Treatments to Enhance Properties of Chrome-free (Wet white) Leather

by

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Abstract

Production of chrome-free or wet white leather, predominantly for upholstery leather, is fast approaching that which has been traditionally tanned with chrome. Recycling of auto parts, specifically the car seats, is driving the momentum towards this type of leather. Wet white leathers are sometimes described as being flat, empty, weak and/or having a poor break and subsequently require more filling than conventional chrome-tanned wet blue. Large amounts of resins, polymers, and syntans are generally used in retanning of wet white leather. In recent studies, we addressed the problems of poor leather quality by utilizing fillers produced from enzymatically-modified waste proteins, specifically those proteins from the leather and dairy industry (low quality gelatins and caseins or whey). We demonstrated that these products did indeed fill the leather, were not removed during washing and while not affecting the mechanical properties, did significantly improve subjective properties, such as handle, fullness, break and color. In this present study, we applied enzymatically modified fillers to wet white leather and evaluated it to see if there was an improvement in the subjective properties. Products were prepared, characterized, and then applied to the wet white tanned stock. After treatment, the hides were retanned, colored, and fatliquored, mechanical properties were determined and subjective analysis was carried out. Scanning electron microscopy (SEM) was also carried out after treatment. As seen in previous studies, the mechanical properties of the treated wet white leather were not significantly different from controls, but there was an improvement in the subjective properties. The data from analysis of the crust will be presented along with microscopy images.

Resumen

La producción de libre de cromo o cuero wetwhite, sobre todo para tapicería de cuero, se acerca rápidamente a lo que tradicionalmente se ha curtido con cromo. El reciclaje de partes de automóviles, específicamente los asientos del auto, conduce el impulso hacia este tipo de cuero. Los cueros wet white son descriptos a veces como chatos y vacíos, débiles y/o por tener un quiebre pobre y, subsecuentemente, requieren más relleno que los convencionales wet blues curtidos al cromo. Grandes cantidades de resinas, polímeros y sintanes se utilizan generalmente en el recurtido de cueros wet white. En estudios recientes, abordamos el problema de los cueros de bajas selecciones mediante la utilización de rellenantes producidos a partir de residuos proteicos modificados enzimáticamente, específicamente aquellas proteínas provenientes de la industria láctea y del cuero (gelatinas de baja calidad y caseínas o suero). Hemos demostrado que estos productos efectivamente rellenan el cuero, no son removidos durante el lavado y sin afectar las propiedades mecánicas, mejoran notablemente las propiedades subjetivas, como el tacto, la plenitud, la soltura de flor y el color. En el presente estudio, se aplicaron rellenantes modificados enzimáticamente a cueros wet white y se evaluaron para ver si hubo una mejora en las propiedades subjetivas. Los productos fueron preparados, caracterizados y luego aplicados sobre los cueros wet white. Después del tratamiento, los cueros fueron recurtidos, coloreados, y engrasados, las propiedades mecánicas fueron determinadas y un análisis subjetivo se llevó a cabo. Microscopía electrónica de barrido (SEM) se llevó a cabo después del tratamiento. Como se ha visto en estudios anteriores, las propiedades mecánicas de los cueros wet white tratados no fueron significativamente diferentes de los controles, pero hubo mejoras significativas en las propiedades subjetivas. Los datos del análisis del semiterminado son presentados junto con las imágenes de microscopía.

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INTRODUCTION

Production of chrome-free or wet white leather is quickly surpassing that which has been traditionally tanned with chrome. Recycling of auto parts, specifically the car seats, is driving the momentum towards this type of leather. One of the common complaints with regards to some of the chrome-free or wet white tannages is the quality of the crust. Invariably, these leathers are described as having characteristics such as being flat, tinny, weak and/or having a poor break. Because the wet white tannage yields an emptier tanned hide it requires significantly more filling than normal conventional chrome tanned wet blue. Heavy amounts of resins, polymers, and syntans thus may be used in retanning of wet white. We have addressed one of the problems of poor leather quality by utilization of fillers produced from enzymatically modified waste proteins, specifically proteins from the leather and dairy industry (low quality gelatins and caseins or whey).[1-5] We demonstrated that these products did indeed fill the leather, were not removed during washing and while not affecting the mechanical properties, did significantly improve subjective properties, such as handle, fullness, break and color. Recently we addressed the problem of loose grain, "spring break" hides[6] and found that, after treatment, more cutting area was presented because of improvements in quality.

In this present study, we applied these enzymatically modified gelatin/whey protein isolate fillers to wet white to determine if we could see an improvement in the subjective properties. Products were prepared and characterized and then applied to the wet white stock. After treatment, the hides were retanned, colored, and fatliquored, subjective analyses was performed, mechanical properties were determined and scanning electron microscopy (SEM) was used to examine the fiber structure of the resultant products. The data from analysis of the crust will be presented along with representative microscopy images.

