Effect of Fillers Prepared from Enzymatically Modified Proteins on Mechanical Properties of Leather*,§

by

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Abstract

In an environment where petroleum feedstuffs are becoming increasingly too expensive for a good cost-effective return, utilization of renewable resources makes economic sense, particularly when these substrates are waste proteins. We have thus proposed the application of enzymatically modified waste proteins from the leather (gelatin) and dairy (casein and whey) industries as fillers in leather production. In previously reported research, we prepared different combinations of these waste proteins, enzymatically modified them, characterized the products and applied them to blue stock using fluorescently labeled proteins to determine how they were distributed in the hide and more importantly not removed by washing. From the data acquired in these previous experiments, we identified potential filling materials. We now have treated various areas in the hide (butt, belly, and neck) with the products, retanned, colored and fatliquored the treated pieces, evaluated them with respect to subjective properties against controls, and finally determined mechanical properties. The results from these tests show that the mechanical properties were not significantly affected by the treatment and subjective properties, e.g., handle, fullness, break and color, were improved over the controls. Fillers thus have the potential to be economically produced from sustainable resources as an alternative to more expensive and increasingly limited conventional products.

Resumen

En un entorno donde las materias primas derivadas del petróleo resultan incrementadamente más caras para poder rendir una efectiva utilidad basada sobre costos, entonces, la utilización de recursos renovables adquier gran sentido económico, especialmente cuando los substratos son derivados de desechos proteínicos. Hemos propuesto entonces el tratamiento por modificación enzimática y uso de proteínas desechadas de las industrias del cuero (gelatina) y derivados lácteos (caseína y suero) como rellenantes en la producción del cuero. En investigaciones previamente publicadas, preparamos diferentes combinaciones de estas proteínas de desecho, las modificamos enzimáticamente, caracterizamos los productos, y los aplicamos a cuero curtido al cromo utilizando proteínas etiquetadas detectables por fluorescencia para determinar la distribución en la piel, y más importantemente, para verificar que no fueran removidas por lavado. Por medio de los datos adquiridos en estos experimentos previos, hemos identificado materiales potencialmente rellenantes. Ahora hemos tratado muestras de diferentes zonas de la piel (croupón, falda y cuello) con los productos, recurtidas, teñidas, y engrasadas y los pedazos evaluados con respecto a las propiedades subjetivas, v.gr. toque, llenura, firmeza de flor y color fueron mejoradas comparadas a los controles. Rellenantes así son potencialmente producibles económicamente provenientes de recursos sostenibles como alternativa a más costosos y incrementadamente limitados productos convencionales.

Introduction

In a prior publication from this laboratory, the use of glutaraldehyde-modified gelatin as a filling agent was described.[1] The results demonstrated, using a fluorescent label attached to the protein, that the polymerized gelatin had indeed entered the loose areas of the hide, acted as a filler, and more importantly, did not wash out after further processing. In

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a more recent study,[2] we expanded on the use of gelatins as fillers by examining whether the extensively reported[3-9] enzymatic polymerization of gelatin using environmentally benign and relatively inexpensive microbial transglutaminase would effectively produce filled products similar to those described in chemical modification. The efficiency of the reaction was again monitored by epi-fluorescent microscopy and it was demonstrated that the enzyme-modified gelatin acted as a filler.

Biopolymers formed by the enzymatic crosslinking of dissimilar proteins have the potential for generating novel products. Many reports have appeared on the properties of a variety of biopolymers synthesized by enzymatic treatment.[10-12] Based on this reported research, we designed experiments in which we enzymatically reacted gelatin with casein[13] or whey proteins;[14] after characterization, we found that unique, highly polymerized products were obtained. We then examined whether these mixed proteins could be applied to enzymatically treated blue stock and be effective as fillers. It was shown, again using fluorescent labels, that indeed these mixed protein products could also be used as fillers, were bound to the leather, and would not easily be removed during further processing [2,14]. We also investigated whether protein combinations could first be enzymatically polymerized (similar to the glutaraldehyde studies), then added to blue stock to give the same filling effect as was found in previous studies.[15] Advantages to producing the products outside the leather would be to allow us to characterize the products with respect to solubility, thermal stability, viscosity and molecular weight distribution (degree of polymerization). Again, with the assistance of the fluorescent labels, it was found that the biopolymers were attached and did not migrate upon washing or further treatments.[15]

In this present study, we treated blue stock with products that were identified, in the above mentioned studies, as potential fillers.[2, 13-15] The treated blue stock was then retanned, colored, and fatliquored (RCF), dried, and subjected to mechanical testing. Percent extractables, subjective evaluation, and yellowing tests were performed. These data along with Scanning Electron Microscopy (SEM) images of blue stock and RCF samples will be presented.

