Studies on the Use of Enzymes in Tanning Process: Part I. High Exhaust Vegetable Tanning

by

Swarna V. Kanth*, P. Ramesh Kannan, N. Usharani, G. C. Jayakumar, A. Yasothai, B. Chandrasekaran

Center For Human And Organizational Resources Development Central Leather Research Institute,

Council For Scientific And Industrial Research

Adyar, Chennai-600 020

Abstract

In the present investigation, an attempt was made to design an eco-friendly vegetable tanning process without pickling process by the application of enzymes to improve the exhaustion of vegetable tannins. Such an approach has resulted in more than 97% tannin exhaustion in the case of the experimental process, an increase of 12% compared with the conventional vegetable tanning process. The tanned leathers showed an improvement in hydrothermal stability, surface color values and there is no surface deposition of tannins in the experimental leathers, although vegetable tanning was carried out at higher pH. This was substantiated through scanning electron microscopic analysis that resulted in opened up, split compact fiber structure that was well coated by tannins, indicating that the enzyme assisted tanning process did not bring about any major change or destruction on the fiber structure of the leathers. The enzyme assisted tanning process is efficient in terms of improved quality of leather and also led to reduction in total solids (TS), chlorides and COD loads. The enzyme assisted tanning system presented here appears to be a viable option for combating pollution arising from the conventional vegetable tanning system.

Resumen

En la presente investigación, se hizo un intento para diseñar un curtido vegetal amigable ecológicamente, sin proceso de pickelado por la aplicación de enzimas para mejorar el agotamiento de los taninos vegetales. Este enfoque ha resultado en un agotamiento del tanino mayor al 97% en el caso del proceso experimental, un aumento del 12% en comparación con el proceso de curtido vegetal convencional. Los cueros curtidos mostraron una mejora en la estabilidad hidrotérmica, en los valores del color superficial y no presenta depósito de taninos en la superficie de los cueros experimentales, aunque el curtido vegetal se llevó a cabo a pH más altos. Esta afirmación fue sostenida a través de análisis de microscopio electrónico de barrido, que resultó en una estructura de fibra compacta y abierta que fue bien recubierta por los taninos, lo que indica que el proceso de curtido asistido por la enzima no supone ningún cambio importante o la destrucción de la estructura de la fibra de la piel. El proceso de curtido asistido por enzimas es eficiente en términos de mejora de la calidad del cuero y conduce a una reducción en los sólidos totales (ST), cloruros y cargas de DQO. El sistema de curtido asistido por enzimas presentado aquí parece ser una opción viable en la lucha contra la contaminación procedente de los sistemas de curtidovegetal convencionales.

*Corresponding author e-mail: chordclri@lycos.com; Tel. +91-44-24911386; Fax: +91-44-24416889

Manuscript received January 12, 2009, accepted for publication August 3, 2009

INTRODUCTION

Vegetable tanning agents are known for low biodegradability due to the presence of phenol content and color in the effluents.[1] At concentration of 15 mg/L and above, vegetable tannins are known to kill fish and methanogenic bacteria inhibit at concentrations ranging from 325 to 3000 mg/L.[1] They also prevent the conversion of urea to ammonia by reducing the activity of urease.[2] Commercial vegetable tannin extracts are often sulfated, concentrated, spray dried powders that contain more than 20% of non-tannins (gums, sugars, mineral salts, organic acids, insoluble matter), which also contribute to high effluent load. Processing technologies that involve rapid drum tannages were developed in recent times to accelerate the vegetable tanning process for the production of medium and light vegetable tanned leathers. Even though drum processes utilize less water than pit processes; they generate effluent contributing to an exorbitant biological oxygen demand (BOD), chemical oxygen demand (COD) and suspended solids due to the high concentration of tanning liquor used. As a result of poor biodegradability of tannins, the treated wastewater retains residual color, leading to constant criticism from the public. Further problem arises due to the phenol content of these effluents. The conventional vegetable tanning system exhibits exhaustion of about 85%. This means that 60,000 – 80,000 tons of vegetable tannins are let out in 10-15 x 10^5 m^3 of spent vegetable tanning liquor in effluents out of 3.5 to 4.0x10^5 tons of vegetable tannins that are being used across the world for leather processing. With stringent discharge norms of effluents in India (2100 mg/L) and world wide[3] it becomes necessary to exhaust the vegetable tannins used in tanning process. Various options like pickle-less vegetable tanning,[4] oxidative degradation of tannin liquors using hydrogen peroxide,[5,6] precipitation using zinc sulphate[7] and oxidative detoxification of bark extracts[8] towards the reduction of pollution load in effluents were reported. All these reported methods do not result in maximum exhaustion or near zero vegetable tannin emission loads. To overcome the problems associated with conventional vegetable tanning process, it is necessary to device suitable strategies for improving the exhaustion of vegetable tannins in the tanning process. Spent pickle liquor has high amount of dissolved solids content, as this partial pickling involves the use of 8-10% sodium chloride salt along with required quantity of sulfuric acid.[9,10] Dissolved solids contributed by salts are not amenable for treatment by the conventional effluent treatment methods. There exist various tertiary treatment methods for the treatment of dissolved solids like electrodialysis, reverse osmosis and thermal distillation. However, the techno-economic feasibility of these methods is low. Hence, there is need for better strategic practices leading to improved process alternatives to decrease the TDS and organic load in wastewater. The use of enzyme options in beam house process as well as post tanning processes were well explored[11-13] and there exists enormous scope for application of the same in tanning processes. In the present study, pickle-free enzyme assisted vegetable tanning using commercial vegetable tanning agent (Wattle GS powder, *(Acacia mollisima)*) at a pH of 7.2 was attempted with the twin objective of decreasing TDS and chlorides using pickle-less vegetable tanning system and enzyme assisted opening up of the fiber matrix with bacterial collagenase to enhance the diffusion of vegetable tannins for achieving better exhaustion of tannins.