EXPERIMENTAL

Materials

Activa TG-TI, a microbial transglutaminase (mTgase) (approximately 100 units/g) containing maltodextrin as a carrier, with activity from pH 4.0 to 9.0, at 0 to 70°C, was obtained from Ajinomoto USA, Inc. (Paramus, NJ), stored at 4°C in a sealed package, and used without further preparation. Commercial Type B gelatin from bovine skin, characterized in this laboratory as 175 grams Bloom, was obtained from Fisher (Fairlawn, NJ). Dithiothreitol (DTT) was obtained from Sigma (St. Louis, MO). Whey protein isolate or WPI (Alacen™ 895) containing 93.2% protein, was generously supplied by NZMP (formerly New Zealand Milk Products) (Lemoyne, PA). Alexa Fluor® 488 and Alexa Fluor® 568 protein labeling kits were obtained from Molecular Probes, Inc. (Eugene, OR). Saform acid, Sellatan CFL, Sellatan RL, Sellatan FL, and Magnapol TGR were obtained from TFL (The Woodlands, TX). Tannigan PAKN was obtained from Bayer (Pittsburgh, PA). Eureka 400R, and Eureka 950R were obtained from Atlas Refinery, Inc. (Newark, NJ). Relugan RV and Lurazol Beige were obtained from BASF (Charlotte, NC). Chrome-free stock (upholstery weight) was purchased from a local tannery; area pieces (butt, belly and neck) were sampled from this stock. All other chemicals were analytical grade and used as received.

Methods

Preparation of fluorescently labeled conjugates

The biopolymers were composed of gelatin/WPI; in order to observe both proteins' contribution to the biopolymer (and migration through the leather), labels with different emissions were added to gelatin and WPI. An Alexa Fluor® 488 protein labeling kit (procedure described in Molecular Probes, Product Information, November 13, 2003) was used to attach a fluorescent label (absorbance at 494 nm and fluorescence emission at 519 nm) to 175 Bloom gelatin. An Alexa Fluor® 568 labeling kit was used to attach a fluorescent label (excitation at 577 nm and fluorescence emission maxima at 603 nm) to WPI (procedure described in Molecular Probes, Product Information, August 23, 2004). Each protein solution (2 mg/ml) was prepared in phosphate buffered saline (PBS), pH 7.2; gelatin was swollen for two hours and then heated at 65°C until melted. To 0.5 ml of the protein solution was added 50 µl of 1 M sodium bicarbonate and the protein solution was added to reactive dye and stirred for 1 h at room temperature (RT). The reaction mixture was added to a column containing Bio-Rad BioGel P-30 suspended in PBS. The sample was eluted using the PBS and the location of the conjugate on the resin was monitored using a handheld UV lamp. The first fluorescent band to elute was collected and the second band (unreacted label) was discarded. The labeled protein was protected from light, stored at 4°C, and aliquots were added to either a stock solution of protein solutions immediately before their use in the preparation of biopolymers using mTgase.

Preparation of biopolymer products

Gelatin samples (175 Bloom) in combination with WPI were suspended in water and allowed to swell for about 2 h at RT; they were stored overnight at 4°C. They were placed in a bath at 65ºC until dissolved. Control samples to which no enzyme was added, were run to monitor changes in physical properties. The pH was adjusted to 7.0-7.5 with 1 N NaOH and 0.5% DTT was then added, and the resulting mixtures were heated at 38°C for one h. MTgase (calculated to be 1 unit/g of total protein for biopolymer reactions) was prepared in 10 ml of water for epi-fluorescent samples, 50 ml of water for area samples, and 100 ml of water for the side studies; the solutions were added with stirring to the protein solutions to give final protein concentrations of 10% w/w for gelatin and 2% w/v for WPI. Aliquots (10 ml) of the reaction mixtures were added to

test tubes for melting point determination and 30 ml aliquot was poured into appropriate containers for determining gel strength. The samples were warmed to 50°C in a shaker bath and the reaction was carried out for 4 h. The enzyme was inactivated by heating the reaction products at 90ºC for 10 min. The samples were cooled to room temperature and then chilled for 17 h at 10ºC in a constant temperature bath. Physical analyses (gel strength, melting point and viscosity) were run on these samples. Aliquots of the samples were lyophilized and molecular weight distribution was determined. Sodium azide (70 µl of 1% solution) was added to the remaining treatment solution as a preservative and the samples were stored at 4°C until use.

