A Novel System of Removing Décorin, A Minor Proteoglycan of Bovine Hides, to Improve the Quality of Leather

by

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Abstract

Décorin is a minor proteoglycan (part protein and part carbohydrate) of the skin that is among the key components that undergo changes and removal during conversion of hides to leather. The majority of décorin removal takes place during the dehairing of hides, either traditionally with sodium sulfide or by an alternative oxidative dehairing procedure. The oxidative dehairing method using sodium hydroxide and percarbonate, developed by Marmer and Dudley, was utilized. Further removal of décorin by exposure to proteolytic enzymes during pretanning was explored to study the effects on the quality of leather. Additional removal of décorin was observed when an alkaline protease was added during the reliming stage and pepsin was added during the pickling stage in the pretanning treatments of the hides. More pronounced improvement in leather quality, due to more décorin removed, was observed in oxidatively dehaired hides than those dehaired traditionally with sulfides. As the décorin content decreased, the leather product became softer, more stretchable, and tougher than the control leather tanned without adding proteolytic enzymes. Employing the alternative oxidative dehairing process can solve the problem of sulfide toxicity to the environment while at the same time improving the quality of leather if co-treated with proteolytic enzymes.

Resumen

Decorina es un proteoglicano menor, cual es un componente de la piel (parte proteína y parte carbohidrato) y es uno de los componentes claves que sufre cambios y remoción durante la transformación de las pieles a cuero. La mayor porción de decorina es removida durante el apelambrado de las pieles, ya sea tradicionalmente con sulfuro de sodio o por la acción alternativa del proceso de pelambre por oxidación. El método de pelambre oxidativo, utilizando hidróxido de sodio y percarbonato, desarrollado por Marmer y Dudley fue el utilizado. Remoción adicional de decorina por acción de enzimas proteolíticas durante el precurtido fue explorado para estudiar sus efectos sobre la calidad del cuero. Remoción adicional de decorina fue observada cuando una proteasa alcalina fue añadida durante la etapa del re-encalado, y pepsina fue añadida durante el piquel en la etapa del precurtido de las pieles. Fue más pronunciada la mejoría en la calidad del cuero obtenido debido a la incrementada remoción de decorina, se observó en las pieles apelambradas por oxidación cuando fueron comparadas a las tradicionalmente apelambradas con sulfuros. A medida que el contenido de decorina disminuyó, el cuero resultante resultó más suave, más elástico, y más tenaz, comparado al control procesado sin la adición de enzimas proteolíticas. Empleando un proceso alternativo de pelambre oxidante entonces se puede resolver el problema de toxicidad por sulfuro al medioambiente mientras que simultáneamente se mejora la calidad del cuero producido, si se co-procesa con enzimas proteolíticas.

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Introduction

Décorin is a small extracellular matrix proteoglycan involved in several fundamental biological functions typically in "decorating" or organizing the collagen fibrils.[1] Its persistence may be related to the stiffness of the final leather product.[2] The efficient removal of proteoglycans from the hide when processing it to leather is generally acceptable and beneficial for leather quality, especially for softness and flexibility when the hides are processed into leather.[3] Our current efforts are focused upon the further removal of décorin which is the predominant and best understood proteoglycan of the skin.[1-4]

One of the initial steps in leather making is removal of hair from the hides. It has been observed that most of the décorin is also removed during this process.[3, 4] Traditional dehairing uses sodium sulfide (Na_2S), lime ($CaO.H_2O$) and soda ash or sodium carbonate (Na_2CO_3).[5, 6] Although sulfide salts are very effective in removing hair and hair roots, these reagents are potential environmental pollutants as main contributor to the high biological and chemical oxidation demand of the resultant waste stream.[7] In addition, sulfide salts that are accidentally exposed to acids may be converted to toxic hydrogen sulfide gas, which poses a severe threat to tannery workers.[8] Due to its health risk and environmental hazard potential, an alternative eco-friendly oxidative dehairing method was developed in our labs at ERRC by Marmer and Dudley using sodium hydroxide and percabonate.[10, 11] They observed that this method gave comparable leather quality to the hide dehaired traditionally with sulfides. Observation also showed that both dehairing procedures remove most of the proteoglycans of the skin, including 60-80% of décorin.[4, 12]

When lime is added in the dehair and relime stages of pretanning, the pH is increased to 12-13 and an "opening-up" or osmotic swelling happens.[6, 13] This can be attributed to the ionic imbalance that builds up in the collagen matrix that cause the removal of the hair, sebaceous materials and some skin proteins from the hide. The other important process in pre-tanning is bating where the removal of additional inter-fibrillary materials, such as proteins and proteoglycans, are taking place.[9, 12] This is facilitated by the use of proteolytic enzymes such as Rohapon 6000.[6] Even though it is well established that during liming and bating stages of pre-tanning, inter-fibrillary materials such as proteins and proteoglycans are removed,[6, 12] no valid quantification of the residual proteoglycan remaining in treated hide has been done. The current work attempts to quantify one of these inter-fibrillary materials by determining the residual amount of décorin, a minor proteoglycan of the skin, in the processed hide resulting to crust leather.

