Studies on the use of Bi-functional Enzyme for Leather Making

by
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Abstract

Preparation of skin or hide for tanning involves several unit processes/operations. This results in the generation of significant quantity of solid and liquid wastes, which is of major concern for the leather fraternity. However, several alternate technologies are available to address this issue. One of the well explored systems is application of enzymes in conventional leather processing. In the present research, application of bi-functional enzyme-fibrozyme (mixture of protease and amylase) for leather processing was studied. The present study relies upon the characteristic evaluation methods to ascertain the efficiency of enzymes in unhairing and fiber opening. Initially, various concentrations of enzymes were applied to cow hides by drum method. In this approach, 3.5% of fibrozyme is optimized for efficient removal of hair and proteoglycans. This is based on the organoleptic evaluation of enzyme treated pelts. The efficiency of enzyme was primarily evaluated through staining technique. Moreover, physical strength parameters were measured to assess the impact on fibers due to enzyme treatment. Morphological evaluation was carried out to confirm that there is no coalescent or distortion of fibers after enzyme treatment. Hydrothermal stability of experimental wet blue leather was found to be 108°C, which confirms better exhaustion and fixation of chromium. The study provides an avenue for integrated enzymatic dehairing and fiber opening using a single formulation of protease and amylase.

Introduction

Development of cleaner technologies for leather manufacture is one of the emerging fields of research to attain the eco-labelling.[1] It is a real challenge to attain paradigm shift in adopting modern technologies in traditional sector like leather.[2] There are several unit processes and operations involved to clean the matrix. Pretanning is carried out primarily to prepare the skins/hides for tanning. In conventional leather making, liming and reliming steps are very imperative as it is prerequisite to remove hair, flesh and to open up the fiber bundles for the diffusion of chemicals. Lime-sulfide process is extensively used in terms of hair removal due to its high efficiency. However, generation of pollution due to this process cause serious health hazard to the workers as well as the environment. Sulfide assisted process also leads to the degradation of hairs, which results in high COD in the effluent. Several alternate technologies have been designed to reduce the pollution load. Application of enzymes in leather processing is one such option, which aids in the reduction of the pollution load, increase the efficiency of the process thereby reducing the duration.[2-3] Protease, amylase and lipase are the three major enzymes that are widely used in bio-processing methods. Conventional reliming process takes a minimum of one day to few days depending on the type of end product. However, enzyme assisted fiber opening considerably reduce the duration and effectively scissors the proteoglycans.[4-5] Amylase helps in the fiber opening and effective removal of the adipose tissue from the skin/hide.

In the present study, a technical evaluation method has been formulated in order to evaluate the efficacy of the enzymes in leather processing. Staining technique is employed to understand the role of protease and amylase in leather making. Protease-amylase mixture is treated with cow hides at various concentrations and their organoleptic properties have been evaluated and the offer of enzyme is optimized. The present study provides a new dimensional approach in analytical methods to assess the leather properties.

Experimental

Materials

Wet salted cow hides were used as raw materials during this 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. Fibrozyme (formulated protease and amylase product having protease activity - 100 U/g, amylase activity - 1000 U/g) was supplied by Southern Petro Chemicals Industries Corporation (United Alacrity), Chennai. Bovine Serum Albumin (BSA), Mucin, Folin ciocelatu reagent, Periodic acid was procured from Sigma-Aldrich, India and other analytical chemicals were procured from SD Fine Chem Ltd., India.

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Quantification on the Release of Protein, Proteoglycan Processes

Five wet salted cow hides were taken and cut in to sides. The left halves were processed for enzymatic unhairing and fiber opening process as given in Table I and the right halves were used as control, where, lime sulphide method was employed for dehairing and conventional reliming using lime was carried out for fiber opening. The pH of the float was adjusted using sodium carbonate to 8.5-9.0 before addition of enzyme for effective unhairing treatment. The spent liquor collected after the unhairing and fiber opening from both control and experimental processes were filtered through Whattman filter paper (cellulose filters, 0.25 psi wet burst, thickness-180 µm, pore size-11 µm). The filtered samples were then estimated for the release of protein and proteoglycan.[6] Proteins are estimated as described by Bradford method,[7] where a standard graph was prepared using BSA and the amount of protein present in the sample sourced from the control and experimental processes was estimated.

