product by the wet blue, 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[11,12] as follows. Either the gallic acid or quebracho standards or the reaction solutions (1 mL) were transferred to borosilicate test tubes. A control blank was prepared and it contained all reagents but standards (gallic acid or quebracho), instead deionized water was added. Azo dye (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/quebracho product remaining in the reaction solution was estimated from the standard curves for gallic acid and quebracho and the latter was chosen as the standard for this study.

### *Subjective Evaluation RCF Leather*

Each treated and untreated leather piece, two samples of each, was evaluated (one evaluator) with respect to handle, fullness, grain (break) 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 ratings, an overall evaluation was determined and this value (from 1 to 5) was reported.

### *Mechanical Properties*

Mechanical properties (tensile, elongation, Young's Modulus, toughness index, tear strength, and thickness) were determined as described in a previous paper.[15]

### *Optical Microscope Equipped with Epi-fluorescent Attachment*

The treated wet blue samples were sectioned, using a razor (grain to flesh) and mounted onto a glass slide. They were examined using an Eclipse 6600 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),[16] and with a digital camera (DXM 1200).

### *Scanning Electron Microscopy (SEM)*

Wet blue samples, after treatment and after RCF, along with their respective control samples were cut into small strips (6.5 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) 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 at least 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 magnification.

## Results and Discussion

In a recent study,[10] we prepared gelatin/quebracho products whose properties made them possible candidates as fillers for leather. We found that when 10% gelatin is reacted with the 2% quebracho, pH 9.0-10.0, at 45°C, for 4 h, the physicochemical properties of the products, when compared to an unmodified control sample, gave products that were amenable to being used as fillers. When quebracho-treated gelatin products were examined using an epi-fluorescence microscope, enhanced fluorescence was observed in the modified gelatin and no significant florescence could be seen in the unmodified control;[10] when samples of blue stock, treated with the gelatin/quebracho products were examined and compared to untreated control samples, the fluorescence images were indicating that the blue stock was filled and that the filler was not removed by washing. SEM analysis showed distinct differences in fiber structure between treated and untreated samples. Samples of treated blue stock along with controls were RCF and were subjectively evaluated; the treated samples showed an improvement in every parameter evaluated. Mechanical properties indicated that there were no adverse differences between tests and controls.

### *Treatment of Wet Blue and Analyses for Phenolic (Quebracho) Content*

In this continuing study, we investigated the rate of uptake by the hide when 10% gelatin/quebracho product (based on blue weight) was added during a 5 h period. The uptake was monitored by utilizing a modification of an analysis for phenolics in food and beverages developed by Medina at ERRC.[11,12] The method, as described in the Experimental section, was modified in that quebracho was used as the standard instead of gallic acid. In Figure 2a, the standard curves that were generated using quebracho as the standard are shown and indicate the reproducibility of the method. The average of the $R^2$ values (n=4) is 0.9933, with a standard deviation of 0.0017. Wet blue was treated with the gelatin/quebracho product and aliquots of the floats were taken each hour from 0 to 5 h. Four trials were carried out. The results from these trials are shown in Figure 2b and indicate that a maximum uptake of the product was about 55% and that the reaction was completed in 3-4 h.

As an additional control, a series was run in which quebracho alone (0.2% based on the wet blue weight which is equivalent to the amount added to react with gelatin in product

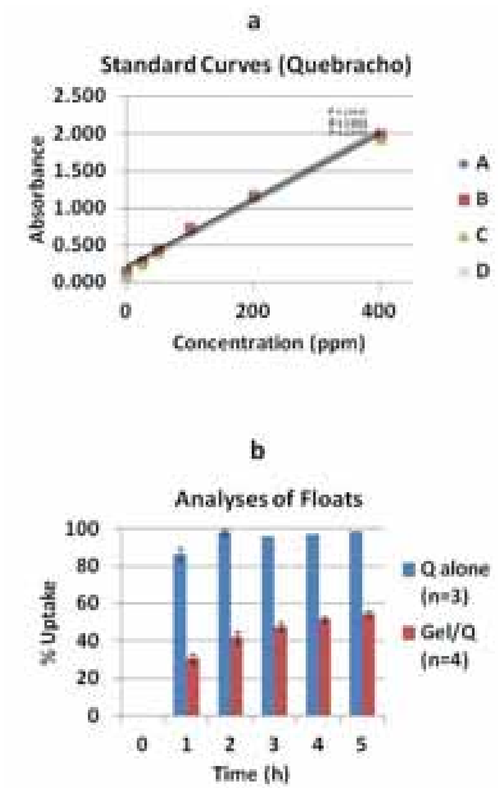


Figure 2. Phenolics assay showing standard curves (n=4) using quebracho (a), and uptake of 0.2% quebracho alone (n=3) and gelatin/quebracho product (n=4) by blue stock (b) (with error bars indicating STD).