It has been reported that the amount of décorin detected in a raw hide depends on what part of the hide it was taken from. However, after subjecting the hide pieces from different parts of the hide to the same tanning treatments, the residual décorin content in the resultant leather was almost identical [4, 12] The objective of this study was to explore ways to improve the quality of leather by exposing the hides to proteolytic enzymes during the pretanning treatments. In order to detect varied removal of décorin during the different pretanning treatments, the Alcian Blue (AB) colorimetric assay of its carbohydrate portion, the SGAG, was utilized.[14, 15] The principle of SGAG assay with AB is based on the specific interaction between the negatively charged sulfated polymers and the tetravalent cationic AB dye. The number of ionic bonds between AB and SGAG are generally thought to be directly proportional to the number of negative charges present on the SGAG chain.[14] This number correspond to the amount of SGAG or décorin present in the sample. The novel system that we have developed incorporated proteolytic enzymes during the reliming, bating and pickling stages of pre-tanning hides to wet blue, the chrome tanned and unfinished leather. The standard chrome tanning procedure was utilized to produce shoe-upper leather products for which the mechanical properties[16] were determined.

Experimental

Materials

The fresh hides were supplied by the local slaughterhouse (JBS, Souderton, PA). We obtained sodium carbonate, sodium hydroxide, sodium percarbonate ($Na_2CO_3 \cdot 1.5H_2O_2$), sodium sulfide in bulk, Pepsin P-7125 and Protease inhibitor cocktail for mammalian tissues, P-8340 from Sigma-Aldrich (St. Louis, MO). Boron TS was from Rohm Tech., Inc. (Malden, MA), Proxel: Chemtan Co. (Exeter, NH); Rohapon 6000, from TFL USA/Canada (Greensboro, NC); Novozym Alkaline protease PN402678 was from Novozymes North America Inc. (Franklinton, NC); Guanidine hydrochloride (Guan·HCl) was from Mallinckrodt #7716, Bio-reagent grade, Thomas Scientific (Swedesboro, NJ). Lime was obtained from Mississippi Lime Company (Genevieve, MS) and Ammonium sulfate ($(NH_4)_2SO_4$) AX1385-9 from EMD Chemicals Inc. (Gibbstown, NJ).

Methods

Thawed fresh bovine hide pieces, cut into 6 in x 8 in sizes with the longer side parallel to the backbone, were chosen as raw materials for the study. Each hide was transferred separately to a dose drum (PFI 300-34; Dose 131 Maschinenbau GmbH, Lichtenau, Germany). The procedure for traditional tanning treatments in converting hides to leather is illustrated in Table I.

For the first set of experiments, four hide pieces near the belly area were taken. Three of the hide pieces were dehaired traditionally (Table I) by using 2% (W/V) Na_2S, 2% (W/V) $CaO.H_2O$ and 1% (W/V) Na_2CO_3 in a 100% float for 4 h (float is the volume of water in milliliter (mL) equivalent to the

TABLE I

The standard tanning treatments[5] from fresh hides (with the novel process included in *bold font)*

(100 % float = volume of water in terms of weight of raw hide in 1:1 ratio)

I. Pretanning steps:

1. Soak*:* 200% float -With 0.15% Boron TS and 0.10% Proxel for dirt removal. (~2 h, 26.7 ºC)

2. Dehair: 100% float-Traditionally, with 2% Na_2S + 2% Lime(CaO.H2O) and 1% Soda ash(Na_2CO_3). (***Oxidatively, with 4-6% (W/V) NaOH and 4% (W/V) sodium percarbonate also in 100% float).*** Both were done at ~ 4-6 h, 29.4ºC and at pH >12

3. Relime*:* 200 % float - with 2 % Lime and 1 % NaHS (***and add 0.2 % Alkaline protease)*** (~20 h, 26.7 ºC) then wash twice @ 100% float and 0.10 % Boron TS; Target pH 8.8 -to- 9.0. Drain.