Application of filler to wet white leather (Epifluorescent Pieces, Area Samples and Sides)

Wet white samples for epi-fluoresecent study (eight pieces, four pieces/drum, ~25g each), wet white stock for area study (six pieces, two pieces each from the butt, belly and neck area, three pieces /drum, ~100g each), and two sides of wet white (one side/drum,~3000 g each), were divided into tests and controls, the former two (small pieces and area pieces) placed in two small Dose drums (Model PFI 300-34, Dose Maschinenbau GmbH, Lichtenau, Germany), the latter sides in larger Dose drum (VGI 1200 by 400), washed (400% float based on wet white weight) by tumbling for 30 min at 50°C, drained and refloated in sodium bicarbonate (1% on wet white weight in 400% float). The samples were tumbled at ambient temperature (25-28°C) until the pH stabilized. The floats were drained, mTgase (5% based on wet white weight in 400% float) was added to the test samples, while water (400% float) was added to the controls and all samples were tumbled for 1 h at ambient temperature. The floats were drained and the prepared biopolymer solutions (diluted to give a 400% float based on wet white weight) were added to the test drums; water (400% float) was added to the control samples. The fillers were applied with 5%-7.5% gelatin and 1-2% WPI loading (based on wet white weight). The samples were then tumbled for 1 h at ambient temperature and then for 4 h at 50°C. The floats were drained and the samples were washed twice for 10 min at 50°C (400% float), drained, patted dry, and stored at 4°C.

Retan/color/fatliquor (RCF) and Drying

The filled samples from the gelatin/WPI treatment of area pieces and sides were retanned, colored and fatliquored using the upholstery formulas as seen in seen in Figure 1.

When completed, area samples (and sides) were toggled and left to dry at ambient temperature and humidity. One set of area samples were placed in the vacuum dryer for 10 min at a temperature of 60°C and then hang dried. The samples from both treatments were rewet, put into plastic bags for one day, then staked twice, and milled for approximately 16-18 h. No finishing operations were done to the hides and they were kept in a shelf in the conditioned room, at 20°C and 65% relative humidity for at least 3 days.

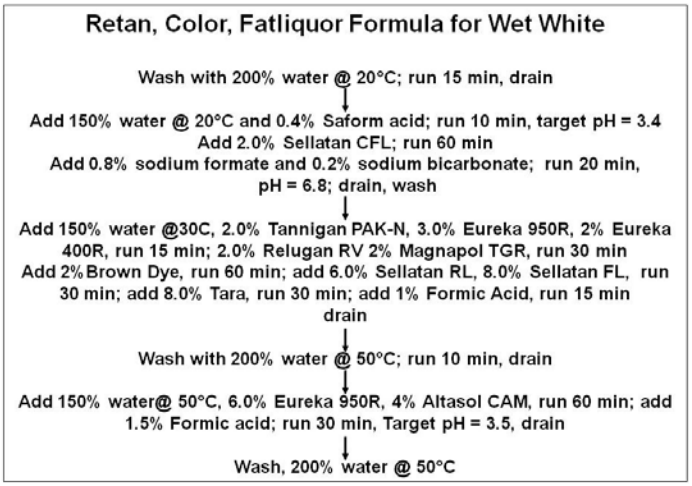

Figure 1: Schematic for RCF of treated (gelatin/WPI biopolymer) and untreated wet white.

Analyses

Physical properties, molecular weight distribution and extractables

Gel strength, melting point, viscosity, and molecular weight distribution (by SDS-PAGE) of the enzyme-treated proteinaceous solutions were determined as described in previous publications.[7,8] Mechanical properties (tensile, elongation, Young's Modulus, toughness index, tear strength, and thickness) were determined as described in a previous paper.[9] Percent moisture in RCF samples was determined as described in ASTM D 3790-79 and percent extractables was determined as described in ASTM D 3495-83.

Protein concentration determination

Protein concentrations in the float, at different stages of the treatment, were determined using the bicinchoninic acid (BCA) assay[10] according to the directions supplied with the kit and with modification in which gelatin was used as standard as opposed to bovine serum albumin (BSA). Samples were centrifuged at 13,400 rpm for 30 min in a microcentrifuge (Eppendorf MiniSpin plus, Westbury, NY). One ml of protein supernatant was removed and typically a 1:25 (v/v) dilution was prepared in order to fall within the linear concentration range for the assay (200 to 1000 µg/ml protein). A 50-µl aliquot of the diluted solution was mixed with 1.0 ml of BCA reagent and incubated at 37°C for 30 minutes. The absorbance of a sample solution at 562 nm minus a reagent blank was compared with a standard curve using known concentrations of gelatin.

Subjective evaluation RCF leather

Each treated and untreated sample was evaluated 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.