EXPERIMENTAL

Reagents and Chemicals

All the chemicals used up to tanning process were of commercial grade and the chemicals used for the analysis of spent tan liquors are of analytical grade. Post tanning chemicals were procured from M/s BASF India Ltd. The bacterial collagenase enzyme used was obtained from BIOSCIENCE Chemicals, Chennai, India, which is of technical grade.

Experimental Trials

Conventionally dehaired and delimed goatskins of area 5 – 6 sq ft from a same lot of similar weight range and grade were selected for the study. Two leathers were taken for each trial – quantity of chemicals calculated on fleshed weight. The samples were processed into upper leathers as per the process described in Table I. The effect of collagenolytic enzyme pretreatment during tanning was studied, employing the bacterial collagenase in the process mentioned in Table I. Experimental trials 1 and trial 2 were carried out at different conditions of enzyme treatment and subsequent to the enzyme treatment, tanning process was followed as mentioned in the table.

Effect of Concentration of Collagenase (trial 1): Fifteen right halves of delimed pelts were equally divided and treated with bacterial collagenase as given in Table I at five different concentrations viz., 0.02, 0.04, 0.06, 0.08 and 0.1% at pH 7.2 at 35°C and the duration of treatment was 30 min.

Effect of Time (trial 2): Fifteen right halves of delimed pelts were equally divided and treated with 0.02% bacterial collagenase at varied running times viz., 15, 30, 45, 60 and 75 minutes, with pH and temperature remaining constant at 7.2 and at 35°C respectively.

Control Process: Thirty left halves of delimed pelts of trial 1 and trial 2 were subjected to tanning process as mentioned in Table I without any enzyme treatment.

The process liquors from all the experimental and control trials were analyzed for the exhaustion of vegetable tannins. All the leathers after vegetable tanning were washed and

piled for 24 h. The leathers were set, hooked to dry and stored at room temperature. Both experimental and control tanned leathers were post-tanned using conventional post-tanning process.

Comparison of Matched Pair of Control and Optimized Experimental Leathers

Matched pair comparison of control and experimental trial at optimized enzyme treatment was carried out using ten delimed Goat skins. The ten left halves of these delimed Goat skins were used for control process and ten right halves were processed using optimized enzyme process. The leathers after vegetable tanning were visually assessed for tannin patches, fullness, case hardening, grain smoothness, fullness and color of tanned leathers. Both experimental and control tanned leathers were converted into crust upper leathers using conventional post tanning and mechanical operations. The leathers were compared for color, fastness, strength and organoleptic properties and subjected to scanning electron microscope (SEM) analysis.

Analysis of Exhaustion of Vegetable Tanning Spent Liquors

Spent tan liquors from control, all pickle-less enzyme tanning experiments and matched pair processes spent tan liquors were analyzed for % uptake of vegetable tannins.[14] Wattle was used for preparation of standard graph. Known concentrations of wattle were prepared. The sample was neutralized to pH of 7 using 0.1N NaOH. From the known concentrations of the sample, 0.5ml was taken and 0.5 ml water was added and made up to 1ml. 5ml of solution A (Solution A: 1ml of (1% $CuSO_4$ and 2% Sodium Potassium Tartrate) in the ratio of 1:1 + 50ml of (1g NaOH in 150ml water and 5g Na_2CO_3 added and made up to 250ml) was added to the samples and allowed to stand for 10 minutes. Then 0.5 ml of Follins reagent (Folin-Ciocalteu reagent-Folin: water (1:1)) was added to the sample and allowed it to stand for 30 minutes and the absorbance was measured at 660 nm using *UV–visible spectrophotometer (Hitachi, Japan)*. The respective absorption value for the particular concentration was plotted. From this plot, the amount of tannins present in the waste liquor (after filtering) was analyzed with the same procedure mentioned above and the exhaustion was calculated as

$$\% \text{ Exhaustion} = \frac{(\text{Concentration of tannin given} - \text{Concentration of tannin in spent liquor}) \times 100}{\text{Concentration of tannin given}}$$

Measurement of Hydrothermal Stability

The shrinkage temperature, which is a measure of hydrothermal stability of leather, was measured using a Theis shrinkage meter.[15] The shrinkage temperature measurements were carried out for all leathers at tanned stage.