For the estimation of proteoglycans, Schiff's assay method was carried out,[8] where the periodate oxidizable glycoconjucates can be estimated. Standard graph was prepared using mucin as working standard and the amount of proteoglycan present in the samples were estimated.

Staining Techniques

Haematoxylin and Eosin (H&E) staining is a routinely used technique in histopathology laboratories as it provides the pathologist/researcher a very detailed view of the tissue. H&E staining is considered to be a critical assay to study the tissue samples. This technique was carried out for staining the relimed pelt (control) and fibrozyme treated pelt, to understand the changes brought about in the fibers. From each sample the color images were acquired with a light microscope and digital camera running under image analysis program.[9]

Chrome Tanning

The enzyme treated pelts were washed thoroughly and pickled. The deliming and bating processes were eliminated for the enzyme based process as the pH of the enzyme treated pelts was around 8±1. In addition, no lime was also used during the fiber opening process. The pickled pelts were subsequently processed for conventional chrome tanning using 8% BCS as given in Table II. The spent liquor was collected to analyze the exhaustion of chromium, which is an indirect measure of fiber opening in the hide matrix. The control pelts were conventionally processed in to chrome tanned leather by carrying out deliming, bating and pickling.

Determination of Shrinkage Temperature

The shrinkage temperature, which is a measure of hydrothermal stability of leather, is determined using a Theis shrinkage tester.[10] A 2 cm^2 samples from control and experimental leathers was cut and clamped between the jaws of the clamp, and was immersed in a solution of water: glycerol mixture (3:1). The temperature of the solution was gradually increased and the solution was kept under stirring using a mechanical stirrer. The temperature at which the leather shrinks was noted and determined as the shrinkage temperature.

Post Tanning Process

Control and experimental leathers were shaved to a uniform thickness of 1.1±0.1 mm and converted in to an upper crust leather

Table I
Process recipe for fibrozyme enzyme application.

Process	Chemicals	%	Time (h)	Remarks
Soaking I	Water	300		
Soaking II	Water	300	2	Adjust the pH of soak liquor to 9-9.5 and left overnight
	Sodium carbonate	0.4		
	Biocide	0.1		
Enzyme treatment	Water	30	6	Adjust the bath pH to 9-9.5. No. of cycles – 6 (10' run and 50' stop and left overnight)
	Fibrozyme	2.5-4		
Washing	Wetting agent	0.2	10	Dry drumming
Washing	Water	100	10	
	Wetting agent	0.2		

by following the post tanning recipe provided in Table III. After post tanning operations, the leathers were piled overnight. Next day, the leathers were set, hooked to dry, staked and buffed.

Evaluation of Physical and Organoleptic Properties of Leathers

The samples for physical testing were obtained as per IULTCS methods.[11] The samples were conditioned at 80°F and 65% R.H. for 48 h.[12] Physical properties such as tensile strength, %elongation, tear strength and grain crack strength were determined as per standard procedures.[13,14] Each value reported is an average of four measurements. The crust leathers were further assessed for softness, grain smoothness, fullness and general appearance by tactile evaluation. Three experienced tanners rated the leathers on a scale of 0-10 points for each functional property.

Scanning Electron Microscopic (SEM) Analysis of Processed Leathers

Samples from control and experimental pelts and from the respective crust leathers were cut from the official sampling position.[11] Samples were first washed in water. Subsequently, the samples were dehydrated gradually using acetone and methanol as per standard procedures.[15] A Quanta 200 series scanning electron microscope was used for the analysis. The micrographs for the grain surface and cross section were obtained by operating the SEM at an accelerating voltage of 5 KV with different magnification levels.

Results and Discussion

Optimization of Enzyme Application

The amount of enzyme used for the experiment has been calculated based on the enzyme activity. Drum based deharing and fiber opening has been employed during the study. After ensuring thorough soaking, 2.5-4% of enzyme (w/w based on soaked weight) has been used for the experiment. The enzyme treatment has been carried out for 12 h with 10 min of intermittent running. The visual and organopleptic assessment of the dehaired and fiber opened pelts have been carried out and the results are depicted in Figure 1. The observations have been compared with that of the control pelts processed employing conventional liming and reliming processes. It could be observed from the figure that at concentrations less than 3.5%, complete hair removal has not been achieved. Hence a minimum of 3.5% of enzyme is required to achieve complete unhairing. During the process, no objectionable odor has been observed. Moreover, the fiber opening and grain smoothness have been observed to be better at higher concentration and has been observed to be better than that of the control pelts. Hence, based on assessment rating, 3.5% of fibrozyme is being optimized for further studies.