preparation) was used to treat the hide. Three trials were carried out and the floats were analyzed for the quebracho content. The hide took almost all of the quebracho up in the first 2 h (Figure 2b). These data from the gelatin/quebracho treatment studies and the quebracho alone trials are indicating that the unbound quebracho (to gelatin) is quickly taken up by the hide, but that the gelatin/quebracho product is picked up to a maximum of 50-55%, indicating that ~40-45% is in excess. This may signify that the hide has reached its maximum absorption of product and that a lower dose of the product may be feasible.

### *Epi-fluorescent and SEM Imaging of Wet Blue*

Epi-fluorescent imaging studies were carried out on representative samples from the above experiments. In a previous study[10] we showed that the gelatin/quebracho product has emission in the 515-555 nm and 600-660 nm range, and this enabled us to monitor the filling capacity of the product in wet blue. In this present study we looked at representative samples of the two controls (one with quebracho and one without) and the gelatin/quebracho-treated samples, and these images are shown in Figure 3 a and b. As seen previously, the images are showing that the control does auto-fluoresce, as does the control that had quebracho alone added to it, but the gelatin/quebracho samples do fluoresce more intensely, showing the distribution of the filler. In Figure 4, the SEM images of blue stock in which a control without quebracho, a control with quebracho, and a gelatin/quebracho treated hide

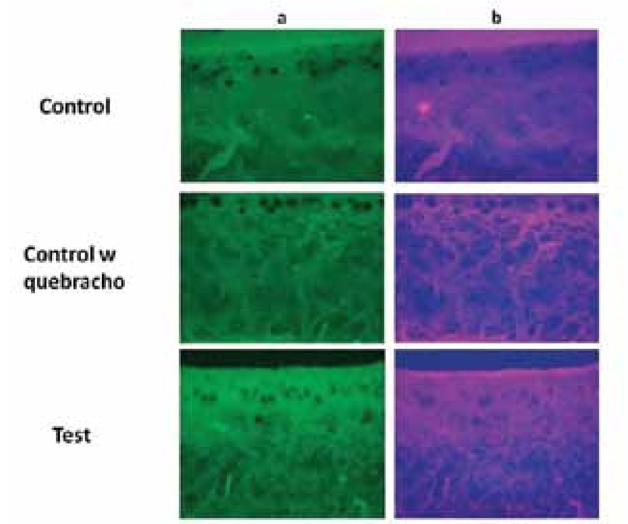


Figure 3. Epi-fluorescent micrographs of blue stock; untreated control sample, quebracho treated control sample, and test sample treated with quebracho-modified gelatin (10% gelatin, 2% quebracho, at pH 9.0, 45°C for 4 h); two emission (barrier) filters, between 515-555 nm (a) and 600-660 nm (b) were used.

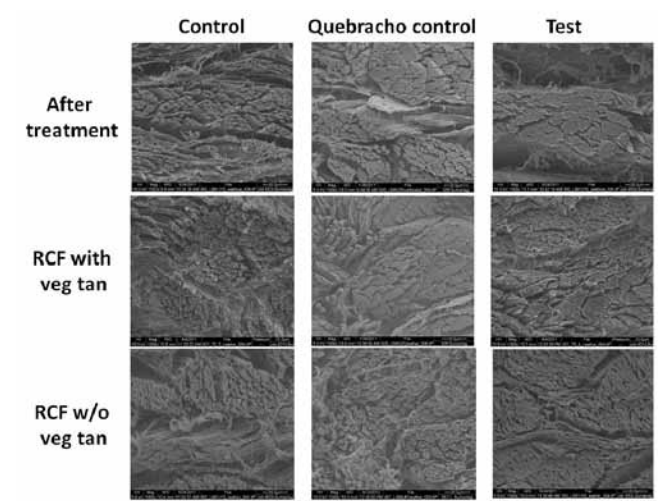


Figure 4. SEM micrographs (1000x) of untreated control sample, quebracho treated control sample, and test samples treated with quebracho-modified gelatin (10% gelatin, 2% quebracho, at pH 9.0, 45°C for 4 h); after treatment and after RCF; (— = 20$\mu$m).