Yellowing Test

Two three-inch (76 mm) square pieces were cut from the each of the treated and untreated samples. One square of each sample was placed in an oven, at 120°C, for 72 h. After this time period, the heated samples were then compared to the unheated samples and evaluated with respect to color change. They were rated on a scale of 1 to 5, with 1 being the worst (highest color change) and 5 being the best (least affect on color).

Optical microscope equipped with Epi-fluorescent attachment

The treated wet white 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 and including an excitation filter, dichromatic beamsplitter (often referred to as a mirror), and a barrier (or emission) filter,[11] and with a digital camera (DXM 1200).

Scanning Electron Microscopy (SEM)

Wet white samples, after treatment and after RCF, along with their respective control samples were cut into small strips (6.5 cm × 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 and 1000× magnification.

RESULTS AND DISCUSSION

Product Preparation, Characterization and Application

Protein products were prepared as described in Experimental section. In this series of experiments, gelatin was used as the major protein and whey protein isolate (WPI) as the minor component. The proteins were reacted with 1 unit of mTgase and the physical properties show that the gel strength has increased from 341 to 365 g, the melting point, slightly from 36.4 to 36.6°C and the viscosity, from 7.7 to 8.7 cP at 60°C. The extent of crosslinking is minimal and the MP and viscosity are reflecting this as well as the minimum changes between test and controls, as shown in the SDS-PAGE gel (Figure 2).

Determination of product uptake (Epi Fluorescence and BCA analysis)

Prior to running the polymerization reactions, the proteins to be used in this study were labeled with fluorescent dyes so that the filler products could be monitored using an epifluorescent microscope. Gelatin was labeled with Alexa Fluor 488®, which has an emission at 519 nm, and WPI was labeled with Alexa Fluor 568®, which has an emission at 603 nm. These conjugates were prepared, and then were kept at 4°C tightly covered until use. Just prior to polymerization with the enzyme, the labeled proteins were added to the stock solutions and the polymerization reactions were run. These solutions were applied to wet white as described in Experimental section and when the treatments were complete, the treated samples, along with a control were examined using an epifluorescent microscope. It was found that the this specific wet white auto fluoresced at the wavelengths we employed (emissions at 519 and 603 nm) and, even though one could suggest that the fluorescence in the test samples is more intense, it was concluded that this was not a reliable technique for evaluating protein uptake.

Since the epi-fluorescent microscopy could not be applied reliably to determine protein uptake and retention, the BCA test was applied to these treatments to see if one could determine rate of pickup. Area samples of wet white were treated with enzyme and gelatin/WPI biopolymer. Aliquots were taken from the floats during the mTgase pretreatment as well as during the protein treatment and enzyme and protein uptake were determined. The results of these analyses can be seen in Figure 3.

The rate of enzyme uptake was quite reproducible with a 30% uptake after 20 min; this value remained constant for duration of the treatment. It appears that the protein uptake after two hours is approximately 40% and it is postulated that in an industrial trial with much more mechanical action, the time for treatment could be reduced considerably.

Subjective Evaluation and Mechanical Properties

In order to evaluate the effectiveness of the product on improving the quality of the wet white, the area pieces (butt, belly and beck), after filler treatment were subsequently RCF as described in Figure 1. Some of the samples were toggled dried and the others vacuum dried. All samples were then subjectively evaluated and mechanical properties were determined.

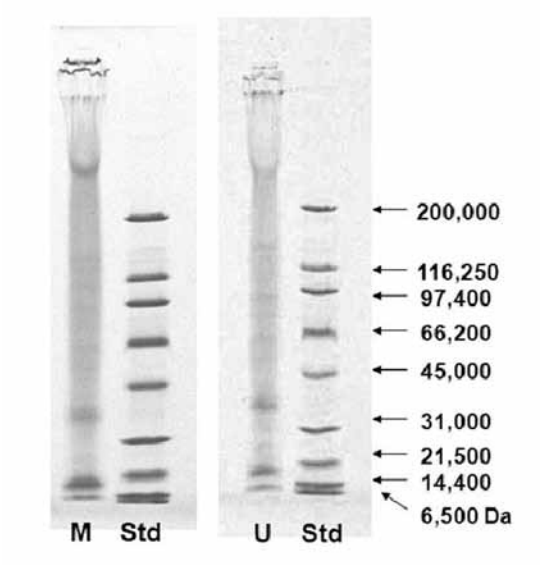

Figure 2: SDS-PAGE of 175 Bloom gelatin, 10% w/w concentration and WPI, 2%,w/v, untreated (U) and modified with 1 unit (per g of protein) mTgase (M); molecular weights are shown in Da.