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) and whole whey (11% protein and approximately 65% lactose) were obtained from Sigma (St. Louis, MO). Sodium caseinate (Alanate® 180), and whey protein isolate or WPI (Alacen™ 895) containing 93.2% protein, were generously supplied by NZMP (formerly New Zealand Milk Products) (Lemoyne, PA). Trutan PA-65 and PRP-77 were obtained from the former Pilar River Plate Corp. (Newark, NJ); Havana Dye (Derma Havana R Powder) was obtained from Clariant Corporation (Charlotte, NC); Altasol-CAM and Eureka 400R were obtained from Atlas Refinery, Inc. (Newark, NJ). Chrome-tanned blue stock was purchased from a local tannery. All other chemicals were analytical grade and used as received.

Preparation of biopolymer products

Gelatin samples (175 Bloom; 30 g) in combination with sodium caseinate, WPI or whey (each, 5.4 g) were suspended in water (220 ml) and allowed to swell for about 2 h at RT; they were stored overnight at 4°C after which they were placed in a bath at 65°C until dissolved. Control samples, to which no enzyme was added, were run to monitor changes in physical properties. The pH was adjusted with 1 N NaOH to 6.5-7.0 for gelatin samples and 7.0-7.5 for gelatin in combination with casein, WPI or whey. To the samples that contained WPI or whey proteins, 0.5% DTT was added, and the resulting mixtures were heated at 38°C for one hr. Varying concentrations of microbial transglutaminase (calculated to be 0-3 units/g of total protein for biopolymer reactions) were prepared in 50 ml of water and these solutions were added with stirring to the protein solutions to give a final protein concentration of 10% w/w for gelatin and 2% w/v for sodium caseinate, WPI and whey. Aliquots of the reaction mixtures were added to test tubes for melting point determination (10 ml) and to appropriate containers for determining gel strength (30 ml). 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 90°C for 10 min. The samples were cooled to room temperature and then chilled for 17 h at 10°C in a circulating 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% soln) was added as a preservative and the samples were stored at 4°C until use.

Application of filler to wet blue leather

As outlined in Figure 1, six pieces of blue stock (~100g each), two pieces each from the butt, belly and neck area, were divided into tests and controls, placed in two Dose drums (Model PFI 300-34, Dose Maschinenbau GmbH, Lichtenau, Germany), washed twice (400% float) by drumming each time for 30 min at 50°C, drained and refloated in 1% sodium bicarbonate solutions (400% float). The samples were drummed at ambient temperature (25-28°C) until the pH of the float stabilized. The floats were drained, 5% mTgase (based on wet weight of the hide) with a 400% float was added to the test samples, while water alone (400% float) was added to the controls and all samples were drummed for 1 h at ambient temperature. The floats were drained and the prepared biopolymer solutions, diluted to give a 400% float based on wet hide weight, were added to the test drums; water (400% float) was added to the control samples. The gelatin/WPI (3 units) sample had a 10% gelatin and 2% WPI loading added to the blue stock, whereas

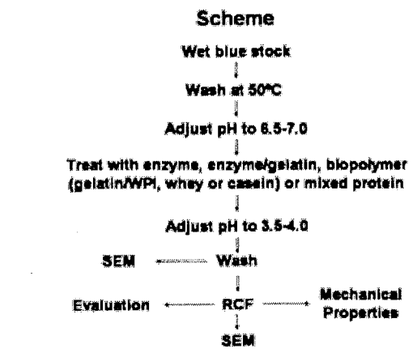

Figure 1: Schematic for treatment, sampling and analysis of blue stock.

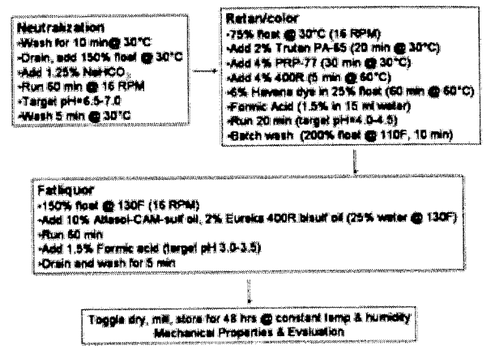

Figure 2: Flow diagram for retan, color and fatliquor of control and treated samples of blue stock.

all other fillers were applied with 5% gelatin alone, or 5% gelatin and 1% WPI, whey, or sodium caseinate loading. The samples were then drummed for 1 h at ambient temperature and then for 4 h at 50°C. In the experiment in which only enzyme was used, the sample was drummed in the enzyme float for a further 4 h at 50°C. In the experiment in which unmodified gelatin was used, the samples were run at RT for one h. The pH was then adjusted to 3.5-4.0 with 4.0 M acetic acid. 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 and the controls were placed in two Dose drums and the samples were retanned, colored and fatliquored as shown in Figure 2. When completed, all pieces were toggled and left to dry at ambient temperature and humidity. They were conditioned, put into plastic bags for one day, then staked twice, and milled for approximately 24 h. No finishing operations were done to the hides and they were kept on a shelf in the conditioning room at 20°C and 65% relative humidity (RH) for at least 3 days.