are shown. The images are indicating that in both controls, a well defined fiber structure is seen, but the treated hide has no separation of fibers

### *RCF of Wet Blue, Subjective and Mechanical Properties, and SEM Imaging*

Wet blue that was treated with 0.2% quebracho alone, a control without quebracho and the gelatin/quebracho product, were brought to the crust. In some cases, a typical RCF for shoe upper was run in which vegetable tannins (mimosa and quebracho) were added in the retan. In some samples, the vegetable tannins were eliminated from the RCF for the purpose of assessing the contribution of the gelatin/quebracho

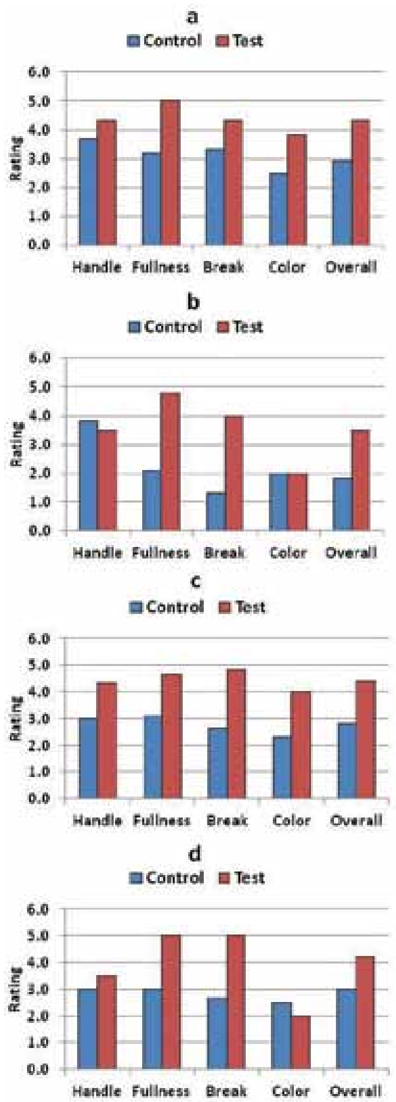


Figure 5. Subjective evaluation (handle, fullness, break, and overall) using rating scale of 1 = worst to 5 = best, of wet blue (a and b), treated with pH-adjusting agents alone (controls) and with quebracho-modified gelatin (tests); RCF with (a) and without (b) added vegetable tannins in the retan; wet blue (c and d), treated with pH-adjusting agents and 0.2% quebracho (controls) and with quebracho-modified gelatin (tests); RCF with (c) and without (d) added vegetable tannins in the retan; all trials were run in duplicate.

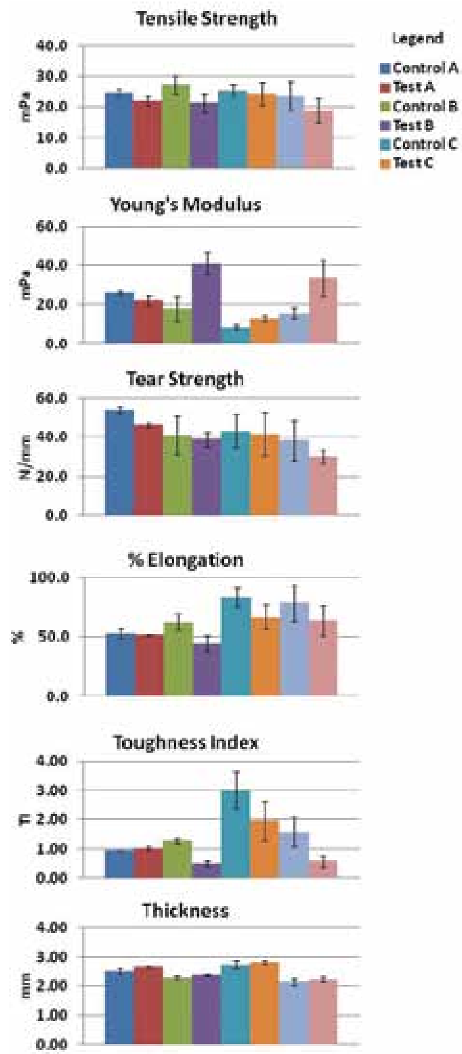


Figure 6. Mechanical properties (with error bars indicating STD) of area pieces of wet blue, treated with pH-adjusting agents alone (Controls A and B) and 0.2% quebracho (Controls C and D); tests A, B, C and D treated with quebracho-modified gelatin at pH 6.5-7.0, 45°C for 4 h; A and C had added vegetable tannins (mimosa and quebracho) in the retan; B and D had no added vegetable tannins.

