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The significance of decorin, a minor proteoglycan of bovine hides, in improving the quality of leather

Mila Aldema Ramos and Cheng-Kung Liu
U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center
600 E. Mermaid Lane, Wyndmoor, PA 19038, USA, Phone: 215 836 6909, Fax: 215 233 6795
email address: mila.ramos@ars.usda.gov.

Abstract

Decorin is a minor proteoglycan (part protein and part carbohydrate) of the skin, which is among the key components that undergo changes and removal during conversion of hides to leather. Decorin removed from each area of the hide (shoulder > butt > belly) is directly proportional to the initial amounts in raw hides; however, the residual decorin content of the leather products are almost the same or uniform. The goal of this research was to explore ways to improve the quality of leather by further removal of decorin and to establish a more reliable decorin assay technique. The two analytical techniques employed were ELISA, an immunoassay based on the analysis of the core protein and Alcian Blue colorimetric assay based on the SGAG, the carbohydrate portion of the decorin molecule. Due to the core protein degradation, as observed in western blotting technique, ELISA was found to be less reliable technique; therefore Alcian Blue assay method was preferably employed. Additional removal of decorin was observed by subjecting the hides to pretanning treatments in the presence of alkaline protease during relime stage and pepsin in the pickling stage. More pronounced improvement in leather quality, due to more decorin removed, was observed in oxidatively dehaired hides (methods developed by Marmer and Dudley) than those dehaired traditionally with sulfides. As the decorin 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.

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1. Introduction

Décorin serves as glue in the fibrillar framework of the collagen matrix in skins and its persistent presence could cause the stiffness of the resultant leather. [1, 2] The majority of décorin removal takes place during the dehairing of hides, either traditionally with sodium sulfide or by an alternative oxidative dehairing procedure (with caustic soda and percarbonate) developed and published by Marmer and Dudley.[3, 4] Decorin removed from each area of the hide (shoulder > butt > belly) is directly proportional to the initial amounts in raw hides; however, the final leather products have almost a uniform residual decorin content.[5 6] Further removal of décorin and improvement of leather product was explored by subjecting the hides to pre-tanning treatments in the presence of selected proteolytic enzymes in the reliming, bating, and in the pickling stages. [7]

The reliability of décorin content measurement is the key in establishing the relationship between residual décorin concentration and the quality of the leather product. The quantitative analysis of decorin can be performed by either analyzing its decorin core protein using the enzyme-linked immunoassay (ELISA) [5] technique or by analyzing the sulfated glucose aminoglycan tail using Alcian blue technique.[6-8] In this report, Alcian blue assay of the sulfated glycosaminoglycan (SGAG) is

utilized to analyze the decorin content of differently treated hide samples. The principle of Alcian Blue (AB) colorimetric assay 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 proportional to the number of negative charges present on the SGAG chain. This number corresponds to the amount of SGAG or décorin present in the sample.[9, 10]

2. Materials and Methods

Materials

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

Methods

Thawed fresh bovine hide pieces (6 in x 8 in) with longer side parallel to the backbone taken from the crop area of the hide were chosen as raw materials for the study. Three of the hide pieces were dehaired traditionally by using 2% (W/V) sodium sulfide (Na_2S), 2% (W/V) lime ($CaO.H_2O$) and 1% (W/V) soda ash (Na_2CO_3) in a 100% float for 4 hours. While a piece of hide was dehaired oxidatively by using 4% (W/V) NaOH and 4% (W/V) sodium percarbonate in a 100% float for 4 hours. All the dehaired samples were lime split and converted to wet blue according to the USDA tanning procedure[11, 12] (Table I). The samples analyzed for décorin content were raw hide and the hides that were unhaired, relimed, delimed/bated and pickled. After being chromed, the steps to make leather were not changed. The wet blue samples were retanned, colored and fatliquored into shoe upper leather and then tested for mechanical properties. [14]

As a control sample, S_{10} followed the traditional pretanning procedure (Table I) where sulfide was used for dehairing[11, 12]. The second sample, S_{11}, was also dehaired with sulfide and relimed with 2% Lime and 1% NaHS but the latter is in the presence of 0.2% alkaline protease, delimed and bated normally with Rohapon and pickled in the presence of 0.1% pepsin during the pretanning of hides to leather. The third sample, O_{12}, was dehaired oxidatively [3, 4], (following the italicized and ***bold font*** steps inserted in the traditional pretanning protocol of Table I) and like S_{11}, it was relimed in the presence of 0.2% alkaline protease, delimed and bated normally, and pickled in the presence of 0.1% pepsin. All the hide samples were chrome tanned according to the protocol outlined in second part of Table I.

