Studies on the Removal of Inter-fibrillary Materials Part II: Removal of Protein, Proteoglycan and Glycosoaminoglycans from Biobased Pre-Tanning Process**

by

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Abstract

Eco-concerns are forcing the leather industry to shift itself from chemical based processing to bio-processing. The use of enzymes for unhairing and bating has been long established and well reported; but the use of α-amylase for fiber opening is a recent development, which advocates the complete elimination of lime. Though the bio-based fiber opening process is established to be an eco-friendly alternative, it is essential to establish its effectiveness versus the conventional liming process by more objective methods. Hence in this work an attempt has been made for quantitative analysis of inter-fibrillary materials viz., proteins, proteoglycans and glycosoaminoglycans (GAGs) released from the skin matrix by the use of complete enzyme based beamhouse process, especially the effect of α-amylase in beamhouse processes. It is observed that treatment of 2% α-amylase for a time period of 180 min is sufficient for the removal of optimum amount of inter-fibrillary materials especially, proteoglycans. Matched pair comparison of experimental bio-based processing has been made with conventional lime based processing and it is observed that the removal of proteoglycans were comparable in both these processes. Also, the crust leathers processed from the matched pair beamhouse processes were compared for their organoleptic and strength characteristics. Both experimental bio-processed leather and conventionally processed control leather were observed to be comparable. This work further authenticates that a completely bio-based beamhouse process is not only eco-friendly but also results in a similar effect on the removal of inter-fibrillary material compared to conventional lime based process.

Resumen

Las preocupaciones ecológicas están obligando a la industria del cuero a un cambio de ser un proceso químico a ser un bio-proceso. El uso de enzimas para el pelambre y rendido ha sido establecido hace tiempo con buenos resultados reportados, pero el uso de α-amilasa para la apertura de las fibras es un desarrollo reciente, que aboga por la eliminación completa de cal. Aunque la base biológica para el proceso de apertura de las fibras se creó para ser una alternativa eco-amigable, es esencial para establecer su eficacia en comparación con el proceso de encalado convencional empleando métodos más objetivos. Por lo tanto, en este trabajo se ha utilizado el análisis cuantitativo de materia interfibrilar como son proteínas, proteoglicanos y glicosaminoglicanos (GAG), obtenidos de la matriz de la piel, por el uso del proceso completo de pelambre basado en enzimas, en especial por el efecto de la α-amilasa en los procesos de ribera. Se ha observado que el tratamiento con 2% de α-amilasa durante un período de tiempo de 180 minutos es suficiente para la eliminación de una cantidad óptima de materiales interfibrilares, en particular, proteoglicanos. En una comparación del bio-proceso experimental y el tratamiento convencional basado en cal, se observa que la eliminación de los proteoglicanos fueron comparables en ambos procesos. Además, el cuero semiterminado elaborado a partir de ambos procesos de ribera equivalentes se comparó por sus características organolépticas y físicas. Tanto el proceso bio-experimental como el proceso convencional de control, se observaron como comparables. Este trabajo confirma además que una ribera basada en un bio-proceso no es solamente eco-amigable sino que resulta en un efecto de remoción de los materiales interfibrilares similar al proceso convencional a base de cal.

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INTRODUCTION

Amongst the four sub groups of leather processing, beamhouse and tanning processes contribute to 80-90% of the total pollution load. Though liming/reliming is an important pre-tanning process it causes ecological concerns as it contributes about 60-70% of the total pollution load in leather processing.[1] Lime has a potential to drive swelling in a gradual manner due to its low solubility but lacks in environmental friendliness. Formation of large amount of lime sludge is the main drawback of lime. Optimum amounts of chemicals need to be employed for the reduction in the TDS, BOD and COD loads. Liming process leads to not only wastewater containing significant amounts of BOD, TDS, S^{2-} but also substantial quantities of solid wastes containing lime sludge, fleshing and hair. The extensive use of sodium sulfide bears unfavorable consequence on environment and the efficacy of effluent treatment plants.[2] Recently there had been good success in making bio-pretanning process based on enzymatic unhairing and fiber opening without lime.[3-6] The use of α-amylase for fiber opening and their optimum conditions were well established.[5,7] Lime-based swelling removes interfibrillar materials (proteoglycans) through osmotic forces, which leads to area reduction and wrinkle formation. The enzymes α-amylase disintegrates the proteoglycans, which opens the fiber matrix and induces swelling. Significant reduction in pollution load has been observed for the amylase based fiber-opening process.[5]