4. Relime and Bate: 125 % float; Add 0.15% $(NH_4)_2SO_4$; then bate with 0.15% Rohapon 6000. (~1.5 h, 32 ºC). The action of the enzyme lowers the alkalinity of the hide.

5. Pickle: 0 % float; Add 3 % NaCl + 2% H_2SO_4 and 8% water for dilution. (*and* ***add 0.1% pepsin)***. (~4 h, 26.7 ºC). Target pH is 1.8.

II. Chrome Tanning and Finishing steps*:*

1. Soak: 25% float - Add 0.75 % sodium formate + 8.0 % Oxochrome (33 % stock solution) + 0.10 % Busan 30 (fungicide) + 12% Water@ 43.3 ºC and + 1% Na Bicarbonate. Hides are soaked in tannin g solution for 8-12 h, at Rm. T. The chemical action of chrome turns hide to leather.

2. Coloring: Dyes added in tanning solution give its color (black in patent leather).

3. Drying: Hang dry to reach ~30% moisture content for ~ 24 h.

4. Finishing: Acrylic or polyurethane (and antioxidant) are added to the leather

weight in gram (g) of the raw hide sample (*e.g.* 500mL water was added to 500g hide =100% float). The control sample, S_{10} followed the traditional pretanning procedure[5] (Table I). The second sample, S_{11}, is the average of two samples that were also dehaired separately with sulfide but relimed with 0.2% alkaline protease and pickled with 0.1% pepsin co-treatment. Consequently, the fourth sample, O_{12}, was dehaired oxidatively[10, 11] (following the italicized and ***bold font*** steps inserted in the traditional pretanning protocol of Table I) and like S_{11}, it was co-treated with the same type and amount of proteolytic enzymes. All the dehaired samples were lime split and chrome tanned according to the first step of part II -Chrome Tanning and Finishing steps in Table I, converting each to wet blue.[5, 6] The samples analyzed for décorin content were raw hide and the hides that were unhaired, relimed, delimed/bated and pickled. Wet blue samples were retanned, colored and fatliquored into shoe upper leather uniformly by following the second part of Table I on chrome tanning and finishing. The leather products are then tested for mechanical properties.[16]

For the second set of experiments, a different bovine hide sample was taken and cut into 6 x 8 in pieces. Eight hide pieces in the crop section were obtained. Three pieces were individually subjected to oxidative dehairing (samples O_{20} - O_{23}). Sample O_{20}, was the control without proteolytic enzyme co-treatment. During relime stage, sample O_{22} was co-treated with 0.2% (W/W) alkaline protease whereas sample O_{23} was with 0.1% (W/W) alkaline protease. Then both O_{22} and O_{23} were co-treated with 0.1% pepsin during the pickle stage. For comparison, S_{20}, a sample dehaired with sulfide and pretanned normally was also included as traditional control. The mechanical properties of the resultant leather from the latter treated samples are also determined and correlated with its corresponding residual décorin content.

Analysis of décorin (SGAG) content

The procedure followed for the analysis of SGAG content in hides is detailed elsewhere.[14, 15] In order to make it more economical and more efficient, the procedure was modified by using only a quarter of the suggested sample amount. The preparation of both stock and working solutions of the reagents are also given in detail by previous workers.[14, 15] For the known SGAG standard preparation and determination of the colored solutions, the SGAG-Kit assay procedure by KAMIYA B Biomedical Company,[17] were followed. Depending on the intensity of the bluish coloration, aliquots of 100 to 240 µL were introduced into the 96-well microplate to read the samples in the ELISA reader, MultiSKan MCC/340 (by Thermo Labsystems for Fisher Scientific), was used. Uniform

aliquot amounts of the standard SGAG, in series of increasing concentration, were introduced into each well, based on the volume of sample used. The absorbance at 605 nm was measured or A_{605} vs. TGS (Tris-glycine sodium dodecyl sulfate (SDS) buffer with 0.15mgAB/L) of the final clear bluish solution (supernatant) of each sample against the blank composed of all the reagents except the sample.