Measurement of Reflectance and Color Difference

The matched pair control and experimental leathers made in this study were subjected to the reflectance measurements using a Milton Roy Color mate HDS instrument. Color measurement (L, a, b, h and C) were recorded and the total color difference (ΔE) and hue difference (ΔH) were calculated as $\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$ and $\Delta H = \sqrt{\Delta E^2 - \Delta L^2 - \Delta C^2}$, where ΔL = Lightness difference; Δa and Δb = difference in a and b values, where 'a' represents the red and green axis and 'b' represents the yellow and blue axis; ΔC, chromaticity difference. $\Delta L < 0$ sample is darker, $\Delta L > 0$ sample is lighter, $\Delta a < 0$ sample is greener, $\Delta a > 0$ sample is redder, $\Delta b < 0$ sample is bluer, $\Delta b > 0$ sample is yellower, $\Delta c < 0$ sample is brighter/more saturated, $\Delta L > 0$ sample is duller/less saturated.

Objective Assessment of Softness through Compressibility Measurements

Softness of leathers can be numerically measured based on their compressibility.[16] Circular leather pieces (2 cm^2 area) from matched pair control and experimental leathers were obtained as per IUP method[17] and conditioned at 20±4°c and 65±2% R.H. over a period of 48 hrs. The samples were spread uniformly over the solid base of the compressibility and resilience (C & R) tester. The initial load acting on the grain surface was 100g. The thickness at this load was measured 60 sec after the load was applied. Subsequent loads were added and the change in thickness was recorded one minute after the addition of each load. Logarithm of leather thickness (Y axis) was plotted against logarithm of load (X axis).

Fastness to Wet and Dry Rub

The matched pair control and experimental samples were cut from the official sampling position.[17] Samples of appropriate size (5 x 14 cm) were cut from the samples and were tested according to IS 6191 – 1971 (LF: 10).[18] This method uses a SATRA Crock meter.

Fastness to Artificial Light of Leathers

The leather specimens from the official sampling position[17] were conditioned at 20±4°C and 65±2% relative humidity for 48 hours. The resistance of the color of the optimized experimental and control leathers to an artificial light source, Xenon lamp was measured using IS 6191-1971 (LF: 4) method.[19] One side of the leather was exposed to light from Xenon arc under prescribed conditions for 20 hrs, along with eight dyed Blue wool standards having increasing levels of fastness. Black panel temperature was maintained at 63±1°C and the relative humidity was 30±5%. Fastness was assessed by comparing the fading of crust leathers with that of the standards, from standard 1 (very low light fastness) to standard 8 (very high light fastness), where each standard being approximately twice as fast as that preceding one. Rating was given on a scale of 1-8 points, where higher

points indicate better fastness. The same methodology was repeated for control and matched pair experimental leathers aged for 6 months.

Physical Testing and Hand Evaluation

Samples for various physical tests from matched pair control and experimental crust leathers were obtained as per IUP method.[17] Specimens were conditioned at 20±4°C and 65±2% relative humidity 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.[20-22] Matched pair experimental and control crust leathers were assessed for softness, fullness, grain flatness, grain smoothness, grain tightness (break) and general appearance by hand and visual examination. The leathers were rated (on a scale of 1 – 10 where 1 is poorest and 10 is the best) for each functional property by experienced tanners.

Analysis of Spent Liquors

Spent tan liquors from matched pair experimental and control tanning experiments were collected and analyzed for COD, chlorides and TS (dried at 103-105°C for 1 hr) as per the standard procedures.[14]

Scanning Electron Microscopic Analysis

Samples from matched pair experimental and control leathers were cut from the official sampling position.[17] The samples were first washed in water. Subsequently, the samples were gradually dehydrated using standard procedure.[23] All specimens were then coated with gold using an Edwards E306 sputter coater. Leica Cambridge Streoscan 440 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 20 kV with different magnification levels.

Results And Discussion

In this study, an attempt was made to improve the exhaustion of vegetable tannins with the use of bacterial collagenase. The approach is based on the concept that the enzymes act as biocatalysts in opening up the fibrous collagen network, thereby enhancing the diffusion of vegetable tannins into the leather matrix. The contact surface areas in the leather exposed for interaction with vegetable tannins also increases. Improved exhaustion of vegetable tannins will not only reduce the pollution, but also result in quality leathers with reduced amount of chemicals used, thereby reducing the cost of production.

Enzyme Application for vegetable Tannins Exhaustion: Optimization of Process Parameters

In order to attain improved exhaustion and distribution of tannins into the leather, conditions on the usage of enzymes has to be optimized. In the process, as mentioned in Table I, 20% concentration of vegetable tannins was chosen for obtaining good quality leather. Experiments were conducted to optimize the important parameters of enzyme treatment, viz., the percentage enzyme concentration and the duration of enzyme treatment. The other parameters such as temperature and pH were kept constant at 35ºC and 7.2 respectively as the bacterial collagenase used for the study has optimum activity under these conditions.

Optimization of Concentration

The exhaustion of vegetable tannins and shrinkage temperature of the tanned leather at different concentrations of bacterial collagenase treatment are given in Table II. From the table, it is seen that the uptake of vegetable tannins by the pelt increases with increasing concentration of bacterial collagenase up to 0.02% concentration of enzyme offered. There was no significant increase in the shrinkage temperature and fixation of the vegetable tannins above 0.02% concentration. Hence, 0.02% percentage concentration appears to be sufficient for maximum uptake of vegetable tannins and was taken as the optimized concentration for better exhaustion of vegetable tannins. The shrinkage temperature of the leathers was found to 86°C and exhaustion of vegetable tannins at this concentration is found to be 98%.