In the present work we have attempted to quantify the removal of inter-fibrillary materials viz., proteins, proteoglycans and GAGs by using a bio-pretanning process. In our earlier paper (Part I),[8] quantification on the removal of protein, proteoglycan and GAGs from conventional pretanning process was standardized. In the present work the comparison on the efficacy of the bio-pretanning process with the conventional pretanning process with respect to the removal of inter-fibrillary materials has been made.

EXPERIMENTAL

Materials

Wet salted goat skins (5-6 sq.ft) were chosen as raw materials for the study. All chemicals used for leather processing were of commercial grade while the chemicals used for the analysis of spent liquors were of analytical grade. α-amylase and protease (biodart) were procured from Southern Petrochem. Ind. Corp. Indian Ltd, Chennai.

Effect of Duration of α-amylase Treatment during Fiber Opening Process

To establish the effect of time of α-amylase treatment on the removal of proteoglycans 8 goat skins were taken for soaking and dehaired using 3% biodart enzyme and 20% water by paint method. After 18 hr the hair was removed and the skin taken for fiber opening experiments for varied time durations viz., 20, 40, 60, 90, 120, 180, 240 and 360 min treatment with 2% α-amylase in a drum along with 100% water at ambient temperature (30°C), pH 8.0. The amounts of protein, proteoglycan, GAGs removed were estimated as per procedure mentioned earlier[8]. The process followed is shown as a flow chart in Figure 1.

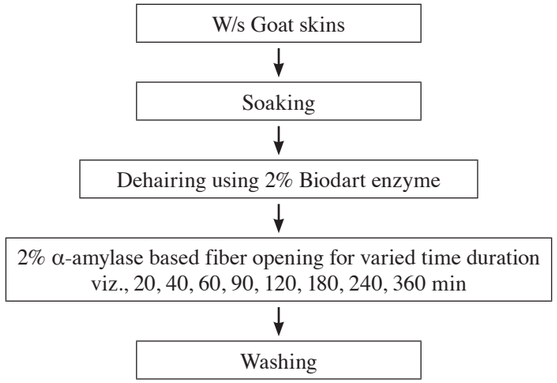

Figure 1. Process flow sheet for experimental trials with 2% α-amylase at varied time intervals viz., 20, 40, 60, 90, 120, 180, 240, 360 min

Effect of Washing after Fiber Opening on the Release of Proteins, Proteoglycans and GAGs

Washing with water in the drum after the enzymatic fiber opening is significant for the removal of the interfibrillary materials adhering physically to the matrix. Hence three goat skins were taken and subjected to experimental fiber opening process after enzymatic unhairing. After the treatment with 2% of α- amylase for 3 hrs, the pelts were subjected to washing and small amount of sample liquor collected at different time intervals viz., 10, 20, 30 and 40 min at ambient temperature (30°C), pH 8.0. The amount of protein, proteoglycan and GAGs were measured at different duration of washings. The process sequence is shown in Figure 2.