Analyses

Physical properties, molecular weight distribution and extractables

Gel strength, melting point, viscosity, and molecular weight distribution (by SDS-PAGE) of the enzyme-treated proteins were determined as described in previous publications.[16,17] Percent extractables in RCF samples was determined as described in ASTM D 3495-83.

Subjective evaluation

Each treated and control sample was evaluated with respect to handle, grain, 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 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 (greatest color change) and 5 being the best (least affect on color).

Mechanical Properties

The samples were stored in a conditioned room at 20°C and 65% RH according to ASTM D1610-01. Mechanical property measurements were performed parallel to the backbone with a strain rate of 10 in/min and a separation between the jaws of 4 inches (10 cm). The mechanical property measurements included: tear strength, tensile strength, elongation, and Young's Modulus. The tear strength, defined as the load at which the initial tear occurs in the sample, was determined according to ASTM D4704 and was normalized by dividing the tear load by the thickness of the sample and is presented with the units of N/mm. Dogbone-shaped samples were cut out and the tensile strength, defined as the stress required to rupture the leather, was determined according to ASTM D2209. Elongation is defined as the maximum strain at rupture. Young's modulus, a physical quantity representing the stiffness of the material, is determined by measuring the slope of a line tangent to the initial stress-strain curve.[18] An upgraded Instron mechanical property tester, model 1122, and Testworks 4 data acquisition software (MTS Systems Corp., Minneapolis, MN) were used throughout this work. Each test was conducted on five samples of untreated and filled RCF blue stock; the average was calculated, and from the mean and standard deviation (STD), error bars were determined.

Scanning electron microscopy (SEM)

The treated blue stock samples were cut into small strips (6.5 cm × 1 cm) and freeze-dried. Two pieces (1.5 mm) were cut from each of the dry samples and 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

TABLE I
Physical Properties of Products

Samples[ab]	Gel Strength (g)	MP (°C)	Viscosity (cP) @ 60°C
Gelatin (0 u)	447.4	35.7	6.33
Gelatin/WPI (3u)	422.2	43.4	84.90
Gelatin/WPI (2u)	501.6	38.7	31.78
Gelatin/WPI (1u)	380.2	37.8	8.45
Gelatin/WPI (0u)	382.1	36.6	6.45
Gelatin/Whey (2u)	363.0	42.2	7.67
Gelatin/Whey[c] (0u)	395.5	35.6	6.52
Gelatin/Casein (1u)	393.4	37.8	12.40
Gelatin/Casein[c] (0u)	235.3	33.0	7.46

[a]Concentration in solution: Gelatin = 10% w/w; WPI, whey or sodium caseinate = 2% w/v
[b]n=1
[c]Control samples

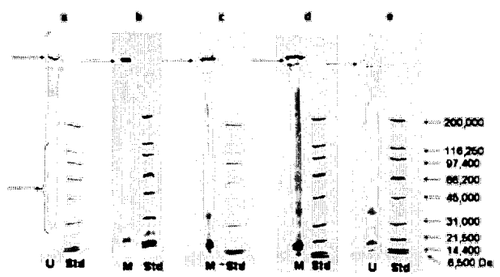

Figure 3: SDS-PAGE of 10% gelatin and 0 units (a) and 10% gelatin with 2% WPI and 3 units (b), 2 units (c), 1 unit (d), and 0 units (e) of mTgase ("U" is unmodified sample and "M" is modified sample; molecular weights are shown in Da).