It was interesting to know the trend of chromium absorption during chrome tanning of the differently treated hides. Therefore, the chromium content of the differently treated samples was also analyzed in the second set of experiments. The analysis of chromium in wet blue samples was performed using atomic absorption spectroscopy. The chromium content was determined using an air/acetylene flame on a Perkin Elmer AAS Model 3300 (Waltham, MA) and expressed in terms of % Cr_2O_3.[5]

Determination of Mechanical Properties:
Mechanical property measurements included tensile strength, elongation-to-break ("stretchability"), Young's modulus ("stiffness"), and fracture energy (the energy needed to fracture leather samples, its "toughness"). Rectangular shaped leather samples (1- × 10-cm) were cut near the standard test area as described in ASTM D2813-03[16] with the long dimension parallel and perpendicular to the backbone. The average thickness of the leather samples varied from 1.7 mm to-2.7 mm. An upgraded Instron mechanical property tester, model 1122 (Instron, Norwood, MA), and Testworks 4 data acquisition software (MTS Systems Corp., Minneapolis, MN) were used throughout this work. The strain rate was set to 25.4 cm/min with a grip distance of 5 cm. Each test was conducted on five samples to obtain an average value.

Results and Discussion

The concentration of décorin in hide samples, with respect to its SGAG content, was calculated from the slope of the standard calibration graph (Figure 1). The graph was prepared by plotting a straight line relating the absorbance to the known amount of standard SGAG. The linearity of the standard graph is good with linear regression of almost equal to unity at R^2 = 0.98. The modified Alcian Blue assay has improved the efficiency, capable of analyzing more samples in shorter period of time. It also required lesser amount of reagents because smaller amount of samples were used.

The reproducibility and reliability of the décorin assay results were improved after the intact hide samples were pulverized and lyophilized first in order to remove the non-uniform amount of moisture initially present in each intact hide sample. The physical properties of the resultant leather were correlated with the residual décorin content measured after subjecting the sulfide dehaired hides to different pretanning treatments

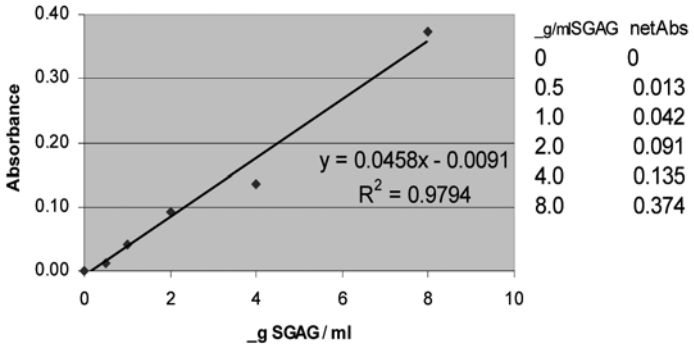

Figure 1. Standard SGAG graph

(Table II). The average moisture content of resultant leather products, according to a Delmhorst moisture meter (Delmhorst Instrument Co., Towaco, NJ), was (15 ± 2)%. An oxidatively dehaired hide from the same hide sample was also included and compared to the control sulfide dehaired hide. The double sided t-test was performed using a p-value of 0.05 or less as an indicator of significance[18]. The t-tests performed for Tables II and III have a corresponding t-value of 2.3 for p-value of 0.05 with 8 degrees of freedom[18]. The t-test gives the probability that the null hypothesis (i.e. no difference) is true. If the calculated t value is smaller than 2.3, then one may assume that there is no significant difference between the means. On the other hand, if the t value is greater than 2.3, an alternative hypothesis is true, i.e. the difference is statistically significant.

The resultant leather products from hides processed traditionally (without proteolytic enzymes) using either of the two dehairing procedures, have been known to give almost the same or comparable physical properties.[6-7] Currently, when compared to the sulfide dehaired hides, the oxidatively dehaired hide showed a more significant improvement in the leather quality when the tanning process was co-treated with proteolytic enzymes in sample O_{12} (Table II). Sample S_{11} (a leather made from a hide dehaired with sulfide but was relimed in the presence of 0.2% alkaline protease delimed and bated traditionally (Table II) and pickled in the presence of 0.1% (W/W) pepsin did not show significant further removal of décorin (t = 0.5) compared to the control sample S_{10} . Sample O_{12} (leather made from the hide dehaired oxidatively, relimed in the presence of 0.2% (W/W) alkaline protease, delimed and bated traditionally, and pickled in the presence of 0.1% (W/W) pepsin) contained approximately 25 % less décorin ~ 40 μg per gram of the tanned hide, compared to the control sample S_{10} (~53 μg per gram). This showed a significant further removal of décorin (t = **4.1**).