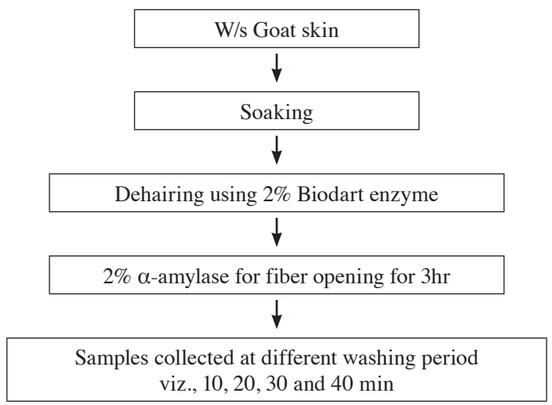

Figure 2. Process flow sheet for experimental trials with 2% α-amylase for 3 hrs and samples collected at varied duration of washing viz., 10, 20, 30 and 40 min

Influence of Varying Percentage of α-amylase on the Removal of Proteoglycans from Beamhouse Bioprocess Method

Eight wet salted goat skins were taken for experimental pretanning process. The skins were unhaired using 3% biodart protease enzyme and subsequently the skins were treated with varying amounts of α-amylase viz., 1, 1.5, 2 and 3% and two skins after unhairing were taken for each fiber opening experimental trial at ambient temperature (30°C), pH 8.0. The fiber opening process was carried out in a drum for 3 hr and then the pelts were washed for 10 min. The sample liquors from fiber opening process and washing were taken for analysis of proteoglycans. Then the pelts were chrome tanned and post tanned into crust leather using the process mentioned in Table I. The crust leathers were analyzed for physical strength properties.

Matched Pair Comparison on the Removal of Protein, Proteoglycans and GAGs from Conventional and Experimental Process

Four goats skin were taken and each skin is cut into two halves. All the four left halves were processed using control process mentioned in Table II and all right halves were processed using bio-pretanning process mentioned in Table III. In control processing samples from liming liquor, lime wash liquor, delime liquor, delime wash liquor, bate liquor and bate wash liquor were collected and filtered using Whatmann filter paper. The pH of the samples were adjusted to 8, which is the pH for α-amylase based fiber opening. In the case of experimental process, samples from fiber opening (using α-amylase) and subsequent washing were collected and filtered using Whatmann filter paper. The amounts of protein, proteoglycan, GAGs were estimated in all the samples. Both the experimental and control pelts were pickled, chrome tanned and post tanned into upper crust leathers using the common process mentioned in Table I.

TABLE I
Process for pickling to crusting

Process	Chemicals	%	Duration (mins)	Remarks
Pickling	Water Sodium chloride H_2SO_4	100 10 1	 10 3×10 + 20	pH adjusted to 2.8-3.0
Chrome tanning	BCS	8	3×10 + 90	
Basification	Sodium formate Sodium bicarbonate	1 1	3×10 + 30	pH adjusted to 3.8-4
Neutralization	Sodium formate Sodium bicarbonate	1 1	3×10 + 30	pH adjusted to 5
Post Tanning	Water Relugan RE Basyntan DI	100 2 4	— — 30	
	Basyntan FB6 Relugan RE	3 2	— 30	
	Lipodermliq SAF Balmol SXE Lipoderm liq FB16	4 4 4	— — 60	
	Wattle GS powder	4	30	
Fixing	Formic acid (1:10 dilution with water)	1	3×10 + 30	pH- 4 Drain
Washing	Water	100	10	Drain, Pile O/N, Set, Dry, Stake, Trim, and Buff

Physical Testing and Hand Evaluation

The samples from bio based beamhouse process (protease based followed by α-amylase based fiber opening) leathers were cut from the official sampling position for physical testing[9] (IUC 2 method). Specimens were conditioned at 80±4°F and 65±2% R.H. over a period of 48 hrs. Physical properties such as tensile strength, % elongation at break, tear strength and grain crack strength were examined as per the standard procedures.[10-12] Experimental crust leather from optimized trials were assessed for fullness, roundness, softness and grain tightness by hand and visual examination. Experienced tanners rated the leathers on a scale of 0-10 points for each property, where higher points indicate better property.

