Rapid Fiber Opening Process for Skins: An Approach for Fail-safe Chemical-free Process

by

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Abstract

Conventional beam house operations adopted by the global tanning industry are coming under the close scrutiny of the environmentalists on account of the possible adverse effect on human health and environment. Lime, which is used to the tune of about 10% on the weight of the raw hides and skins, in re-liming stage contribute to generation of large quantities of sludge, the disposal of which is a serious concern. Also the process duration to achieve the required fiber opening in the re-liming process is very long, ranging from 24-72 hrs for different substrates and end products. Enzyme based beam house processes are being advocated to overcome the problems associated with the conventional processes. However, enzyme based methods need greater control over process parameters to achieve satisfactory results in leather processing and avoid damage to the pelt. To overcome the problems associated with the conventional re-liming process, an attempt has been made in the present investigation to utilize cocktail of carbohydrases for fiber opening of hides and skins. Fiber opening at optimized concentration of the above enzyme has been studied for its efficiency on goatskins. A process using optimum quantity of enzyme led to fiber opening in 30 minutes. The efficacy of enzyme on rapid fiber opening of dehaired skin was studied at different experimental conditions of pH, time and enzyme concentration. The performance of the enzyme in the given experimental conditions was assessed by quantifying the removal of carbohydrate and proteoglycan and examining the fiber opening by scanning electron microscopic studies. Effect of enzyme on the final quality of the leather was evaluated by changes in chemical characteristics, physical properties and visual examination.

Introduction

The conventional liming and re-liming processes followed globally in the tanning sector contribute high amount of COD, BOD, TDS etc., to the effluent.[1-3] Most of the enzymatic or enzyme assisted chemical methods developed in the past are applicable only for unhairing of hides/ skins in leather processing. The unhaired skins however require fiber opening subsequently and this is conventionally accomplished using lime. Treatment of hides/skins with lime removes inter-fibrillar materials present in the collagen matrix. Generation of large amount of lime sludge is the major drawback associated with use of lime. In principle, fiber opening is the process that removes the proteoglycans and glycosaminoglycans from the skins and it can be achieved using enzymes or chemical auxiliaries. Few researchers have reported about the application of enzymes in fiber opening.[4] Xylanase enzyme has been used to act upon the linear carbohydrate polymer to achieve opening up of the rigid fiber structure of stingray skin.[5] In conventional process it is necessary to treat the skins with lime for several hours or days to achieve the required level of fiber opening. To avoid this time-consuming re-liming process, a detailed study on rapid fiber opening process by using cocktail of carbohydrate cleaving enzymes, replacing conventional lime, was carried out in this work and the results are discussed in the direction of advantages of the present work.

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MATERIALS AND METHODS

Cocktail of carbohydrase enzymes produced by solid-state fermentation (SSF) of *Aspergillus terreus* was used for the fiber opening study. The carbohydrase activity [6-7] of the enzymes solution was found to be 40,000U/g of substrate. The enzyme used in the study is stable over a temperature range of 25-40°C and exhibited activity over a pH range of 6.0-11.0. All the other chemicals used were of commercial grade.

EXPERIMENTAL

Optimization of Enzyme Concentration

Goatskins were used to study the efficacy of the enzymatic fiber opening process. Three sets of trials were carried out. In the first set, three wet salted goatskins were taken and vertically cut into left (L) and right (R) pieces, and were marked as 1L 1R, 2L 2R, 3L and 3R. The skins marked as 1L, 2L and 3L were processed by conventional fiber opening process, i.e. by using lime and their respective right pieces - 1R, 2R and 3R were processed with fiber opening enzyme.

In the case of conventional liming process, the skins after unhairing were treated with 10% lime and 150% water for 48 hrs. Whereas in case of enzymatic process, the skins after unhairing were treated with varying amounts viz., 1%, 2%, 3% (v/w) of enzyme on the basis of the weight of the skin with 100% water for a period of 30 min.