Optimization of Duration of Treatment of Enzymes

The fixation of vegetable tannins to the leather in terms of percentage exhaustion and shrinkage temperature for various treatment durations are given in Table II. It is evident from the table that the uptake of vegetable tannins increases gradually with time. It requires minimum of 30 minutes to bring about significant exhaustion in tanning bath. At time intervals beyond 30 minutes, there was slight increase in exhaustion of vegetable tannins, however longer time of exposure may result in damaging the collagen pelt that results in decrease in strength of the matrix due to high fiber splitting. Hence, 30 minutes of enzyme treatment was taken as the optimum duration. The treatment of acid protease for time period of 30 minutes resulted in vegetable tannins uptake of 98%. Hence pH 7.2 at 0.020% concentration of bacterial collagenase for 30 minutes was taken as optimized conditions for better exhaustion of vegetable tannins.

Mechanism of Pickle-less Enzyme Assisted Vegetable Tanning of Leathers

The approach of developing pickle-less enzyme assisted vegetable tanned leathers is based on the concept that the enzyme helps in opening up of the fibrous collagen network, thereby enhancing the diffusion of vegetable tannins into the

TABLE I

Control and Experimental Vegetable Tanning Process

Process	%	Chemicals	Duration (min)	Remarks
Material: Delimed Goatskins from a similar lot				
Control Tanning Processes				
Control 1	100	Water		
	5	Sodium Chloride	30	pH 4.5 – 4.7
	0.75	Sulphuric acid	3X15 + 30	
	1	Basyntan P	60	
	10	Wattle	60	
	10	Wattle	180	Check penetration
Ascertain complete penetration of chemicals				
	0.1	Formic acid	45	pH 3.8 – 4.0
Experimental Tanning Processes				
Experiment	100	Water		pH 7.2
Enzyme Treatment (Experimental trial groups) Trial 1 – Treatment at varying concentration (0.01, 0.02, 0.03, 0.04 and 0.05 %) Trial 2 – Treatment at varying running time (15', 30', 45', 60', and 75') **Optimized enzyme treatment at 0.020% enzyme, pH 7 for 30 min** Drain liquor				
	50	Water		
	0.1	Oxalic acid	10	
	0.1	Non swelling acid	10	
	1	Basyntan P	60	
	10	Wattle	60	
	10	Wattle	180	Check penetration
	0.25	Formic acid	2X15 + 15	pH 3.8-4.0
All control and experimental leathers were sammed, split and shaved to uniform thickness (1.0-1.1 mm)				

leather matrix; also the contact surface areas in the enzyme treated collagen available for interaction with vegetable tannins increases. The primary objective of this work is to open up the collagen matrix by enzyme treatment for improved exhaustion of vegetable tannins and the secondary objective is to decrease TDS and chlorides using pickle-less vegetable tanning by avoiding the use of salt in the pickling process. This will result in better penetration of vegetable tannins as the collagen matrix will also be well opened up by the enzyme treatment. The approach of this study is based on the fact that the vegetable tannins can penetrate rapidly at higher pH. It was reported from the fixation profile of vegetable tannins at various pH values that penetration is facilitated at higher pH, while fixation is promoted at lower pH and maximum fixation of vegetable tannins on collagen occurs at pH around 3.5.[24] Since the pH of the aqueous solution of vegetable tannin is around 4.0 - 4.2, the penetration of vegetable tannins is facilitated at this pH.[25] Vegetable tannins result in surface fixation or case hardening if used directly after deliming.[25] On the application of vegetable tannins on delimed pelt after enzyme treatment in fresh float of water containing oxalic and organic non swelling acid, maximum penetration of vegetable tannins can be achieved by three factors: high concentration, pH and opened up fibre matrix. In addition, the enzyme containing float is drained and a fresh float at pH 4.5 – 5.0 is maintained to ensure the enzyme activity is reduced that can result in damaging the pelt at pH 7.2. Hence, the pH of the pelts were reduced to pH

TABLE II

Exhaustion of Vegetable Tannins, Shrinkage Temperature and Time Taken for Penetration of Tannins at Varied Concentration and Duration of Enzymes

Process Parameters		Exhaustion of Vegetable Tannins (%)	Shrinkage Temperature Ts (°C)	Time Taken for Penetration of Vegetable Tannins (Hrs)
Control		85.2±2.14	82±2	5.5
Experimental – concentration (30 min)	0.01%	90.5±1.68	82±2	4.2
	0.02%*	98.1±1.91*	86±1*	3.2*
	0.03%	98.3±0.51	86±1	3.0
	0.04%	98.6±1.88	86±1	2.7
	0.05%	99.6±1.41	86±1	2.3
Experimental – Duration (0.02%)	15	90.2±0.88	82±1	4.5
	30*	98.2±1.84*	86±2*	3.2*
	45	98.8±1.45	86±2	2.8
	60	99.2±1.82	86±2	2.6
Optimized experimental conditions (0.020% enzyme offer and 30 min)		97.9±0.88	86±2	4.2

* Optimized experimental conditions

TABLE III

Measurement of Color Difference of Control, Experimental and Optimized Matched Pair Leathers