The elongation-to-break of ~70 % in sample $O_{12,}$ was significantly (p = **6.3**) improved to about 1.5 times greater than the control sample $S_{10.}$ "Toughness" or fracture energy results follow almost the same trend, sample O_{12} was also about 1.5 times greater than the control sample, S_{10} (t = **3.7**). The Young's modulus or "stiffness" value of sample O_{12} is about half,

TABLE II

Residual décorin content and corresponding physical properties of the resultant leather from first set of experiments

Sample code	Dehaired By	Different treatment1	µg décorin /g hide	Elongation-to-break (%)	Fract Energy "toughness" (J/cm³)	Young's modulus (MPa)	Tensile strength (MPa)
S_{10}	sulfide	control	53 ± 5	43 ± 6	2.2 ± 0.3	19 ± 3.6	10.6 ±0.5
S_{11}	sulfide	AP+Pn*	51 ± 7	50 ± 8	2.7 ± 0.6	18 ± 3.5	10.2 ± 2
O_{12}	oxidative	AP+Pn*	40 ± 5	69 ± 7	3.3 ± 0.6	10 ± 2	11.4 ± 1
$t\ S_{11}$ ** $t\ O_{12}$ ***	S_{11} vs. S_{10} O_{12} vs. S_{10}		0.5 4.1	1.6 6.3	1.7 3.7	0.4 4.9	0.4 1.6

* AP + Pn = co-treatment with 0.2% alkaline protease (AP) in relime and 0.1% pepsin(Pn) in pickle stage
** where $t\ S_{11}$ is the t-test for S_{11} vs. control S11; t-test = $[S_{10} - S_{11}] / [(SD^2/5 + SD^2/5]^{1/2}$
*** where $t\ O_{12}$ is the t-test for O_{12} vs. control S_{10} (with no proteolytic enzyme (no PE)

TABLE III

Residual décorin content and corresponding physical properties of the resultant leather from second set of experiments (oxidatively dehaired hides)*

Sample code	Different treatment[1]	µg décorin /g hide	Elongation to- break (%)	Fracture Energy (MPa)	Young's Modulus (MPa)	Tensile strength (MPa)	Cr_2O_3 (%)
O_{20}	Oxid-noPE*	55 ± 4.9	44 ± 1.0	1.6 ± 0.3	18.3 ± 3.3	6.9 ± 1.1	3.3 ± 0.2
O_{22}	(0.2%AP+ 0.1%P)[I]	43 ± 1	44 ± 5.7	2.1 ± 0.3	10 ± 1.4	7.6 ± 0.9	3.6 ± 0.3
O_{23}	(0.1%AP+ 0.1%Pn) [II]	37 ± 1	51 ± 2.7	1.7 ± 0.3	13.6 ± 0.7	7.1 ± 0.7	3.6 ± 0.5
S_{20}	Standard Control **	56 ± 1	34.4 ± 2.4	1.24 ± 0.2	17.8 ± 4	4.9 ± 0.5	4.4 ± 0.2
$t\ O_{22}$ $t\ O_{23}$	$(O_{20}$ vs $O_{22})^{III}$ $(O_{20}$ vs $O_{23})_{IV}$	**5.3** **8**	0 **5.4**	1.9 0.5	**5.2** **3.1**	1.0 0.3	2.1 1.3
$t\ O_{20}$-S_{20} $t\ O_{22}$-S_{20} $t\ O_{23}$-S_{20}	$(O_{20}$ vs $S_{20})^{V}$ $(O_{22}$ vs $S_{20})^{VI}$ $(O_{23}$ vs $S_{20})^{VII}$	0.4 **20** **30**	**8.3** **3.5** **10.3**	2.2 **5.4** **2.9**	0.2 **4.1** **2.3**	**3.6** **5.9** **5.8**	**9.2** **5.0** **3.2**

*control I, sample O_{20}; hide is oxidatively dehaired and pretanned traditionally, no P.E.
**control II, sample S_{20}; hide is dehaired with sulfide and pretanned traditionally, no P.E.
[I] AP + Pn = 0.2% AP added in relime and 0.1% pepsin (Pn) added in pickle stage of sample O_{22}
[II] AP + Pn = 0.1% AP added in relime and 0.1% Pn added in pickle stage of sample O_{23}
[III] t-test for O_{22} and [IV] t-test for O_{23}, both vs. control O_{20}.
[V-VII] t-test for O_{20}, O_{22}, and O_{23} vs. control S_{20}, respectively

thus making it doubly softer than the control sample, S_{10} (t = **4.9**). In short, the improvement in quality is significantly greater in the oxidatively dehaired sample $O_{12,}$ than in the traditionally sulfide dehaired sample S_{11}. These physical characteristics were indicative of an improvement in the quality of the resultant leather using our novel system and appeared to be due to the further removal of décorin compared to the control.