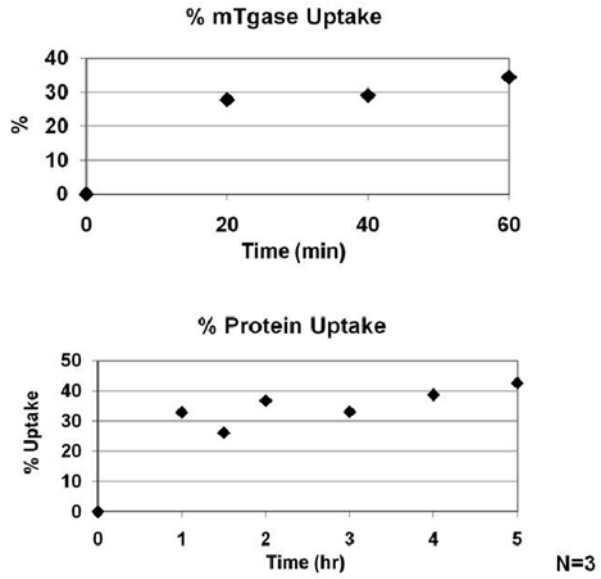

Figure 3: mTgase and protein uptake profiles of wet white pretreated with mTgase and then treated with gelatin/WPI biopolymer.

The results of subjective evaluation are shown in Figure 4 and these ratings are the average of data from five runs. As indicated, nine (60%) of the 15 test evaluations exceed the control samples, four (27%) test evaluations are equal to control sample and two (13%) of the test evaluations rated less than the control samples.

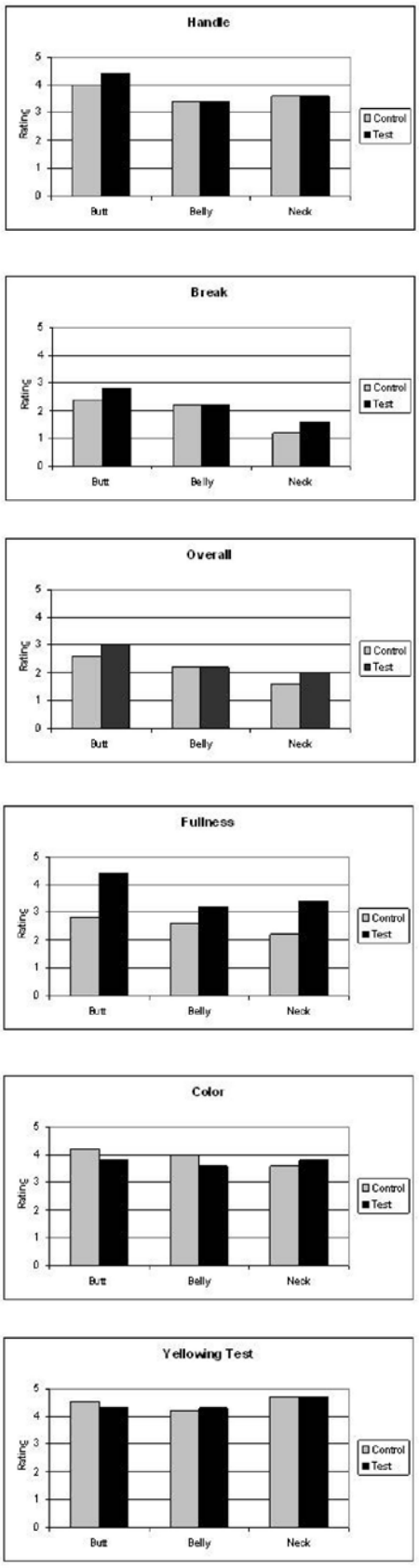

Figure 4: Subjective evaluation (handle, fullness, break, color and overall rating) using rating scale of 1 = worst to 5 = best, of wet white stock, treated with pH-adjusting agents alone (controls) and with mTgase and gelatin/WPI biopolymer (tests), then RCF; data are from five trials.