Process Parameters		Δ_L	Δ_C	Δ_H	Δ_a	Δ_b	Δ_E
Experimental Concentration	0.01%	d=-2.798	S=0.053	I=2.047	G=-0.902	B=-1.712	5.927
	0.02%#	d=-5.129	S=0.119	D=-3.119	MR=-1.123	MB=-1.697	8.886
	0.03%	d=-5.145	S=0.142	D=-3.128	MR=-1.155	MB=-1.705	8.899
	0.04%	d=-6.387	S=-0.250	D=-0.201	MR=0.409	MY=0.162	10.013
	0.05%	d=-6.999	S=-0.298	D=-0.292	MR=0.488	MY=0.315	13.018
Experimental Time	15’	l=8.698	S=-0.081	D=-0.398	MR=-0.892	B=-0.426	5.848
	30’@	d=-5.179	S=-0.409	D=0.417	MR=0.592	MB=-1.122	8.689
	45’	d=-6.421	S=-0.260	D=-0.211	MR=0.426	MY=0.158	9.789
	60’	d=-6.862	S=-0.281	D=-0.242	MR=0.429	MY=0.209	12.818
Optimized Experimental Conditions	0.020% 30’	d=-5.428	S=-0.268	D=-2.286	MR=0.126	MB=-1.158	9.126

#,@ Optimized experimental conditions

4.5-5.0 using very low concentration of organic non swelling acid and oxalic acid as well as the pH of vegetable tannin liquor itself will make the pH of the residual float in the drum to be 4.5-5.0. Under these conditions the inner cross-section of the pelt cannot have pH less than 4.5 or 5.0. This prevents the surface fixation on the skins and aids proper penetration of vegetable tannins as the collagen matrix is well opened up. Finally, after completing the tanning process, the pH is adjusted to 3.5 - 3.7 using minimum amount of formic acid to fix the penetrated vegetable tannins. An interesting observation was also made regarding the duration of tanning. The time taken for penetration and completion of tanning is comparatively lower than the time taken for conventional vegetable tanning as given in Table II. This is due to the fact that the diffusion of vegetable tannins is faster in enzyme assisted process as the fiber matrix in the pelt is well opened up and the vegetable tannin particulates with higher molecular weight[26] can penetrate rapidly after enzyme treatment.

Performance of Leathers: Bulk Properties of Leathers – Hand Evaluation of Leathers

It is known that the results of hand and visual evaluation method are not objective but subjective, that varies from person to person. Yet it could be taken as reliable, if carried out by experienced persons and averaged. The hand and visual evaluations were carried out for both tanned and crust leathers. It is seen that the experimental leathers have good bulk properties, which are better in comparison to that of control leather. Vegetable tannin patches and case hardening were not observed for both control and experimental leathers. Fullness and color of tanned leathers is good for experimental leathers with grain smoothness slightly better for control leathers. Crust leather from both control and experimental processes was evaluated for various bulk properties by hand and visual evaluation. The experimental leathers exhibit better fullness compared to control leathers. This is primarily due to improved penetration and fixation of vegetable tannins in the experimental process, compared to control process. Other properties such as softness, grain tightness and smoothness are comparable to that of conventionally processed leathers. Uniformity of color and overall appearance of experimental leathers is better than the control leathers.

Effect of Pickle-less Enzyme Treatment on Surface Color

The reflectance measurement at visible wavelength for control and matched pair experimental crust leathers was measured and the absorbance maxima were 450 nm. Absorbance maximum is the wavelength at which, the reflectance is minimum. Since the absorbance maxima are similar for both control and matched pair experimental leathers, it can be concluded that there is no major significant variation in the color or shade between control and matched pair experimental leathers. The color difference values with respect to ΔL, Δa, Δb, ΔC, ΔH and ΔE of all the experimental leathers, control and matched pair experimental leathers are given in Table III. It is observed that the matched pair experimental samples show a total color difference (ΔE) of 9.126 compared to control leather, which means the overall color difference of the enzyme assisted leathers, is higher, significant and visible than control leathers. From the ΔL and ΔC values shown in the Table III, it was observed that there was an increase in the darkness value (ΔL) and color intensity value (ΔC) for control and enzyme assisted leathers. The increase in color values is also proportionate to the uptake of vegetable tannins in leathers as observed in Table III. Enzyme assisted vegetable tanned leathers exhibited maximum increase in color intensity and darkness values. With enhanced fiber opening and splitting, increase in vegetable tannin content of leathers, dye uptake and other post tannin chemicals facilitates better darkness values and color intensity. However, there was not much of hue difference in the enzyme treated leathers as compared to control. These results are similar to that observed in the visual assessment.

Effect of Pickle-less Enzyme Treatment on Strength Properties

It is essential to analyze the strength characteristics upon treatment with enzymes as one may expect, opening up of fiber structure influencing the strength of the leather. Tensile, tear strength and grain crack tests were carried out for the matched pair control and matched pair experimental crust leathers both along and across the backbone line. The mean values corresponding to along and across of backbone was calculated. The average values are given in Table IV. The values of various strength properties of matched pair experimental leathers are found to be comparable to that of the control leathers. It was observed that the strength characteristics of the experimental leathers are not appreciably affected due to enzyme treatment.