Table II
Process recipe for chrome tanning process.

Chemicals	% Offer	Time	Remarks
Pickle liquor	50		Check pH 2.8-3.0
Chrome tanning agent	8	2-3 h	Check penetration
Water	50	30 min	
Sodium formate	1	15 min	
Sodium bicarbonate	1-1.5	4x15 min + 1 h	Check pH 3.8-4.0
Water	50		Drain/washed Aged for 48 h

Proteoglycan Release After Enzyme Treatment

Opening up of fiber bundles is a crucial step in leather making. Ever since, the quote "Leathers are made in lime yard" always signifies the importance of opening up of fiber bundles to fibrillar level in order to enhance the uptake of chemicals added during tanning and post tanning processes. Conventional reliming process depends on the plumpness brought about due to the difference in concentration gradient inside and outside the pelts. However, in the case of enzyme assisted process, release of proteoglycans is considered to be the indirect measurement of fiber opening. As, in the latter case, no visual changes can be observed except for a clean pelt after removal of short hairs. The amount of proteins and proteoglycans released during the control and experimental trials are provided in Table IV. It could be observed that a significant quantity of protein and proteoglycans have been released from the skin, which is an

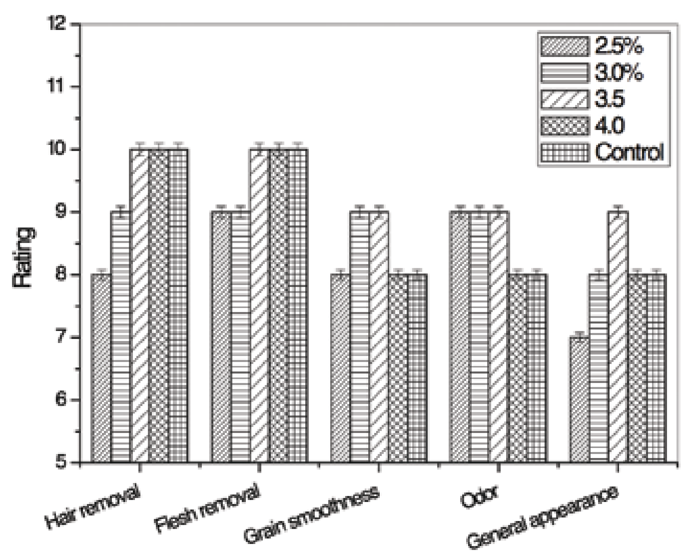

Figure 1. Assessment rating of fibrozyme treated and control pelts

Table III
Post-tanning recipe for the manufacture of upper leather from wet blue.

Process/chemicals	%	Duration (min)	Remarks
Washing			
Water	100	10	Drained
Neutralization			
Water	150		
Sodium formate	1.0	10	
Sodium bicarbonate	1.0	3x15+45	pH – 5.0 - 5.2, Drained.
Washing			
Water	200	15	Drained
Retanning, Dyeing and Fat liquoring			
Water	100		
Grain tightening acrylic syntan	4.0	30	
Semi-synthetic fatliquor	3.0	45	
Acid dye	3.0	30	
Synthetic fatliquor	4.0		
Phenol –naphthalene based syntan	5.0		Mixed in hot water
Melamine syntan	6.0	60	
Semi-synthetic fatliquor	4.0	30	
Wattle	4.0	30	
Formic acid	1.5	4x10+20	The dye exhaustion was checked. Drained.
Washing	100	15	Drained. Crust leathers were set twice, hooked to dry, conditioned, and staked.

indication that the enzyme has cleaved proteoglycans and aided in opening up of the fiber bundles. This is in accordance with the visual assessment made in the earlier section.

