Whey Protein Isolate: A Potential Filler for the Leather Industry

by

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Abstract

The upgrading of leather that presents loose areas and poor grain break is one of the most value adding opportunities for a tanner. Typically, petroleum-based products are used to improve the final appearance and feel of crust leather. In this study, we demonstrate that blends composed of whey protein isolate (WPI), a byproduct of the cheese industry, and small amounts of gelatin, a byproduct of the leather industry, could be effectively used as filling agents for both shoe upper and upholstery leather. Wet blue leather from three different areas in the hide (butt, belly and neck) was treated with the WPI-gelatin blend, retanned, colored and fatliquored, and their subjective and mechanical properties evaluated. The effect of pretreatment of the wet blue samples with various concentrations of the enzyme microbial transglutaminase (mTGase) was also examined. It was found that the rate of uptake of the WPI-gelatin blend by upholstery wet blue increased four-fold when it was pretreated with a 2.5% mTGase solution. Conversely, this rate was decreased when shoe upper was pretreated with increasing amounts of mTGase. The subjective properties (e.g. handle, fullness, color and grain break) of both shoe upper and upholstery leather that were treated with the WPI-gelatin blend were significantly improved over the controls. Importantly, the grain break of the belly area of samples that were pretreated with enzyme (both upholstery and shoe upper) was remarkably improved. Hence, fillers mainly composed by the less expensive WPI were demonstrated to be effective filling agents for both upholstery and shoe upper leather.

Resumen

La mejoría en pieles que presentan áreas vacías y poca firmeza de flor es uno de los mayores desafíos que tiene el curtidor para añadirles valor. Típicamente, materias primas derivadas del petróleo son utilizadas para mejorar el aspecto final y tacto de la piel. En el presente estudio, demostramos que mezclas compuestas por suero concentrado de proteína (WPI, de sus siglas en inglés), un subproducto de la industria del queso, junto con pequeñas cantidades de gelatina, un subproducto de la industria del cuero, se pueden utilizar de forma efectiva como agentes de relleno para cueros de tapicería y de empeine (calzado). Diferentes zonas de pieles curtidas al cromo (croupón, falda y cuello) fueron tratadas con la mezcla de WPI-gelatina, recurtidas, teñidas y engrasadas y las muestras evaluadas con respecto a las propiedades subjetivas y mecánicas. El efecto del pretratamiento de la piel con varias concentraciones de la enzima transglutaminasa microbial (mTGase) fue también examinado. Se halló que la velocidad de absorción de la mezcla de WPI-gelatina por parte del cuero de tapicería incrementaba cuatro veces cuando era pretratado con una solución de 2.5% mTGase. En cambio, esta velocidad disminuía cuando el cuero

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de empeine era pretratado con cantidades crecientes de mTGase. Las propiedades subjetivas (toque, llenura, firmeza de flor y color) de las pieles de tapicería y de empeine que fueron tratadas con la mezcla de WPI-gelatina mejoraron sensiblemente en comparación a los controles. En particular, la firmeza de flor de las muestras de la falda que fueron pretratadas con la enzima (ambos tapicería y empeine) fue mejorada notablemente. Por consiguiente, agentes de relleno compuestos por la más económica WPI fueron eficazmente comprobados de incrementar el rendimiento, tanto para cueros de tapicería como de empeine.

Introduction

The presence of loose areas with poor grain break in finished leather is one of many concerns that tanners are facing in today's leather processing. This problem becomes particularly significant in the neck and belly areas of the hide with the belly area exhibiting a looser break.[1] Fillers are materials used to fill the interstices of the leather and make the looseness less pronounced which in turn should improve cutting yields.

Over the last several years, this laboratory has explored alternatives to petroleum-based fillers. Chen et al.[2] demonstrated that collagen hydrolysate crosslinked with glutaraldehyde could be suitable for filling low quality leather. More recent research has focused on the use of gelatin polymerized by the action of microbial transglutaminase (mTGase) (EC 2.3.2.13), an enzyme capable of forming crosslinks in a wide variety of proteins. Both commercial and experimental alkali-extracted gelatins were effectively crosslinked with mTGase, yielding products with improved functional properties.[3-6] This enzyme also proved to be effective in the crosslinking of gelatin with sodium caseinate, a dairy industry byproduct.[7] Enzymatically modified gelatin and casein were successfully applied as fillers in wet blue leather. It was later found that these modified proteins were not removed during the washing process.[8] Nevertheless, the relatively elevated cost of gelatin and casein encouraged the search for a cheaper source of renewable proteins. Whey and whey protein isolate (WPI), byproducts of the cheese manufacturing industry, fulfill that condition and were also effectively reacted with mTGase yielding viable products for use as filling agents.[9,10]