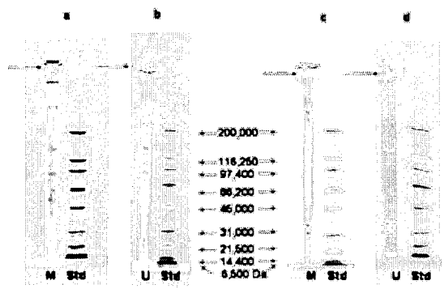

Figure 4: SDS-PAGE of 10% gelatin and 2% whey with 2 units (a) and 0 units (b) of mTgase and 10% gelatin and 2% sodium caseinate with 1 unit (c) and 0 units (d) of mTgase ("U" is unmodified sample, "M" is modified sample; molecular weights are shown in Da).

coater (180 sec). 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

Polymer Preparation and Characterization

From previous studies,[2, 13-15] we determined, based on physical properties, degree of polymerization and epi-fluorescent images of treated blue stock, that the products from enzymatic modification of gelatin in combination with WPI, whey or casein, should warrant further investigation as fillers. In this present study, we scaled up these various treatments and applied them to the butt, belly and neck areas of the hide. Subsequently, the pieces were retanned, colored and fatliquored, mechanical properties were determined and subjective evaluation was performed.

Characterization of the polymers with respect to physical properties and degree of polymerization is essential in determining the optimal products to be used as fillers. Table I shows the various combinations of gelatin and mixed proteins that were prepared and subsequently characterized with respect to their physical properties; the properties of the unmodified proteins are also included. If one compares the physical properties of the modified and unmodified samples, one can see that the addition of enzyme increased the gel strengths, melting points, and viscosities of the gelatin and WPI or casein combinations. The gel strength of the gelatin/whey combination has decreased, a phenomenon we have previously observed,[14] and may be due to dilution of the sample with lactose in the whey, however, the melting point and viscosity did increase.

The gelatin/WPI product with 3 units of enzyme had a melting point of 43.4°C and a viscosity of 84.9 cP; 10% gelatin/2% WPI (based on the blue weight) was offered. This product was difficult to handle during treatment, for it was necessary to heat the solution and then emulsify using a hand blender. We surmised that the product had too high a degree of polymerization, so our second gelatin/WPI product was prepared with only 2 units of enzyme added to polymerize the protein. As seen in Table 1, the melting point was lower (38.7°C) and the viscosity at 31.8 cP was less than half that of the previous preparation. From the previous gelatin/WPI experiment, we also surmised that we overloaded the offering, for a gelled product remained after treatment; we reduced the loading so only 5% gelatin/1% WPI (based on blue weight) was offered in this trial and in all subsequent trials using various treatments. We had no problems in application of this product, and, from data obtained in this trial, we investigated the feasibility of products that were even less polymerized. In the final gelatin/WPI experiments we added 1 unit and 0 units respectively to the protein solutions. The physical properties data (Table 1) show that the melting point and viscosity of the 1 unit enzyme addition is only slightly higher than the 0 unit product.

At the same time, we examined the molecular weight distribution of the products prepared. In Figure 3, the SDS-PAGE gels of unmodified gelatin, and effect of increasing enzyme concentration on the degree of polymerization of gelatin and WPI combinations are shown. As the amount of enzyme added to the gelatin/WPI increased from 0 to 3 units (e to b), the band that will not enter the gel (arrow) becomes darker, indicating that an increasing amount of a high molecular weight moiety is being formed. In Figure 3a, the broad bands that represent gelatin (from 21,000 to 116,000Da) are decreasing in intensity, and likewise to a slighter extent for the bands between the 6,500 to 45,000 Da molecular weight range that are characteristic of WPI. It is interesting to note that the molecular weight distribution for gelatin/WPI treated with 3 units of enzyme (Figure 3b) indicates a high degree of polymerization, thus correlating with the melting point and viscosity data shown in Table 1. As was reported in a previous study,[13] when gelatin and WPI combinations are treated with mTgase, the gelatin is preferentially affected over WPI. This can be seen also in this study.

In the next experiment, we prepared products from gelatin and low-cost whey. To a solution containing 10% gelatin and 2% whey, we added 2 units of enzyme, and the physical properties are shown in Table 1. With a melting point of 42.2°C and viscosity of 7.67 cP, we had no difficulty preparing a treatment solution with this product. Figures 4a and b show the molecular weight distribution of the modified and unmodified gelatin/whey combinations. As indicated by the arrow, the high molecular weight band that does not enter the gel has become a little more intense than in the control sample. Whey contains 65% sugar and only 11% protein and the bands contributed by this product are difficult to see.

In the final experiment, we prepared a gelatin/casein biopolymer using 1 unit of enzyme/gm total protein. With a melting point of 37.8°C and viscosity of 12.4 cP (Table I), the product proved easy to handle, with respect to solubility and uptake by the blue stock. Figures 4c and d are showing the molecular weight distribution of the modified and unmodified gelatin/casein combinations, and the high molecular weight band that does not enter the gel has become darker. It also appears that molecular weight bands between 45,000 and 200,000 Da have become more intense, further indicating the gelatin/casein reaction with the enzyme.