Effect of Pickle-less Enzyme Treatment on Light Fastness

Vegetable tannins are well known to reduce the light fastness characteristics of leathers. Hence fastness to light and rubbing of both control and experimental crust leathers under artificial light (Xenon lamp) was studied and is given in Table IV. The leathers from both control and matched pair experimental processes exhibit similar values. The effect of ageing of crust leathers for six months on the fastness properties was also studied and the values are given in Table IV. It is observed that both control and experimental samples do not show significant change in the fastness properties upon ageing and are equivalent to the Blue wool standards 4.5 (given in parenthesis in Table IV).

TABLE IV

Physical Testing, Fastness to Light and Rubbing of Control (C) and Matched Pair Experimental (E) Leathers

Sample	Tensile Strength	Extension at Break	Tear Strength	Grain Crack Resistance		Fastness (Ageing)		
	(Kg/cm^2)	%	(Kg/cm)	Load (Kg)	Distension (mm)	Wet Rub	Dry Rub	Light[a]
C	223±12	54±6	36±6	32±3	11.8±0.6	4.0-4.5	4.5-5.0	3.0-3.5 (4.0-4.5)
E	216±9	52±4	34±5	34±4	12.4±0.8	4.5-5.0	4.5-5.0	3.0-3.5 (4.5 5.0)

[a] Value in parenthesis indicates the corresponding blue wool standard

TABLE V

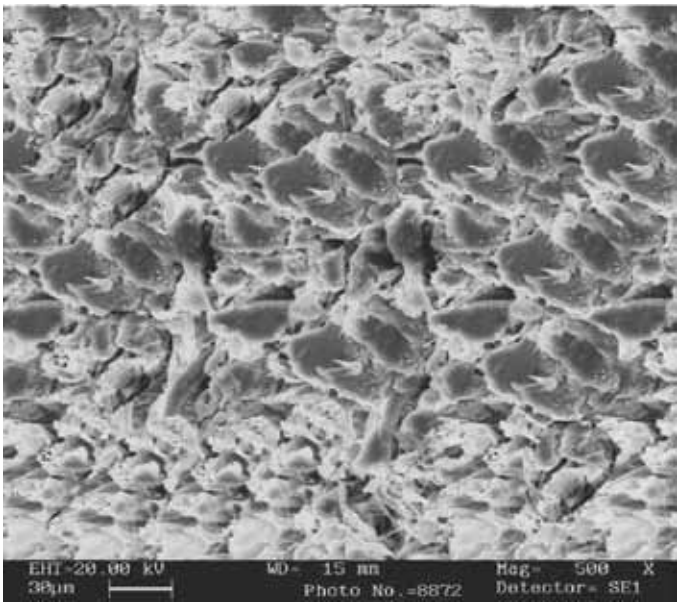
Environmental Impact of Control and Matched Pair Experimental Processes

Parameters	Control[a]	Experimental[b]
% Uptake of vegetable tannins	84.4±3.08	97.9±0.88
Volume of effluent (L/metric ton of raw skins)	608	714
COD	20146±340	9525±190
TS	85788±680	12586±310
Chlorides	25120±60	540±40
Emission load (Kg/metric ton of raw skins)		
COD	12.2	6.8
TS	52.2	8.9
Chlorides	15.3	0.38
Cost Estimate – Chemicals/Bio-products (US$/t of Raw skins)	221.45	223.61

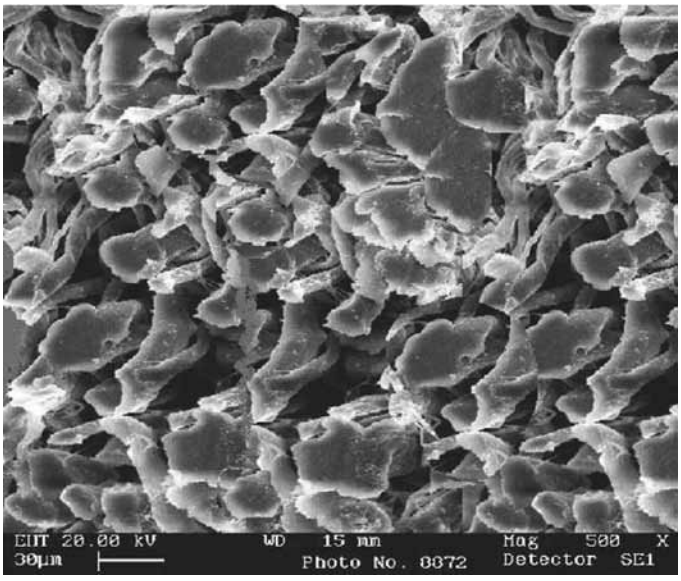
[a] Composite of spent pickle and tan liquor
[b] Spent tan liquor alone considered as composite

Effect of Enzyme on the Structure of Leather Matrix: Assessment of Opening up of Fibre Bundles: Implicit approach

Scanning electron micrograph analysis was performed to investigate fiber structure of the tanned leathers as the enzyme treatment was carried out at pH 7.2 on delimed pelts as against conventional vegetable tanning in salt at pH 4.5. The scanning electron micrographs of the cross section of tanned samples from control and experimental tanning processes at a magnification of 500X are given in Figure 1a and Figure 1b, respectively. Both the control and experimental leathers show compact fiber structure, which is well coated that is characteristic of vegetable tanned leather. It is clear from the photomicrograph of the experimental leather, enzyme assisted process has opened up the fiber matrix and also well coated by vegetable tannins. There is also no major change or destruction of the fiber structure in the enzyme assisted processed leather.