TABLE II

Control pretanning process (Soaking to bating)

Raw material: W/S Goat skins

Process	Chemicals	%	Duration	Remarks
Soaking	Skins were soaked conventionally. Soaked weight was noted. All the addition of chemicals was based on soaked weight			
Liming	Water Limse Na_2S	10 6 2	O/N	Chemicals were applied as paste on the flesh side. N/D skins were dehaired.
Reliming	Water Lime	300 10	4 days	Fleshing is done after 4th day. The pelt weight was noted. Amount of chemicals added for subsequent processes were based on pelt weight.
Washing	Water	200	20 min	Drain the bath.
Deliming	Water NH_4Cl	100 1.5	60 min	Completion of deliming was checked.
Washing	Water	200	20 min	Drain the bath.
Bating	Water Microbate	100 0.5	60 min	Extent of bating ascertained.
Washing	Water	200	15 min	Drain the bath.

* - Process carried out at ambient temperature (30°C)

TABLE III

Experimental pretanning process (Soaking to fiber opening)

Raw material: W/S Goat skins

Process	Chemicals	%	Duration	Remarks
Soaking	Skins were soaked conventionally. Soaked weight was noted. All the addition of chemicals was based on soaked weight.			
Enzymatic unhairing	Water Biodart	10 3	18 hrs	Chemicals were applied as paste and applied on the flesh side. N/D skins were dehaired.
Enzymatic fiber opening	Water α-amylase	100 2	3 hrs	Drum was run for 3 hours continuously.
Washing	Water	200	15 min	

* - Process carried out at ambient temperature (30oC), pH 8.0

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RESULTS AND DICUSSION

Effect of Duration of α-amylase Treatment on the Removal of Proteoglycan

The influence of duration of α-amylase treatment on the removal of proteoglycan was carried out using 2% α-amylase treatment on goat skins. The process liquor estimated for proteins, proteoglycans and GAGs at different time interval is given in Table IV. From the table it is seen that the amount of proteoglycan released is increasing with increasing time of treatment of α-amylase. After 180 min of treatment of α-amylase there is no significant increase in the removal of proteoglycans at it is observed that 4.09 and 4.12 mg of proteoglycan removal at 180 and 240 min. But with further increase in the treatment time of α-amylase to 360 min, the proteoglycan removal has been found to decrease. Amylases are highly specific in catalyzing the hydrolytic scission of a specific glycosidic bond. Amylases are carbohydrates that catalyze the digestion of the α-D-1,4 glycosidic bonds. When the skin matrix is treated with amylase, the initial effect could predominantly be on the scission of the glycosidic linkages of proteoglycan conjugated with skin matrix, especially with collagen. The amylases can also attack the glycosidic linkages between the protein and glycan moiety of the proteoglycan resulting in the separation of protein and glycan moiety. Though initial amylase treatment is dominated by the attach of glycosidic linkage conjugated with skin matrix, decrease in the amount of proteoglycan observed for longer duration of amylase treatment could be due to breakdown of the proteoglycan. When the pelt treated with amylase made into crust leather after chrome tanning, the one treated with amylase for time period of 360 min resulted in slight looseness of the grain. This could be due to the high activity of the amylase resulting in the extensive break down of the glycan conjugation. The treatment of α-amylase for a time period of 180 min is found to be ideal with respect to the removal of proteoglycan being saturated at this time period.

TABLE IV

Protein, proteoglycan and GAGs by treatment with α-amylase (2%) at varied time interval (without wash liquor)

Time interval (min)	Protein (mg/g)	Proteoglycans (mg/g)	GAGs (mg/g)
20	2.28±0.09	2.21±0.08	0.22±0.009
40	2.26±0.093	2.50±0/12	0.22±0.009
60	2.31±0.1	2.74±0.13	0.23±0.01
90	2.43±0.11	3.08±0.15	0.25±0.011
120	2.45±0.12	3.52±0.17	0.25±0.012
180	2.77±0.13	4.09±0.2	0.27±0.013
240	2.88±0.15	4.12±0.23	0.27±0.015
360	3.08±0.17	3.72±0.18	0.29±0.017

Removal of Protein, Proteoglycan and GAGs: Influence of Washing (time) after α-amylase Based Fiber Opening

After fiber opening treatment with 2% of α- amylase for 3 hrs, the pelts were subjected to different washing periods and the amount of protein, proteoglycan and GAGs measured at different duration of washings are given in Table V. Significant amount of protein, proteoglycan and GAGs are found to be removed during washing. From the table, it is clear that increasing the time of agitation is favoring the removal of interfibrillary materials, which were broken down during the treatment with α- amylase is enhanced.