Histological Examination of the Control and Experimental Pelts

The pelts obtained after fiber opening by employing both conventional reliming and fibrozyme treatment have been subjected to histological examination by adopting Hematoxylin and Eosin (H&E) staining technique. By employing this technique, a clear picture about the opening up of the fiber bundles could be obtained. The H&E stained images of raw cow hide, conventionally relimed and enzyme treated cow pelts are shown in Figure 2 (a-c). Collagen fibers being acidophilic, reacts with the eosin dye and are stained pink. From the figure a well-organized collagen fiber could be seen in all three raw materials. Apart from this, it is also clearly observed that the level of separation/opening of the fibers varied distinctly with respect to the type of sample. Stained image of raw hide (2a) showed compact fiber orientation as compared to relimed (2b) and enzyme treated pelt (2c). Moreover, the splitting of fibers in case of the enzyme treated pelt was better, which is evident from the higher distance between the collagen fibers.

Stratigraphic Distribution of Chromium in Wet Blue Leathers

The layer wise distribution of chrome content in both control and experimental wet blue leathers was assessed to estimate the chrome penetration and distribution, which would in turn provide a substantiate evidence on the level of opening up of the fibers. Chrome content in grain, middle and flesh layer has been determined to be 3.59, 3.51 and 3.64% Cr_2O_3, respectively and the average chrome content in the leather has been found to be 3.62% Cr_2O_3. The uniform distribution of chrome content in the leather

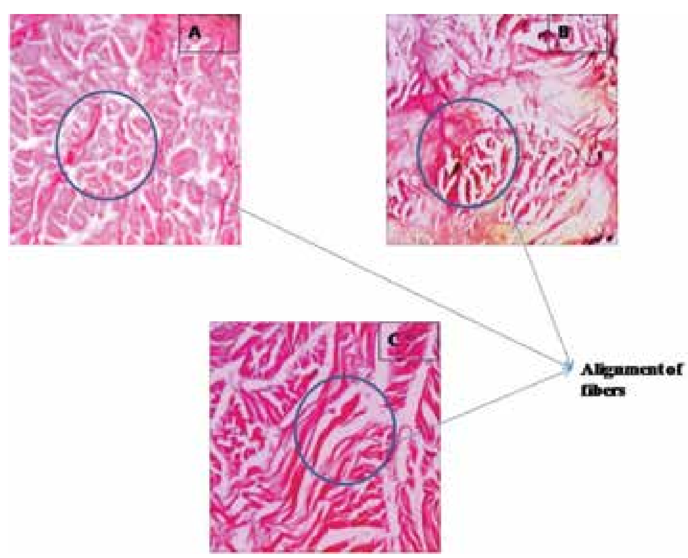

Figure 2. Photomicrographs of H&E stained raw (A), relimed (B) and fibrozyme (C) treated cow hides

confirms the through-through penetration. This is possible only when the uniform and efficient opening up of the fiber bundles have been achieved. Also, the chrome exhaustion has been found to be 75% with the shrinkage temperature of 108°C.

Visual Assessment of Wet Blue Leathers

The wet blue leathers obtained from control and experimental trials were visually assessed by experienced tanners. Various parameters such as color of the wet blue, chrome patches, grain smoothness and general appearances have been used for assessing the wet blue leathers. The results are provided in Table V. It could be observed from the table that the rating of the experimental wet blue is in the range of 8-9, which indicates that the fibrozyme based deharing and fiber opening has not deteriorated the quality of the final leather. Specifically, no grain damage has been observed in the case of enzyme treated (Experiment) wet blue leathers. The general assessment also indicates that the enzyme processed leathers are on par to that of conventionally chrome tanned leathers.

Physical Characteristics of Crust Leather

The strength characteristics like tensile, tear and grain crack strength of the crust leathers processed from control and experimental wet blue leathers have been analyzed. The tensile, tear and the grain crack strength of the experimental upper leather have been determined to be 270 Kg/cm^2, 50 Kg/cm, 43 Kg with distension of 9.28 mm, respectively. Similarly values of 265 Kg/cm^2, 40 Kg/cm, 32 Kg with distension of 9.00 mm, respectively has been obtained for control leathers. Hence, it could be inferred that the fibrozyme treatment has not affected the final leather quality, rather proper opening up of the fiber bundles has aided in obtaining higher strength characteristics.

Organoleptic Properties of Crust Leathers

The control and experimental crust leathers have been assessed by experienced tanners for their organoleptic properties.

Table IV
Release of protein and proteoglycans in the spent liquor.