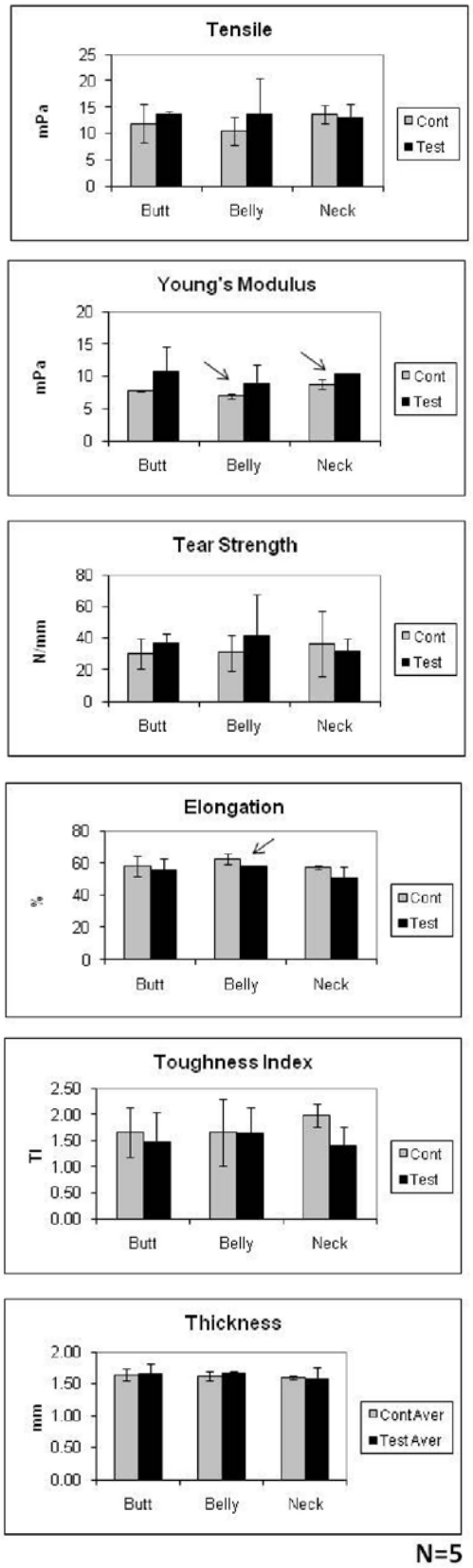

Figure 5: Mechanical properties (with STD Dev) of wet white stock, treated with pH-adjusting agents alone (controls) and with mTgase and gelatin/WPI biopolymer (tests), then RCF; data are from five trials.

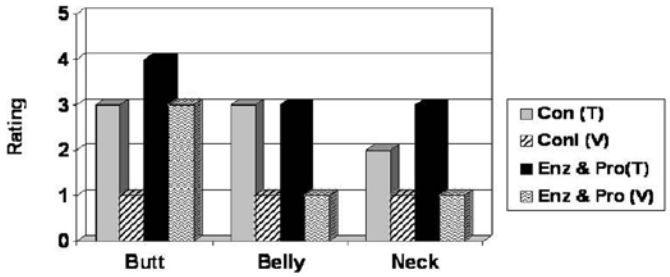

Figure 6: Subjective evaluation (overall rating) using rating scale of 1 = worst to 5 = best, of wet white samples, toggle dried (T) or vacuum dried (V), treated with pH-adjusting agents alone (controls) and with mTgase and gelatin/WPI biopolymer (tests), then RCF.

Thus there appeared to be significant improvement in the fullness, the break in the control and test samples was not good as indicated by the lower ratings of both, however the test samples did a little better than control. It has been reported[12] that protein additions traditionally have a detrimental effect on color change on the wet white samples. However, in this series of experiments, both control and test samples did very well in the yellowing test (Figure 4), for there did not appear to be any significant deterioration.

Mechanical properties were determined on butt, belly and neck area samples, and the results from these analyses are shown in Figure 5 and are the average of data from five trials.

For the most part there are no significant differences in the mechanical properties between the test samples and the controls. Exceptions are the higher percent elongation in the belly area of the control samples and higher Young's modulus in the belly and neck area of the test samples, indicating stiffer leather.

We carried out a trial in which the samples were vacuum dried as opposed to toggle dried. Vacuum drying traditionally has been avoided in wet white processing, for while it will improve the mechanical properties of wet white, it has a detrimental effect on subjective properties.

The overall rating, as indicated (Figure 6), substantiates this and rather dramatically shows the negative effect of vacuum drying on the subjective evaluation of the product while the mechanical properties (Figure 7), specifically the tensile strength, have improved.

Recent research by Liu et al at this laboratory, examined the effects of simultaneously toggling and vacuum drying wet white on the mechanical properties of the resulting leather.[13] This type of drying could be also beneficial to improving the subjective properties and thus has the potential to producing better wet white products.