Influence of Varying Percentage of α-amylase on the Removal of Interfibrillary Material and Physical Properties of the Leathers

In order to study the removal of protein, proteoglycans and GAGs at varying amounts of α-amylase treatment, four trials were carried out with two goat skins each with different % of α-amylase viz., 1, 1.5, 2 and 3 for a time period of 180 min. The amount of protein, proteoglycan and GAGs at varied amounts of α-amylase is given in Table VI. Increase in the interfibrillary material released is observed with increased amounts of α-amylase. The strength properties of the crust leathers treated with varying amounts of α-amylase is given in Table VII. The crust leathers treated with 2% α-amylase results in higher tensile strength and grain crack resistance compared to other leathers. However, the tear strength is found to be higher for the leathers treated with 3% α-amylase. The leathers treated with 1% α-amylase for fiber opening are observed to be relatively harder compared to leathers treated with higher amounts of α-amylase. The leathers treated with 3% α-amylase are slightly pipey compared to other leathers. The leathers treated with 1.5 and 2% α-amylase found to posses comparable organoleptic properties. However, the treatment with 2% α-amylase for fiber opening resulted in the release of optimum amount of proteoglycan and hence can be considered as optimum amount for fiber opening experiments.

Matched Pair Comparison on the Removal of Protein, Proteoglycans and GAGs from Conventional and Experimental Process

Four goats skin were taken and each skin cut into two halves. All the four left halves processed using control process and all right halves processed using bio-beamhouse process using 3%

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biodart for dehairing and 2% α-amylase for fiber opening. Both the experimental and control pelts have been subsequently pickled, chrome tanned and post tanned into upper crust leathers using common recipe (Table I). The proteoglycan removal for both control and experimental process has been measured and the values are shown in Table VIII. From the table it is observed that the total amount of proteoglycan removed from both control and experimental process have been found to be comparable. The crust leathers from both control and experimental process have been compared for strength and organoleptic properties. Different strength parameters are given in Table IX and it is observed that the tensile strength and grain crack resistance characteristics of experimental leathers are better than control leathers whereas tear strength of control leathers were found to be slightly better than the experimental leathers. However, the differences in the strength characteristics are not very significant. The organoleptic properties of the leathers are shown in Figure 3. Both the experimental and control leathers showed comparable organoleptic properties.

TABLE V

Release of protein, proteoglycan and GAGs after treatment with 2% α-amylase for 180 min and at varied time interval of washing

Washing Time (min)	Protein (mg/g)	Proteoglycans (mg/g)	GAGs (mg/g)
10	2.75±0.11	4.52±0.18	0.20±0.01
20	2.90±0.13	5.58±0.25	0.20±0.01
30	3.12±0.156	6.14±0.31	0.21±0.01
40	3.36±0.18	6.23±0.34	0.21±.01

TABLE VI

Release of protein, proteoglycan and GAGs at varied amounts of α-amylase for fiber opening

% α-amylase	Process	Protein (mg/g)	Proteoglycan (mg/g)	GAGs (mg/g)
1	Fiber opening spent liquor	2.00±0.08	2.08±0.08	0.07±0.00
	Wash liquor	2.34±0.10	3.64±0.16	0.14±0.01
1.5	Fiber opening spent liquor	2.41±0.10	3.49±0.15	0.13±0.01
	Wash liquor	2.64±0.11	3.92±0.18	0.15±0.01
2	Fiber opening spent liquor	2.72±0.12	4.31±0.21	0.22±0.01
	Wash liquor	2.93±0.14	5.61±0.28	0.24± 0.10
3	Fiber opening spent liquor	2.81±0.13	4.23±0.21	0.22±0.01
	Wash liquor	2.95±0.14	5.63±0.30	0.23±0.01