The pelts of both conventional and enzymatic treatments were monitored intermittently by visual and feel assessments for fiber opening. Both control and experimental leathers were subjected to hide substance analysis. Also, the carbohydrate and proteoglycan levels in the samples were assayed by following standard procedures. For further experiments, the optimized enzyme concentration was used to standardize other parameters such as pH and treatment duration.

pH Optimization

Second set of experiments was carried out to determine the optimum pH for maximum fiber opening with the standardized quantity of the enzyme. The enzyme of fixed concentration was given at different pH condition, viz. 6.0, 8.0, 10.0 and 12.0 without changing other parameters. At the end of predetermined time, the carbohydrate and proteoglycan levels in the pelt samples were quantified spectrophotometrically to determine the optimum pH condition required for maximum removal of interfibrillarly materials.

Time Course Study

Third set of experiments was carried out to find out the optimum time duration required for obtaining the maximum fiber opening at conditions of optimum pH and enzyme concentration. The skins were treated with fiber opening enzyme for varied length of time period, i.e., 30, 60, 90 and 120 mins, followed by which, the carbohydrate and proteoglycan assay were carried out on the samples to ascertain optimum period required for maximum removal of the saccharide moieties and satisfactory fiber opening. The pelt thus produced with standardized quantity of enzyme, pH and time duration along with control samples were processed in to wet blue leathers following a standard chrome tanning process. The process followed is given in Appendix I. The chromium content in both the control and experimental tanned leathers (wet blue) were analyzed by following IUC method. The wet blue leathers were converted into crust leather following standard procedure given in Appendix II.

Carbohydrate Assay

Total carbohydrate content of the pelt samples, both the experimental and control were determined according to the phenol-sulphuric acid method,[8] using D-glucose as standard. Dehaired pelt before fiber opening treatment was used as blank sample to compare the remaining sugar content of the lime or enzyme treated samples. Sample for assay, was prepared by hydrolyzing 100 mg each of lyophilized sample with 0.5 N sulphuric acid solution at 100°C in sealed tube for 4 hrs. Assay was carried out by using 1ml of hydrolyzed aliquot of pelt sample mixed with 5% (v/v) phenol. And then the tubes were cooled in ice for 10 minutes and 5ml of concentrated sulphuric acid was added through the sides of tubes. The contents were thoroughly mixed and the tubes were heated in a water bath at 80°C for 20 minutes. After cooling the tubes to room temperature, the absorbance was noted at 490nm using a spectrophotometer. A reagent blank was prepared in the same manner using distilled water. The amount of carbohydrate remained in the pelt samples was calculated as glucose from the standard curve drawn using glucose solution of known concentration.

Estimation of Proteoglycan

In order to estimate the amount of proteoglycan in the pelt sample both the experimental and control samples were assayed by Schiff's colorimetric reaction.[9] Initially, 100mg of sample was hydrolyzed using 0.5 N sulphuric acid solution at 100°C in sealed tube for 16-18 hrs and allowed to cool to room temperature. To 1ml of hydrolyzed sample, 100ul of decolorized Schiff reagent was added and incubated at 37°C for 2 hours. After wards the reaction mixture was mixed and allowed to remain at room temperature for 30 minutes for color development. Absorbance of the reaction product was measured at 555 nm using UV-Vis spectrophotometer and the total amount of proteoglycan present in the sample was calculated using mucin as standard.

Scanning Electron Microscopic Analysis

In order to study the effect of enzyme on the fiber opening and the structural characteristics of the biological material produced, the specimens of control and experimental pelts

were examined microscopically. Briefly, samples measuring 5mm x 2mm were cut from the crust leathers using fresh stainless steel blades. The samples were mounted both vertically and horizontally on aluminum stubs using an adhesive. These were then coated with gold using an Edwards E-306 sputter coater. The stubs were introduced into the specimen chamber of a FEI-Quanta 200 scanning electron microscope. The stubs mounted on the stage could be tilted, rotated and moved to the desired position and orientation. The micrographs for the cross-section were obtained by operating the microscope at higher voltage.