We recently demonstrated that the addition of small amounts of gelatin to whey protein isolate (WPI) in the presence of mTGase and the reducing agent dithiothreitol (DTT) yielded novel products with improved physical properties (e.g., viscosity, gel strength, degree of polymerization) over either protein component.[11] The main goal of that study was to obtain biopolymers with unique properties at low cost, hence using WPI as the majority component of the WPI-gelatin blend.

In the present study, we examine the suitability of biopolymers produced by combining WPI with small amounts of commercial low Bloom gelatin as a filling agent for shoe upper or upholstery leather. The effectiveness of the various treatments was assessed by measuring the physical, mechanical and subjective properties of crust leather from three different areas of the hide (butt, belly and neck).

Experimental

Materials

Microbial transglutaminase, Activa TG-TI (approximately 100 units/g), a commercial mTGase formulation containing 99% maltodextrose as a carrier, with an active range of pH 4.0 to 9.0 at 0 to 70°C, was obtained from Ajinomoto USA Inc. (Paramus, NJ) and used without further purification. Type B gelatin, alkaline extracted from bovine skin, and characterized in this laboratory as 115 g Bloom, was obtained from Sigma (St. Louis, MO). WPI, Alacen 895, containing 93.2% protein (manufacturer's data), was generously supplied by NZMP (formerly New Zealand Milk Products; Lemoyne, PA). Dithiothreitol (DTT) was obtained from Calbiochem (San Diego, CA). A Bicinchoninic Acid (BCA) Kit for protein determination was purchased from Sigma (St. Louis, MO). Trutan PA-65 and Trutan 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, Altasol 310-L and Eureka 400R were obtained from Atlas Refinery, Inc. (Newark, NJ). Basyntan NNOL and Basyntan DLE were obtained from BASF Corporation (Charlotte, NC). Upholstery and shoe upper wet blue leather was obtained from commercial tanneries. All other chemicals were reagent grade and used as received.

Preparation of WPI-Gelatin Blends

One day prior to the treatment, the required amounts of WPI and gelatin powders were suspended in water (200% float), mixed well and allowed to sit at room temperature for at least two h. The amount of protein powder was calculated on the basis of the weight of wet blue. Next, a 10% DTT (w/v) solution was prepared and the volume necessary to give a concentration of 10 mg DTT per g of WPI was added. The pH was then adjusted to 7.5 with 1 N NaOH or 1 N HCl and heated at 38 ºC for one h, cooled to room temperature and stored overnight at 4 ºC. It is worth noting that all the proteinaceous blends discussed in the present paper were prepared without adding mTGase to the mixture. By doing this, the WPI-gelatin blend can be stored for an extended period of time without danger of it becoming a permanent gel.

Application of WPI-Gelatin Blends to Wet Blue Leather

Wet blue samples from the butt, belly, and neck were tumbled with water in a Dose drum (Model PFI 300-34, Dose Maschinenbau GmbH, Lichtenau, Germany) for 30 min at 50°C, drained and refloated (200% float, 50°C) in 4%

sodium bicarbonate solution, based on the wet blue weight. Upon pH stabilization, the float was drained, mTGase solution (2.5% or 5% mTGase) with a 200% float was added and samples were drummed for one h at room temperature (22 ± 4°C). Note that the mTGase concentrations stated throughout the manuscript are mTGase + carrier concentration. The float was drained and a WPI-gelatin solution was added (200% float). Control pieces to which no enzyme or protein mixture was added were also run with a 200% float of water. The samples were tumbled for one h at RT and then for five h at 45°C. The floats were then drained and the samples washed twice for 10 min at 50°C (200% float), drained, patted dried and stored at 4°C. Previous work carried out in our laboratory typically employed a 400% float in all the above mentioned processing steps.[8-10,12] By cutting back the float to a half we reduce the consumption of water and also increase the concentration of protein in the solution, which ultimately leads to a higher concentration gradient between the solution and the leather.