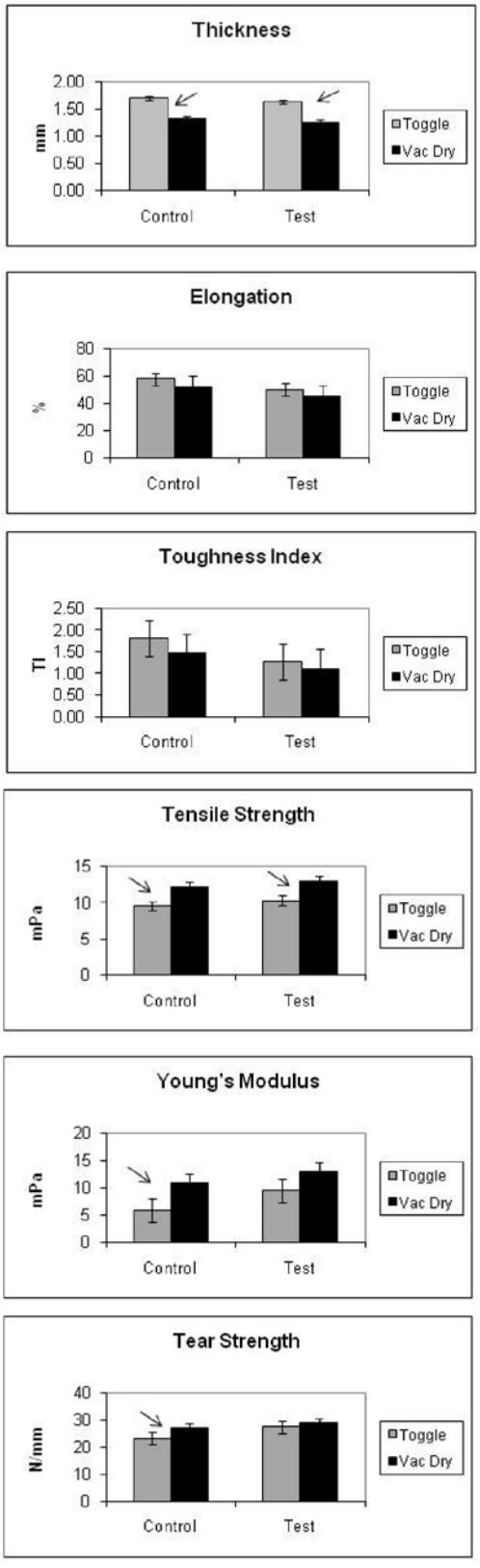

Figure 7: Mechanical properties (with STD Dev) of wet white stock, toggle dried (T) or vacuum dried (V), treated with pH-adjusting agents alone (controls) and with mTgase and gelatin/WPI biopolymer (tests), then RCF; toggle data is averaged from 5 trials, all areas, vacuum data is from one trial, areas averaged.

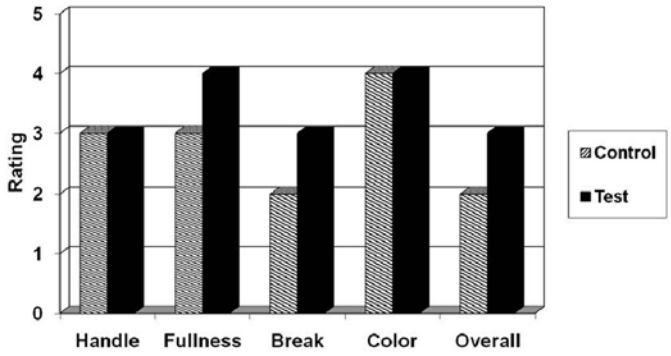

Figure 8: Subjective evaluation (handle, fullness, break, color and overall rating) using rating scale of 1 = worst to 5 = best, of wet white sides, treated with pH-adjusting agents alone (controls) and with mTgase and gelatin/WPI biopolymer (tests), then RCF.

Treatment of Sides

The butt, belly and neck areas give a good indication of biopolymer effectiveness on subjective evaluation of treated wet white. To get a better overall evaluation, the final run in this series was carried out on sides of wet white. The subjective evaluations of these sides are shown in Figure 8 and one can observe a further indication of biopolymer effectiveness.

In this experiment one can see that the break and overall evaluation have improved over the small sample runs while the fullness remains higher as seen before. Composite samples were taken from these sides and moisture and extractable analyses were performed. It was found that the moisture content of the control was 8.74% and that of the test was 10.6%, and based on standard deviations, these results were significantly different. The extractable values were 11.1% and 11.5% respectively for control and test samples and no significant difference was found. Finally, samples were taken from these sides for SEM analyses and representative images are shown in Figure 9. As has been seen in previous trials, the structure of the test samples appears to be more open than the controls.

CONCLUSIONS

Products prepared from enzyme-modified gelatin and whey protein isolate were applied to wet white leather. Since it was found that the biopolymer treatments, using labeled proteins, could not be monitored by epi-fluorescence microscopy because wet white hide samples auto-fluoresce at the same wave lengths as the Alexa Fluor® labels, a modified BCA test (using gelatin as a standard) was used effectively to monitor both enzyme and protein uptake. Subjective evaluation of treated butt, belly and neck areas and treated full sides showed that, for the most part, the fullness, color, and overall