Physical Testing and Visual Assessment

The samples for physical testing were cut from both the enzyme treated and conventional crust leathers from the official sampling position.[10] The samples were conditioned to the required relative humidity of 65±2% at 20±2°C for 48 h as per IUP 3.[11] The tensile and tear strengths were measured as per the standard procedures.[12-14] A panel of experienced leather technologists assessed the organoleptic properties such as general appearance, grain smoothness, feel and area yield. The leathers were rated on a scale of 0-10 points for each functional property, where higher points indicate better property.

RESULTS AND DISCUSSION

Effect of Enzyme Concentration on Fiber Opening

In order to ascertain the effect of enzyme on the major leather making protein, samples were taken after enzyme treatment along with corresponding control and subjected to hide substance analysis. According to the results given in Table I, it is evident that no loss of hide substance due to enzyme action was noticed, when compared to control.

Removal of glycosoaminoglycan particularly proteoglycan is necessary to allow the fiber structure open up to ensure good quality leather. The carbohydrate assay carried out before and after lime/enzyme treatment indicated removal of carbohydrate to an extent of 80% in 30 min with 1% enzyme compared to that of lime in 24 hrs with the removal of 77%. Increase in enzyme concentration produced marginal improvements in carbohydrate removal as seen from results in Table II. Similarly in the case of removal of proteoglycan, the enzyme was able to remove comparable levels of GAGs as that of lime (78% Vs 77%). Increase in enzyme concentration to more than 1% led to the same observation that of carbohydrate removal, producing marginal improvements.

Effect of pH on Fiber Opening

The fiber opening trials were carried out with the carbohydrase enzyme solution at various pH conditions ranging from 6.0 - 12.0 at 2 pH units interval. The results, as inferred from carbohydrate and proteoglycan removal are given in Table III and Table IV respectively. It is seen that optimum activity is achieved at pH 8.0 compared to other pH conditions such as 6.0, 10.0 and 12.0.

TABLE I
The hide substance analysis.

Sample	% Hide substance (on dry basis)
Control	72.0±1.3
Experiment 1	70.8±1.7
Control	71.6±2.2
Experiment 2	71.8±2.1
Control	74.7±1.5
Experiment 3	75.9±2.1

*Average value of 3 determinations

TABLE II
Extent of carbohydrate and proteoglycan removal with lime or enzyme.

Sample	Sugar Removed (%)	GAG Removed (%)
Control	77±1.2	78±1.3
1.0% Enzyme	80±1.3	77±1.3
2.0% Enzyme	83±1.7	87±1.7
3.0% Enzyme	86±1..3	85±1.3

*Average value of 3 determinations
GAG- glycosaminoglycan

Effect of Time on Fiber Opening

The unhaired skins were treated with 1% carbohydrase solution with 100% water at pH 8.0 for different periods of time varying from 30, 60, 90,120 minutes. It was found that optimum opening up of fiber structure was obtained within a period of 30 minutes as indicated by the result of carbohydrate and proteoglycan removal presented in Tables V. By extension of treatment time from 30 min to 60 minutes did not lead to any significant change. On the contrary, extending the enzyme treatment time beyond 60 minutes marginally affected the opening up of fiber bundles as observed from the relatively decreased levels of interfibrillary materials. This may be due to the reglycation of collagen fibers, non-enzymatically, upon maintaining the contact of enzymatically released sugar moieties with collagen matrix.

TABLE III
Extent of Carbohydrate removal at different pH conditions.

Sample	Sugar Removal (%)
pH 6.0	84±1.3
pH 8.0	96±1.7
pH 10.0	93±1.3
pH 12.0	74±1.3

*Average value of 3 determinations

TABLE IV
Extent of Proteoglycan removal at different pH conditions.

Sample	Sugar Removal (%)
pH 6.0	78±1.8
pH 8.0	91±2.0
pH 10.0	87±2.1
pH 12.0	76±1.8

*Average value of 3 determinations

TABLE V
Extent of Carbohydrate removal for different time periods.