Aliquots of 3 ml were extracted from the drum after varying time intervals throughout the treatment of the samples with the mTGase or the WPI-gelatin solutions. Aliquots of water after each wash were also collected to estimate the amount of protein that was removed from the wet blue. One drop of 5% sodium azide solution was added to each aliquot and they were stored at 4°C until needed to run the protein determination assay. The following day, both treated and untreated samples were weighed and the amount of reagents needed for the Retan-Color-Fatliquor (RCF) calculated.

Retan/Color/Fatliquor (RCF)

Control and test samples were retanned, colored and fatliquored in separate drums. Shoe upper and upholstery samples followed slightly different procedures (Figure 1a and 1b, respectively).

Figure 1a

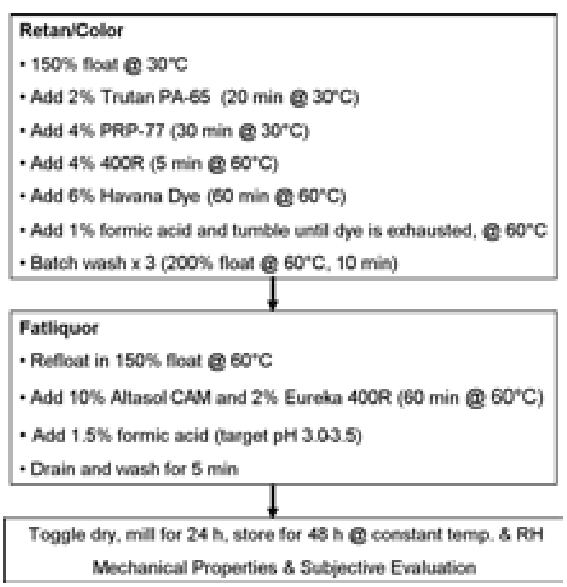

Figure 1b

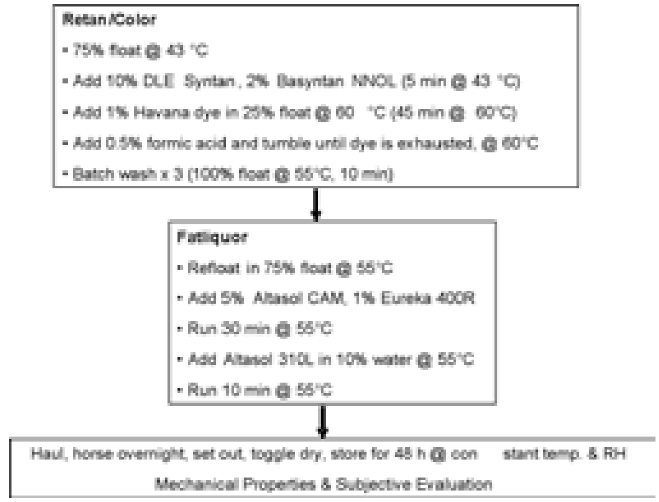

Figure 1: Flow diagram for retan, color and fatliquor formulation of (a) upholstery and (b) shoe upper wet blue.

Drying

After RCF, samples were removed from the drum and allowed to dry. Shoe upper crust leather was allowed to hang freely, whereas stretching (toggling) was applied to upholstery crust leather. Once dry, the leathers were conditioned, put into plastic bags for one day and then staked twice. Shoe upper crust samples were not milled; upholstery crust samples were milled for about 24 h. All samples were then kept on a shelf in the conditioning room at 20°C and 65% relative humidity for at least three days.

Mechanical Properties

Tensile strength, Young modulus and tear strength were determined as described in a previous publication.[12]

Subjective Evaluation

Experimental and control crust leathers were assessed for handle, fullness, grain tightness (break), color and general appearance by hand and visual examination. Handle is defined as the sensation or feeling of certain physical properties of leather, such as flexibility and smoothness, which can be perceived by touch with fingers and hands. Fullness refers to the way a loop of leather feels in the palm of the hand when compressed. A full leather fills the palm while a flat leather has more of a cardboard effect. Grain break is the pattern of tiny wrinkles formed when the leather is bent grain inward. Leather was rated on a scale of 1 to 5 for each functional property by two experienced tanners, where higher numbers indicate a better property.

Protein Concentration Determination

Protein concentrations in the float, at different stages of the treatment, were determined using the bicinchoninic acid (BCA) assay[13] according to the directions supplied with the kit. 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 I l 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 bovine serum albumin.