In summary, the data from the physical properties study and the SDS-PAGE gels correlate quite well. These data are indicating that the degree of polymerization of these products, except for the gelatin/WPI (3 units), is not too extensive, and, along with the ease of handling and uptake of the protein, point toward making products that have viscosities and melting points that would be manageable in the treatment temperature range (ambient to 50°C).

Application of fillers to wet blue and RCF

As shown in the scheme outlined in Figure 1, the blue stock pieces were washed thoroughly several times. Control pieces from the butt, belly, and neck areas, to which no filler was added, were run in a separate drum; these samples were only subjected to pH adjustment. When the appropriate pH for optimal enzyme activity was reached (about 6.5-7.5 after pretreatment with 1% $NaHCO_3$), the floats were drained and 5% mTgase was added to the test pieces; the samples were then drummed, at ambient temperature, for 1 h. The purpose of this step was to allow the enzyme to be distributed uniformly inside the hide pieces but not react to a great extent with the hide because the temperature was not optimal. In order to determine the effect, if any, of enzyme on mechanical and subjective properties, and gelatin on subjective properties (e.g. dye uptake) two experiments were carried out in which enzyme only and enzyme with unmodified gelatin were used to treat the blue stock. In the remaining experiments, the floats were drained after enzyme treatment and the protein biopolymer solutions were diluted to give appropriate float and concentration and then added to the test blue stock pieces.

The gelatin/WPI with 3 units of mTgase was offered at 10% and 2% based on the blue weight, but, after the treatment was complete, the float contained unabsorbed product so all other polymer treatments were offered at half this amount. The samples were drummed for 1 h at ambient temperature, again to distribute the protein uniformly within the hide; and then for 4 h at 50°C, which is the optimal temperature for enzyme reaction. (In the enzyme-only treatment, after 1 h at room temperature, the pieces were drummed for a further 4 h at 50°C. In the unmodified gelatin treatment, the samples were drummed for 1 h at RT.) The pH was then adjusted to below 4.0 using acetic acid (this pH will inactivate the enzyme); the floats were drained and the samples were washed twice at 50°C to remove any unattached protein.

All samples were then RCF as outlined in Figure 2. The control samples and the filled samples were again run in separate drums, so as to prevent competing uptake of retan, dye, and fatliquor.

TABLE II
Subjective Evaluation[ab]

Treatment	Butt		Belly		Neck	
	Control	Test	Control	Test	Control	Test
Enzyme alone[c]	2	3	3	3	4	3
Enzyme and gelatin[cd]	3	4	4	4	2	3
Gelatin/WPI (3 units)[ce]	2	2	3	3	3	2
Gelatin/WPI (2 units)[cf]	2	3	1	4	2	4
Gelatin/WPI (1 unit)[cf]	2	4	2	4	2	4
Gelatin/WPI (0 units)[cf]	4	5	3	5	4	5
Gelatin/whey (2 units)[cf]	3	5	2	3	3	4
Gelatin/casein (1 unit)[cf]	4	5	2	4	3	5

[a]Scale 1-5, 1=worst, 5=best
[b]n=2
% offered, based on blue weight: [c]5% mTgase; [d]5% gelatin; [e]10% gelatin, 2% WPI; [f]5% gelatin, 1% WPI, whey, or sodium caseinate

TABLE III
Yellowing Test[ab]

Treatment	Butt		Belly		Neck	
	Control	Test	Control	Test	Control	Test
Enzyme alone[c]	3	3	2	3	2	3
Enzyme and gelatin[cd]	4	4	4	3	3	3
Gelatin/WPI (3 units)[ce]	4	3	3	3	2	3
Gelatin/WPI (2 units)[cf]	4	2	4	3	3	2
Gelatin/WPI (1 unit)[cf]	4	4	4	3	3	2
Gelatin/WPI (0 units)[cf]	3	3	3	2	2	3
Gelatin/whey (2 units)[cf]	5	3	4	2	3	3
Gelatin/casein (1 unit)[cf]	4	5	3	4	4	4

[a]Scale 1-5, 1=worst, 5=best
[b]n=2
% offered, based on blue weight: [c]5% mTgase; [d]5% gelatin; [e]10% gelatin, 2% WPI; [f]5% gelatin, 1% WPI, whey, or sodium caseinate

It was observed that the floats for the filled samples were lighter after coloring, indicating better uptake of the dye. After drying at ambient temperature and conditioning, the samples were milled for 24 h; we increased the time to accentuate any differences between the test pieces and the controls.