A different bovine hide sample was taken and cut into 6 x 8 in. dimension and eight hide pieces in the crop section were obtained. Three pieces were subjected to oxidative dehairing (Table III). Control I, Sample O_{20}, is the control without proteolytic enzyme co-treatment. During relime stage, sample O_{22} is treated with 0.2% (W/W) alkaline protease whereas sample O_{23} is incorporated with 0.1% (W/W) alkaline protease. Then both O_{22} and O_{23} are co-treated with 0.1% pepsin during the pickle stage. Sample S_{20} is traditionally dehaired with sulfides and pretanned without proteolytic enzyme co-treatment. The mechanical properties of the resultant leather from the later treated samples are also determined and correlated with its corresponding residual décorin content (Table III). The corresponding t-test values shown in **bold font** are for the means that are significantly different (t > 2.3) when the samples co-treated with proteolytic enzymes are compared to the control without proteolytic enzymes and the traditionally tanned hides.

From Table III, the residual décorin content of oxidatively dehaired control sample O_{20} has a significant difference (t = **5.3**) from sample O_{22}, and is significantly lowered (t = **8**) in sample $O_{23.}$ The results suggest that the addition of 0.1% (W/W) alkaline protease in the relime and 0.1% pepsin in the pickle stages of pretanning hides to leather work better than using 0.2% (W/W) alkaline protease with the same amount of pepsin. In both cases, the amount of chromium absorbed do not increase significantly from the control (t = 2.1 and t =1.3, respectively). The increase in % elongation-to-break from ~44% in sample O_{20} to ~51% in O_{23}, appears to be significant (t = **5.4**). Also, the Fracture energy in both samples co-treated with the proteolytic enzymes (t = **5.4** for O_{22} and t = **2.9** for O_{23}) have shown significant improvement compared to the traditional control, S_{20}. The tensile strength with t-test values ranging from **3.6** to **5.8** is showing significant improvement when the oxidatively dehaired hides, with and without proteolytic enzyme, are compared to the traditionally pretanned hide. The changes in the mechanical properties signified that a softer and yet much tougher leather product can be obtained when the hides are co-treated with proteolytic enzymes. The amount of chromium is significantly decreased from the traditionally tanned hides to those with and without proteolytic enzymes incorporation during the beamhouse operations when the hides are dehaired oxidatively as shown by all bold t-test values (**9.2, 5** and **3.2,** respectively). Overall, a significant improvement is observed in leather quality when the resultant leather from the hide that as dehaired oxidatively and co-treated with proteolytic enzymes during pretanning is compared to the control leather obtained from the hide dehaired traditionally with sulfides and no proteolytic enzyme co-treatment during the pretanning stage of hides to leather as shown in Tables II and III.

Conclusion

Our results indicated that the lower the residual décorin content, a softer, more stretchable and yet tougher leather product can be obtained when the hides are co-treated with proteolytic enzymes. Further removal of décorin and improvement of leather product can be obtained by subjecting the hides to pre-tanning treatments in the presence of selected proteolytic enzymes in the reliming, bating, and/or in the pickling stages. The novel recipe consisted of adapting the oxidative dehairing protocol, and then incorporating alkaline protease (preferably 0.1 to 0.2% (W/V) in the reliming step and pepsin (preferably ~ 0.1% (W/V) in the pickling step. Tests showed that the elongation-to-break ("stretchability") and the fracture energy ("toughness") measurements of the leather using our novel system were about 1.5 times greater than the control sample (leather made by traditional tanning using sulfide for dehairing and without alkaline protease and pepsin). The Young's Modulus or "stiffness" of samples with proteolytic enzymes was about one half to two thirds that of the control sample, either dehaired with sulfide or oxidatively. The corresponding t-test values shown in **bold fonts** were for the means that were significantly different (t > 2.3) when the samples co-treated with proteolytic enzymes were compared to the control without proteolytic enzymes and the traditionally tanned hides. Our novel system therefore can make it possible to produce high-quality leather that is softer, more stretchable, and tougher than the control leather made from sulfide dehaired hides with no enzyme co-treatment during the pretanning stages. Another advantage of replacing sulfides by percarbonates and other oxidative chemicals during the dehairing step is that it makes this process more eco-friendly. The potential health risk and environmental hazard of sulfides could diminish and eventually be eliminated.

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