(a)



(b)

Figure 1. – Scanning electron micrograph of vegetable tanned leather sample showing the cross section of (a) control at X500 magnification and (b) matched pair experiment at X600 magnification.

Effect of Pickle-less Enzyme Treatment on Softness

The enzyme assisted vegetable tanning system employs vegetable tannins at an opened up fiber structure, which resulted in uptake of vegetable tannins and results in better quality leathers. Vegetable tannins are known to produce hard leathers and generally employed for producing heavy duty leathers. Hence, it is important to evaluate the extent of softness on the final leather. Quantitative assessment of softness for both control and matched pair experimental leathers was made through compressibility measurements.

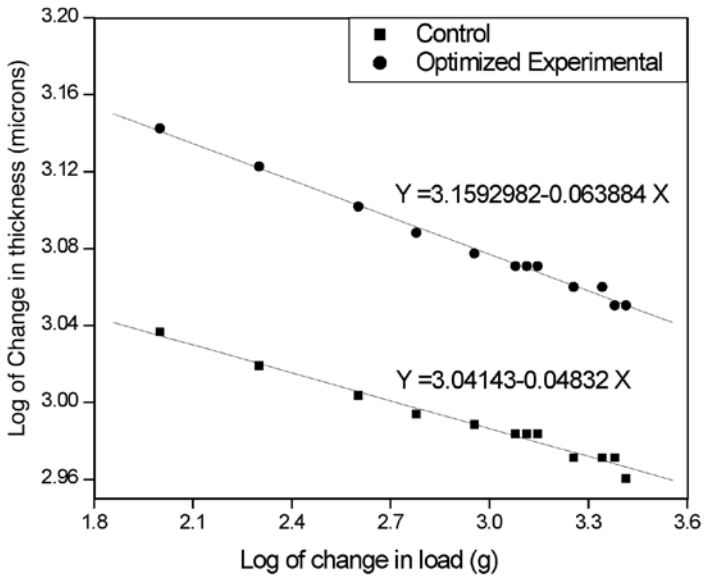


Figure 2. – Plot of log of change in load vs log of change in thickness for control and matched pair experimental Leathers.

The plot of logarithm of thickness vs. logarithm of load for the control and matched pair experimental leathers exhibited linear fit[16] as shown in Figure 2. The corresponding equation of the line was obtained. Based on the equation negative slope angles (compressibility index, CI) were calculated and the values are 8.89° and 8.57° for the control and matched pair experimental leathers. Higher values signify more softness in the leather. It is evident that the experimental leather exhibit comparable negative slope (CI) angle with that of control leather. This shows that the experimental leathers are comparable but slightly of lower softness to that of the control leathers.

Environmental Tolerability, Financial and Technical Practicality on Enzyme Treatment

The spent vegetable tan liquor contains highly non biodegradable matter and it contributes to exorbitantly high COD, dissolved and suspended solids. Composite liquor from the conventional process was prepared by mixing spent liquors from pickling and vegetable tanning processes in control. It should be noted that experimental vegetable tanning process does not have pickling process and hence spent vegetable tan liquor alone was considered as composite, which contained enzymes in the spent vegetable tan liquor. COD, TS, chlorides and % up take of vegetable tannin are the parameters that were chosen for analyzing the environmental impact. The emission loads of control and enzyme assisted spent tan liquor was calculated by multiplying COD/TS values (mg/lit) with volume of effluent (lit) per metric ton of raw skins processed. It is seen from Table V, that COD, chlorides and TS values of matched pair experimental spent tan liquor are lower than that of control. It is evident that the enzyme assisted vegetable tanning method reduces the COD, TS and chloride loads by 44, 83

and 98%, respectively. The reduction in COD, chlorides and TS loads helps in achieving cleaner vegetable tanning. Especially, the reduction of chlorides by 98% is a significant achievement in avoiding pollution due to chlorides. Implementation of pickle-less enzyme assisted vegetable tanning method could bring considerable change in the tanning industry making the experimental leather process to have the benefit of zero vegetable tannin emission in the composite liquor. Development of any new process requires commercial feasibility and cost effectiveness. The experimental process developed in this work primarily involved the use of biological materials like enzymes in order to achieve lower TDS, chlorides and COD of effluent as well as better quality leather. The total chemical costs for processing 1 ton of goatskins through conventional and experimental process schemes are given in Table V. The total cost for processing 1 ton of goatskins through conventional vegetable tanning process is about US$ 221.45, assuming partial pickling and 20% wattle GS powder, whereas experimental pickle-less enzyme assisted vegetable tanning process is about US $223.61. Hence, the possible increase in chemical cost is about US $2.16**,** for processing 1 t of raw goatskins. The reduction in discharge of effluent, BOD, COD, TDS, chloride and TS loads would provide additional benefit in effluent treatment costs. Apart from this, the disposal of sludge and waste generated through control-based process causes both ecological and economic concerns. Similarly, the formation of dry sludge can also be avoided. The combination of bio-products (enzymes) in tanning with natural tanning material like vegetable tannins leads to an achievable eco-option to the conventional intricate leather processing. Such eco-option has potential in both developed as well as developing countries with greatly reduced pollution loads from tanning operations without affecting the leather qualities.