Sample	Sugar Removed (%)	GAG Removed (%)
½ hr	95±1.2	90±1.2
1 hr	93±1.3	88±1.3
1 ½ hr	89±1.3	86±1.3
2 hr	79±1.2	72±1.2

*Average value of 3 determinations

Chromium Content of Wet Blue Leather

The chromium content of both control (4.1±0.1%) and experimental leathers (4.4±0.2%) indicate comparable fiber opening and availability of functional groups for interaction with chromium in the process of tanning. Opening up of fibers of the collagen matrix, results in exposure of increased number of interacting functional groups such as –COOH or –NH2 leading to relatively increased to chromium uptake. This is reflected by decreased chromium content in the waste liquor that is discharged. The conventional fiber opening treatment, required a 10% (w/w) $Ca(OH)_2$ in 300% (v/w) water for 24 hrs to achieve a chromium absorption of 4.1% as against the enzymatic process that required only 1% (v/w) enzyme solution (without lime) and a treatment time of only 30 min to uptake marginally increased level of chromium (4.4%),which is about 7% more compared to the chromium level of the conventional process.

Scanning Electron Micrography

Scanning electron micrograph of lime treated and enzyme treated goatskin specimens showing their cross section at a magnification of 250x are presented in Figures 1a and 1b respectively. The micrographs confirm that the enzyme was able to bring out a conspicuous opening up of fiber structure, compared to control in the shortest possible time. Glycosaminoglycans are strongly anionic, un-branched long-chain polysaccharides containing aminated monosaccharide. The complex molecules formed by the attachment of glycosoaminoglycan chains to a protein core constitute the proteoglycans. Some of the significant constituents of connective tissue matrices of skin are proteoglycans. The carbohydrase treatment removes interfribilary materials relatively to a greater extent in a period of 30 minutes compared to 24 hrs of conventional lime treatment process.

Physical and Organoleptic Properties

The physical and organoleptic properties of the control and enzyme treated skin samples, after conversion into crust, are presented in Tables VI and VII respectively. It is apparent from the data that the strength properties of the enzyme treated sample are better than the control as was also inferred from the organoleptic assessments. Both the visual and feel test suggested that the crust leather made out of enzyme treated pelt was more filled with increased fluffiness. The texture of the leather was also relatively good in softness with smooth grain. These properties and the observation of SEM studies are in conformity to the physical testing data. Increased tensile strength and grain crack strength observed in enzyme treated sample were due to the enhanced chromium penetration and interaction that was facilitated by the enzyme mediated removal of inter-fibrillar component. The decreased percent elongation and grain distension (in case of enzyme processed sample compare to lime treated), which is inversely proportional to the tensile strength and grain crack, also

TABLE VI
Physical testing data of conventional and experimental skin.

Experiment	Tensile strength (Kg/cm^2)	% Elongation at break	Tear strength (Kg/cm)	Grain crack strength	
				Load (Kg)	Distension (mm)
Control	215.5±13.3	58.8±2.7	43±4.2	42±0.7	9.94±0.9
Enzyme	218±12.5	47.8±1.9	41.9±2.2	45.2±2.5	8.37±0.4

*Average value of 3 determinations

TABLE VII
Visual Assessment Data.*
(scale 1-10)

Properties	Enzyme Treated	Conventional
General Apperance	8±1	8±1
Grain smoothness	9±0.5	8±1
Feel	8±1	7±1
Area yield	8±1	7±1

*Assessment by experienced leather technologists

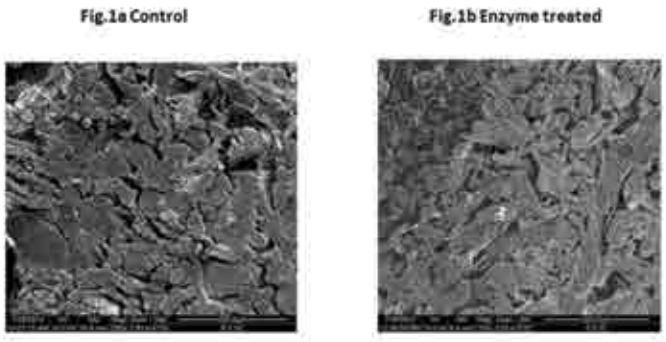

Figure 1. SEM image of enzyme treated goatskin specimens – cross section at a magnification of 250x.