Subjective Evaluation

All samples were evaluated with respect to handle, grain (break), 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 are the values (average of two separate evaluations) that are reported in Table II.

Test values that were equal to or better than controls are underlined. In almost all cases, except for enzyme treatment alone in the neck and gelatin/WPI with 3 units treatment, also in the neck, the test pieces were found to be equal to or superior to the control pieces. This evaluation also demonstrated that the enzyme and gelatin treatment did not adversely affect these properties.

Yellowing Test

The yellowing test was run on all samples and the results can be seen in Table III. Again, the test values that were equal to or better than controls are underlined. The enzyme treatment

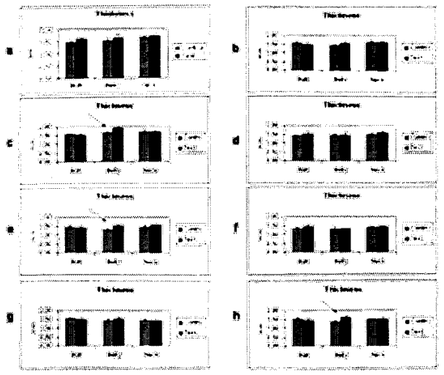

Figure 5: Thickness (mm) of blue stock after various treatments; enzyme alone (a), gelatin (0 units) (b), gelatin/WPI (3 units) (c), gelatin/WPI (2 units) (d), gelatin/WPI (1 unit) (e), gelatin/WPI (0 unit) (f), gelatin/whey (2 units) (g), gelatin/casein (1 unit) (h).

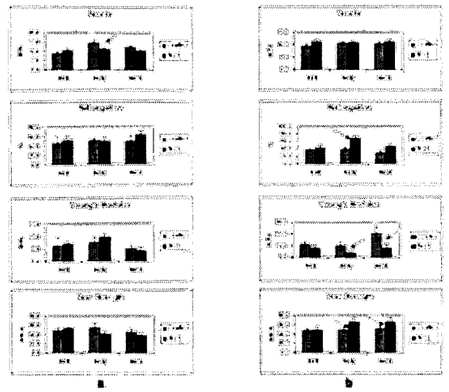

Figure 7: Mechanical properties (tensile, elongation, Young's Modulus and tear strength) of blue stock treated with gelatin/WPI (3 units) (a) and (2 units) (b).

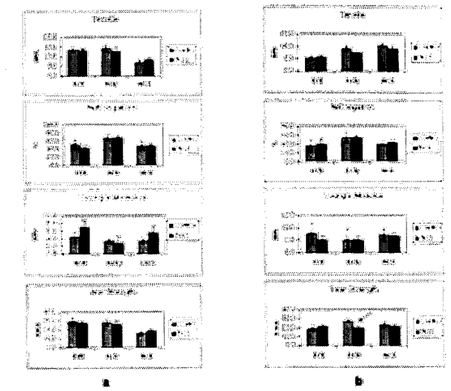

Figure 6: Mechanical properties (tensile, elongation, Young's Modulus and tear strength) of blue stock treated with enzyme alone (a) and gelatin (0 units) (b).

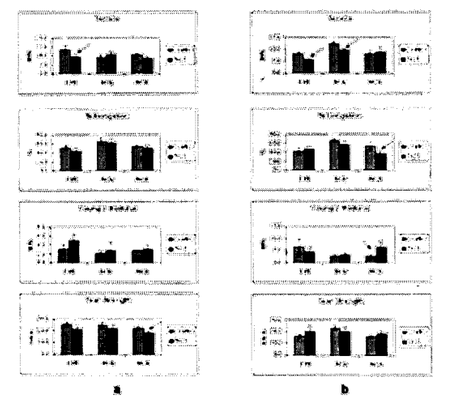

Figure 8: Mechanical properties (tensile, elongation, Young's Modulus and tear strength) of blue stock treated with gelatin/WPI (1 unit) (a) and (0 units) (b).

alone, enzyme and gelatin alone, except in the belly area, gelatin/WPI (3 units), except in the butt area, gelatin/WPI (1 unit and 0 units) except in the belly and neck areas, and gelatin/casein (1 unit) were equal to or better than the controls. The majority of the filled samples did not perform as well as the controls; the results of this test (yellowing after heating) are characteristic of the results found when proteins are incorporated in the leather finishing process. Again, in general, the enzyme and gelatin treatments did not adversely affect the leather.