Results and Discussion

Shoe Upper Wet Blue

We first investigated the uptake of mTGase and WPI-gelatin blends by shoe upper wet blue at both 2.5% and 5% mTGase concentration levels. Similar trends for the uptake of mTGase were obtained for samples that were pretreated with 2.5 or 5% mTGase. In both cases, the curve leveled off after only 20 minutes and the bath was not exhausted after one hour of drumming (Figure 2a). After draining the mTGase solution, a blend of 5% WPI and 0.5% gelatin, with respect to weight of wet blue, was added and drummed one hour at RT followed by 5 h at 45°C. A protein uptake of 98% was achieved with wet blue that was not treated with mTGase, whereas wet blue pretreated with 2.5% and 5% mTGase reduced the percentage to 86% and 83%, respectively (Figure 2b).

The absorption of the protein by the wet blue follows first order reaction kinetics. Hence,

$$\ln\left(\frac{[A]}{[A]_0}\right) = -\kappa \cdot t$$

where [A] and $[A]_0$ are the protein concentration remaining in the drum at time *t* and *t* = 0, respectively and *k* is the uptake rate coefficient. Table I shows *k* and the correlation coefficient values for the uptake of WPI-gelatin by shoe upper wet blue and shoe upper wet blue pretreated with 2.5% or 5% mTGase. The most rapid absorption, reflected by the highest value of *k*, was obtained for the wet blue that was not pretreated with enzyme ($k = 0.608\ h^{-1}$), followed by the one pretreated with 2.5% ($k = 0.413\ h^{-1}$) and 5% mTGase ($k = 0.323\ h^{-1}$), respectively.

The wet blue was washed twice immediately after draining the proteinaceous solution. No detectable level of protein was found in washes of wet blue pretreated with 5% mTGase. At 2.5% and 0% mTGase there was a protein removal of approximately 6% and 8%, respectively, and approximately 75% of that protein was washed out in the first wash. Part of that washed out protein could be due to the unbound protein adhered to the hide or to the insufficient draining of the drum before the addition of water.

All crust samples were evaluated with respect to handle, fullness, grain tightness (break) 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 in which the

Figure 2a

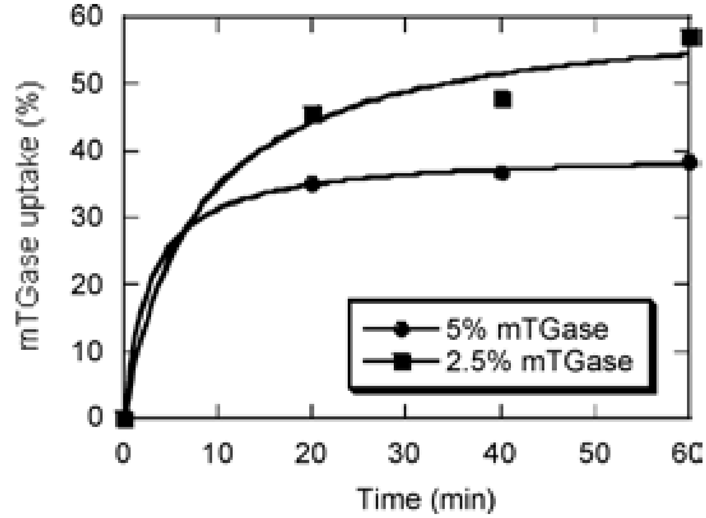

Figure 2b

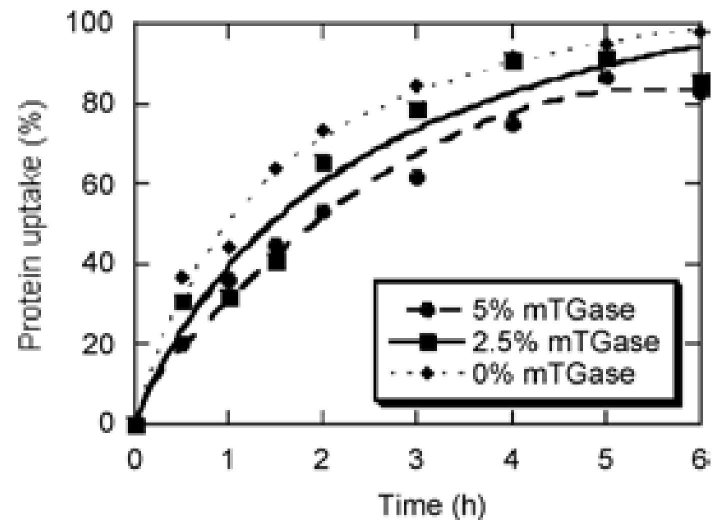

Figure 2: (a) mTGase and (b) protein uptake profiles by shoe upper wet blue pretreated with a solution containing 0, 2.5 or 5% mTGase and treated with a solution of 5% WPI + 0.5% gelatin. All percentages were calculated with respect to the weight of wet blue and added in a 200% float.