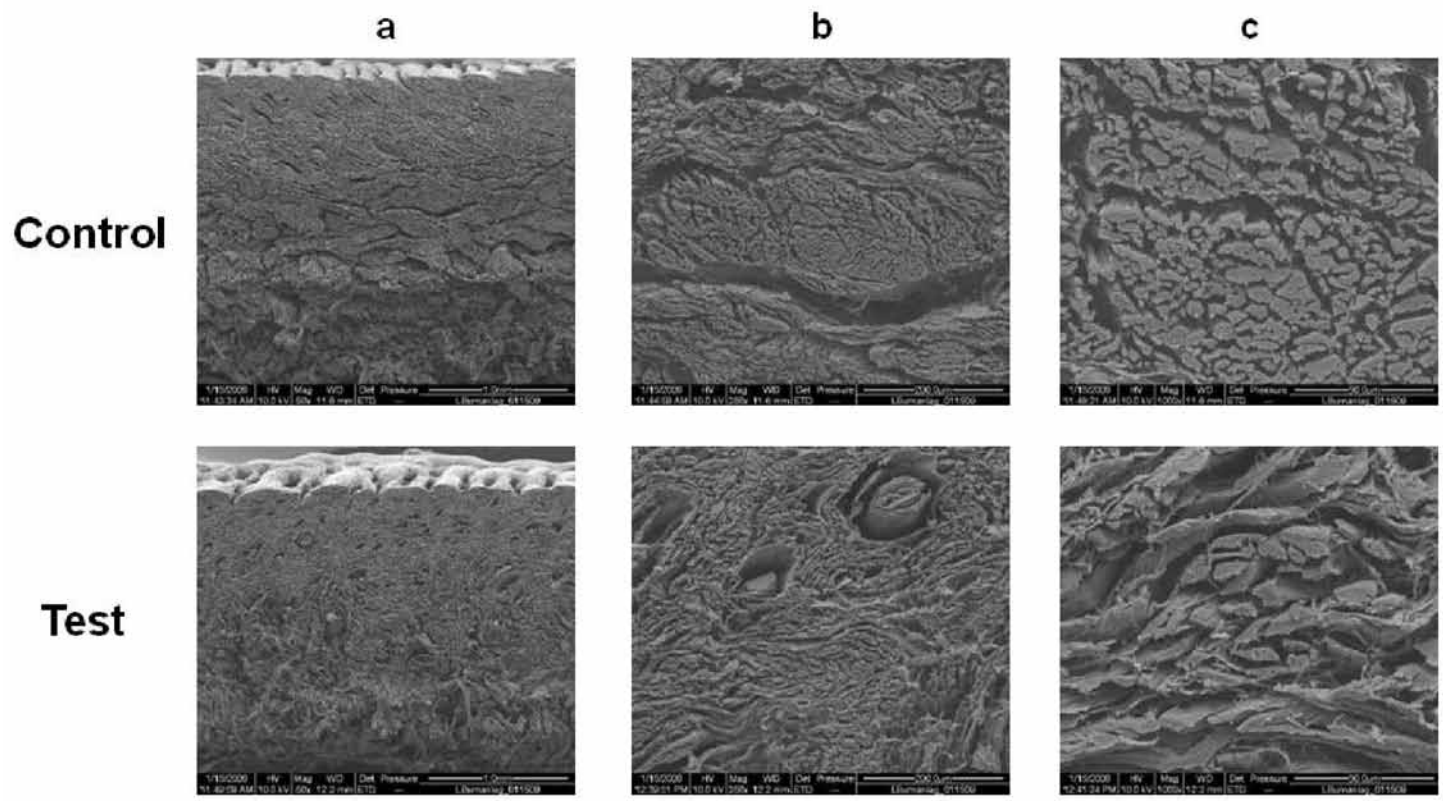

Figure 9: Scanning Electron Microscope (SEM) images of wet white stock from sides; control, treated with pH-adjusting agents alone, and test, treated with mTgase and gelatin/WPI biopolymer; (a), 50X (— = 1 mm), (b), 250X (— = 200 um) and (c), 1000X (— =50um).

evaluation improved, but perhaps a more robust filler could be employed to facilitate a better break. The yellowing test showed, in the combined evaluation of six studies, that the tests for belly and neck area were better than or equal to control; the test butt was slightly lower. As seen in previous studies, the mechanical properties of the treated wet white leather were not significantly different (other than in thickness) from controls. SEM images of the wet white have suggested a difference in fiber structure between controls and treated samples, with the latter having a more open structure. When vacuum dried and toggled dried wet white sample were compared, the subjective evaluation of the vacuum dried samples was poorer in all respects. With respect to mechanical properties the thickness is less in the vacuum dried samples (which one would expect) and the tensile strength of the vacuum dried samples was improved in both test samples and controls. Perhaps a combination toggle and vacuum dry operation would allow wet white to have benefit of both.

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