Mechanical Properties

As mentioned in the Experimental section, the controls for the filled samples were run so the same process of pH adjustment could be applied to them as the filled samples. The only difference was that no enzyme alone, gelatin/enzyme, or biopolymer was added to them. In Figure 5 we are showing the thickness of the control and filled samples in three different areas. For the most part, there appears, based on the error bars, not to be any significant difference in these samples, except (as shown by the arrows) for the belly areas in Figures 5c, 5d, and 5h, which represent blue stock that had been treated with gelatin/WPI (3 units), gelatin/WPI (2 units) and gelatin/casein (1 unit), respectively. Gelatin/WPI (3 units) had a 10%/2% loading of biopolymer added to the blue stock, whereas the other two biopolymers were applied in 5%/1% loading.

Figures 6 through 9 show the tensile strength, percent elongation, Young's Modulus and tear strength for samples from the different treatments. Arrows point to data that are significantly different as indicated by the error bars. In Figure

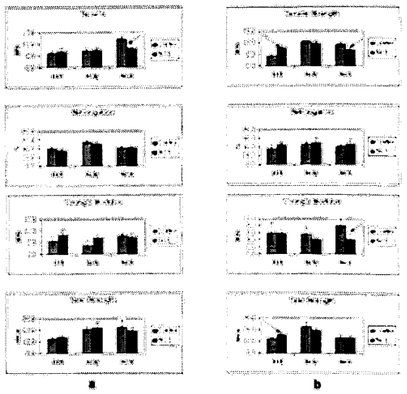

Figure 9: Mechanical properties (tensile, elongation, Young's Modulus and tear strength) of blue stock treated with gelatin/whey (2 units) (a) and gelatin/casein (1 unit) (b).

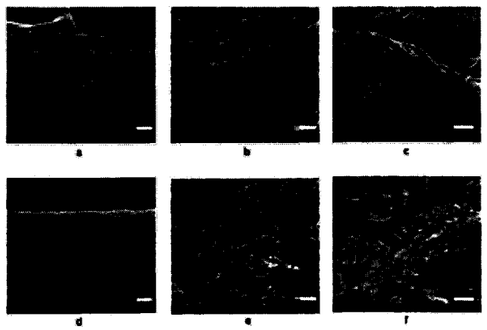

Figure 10: Scanning Electron Microscope images of blue stock from neck area: controls (treated with pH-adjusting agents alone) 100X (– = 300 μm) (a), 250X (– = 50 μm) (b), and 1000X (– = 20 μm) (c); filled blue stock, treated with gelatin/whey (2 units) 100X (– = 300 μm) (d), 250X (– = 50 μm) (e), and 1000X (– = 20 μm) (f).

6, which represents the blue stock that had been treated with enzyme alone (Figure 6a) and with enzyme then gelatin (Figure 6b), the only significant difference can be seen in the belly area for the tear strength of the enzyme/gelatin treated sample. Figure 7 shows the mechanical properties of blue stock that had been treated with gelatin/WPI (3 units) (Figure 7a) and gelatin/WPI (2 units) (Figure 7b). With respect to the former, the tensile for the control in the belly area is higher, but there are no significant differences in the other tests; in the latter, the percent elongation and the tear strength appear to be better in the test samples, whereas the Young's Modulus for the test sample, being lower, indicates that the samples are softer than the control.

Figure 8 shows the mechanical properties of blue stock that had been treated with gelatin/WPI (1 unit) (Figure 8a) and gelatin/WPI (0 units) (Figure 8b). For the most part there does

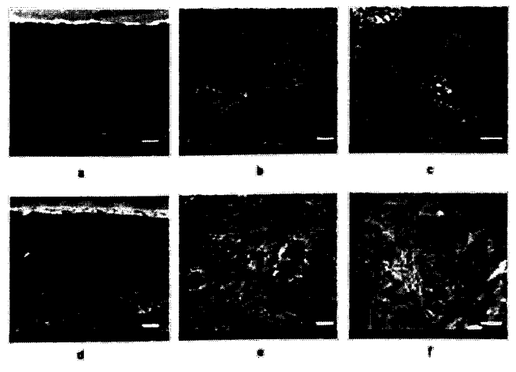

Figure 11: Scanning Electron Microscope images of retanned, colored, fatliquored blue stock from neck area: controls (treated with pH-adjusting agents alone) 100X (– = 300 μm) (a), 250X (– = 50 μm) (b), and 1000X (– = 20 μm) (c); filled blue stock, treated with gelatin/whey (2 units) 100X (– = 300 μm) (d), 250X (– = 50 μm) (e), and 1000X (– = 20 μm) (f).

not appear to be any significant differences in the gelatin/WPI (1 unit) samples except for the tensile strength for the control in the butt and the tear strength for the control in the belly. With respect to the gelatin/WPI (0 units), the tensile strength of the controls in the butt and belly are higher than the tests, the elongation is higher in the neck control and for the Young's Modulus in the belly area, the control is softer than the filled leather. Figure 9 shows the properties of the gelatin/whey (2 units) (Figure 9a) and gelatin/casein (1 unit) (Figure 9b). In the former, there are no significant differences found except in the tensile strength for the control in the neck area. In the latter, the tensile strength is higher for the filled in the butt but lower in the neck. The Young's Modulus for the neck test is low, indicating softer leather, and the tear strength is higher for the filled leather in the neck area.