grain break was weighted more than the other ratings was also presented. Table II reports the results on the above mentioned subjective properties of shoe upper wet blue subjected to treatments A, B or C. Values that were equal to or better than controls are underlined. In all treatments, the test pieces were found to be equal to or superior to the control pieces. Only the handle of the leather pretreated with 2.5% mTGase was rated slightly lower than the control. Focusing on the grain break, it is important to note that the wet blue samples used for the 0% and 2.5% mTGase batches had a good break before the treatment while the 5% mTGase wet blue samples exhibited a poor break. The samples that already exhibited a good break showed neither a significant improvement in break nor any detrimental effect. The 5%

TABLE I

Uptake Rate Coefficient *k* for Various Treatments

Treatment	Wet blue	% mTGase[a]	% WPI[a]	Gelatin[a]	k (h^{-1})	R^2
A	Shoe upper	0	5	0.5	0.608	0.994
B	Shoe upper	2.5	5	0.5	0.413	0.851
C	Shoe upper	5	5	0.5	0.323	0.950
D	Upholstery	0	2.5	0.25	0.365	0.830
E	Upholstery	2.5	2.5	0.25	1.377	0.956

[a]Percentages calculated with respect to weight of wet blue.

TABLE II

Subjective Evaluation[a,b,c]

		Treatment A		Treatment B		Treatment C	
Hide area	Property	Control	Test	Control	Test	Control	Test
Butt	Handle	4	4	4	3	3.5	4.5
	Fullness	4	5	4	5	4	4.5
	Break	5	5	5	5	1.5	3.5
	Color	3	5	3	5	4	4
	Overall	4	5	4	5	3	4
Belly	Handle	3	4	3	2	2	4.5
	Fullness	3	5	4	5	2	4.5
	Break	4	5	5	5	1	4
	Color	3	5	3	5	2.5	3.5
	Overall	3	5	4	5	1.5	4.5
Neck	Handle	3	4	3	2	3.5	4.5
	Fullness	4	5	4	5	4	4.5
	Break	5	5	5	5	2.5	4
	Color	2	5	2	5	3	3.5
	Overall	3	4	1.5	4.5	2.5	4.5

[a]Scale 1-5, 1=worst, 5=best

[b]n=2

[c]A, B and C stand for treatments of shoe upper with a solution containing 5% WPI + 0.5% gelatin and pretreated with a solution of 0, 2.5 or 5% mTGase, respectively. All percentages were calculated with respect to the weight of wet blue and added in a 200% float.

mTGase wet blue sample clearly showed significant improvement in leather from all areas of the hide when comparing the break to the control.

Next, we examined the effect of reducing the WPI to 2.5% and gelatin to 0.25% and mTGase to 2.5%, for samples that exhibited a poor break. Approximately 80% of the protein was taken up by the wet blue, with a rate coefficient rate of $k = 0.262\ h^{-1}$. During the wash procedure, approximately 6% of the protein was removed, all of it in the first wash. Although the break of the crust leather fared better than the control, the improvement was not as dramatic as when a 5% mTGase treatment followed by 5%WPI + 0.5% gelatin was used (data not shown). These results suggest that 5% WPI + 0.5% gelatin filled the leather better than 2.5% WPI + 0.5% gelatin, particularly in the belly area.