Process	Protein (mg/g of raw wt)	Proteoglycans (mg/g of raw wt)
Enzyme Liquor	26.8±0.5	4.7±0.3
Wash liquor (10 min) I	9.2±0.3	1.8±0.2
Wash liquor (10 min) II	3.2±0.4	1.1±0.3

Table V
Visual assessment data of the wet blue leather.

Parameter	Control	Experimental
Color of the wet blue	8±1	8±1
Chrome patches	Nil	Nil
Grain smoothness	8±0.5	9±0.5
General appearances	8±0.5	8±0.5

*rating on a scale of 1–10 with 10 being the best

Table VI
Organoleptic property of cow upper leather.

Parameters	Control	Experimental
Softness	8±1	8±1
Roundness	8±1	9±0.5
Fullness	7±1	8±1
Grain tightness	8±1	8±1
Color uniformity	7±0.5	7±1
Strength	8±0.5	8±1
Grain smoothness	7±1	8±1
General appearance	7±1	7±2

*rating on a scale of 1–10 with 10 being the best

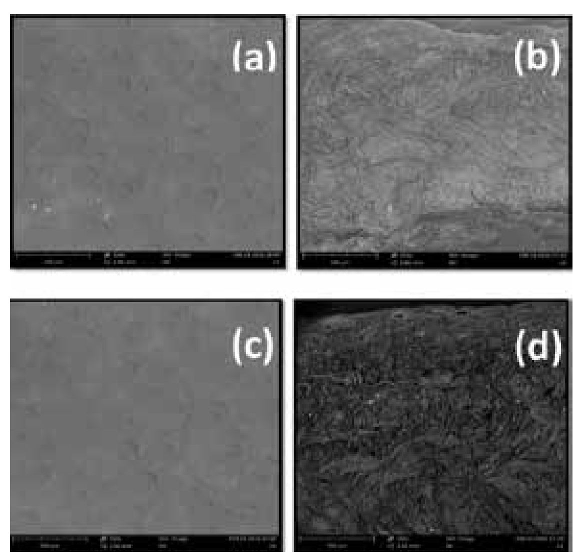

Figure 3. Scanning Electron Microscopy images of curst leathers of cow hide: a) Grain and b) Cross-section of fibrozyme treated leathers c) Grain and d) Cross-section of control leathers

The leathers have been assessed for various organoleptic properties such as softness, roundness, fullness, grain tightness, color uniformity, strength, grain smoothness and general appearance. The results are provided in Table VI. It could be observed from the table that the experimental leathers are on par with that of the control leathers in terms of grain tightness, grain smoothness, roundness, dye affinity and strength. In addition, it was also observed that there were no grain damage and the experimental leathers are softer than the control leathers.

Scanning Electron Microscopy Evaluation

Scanning electron microscopic (SEM) images of the control and experimental pelts are provided in Figure 3 (a-d). Figure 3a and c shows the grain pattern of the control and experimental pelts, respectively. It could be observed that the grain layer of both the pelts is devoid of any hair. Also, the grain is found to be smooth and does not show any damage. Fig. 3b and d shows the cross sectional view of the control and experimental pelts, respectively. It could be observed from the figure that the fiber bundles have better opened up structure. Hence, it could be inferred that the fibrozyme could be effectively used for both dehairing and fiber opening of cow hides in a single step.

Conclusions

In the present study, a single step enzymatic dehairing and fiber opening of cow hides has been established. Offer of enzyme has been optimized to 3.5% (w/w) based on the soaked weight of the cow hides. From the hand evaluation properties, the enzyme treated pelts have shown clean and complete removal of hairs and the skin is devoid of any scud or short hairs. The stratigraphic distributions of chromium in control and experimental wet blue leathers have been found to be uniform throughout the cross section of the leather. An average chrome content of 3.62% and shrinkage temperature of 108°C has been obtained for the experimental leathers. The visual and organoleptic assessment of the experimental leathers has been rated high by the experienced tanners. The physical strength met the upper leather norms. The morphological evaluation of experimental pelts through SEM also substantiates that there is no coalescence and distortion of fibers due to enzyme treatment. The H&E staining technique also confirmed the complete opening up of the fiber bundles in the experimental leathers. Thus this single step methodology could be successfully implemented in tanneries. This method not only saves time but also completely eliminates the use of lime and sulphide in leather processing.

Acknowledgement

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