To verify the results, the residual décorin content and the corresponding physical properties of the resultant leather from another hide source were also investigated with the inclusion of sample O_{20} as control I, dehaired oxidatively and pretanned without proteolytic enzymes co-treatment. Sample O_{22} was treated with 0.2% alkaline protease during the relime step, while only 0.1% alkaline protease was added to sample O_{23}. 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 and pretanned traditionally is also included as control II. To investigate the chromium absorption and retention in the differently treated samples, chromium content was also analyzed using Atomic absorption spectroscopy. [13]

TABLE I. The standard tanning treatments[11, 12] from fresh hides (with novel process 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 and 1% Soda ash.
(***Oxidatively, with 4-6% (W/V) NaOH and 4% (W/V) sodium percarbonate).***
Both done at ~ 4-6 h, 29.4ºC and at pH >12
3. Relime*:* 200 % float - with 2 % Lime and 1 % NaHS (~20 h, 26.7 ºC) (***and add 0.2 % Alkaline protease)*** 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, 32.2 ºC). The action of the enzyme lowers the alkalinity of the hide.
5. Pickle: 0 % float; Add 3 % Sodium chloride (NaCl) + 2% Sulfuric acid and 8% water
for dilution. (***Add 0.1% pepsin)***. Target pH is 1.8.
II. Chrome Tanning and Finishing steps*:* (Table I continuation)
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 tanning solution for 8-12 h, at Rm T. The chemical action of chrome turns hide to leather.
2. Colouring: Dyes added in tanning solution give its color (usually 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

Determination of decorin content

Modified procedure for the analysis of SGAG content

A 50mg of lyophilized, pulverized hide (raw and/or pretanned) was weighed into a 15 ml plastic tube with calibration and screw cap (from Globe Scientific, Paramus, NJ). 1.9 ml of Ca Tris Buffer (pH 6.8) and 50 µl (2500 U) [13] of Collagenase preparation were added and mixed well by shaking at 27 °C for 10 minutes in water bath. The protein was extracted with 1.25 ml Guan·HCl (8.0 M)[13] and a protease inhibitor cocktail was added to protect the protein from degradation. The whole mixture was gently mixed overnight to obtain effective protein extraction. The rest of the protocol was the same as the one illustrated and published in detail in previous papers. [6, 9] The Alcian Blue Assay Kit from Kamiya Biochemicals, Inc[10] has also been utilized in order to save time and chemicals. This allowed us to assay more samples in much shorter period of time and obtained better repeatability. For calibration purposes, the set of known concentration of SGAG that was provided with the Kit was analyzed by taking absorbance readings at 605nm in order to generate a standard curve.

3. Determination of Mechanical Properties

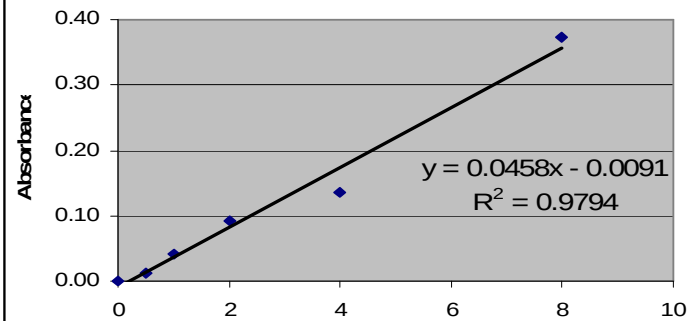
Mechanical property measurements included tensile strength, elongation-to-break ("strechability"), Young's modulus ("stiffness"), and fracture energy (the energy needed to fracture leather samples, its "toughness"). Rectangular shaped leather samples (1- × 10-cm) were cut near the standard test area as described in ASTM D2813-03[14] 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.

4. Results and Discussion

The concentration of decorin in hide samples, with respect to its SGAG content were calculated from the slope of the standard calibration graph (Fig 1). The graph is prepared by plotting a straight line relating the absorbance to the known amount of standard SGAG. The modified Alcian Blue assay has improved efficiency, capable of analyzing more samples and requiring lesser amounts of reagents because small aliquot amounts from each sample were used.