Upholstery Wet Blue

The ability of WPI and gelatin to fill and improve upholstery wet blue was examined. Given the smaller thickness of upholstery (1.0-1.2 mm) compared to shoe upper (2.0-2.4 mm), a lower concentration of WPI and gelatin was selected to make up the proteinaceous blend (2.5% WPI + 0.25% gelatin). The effect of an enzymatic pretreatment of the samples with 2.5% mTGase prior to the addition of the proteinaceous blend was also evaluated. About half the amount of the enzyme was picked up by the wet blue within the first 30 minutes, and the curve leveled off thereafter (Figure 3a). A complete uptake of protein was reached within the first 3 h of tumbling for wet blue that was pretreated with 2.5% mTGase. Conversely, the protein uptake trend for samples that were not pretreated with the enzyme leveled off at approximately 90% after 4 h of drumming (Figure 3b). A remarkably faster uptake of protein was observed for samples that were pretreated with mTGase, as can be seen from the uptake coefficient values (Table I). Approximately 5% of the protein was removed in the first wash regardless of the enzymatic pretreatment. A non detectable amount of protein was removed during subsequent washes.

The treatment of upholstery wet blue with 2.5% WPI + 0.25% gelatin considerably improved the handle, fullness, and color of the resulting crust leather. Most importantly, the break of the belly and butt areas was significantly improved when the wet blue was pretreated with 2.5% mTGase prior to the addition of the proteinaceous blend (Figure 4).

Mechanical Properties

Leathers from three different areas of the hide, neck, belly and butt, were tested for mechanical properties. This report will present the test results from belly area only, the primary area of concern. The three areas demonstrated the same tendency towards the change of two major variables: percent WPI and percent mTGase. A 3-D regression plot of the resultant tensile strength as a function of percent WPI and

Figure 3a

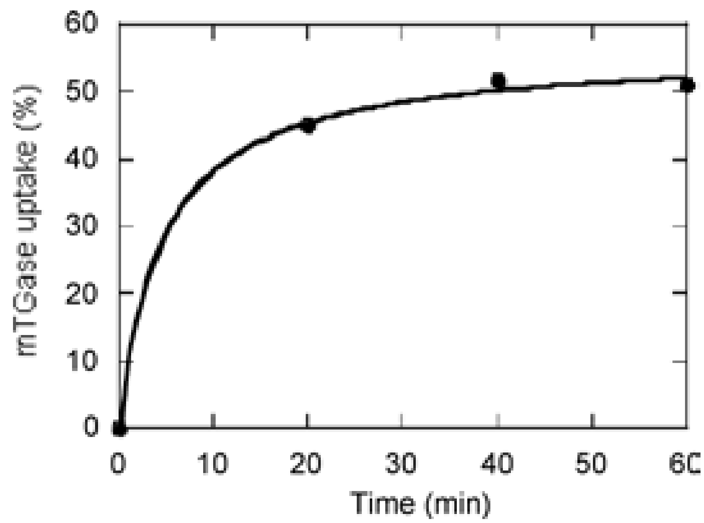

Figure 3b

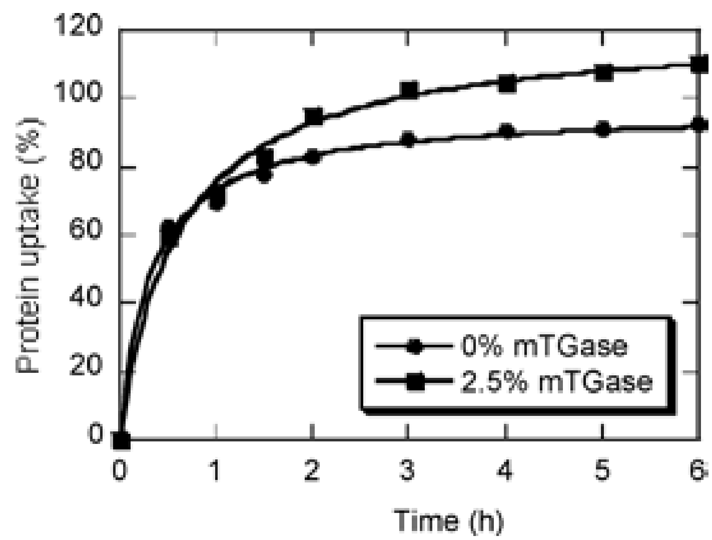

Figure 3: (a) mTGase and (b) protein uptake profiles by upholstery wet blue pretreated with a solution containing 0 or 2.5% mTGase and treated with a solution of 2.5% WPI + 0.25%. All percentages were calculated with respect to the weight of wet blue and added in a 200% float.

percent mTGase simultaneously for both upholstery and shoe upper leather samples was developed (Figure 5). The tensile strength decreases slightly with increasing percent WPI for both upholstery and shoe upper leather. However, for upholstery leather, the tensile strength increases significantly with percent mTGase, whereas for shoe upper leather, the tensile strength shows little change with percent mTGase. It is worthy to note that as demonstrated in Figure 5, shoe upper leather has greater tensile strength than upholstery leather and this is ascribable to the fact that shoe upper leather is thicker and has more fiber network to resist the fracture.