In general, it does not appear that the mechanical properties of the filled leather samples are significantly different from those of the control samples, indicating that the addition of the filler and the treatment with the enzyme and/or gelatin does not adversely affect these properties.

Extractables

Percent extractables were carried out on all the RCF samples and the results can be seen in Table IV; test values that were better than controls are underlined. In almost all cases the percent extractables were higher in the filled samples than in the untreated samples. The only exceptions were the enzyme/gelatin samples from the butt area, the gelatin/WPI (3 units) from the belly and neck area and the gelatin/WPI (0 units) samples from the neck area. The enzyme-alone treatment gave slightly lower values in the belly and neck areas. Apparently, the addition of the protein improves the fatliquor pick-up, which would ultimately lead to a product with enhanced properties as noted in the subjective evaluation.

TABLE IV
% Extractables[a]

Treatment	Butt		Belly		Neck	
	Control	Test	Control	Test	Control	Test
Enzyme alone[c]	10.9	11.9	14.7	13.6	14.8	13.2
Enzyme and gelatin[bc]	11.3	10.9	9.2	15.6	12.4	14.7
Gelatin/WPI (3 units)[bd]	10.6	12.3	15.8	13.2	15.6	11.8
Gelatin/WPI (2 units)[be]	12.3	13.4	10.5	18.8	13.5	16.8
Gelatin/WPI (1 unit)[be]	9.8	13.6	14.1	16.0	9.5	15.2
Gelatin/WPI (0 units)[be]	11.0	11.3	13.4	14.9	13.2	9.9
Gelatin/whey (2 units)[be]	7.7	11.3	11.2	13.4	10.6	13.4
Gelatin/casein (1 unit)[be]	8.7	13.6	10.1	12.0	8.7	10.1

[a]MFB, n=1
% offered, based on blue weight: [b]5% mTgase; [c]5% gelatin; [d]10% gelatin, 2% WPI; [e]5% gelatin, 1% WPI, whey, or sodium caseinate

SEM Examination

All filled blue stock and RCF samples as well as their controls were sampled for SEM investigation. Figure 10 is representative of the micrographs of blue stock that were examined; this sample, of the neck area, was treated with gelatin/whey (2 units). If one compares the control samples (Figures 10a, b, and c) to the tests (Figures 10d, e, and f) in the three magnifications, one can see that the structure of the filled samples is more open; the fibers appear to be separated. In a previous study,[15] we had observed that the treated samples, as compared to the control, have more regularity to their structure; the fibers appear to be larger in diameter, which may indicate that fibers are being coated with the polymer. RCF polymer-treated blue stock and controls (from gelatin/whey, 2 units, neck area) were also examined using SEM (Figure 11), but these samples demonstrated that it was difficult, in most cases, to distinguish differences between control (11a, b, and c) and test (11d, e, and f) after the samples were RCF.

CONCLUSIONS

When one prepares fillers from gelatin alone or from enzymatically modified mixed proteins, products that do not have a high degree of polymerization as indicated by physical properties and molecular weight distribution are desirable. It was found that 5% gelatin/1% WPI, whey or casein in a 400% float, based on blue weight, gave satisfactory results, but less protein offered, in a shorter float, might also be satisfactory. Almost all treatments gave products that, when evaluated subjectively with respect to grain, handle, fullness and color, were superior to controls. However, even though the yellowing test results for the gelatin/casein (1 unit) treatment and the enzyme treatment alone were either equal to or better than the controls, in most cases the results from other treatments did not fare as well. The filled samples essentially had mechanical properties similar to controls, indicating that the addition of the filler and the treatment with the enzyme do not adversely affect these properties. In almost all cases the percent extractables was higher in the filled samples than in the untreated samples, which would ultimately lead to products with enhanced properties as noted in the subjective evaluation. SEM investigation showed that the fibers in the filled blue stock were separated and more regular than the controls; no differences could be seen in RCF samples. Thus these reactions between multiprotein biopolymers have the potential to be used in leather processing as economical replacements to petroleum-based fillers and potentially as coatings.

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