confirm that the increased rigidity was contributed by increased chromium uptake as the result of loss of elasticity. In spite of all these, the enzyme treated specimen showed a small decrease in the tear strength - the force required to tear the leather sample across the given width, compare to that of the control. On the other hand a slight increase in this property observed in the case of conventionally fiber opened leather sample is attributed to the existence of fibers in the interlocked state particularly in the region below the grain. These interlocked fibers, which are densely present, would have contributed to the resistance exerted by the leather when it was subjected to tearing. Such interlocking pattern of fibers was not seen in the enzymatically fiber opened leather; instead the fibers were well stretched and are uniformly oriented. Because of this simple architecture, the enzyme treated samples would have displayed slightly lower tear strength. However, all these parameters may well accepted as they fulfill the standards and meet the functional requirements.

CONCLUSION

Enzymatic fiber opening process based on cocktail of carbohydrase enzymes has been attempted to facilitate rapid fiber opening in about thirty minutes. Use of lime in fiber opening, which requires a 48-fold increase in time, has been eliminated. The advantages of this enzymatic process include the elimination of lime, judicial usage of water and cutting short the length of the process time from 24 hr to 30 min. The direct benefits are: avoidance of chemical sludge formation and its disposal, reduced water and electricity consumption, quick preparation of substrate for tanning etc. This enzyme assisted fiber opening beam house process afforded crust leathers that are comparable to, or better than, those made by conventional liming method. Enhanced exhaustion and decreased discharge of chromium in the post fiber opening effluent is the added advantage. Since the enzyme is produced through solid-state fermentation technology, it is envisaged to be cost effective. The approach would provide improved productivity with enhanced product quality in a given process time through eco benign process.

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Appendix- I

The dehaired skins, after defleshing were de-limed with 1% (w/w) ammonium chloride with 100% (w/v) water for 45 min in a drum followed by treatment with bating enzyme for 30 minutes and washed. The skins were treated with 8% (w/w) salt and 80% (w/v) water for 10 min and 1% (v/w) sulfuric acid was added in 4 feeds at 10 min interval and tumbled in a drum for 60 min to obtain pickled skin at pH of 2.8. Thus obtained pickled skins were tanned using 8% (w/w) basic chromium sulphate (BCS) in 50% pickle water for 90 min. Then 50% (w/w) water was added and the drum was run further for 30 min. To the running drum, 1% (v/w) sodium formate (mixed with 10% w/v water) was added. After 30 min 1% (w/w) sodium bicarbonate (mixed with 10% w/v water) was added in 3 feeds at 10 min interval and continued the tumbling for 60 more minutes to bring the pH to 3.8.

Appendix-II

The wet blue leathers, obtained by the procedure under Appendix- I were shaved to 1.0mm thickness. Each of these samples was washed in 100% (w/v) water in a drum for 10 min. After draining the wash water, the wet blue leathers were treated with neutralizing syntan 1.0% (w/w) in the drum for 20 min with 100% water. Followed by this, sodium formate 0.5% (w/w) and 0.5% (w/w) sodium bicarbonate were added to the drum in 3 feeds at 10 min interval, while the drum was in running mode. After ensuring the pH of the liquor at 5.0, the leather samples were washed twice with 200% (w/v) water float for 10 min. The individual experimental samples were then given a fresh float of water (100%) followed by resin syntan (3% w/w) and allowed to run in the drum for 20min. After this, dying (2% w/w acid dye) for 30 min and fat liquoring (4% w/w synthetic fat liquor) for 30 min were carried out in the drum. Subsequent treatments using melamine based retaining syntan (8% w/w) and naphthalene based re-tanning syntan (8% w/w) for 40 min, followed by successive addition of fat liquor based on sulfochlorined hydrocarbons (4% w/w), polymeric fat liquor (3% w/w), and fat liquor based on synthetic and neutral oils (4% w/w) accompanied by drumming for 40 min. Finally, fixing was carried out using formic acid (2% v/w) and water (20% v/w) given in 3 feeds at 10 min interval and run for 30 min and piled overnight. The leather samples were set, conditioned, again set with reversible setting machine and hooked for drying. Thus dried leathers were staked and buffed using 400 grit emery paper.

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