Figure 4

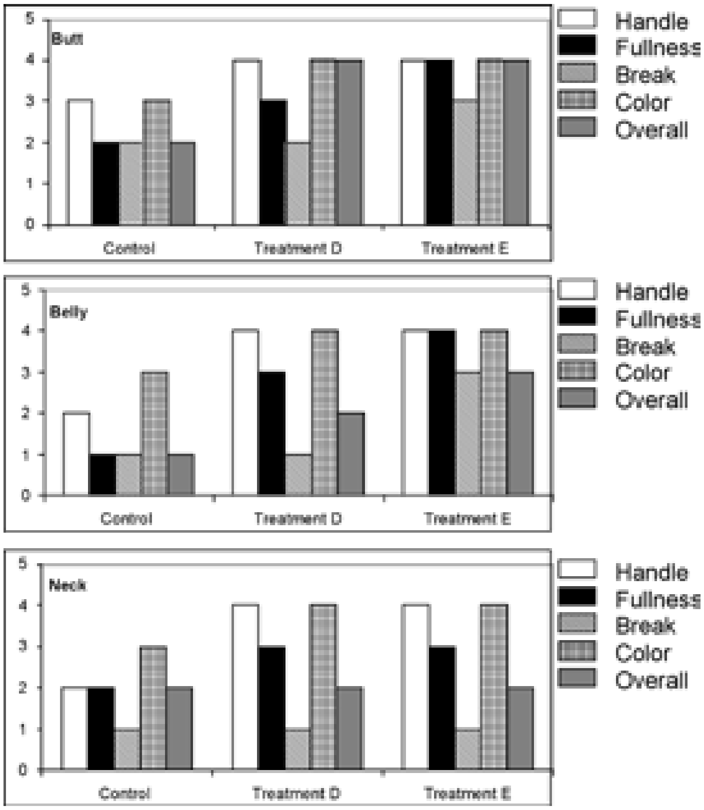

Figure 4: Subjective properties of upholstery crust leather. D and E stand for treatments of upholstery with a solution containing 2.5% WPI + 0.25% gelatin and pretreated with a solution of 0 or 2.5 mTGase, respectively. All percentages were calculated with respect to the weight of wet blue and added in a 200% float.

Figure 5

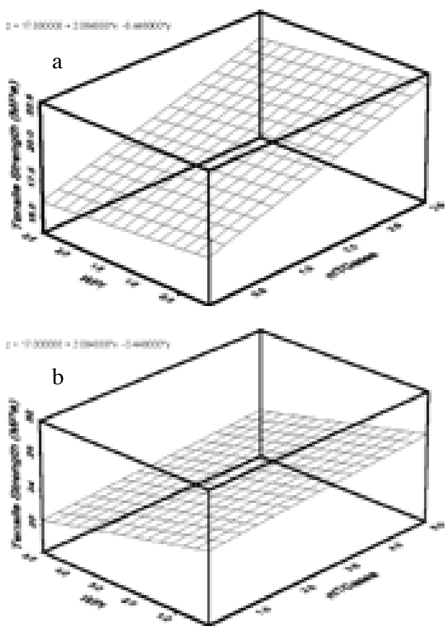

Figure 5: Effect of the various treatments of leather with WPI and mTGase on the tensile strength of (a) upholstery and (b) shoe upper crust leather. The regression graphic corresponds to samples from the belly area.

Figure 6

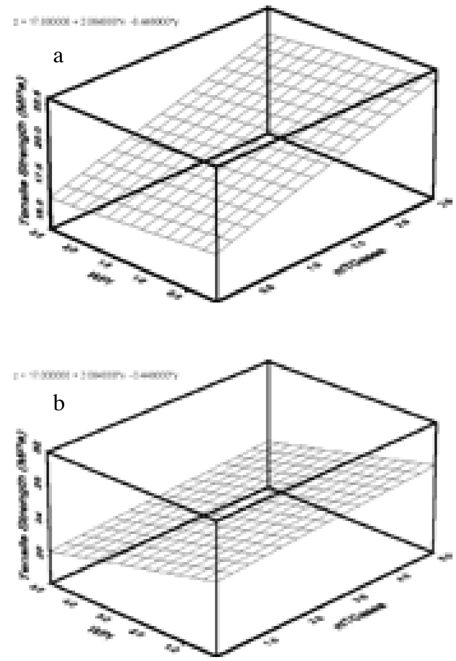

Figure 6: Effect of the various treatments of leather with WPI and mTGase on the Young's modulus of (a) upholstery and (b) shoe upper crust leather. The regression graphic corresponds to samples from the belly area.