Figure 1. Standard SGAG graph



µg/mlSGAG	netAbs
0	0
0.5	0.013
1.0	0.042
2.0	0.091
4.0	0.135
8.0	0.374

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 (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[15]. The t-tests performed for Tables II and III have a corresponding t-value of 2.308 for p-value of 0.05 with 8 degrees of freedom[15]. The t-test gives the probability that the null hypothesis (i.e. no difference) is true. If the calculated t-test value is smaller than 2.308, then one may assume that there is no significant difference between the means. On the other hand, if the t-test value is **greater** than 2.308, an alternative hypothesis is true, i.e. the difference is statistically significant.[15]

Our results showed that in general, the quality of the resultant leather is improved consistently as further removal of decorin is observed when the tanning process is treated with proteolytic enzymes, such as alkaline protease and pepsin.

The overall observation is that; as the decorin content decreases, the Toughness or fracture E, increases, the Elongation increases (more stretchable), the Young's Modulus decreases (softer) and Chromium content also slightly increases.

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

Sample code	Dehaired By	Different treatment[1]	µgdecorin /g hide	Elongation to-break (%)	Fract. Energy "toughness" (J/cm^3)	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% AP in relime and 0.1% pepsin(Pn) in pickle stage

** where t S_{11} is the t-test for S_{11} vs. control S_{11}; 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}.

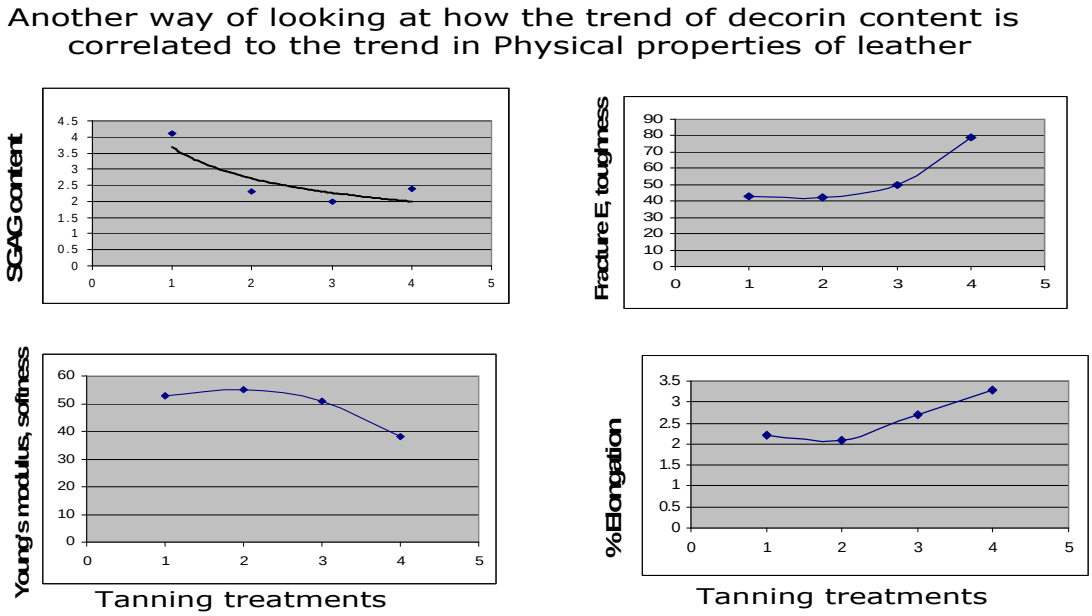


Figure 2. As the concentration of decorin decreases, the overall mechanical properties showed improvement; the young's modulus is decreasing (softer) while the elongation (strechability) and fracture energy (toughness) increases accordingly.

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

Sample code	Different treatment[I]	µg decorin /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,
**control II, sample S_{20}; hide is dehaired with sulfide and pretanned traditionally,
[I] AP + Pn = 0.2% AP added in relime and 0.1% pepsin (Pn) added in pickle stage of 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

6. Conclusions

A softer, more stretchable and yet tougher leather product can be obtained when the hides are co-treated with proteolytic enzymes during the pretanning stages of tanning hides to leather. And this is directly related to the additional removal of décorin. Additional removal of décorin was observed when an alkaline protease was added during the relime stage and pepsin was added during the pickle stage in the pretanning treatments of the hides. The novel recipe consisted of adapting the oxidative dehairing protocol[3, 4], and then incorporating alkaline protease (preferably 0.1 % (W/V) in the reliming step and pepsin (preferably ~ 0.1% (W/V) in the pickling step. Tests showed that the elongation-to-break ("strechability") 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 in pretanning). 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 by oxidation. 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 traditional methods. Another advantage of replacing sulfides by caustic soda with 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 can be diminished and eventually eliminated.

More pronounced improvement in leather quality, due to more decorin 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 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.

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