TABLE VII

Physical strength characteristics of crust leathers processed from varying amounts of α-amylase for fiber opening

% α-amylase	Tensile strength (N/mm)	% Extension at Break	Tear strength (Kg/Cm)	Grain crack resistance	
				Load (Kg)	Distension (mm)
1	28.85±6.15	60.07± 2.48	46.67±5.22	43±1	9.40±0.35
1.5	29.07±6.23	68.93±3.1	44.69±5.21	46±2	9.54±0.42
2	30.25±7.71	68.5±3.43	43.72±6.12	50±2	10.20±0.4
3	22.76±5.74	83.5±4.59	51.20±7.43	44±1	9.43±0.36

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CONCLUSIONS

In this work, the effect of α-amylase on the removal of inter-fibrillary materials has been studied. It is observed that 2% α-amylase for a time period of 180 min is sufficient for the removal of optimum amount of inter-fibrillary materials. Matched pair comparison of conventional limed process and bio-based beamhouse process using α-amylase resulted in comparable removal of inter-fibrillary materials. Also, the leathers made from the matched pair comparison of control liming and experimental bio-based process using 2% α-amylase are observed to have comparable strength and organoleptic properties. The work establishes that the bio-based beamhouse process is comparable to that of conventional process. This substantiates that the eco-friendly bio beamhouse process is a commercially viable option.

TABLE VIII

Matched pair comparison on the removal of proteoglycan from conventional & α-amylase (2% and 180 min) based fiber opening

Conventional Process		Enzymatic process	
Process	**Proteoglycans (mg/g of raw wt)**	**Process**	**Proteoglycans (mg/g of raw wt)**
Liming liquor (3 days)	3.46±0.21	α-amylase fiber opening spent liquor	4.39±0.34
Wash liquor (10 min)	2.10±0.12		
Delime liquor	3.77±0.31		
Wash liquor (10 min)	1.10±0.08	Wash Liquor	5.74±0.41
Bate liquor	1.50±0.03		
Bate wash liquor	0.63±0.02		
Total	12.56		10.13

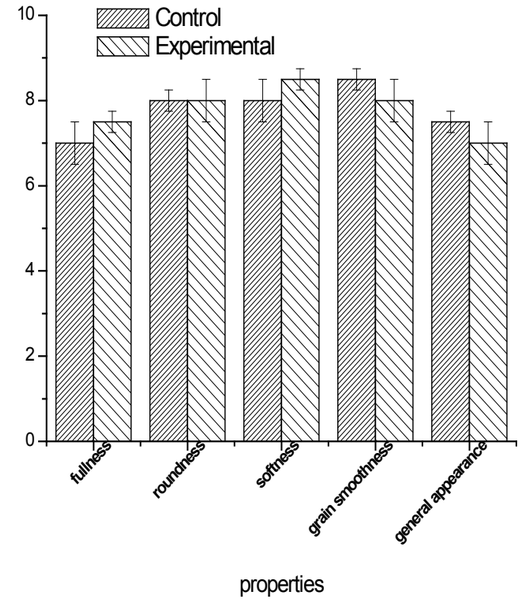

Figure 3. Organoleptic properties of crust leathers processed from matched pair experimental leathers (α-amylase 2%) and control leathers (lime)

TABLE IX

Physical strength characteristics of crust leathers processed from matched pair experimental leathers (α-amylase 2%) and control leathers (lime)

Process	Tensile strength (N/mm)	% Extension at Break	Tear strength (Kg/Cm)	Grain crack resistance	
				Load (Kg)	Distension (mm)
Experimental	32.66±3.15	68.93±3.1	36.63±2.72	49±3	9.61±0.29
Control	29.07±2.23	70.56±2.83	44.67±2.91	32±2	10.54±0.42

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