Figure 7

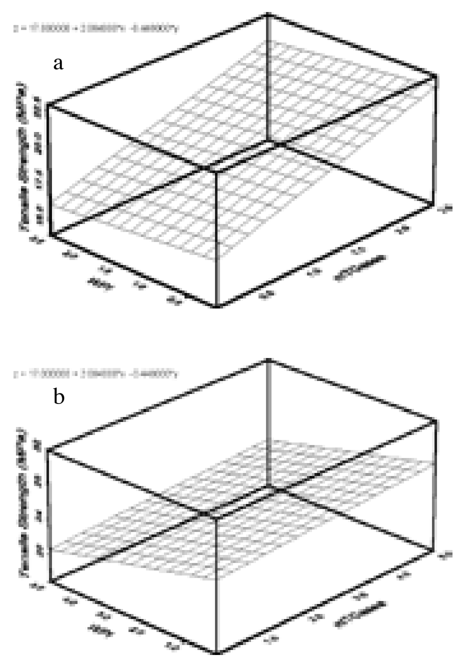

Figure 7: Effect of the various treatments of leather with WPI and mTGase on the tear strength of (a) upholstery and (b) shoe upper crust leather. The regression graphic corresponds to samples from the belly area.

Young's modulus is a value indicating the stiffness of leather. Young's modulus of upholstery leather increases significantly with both percent WPI and percent mTGase (Figure 6a). On the other hand, for shoe upper leather Young's modulus also increases significantly with percent mTGase, but changes very little with percent WPI (Figure 6b). Looking closely at Figure 6, one can notice that the ranges of Young's modulus values are higher for shoe upper leather than upholstery leather. Besides the fact that shoe leather is thicker, this variation could be due to the use of different types of fatliquors.

Tear strength decreases with both percent WPI and percent mTGase for upholstery leather (Figure 7a). However, for shoe upper leather the tear strength responds quite differently to the changes of percent mTGase and percent WPI. As demonstrated in Figure 7b, tear strength of shoe upper leather decreases pronouncedly with percent mTGase but is relatively unchanged with percent WPI.

Conclusions

Current prices of sodium caseinate ($5.8/lb)[14] and gelatin ($2.6/lb)[15] emphasized the need for research into cheaper sources of protein to generate fillers for leather. The less expensive whey protein isolate ($1.05/lb)[14] along with small amounts of gelatin were successfully applied as a filling agent for upholstery and shoe upper leather. Subjective properties such as fullness, handle and color of the resulting crust leather were significantly improved. Furthermore, grain break for upholstery and shoe upper leather fared markedly better when samples were pretreated with mTGase. The enzymatic pretreatment of wet blue leather with mTGase also affected the protein uptake ratio. The uptake coefficient for upholstery leather pretreated with 2.5% mTGase increased four-fold over samples that were not enzyme pretreated. The trend was reversed for shoe upper leather with a drop in the uptake coefficient value from 0.608 h^{-1} to 0.323 h^{-1} for samples that were not pretreated with mTGase or pretreated with 5% mTGase, respectively. We further demonstrated that a 200% float satisfactorily enabled the proteins to be taken up by the wet blue. If this technology is to be transferred to the industry, use of a shorter float could be feasible due to a stronger mechanical action. Importantly, the proteins are not considerably removed by washing, regardless of the enzymatic pretreatment. Another advantage presented herein is that the proteinaceous blend was added to the drum without any enzyme pretreatment, thus its preparation becomes more convenient and feasible. Even though the various treatments did not negatively affect the mechanical properties of the crust leather, filled samples were a little stiffer and presented slight lower tear strength than the controls. Further research that explores the possibility of using even cheaper sources of protein as a raw material for bio-based leather products is an interesting option currently being examined in our laboratory.

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