product to the quality of the crust. In Figure 5, the subjective properties of those samples that had no quebracho added to the blue stock are shown and indicate that in all cases the gelatin/quebracho-treated samples, whether vegetable was added to the retan (Figure 5a) or not (Figure 5b), were far superior to the control samples. The evaluation of those samples in which the blue stock was pretreated with 0.2% quebracho (to assess whether the quebracho alone was contributing to superior properties) are shown in Figure 5, and again in almost all cases the gelatin/quebracho-treated samples were improved, whether vegetable tannins (mimosa and quebracho) were used (Figure 5c) or eliminated (Figure 5d). In summary, subjective properties of the samples show unequivocally that the gelatin/quebracho-treatment significantly improves the subjective properties over control samples with and without the addition of quebracho; the same holds true if vegetable tannin is not added in the RCF. The mechanical properties of wet blue that was treated with 0.2% quebracho alone, a control without quebracho and the gelatin/quebracho product and brought to the crust (as described above) were determined.

In Figure 6, the mechanical properties of those samples with no quebracho added to the blue stock (trials A) are shown and indicate that there are no significant differences in the tensile strength, that in the samples without the vegetable tannins (mimosa and quebracho) added in the retan (trials B), there are significant differences between the tests and the controls in the percent elongation, Young's Modulus and Toughness Index, possibly reflecting the absence of added vegetable tannins; the tear strength of the control in trials A shows significant difference, and finally, the thickness of the test samples, in both trials A and trials B, are significantly higher, as one might expect. In all trials, mechanical property data are averaged from two trials each and within the trials, each mechanical property is average of five determinations.

The mechanical properties of those samples in which the blue stock was pretreated with 0.2% quebracho (to assess whether the quebracho alone was contributing to superior properties) are also shown in Figure 6. The tensile strength, percent elongation, tear strength, and thickness are not showing significant differences in trials C, but some differences are noted when samples were treated (trials C) or not treated (trials D) with additional tannins (e.g. thickness). Young's Modulus and Toughness Index are showing significant differences in trials D, when tannins were not added. In summary, the mechanical properties of the samples (quebracho/control, control no quebracho and gelatin/quebracho) show when vegetable tannins (mimosa and quebracho) are added, a few significant differences are seen, even when a quebracho control is used. However when vegetable tannins are eliminated in the RCF, more significant differences are noted.

SEM images (Figure 4) of the RCF samples (quebracho/control, control no quebracho and gelatin/quebracho) show that the control without quebracho and control with quebracho have similar fiber structure (more defined), whereas the fiber structure of the blue stock treated with the gelatin quebracho product is not resolved.

## Conclusions

In this study, we have shown that treatment of blue stock with gelatin/quebracho can be monitored for optimal conditions by using a modification of a phenolic assay developed for food and beverages. Furthermore, the presence of the filler gives crust leather with superior handle, fullness, break and color, whether vegetable tannins (mimosa and quebracho) are added or omitted in RCF, compared to samples not treated with the gelatin/quebracho product, and particularly over samples that had just quebracho alone added as an additional control. This is all further confirmation that a filler produced from a common vegetable tannin (quebracho) and a waste product from the leather industry (gelatin) can add economic value to leather by improving its quality.

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## References

1. Haslam E.; Vegetable tannage - Where do the tannins go? *J. Soc. Leather Techn. Chem.* **81**, 45-51, 1997.
2. Slabbert, N. P.; The basics of practical tanning systems reconciled with vegetable tanning theories. *JALCA* **94**, 1-7, 1999.
3. 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.
4. Cao, N., Fu, Y., and He, J.; Mechanical properties of gelatin films cross-linked, respectively, by ferulic acid and tannin acid. *Food Hydrocolloids* **21**, 575-584**,** 2007.
5. 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.
6. 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.
7. 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.
8. Kosaraju, S. L., Puvanenthiran, A., and Lillford, P.; Naturally crosslinked gelatin gels with modified material properties. *Food Research International* **43**, 2385-2389, 2010.
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. 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.
12. 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.
13. 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.
14. 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.
15. Liu, C.-K., and McClintick, M.D.; Measurements of the initial strain energy of leather. *JALCA* **92**, 157-171, 1997.
16. http://www.microscopyu.com/articles/fluorescence/filtercubes/filterindex.html