Conclusions

Enzymes were used in tanning as an eco-friendly approach for achieving better exhaustion of vegetable tannins. The approach is based on the concept that the enzymes act as biocatalysts in opening up the fibrous leather network, which enhance the diffusion of vegetable tannins into the leather matrix and also the contact surface area in the leather exposed for interaction with vegetable tannins increases. 0.20% concentration of enzyme, at pH of 7.2 for 30 minutes with 20% vegetable tannins was found to be optimum with respect to the uptake of vegetable tannins and achieving higher hydrothermal stability with reduced processing time. The enzyme treatment resulted in leathers with fullness and no major significant variation in the properties as compared to the conventional vegetable tanned leathers. The scanning electron micrographs of enzyme assisted vegetable tanned leathers exhibited better opening up of fiber bundles as well as separation of fibers. The main advantages of the current approach is the unprecedented environmental benefits achieved with reduction in TS, chloride and COD loads by 83, 98 and 44 % as compared to control process, as well as achieving better uptake of vegetable tannin and quality improvement in finished leathers. The experiment tanning process has the cost advantage due to net saving from the reduced effluent treatment cost. In general, this approach provides an abundant scope for decreasing the pollution load. Analyzing the various results from the experiments, an outlook at the use of enzymes in tanning process is indispensable for vegetable tanning.

References

1. Field, J.A. and Lettinga, G.; *Biodegradation of tannins, metal ions in biological systems: Degradation of environmental pollutants by microorganisms and their metalloenzymes*, New York: Marcel Dekker Inc. 1992.
2. Sivapalan, K., Fernando, V. and Thenabadu, M.W.; *Soil and Biology Biochem.* **17,** 547–551, 1985.
3. Buljan, J.; *World Leather* **11,** 65–68, 1996.
4. Saravanabhavan, S., Thanikaivelan, P., Rao, J.R. and Nair, B.U.; *JALCA* **99,** 281–289, 2004.
5. Ali, S. J., Rao, J. R. and Nair, B.U.; *Green Chemistry* **6**, 298–302, 2000.
6. Sekaran, G., Chitra, K. and Madhavakrishna, W.J.; *JALCA* **83,** 5–16, 2004.
7. Sharli, A., Madhan, B., Rao, J.R. and Nair, B.U.; *JALCA* **98**, 381–389, 2003.
8. Field, J. A., Lettinga, G.J. and Habets, L. H. A.; *Chem. Tech. Biotech.* **49,** 35–43, 1990.
9. Thanikaivelan, P., Rao, J.R. and Nair, B.U.; *JSLTC* **84**, 276-284, 2000.
10. Aloy, M., Folachier, A. and Vulliermet, B.; *Tannery and Pollution, Centre Technique Du Cuir*, Lyon, France 1976.
11. Thanikaivelan, P., Rao, J.R., Nair, B.U. and Ramasami, T.; *Critical Rev. Environ. Sci. Tec.,* **35,** 37–79, 2005.
12. Kanth, S.V., Venba, R.**,** Madhan, B., Malathy, J., Chitra, P., Yasothai, A., Chandrababu, N.K. and Sadulla, S.; *JALCA* **101**, 435-443, 2006.
13. Kanth, S.V., Venba, R., Madhan, B., Chandrababu, N.K. and Sadulla, S.; *Dyes and Pigments* **76,** 338-347, 2008.
14. Clesceri, L.S., Greenberg, A.E. and Trussel, R.R.; *Standard methods for the examination of water and wastewater*. Washington, DC: American Public Health Association, 1989.
15. McLaughlin, G. D., and Theis, E.R.; *The chemistry of leather manufacture*. New York: Reinhold, 1945.
16. Lokanadam, B., Subramaniam, V. and Nayar, R.C. ; *JSLTC* **73,** 115–124, 1989.
17. IUP 2: Sampling; *JSLTC* **84**, 303-309, 2000.

18. Determination of color fastness to rubbing (Croaking test); *Indian Standards* IS 6191 (LF: 10), 1971.
19. S 6191, LF: 4, Determination of fastness to artificial light - Xenon lamp of colored leather; *Indian Standards*, 1971.
20. IUP 6: Measurement of tensile strength and percentage elongation; *JSLTC* **84,** 317, 2000.
21. IUP 8. Measurement of tear load - Double edge tear; *JSLTC* **84,** 327–332, 2000.
22. IUP 9: Measurement of distension and strength of grain by the ball burst test; *JSLTC* 1996.
23. Echlin, P. and Heywood, V.H; *Scanning electron microscopy. London: Academic- Press,* 1971.
24. Gustavson, K.H; *The Chemistry of Tanning Processes, New York: Academic Press Inc,* 1956.
25. Covington, A.D. and Shi, B.; *JSLTC* **82**, 64-72, 1998.
26. Hagerman, A. E., Rice, M.E. and Ritchard, N.T.; *J Agri. Food Chem*. **46,** 2490–2497, 1998.