Novel Approach towards High Exhaust Chromium Tanning – Part I: Role of Enzymes in the Tanning Process

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Abstract

Stringent environmental regulations on the discharge of pollutants from various industries have prompted researchers to seek the development of eco-benign technologies and process innovations aimed at low-waste and high exhaust chrome tanning systems. However, an effective biotechnological solution for reducing chromium pollution that is cost effective, commercially adaptable high exhaustion chrome tanning process has not been explored. In the present investigation, an attempt has been made to bring about enzyme assisted chrome tanning process using acid protease. The effect of varying conditions of enzyme treatment on the exhaustion of the chrome tanning agent have been studied and the conditions optimized. Three different control chrome tan ning systems have been chosen with and without float in the tanning process. In order to have a comparative study, chrome tanning at an offer of 6% and 8% basic chromium sulphate (BCS) in experimental processes has also been performed. It has been observed that the penetration and distribution of chromium is much better for enzyme assisted chrome tanning process at pH 4.5 compared to other systems. There is no surface deposition of chromium in the experimental leathers, although chrome tanning has been carried out at higher pH of 4.5. This has been substantiated through scanning electron microscopic analysis. Chromium exhaustion greater than 95% has been achieved in optimized enzyme assisted chrome tanning systems. The amount of chromium present in these leathers has been relatively higher compared to conventional chrome tanned leathers. The performance characteristics assessed by physical and tactile evaluation of the enzyme assisted leathers has been on par with conventional leathers.

Resumen

Estrictas regulaciones ambientales sobre el vertido de contaminantes de diversas industrias han llevado a los investigadores a buscar el desarrollo de tecnologías ecológicas benignas y procesos innovadores destinados a obtener menores residuos y sistemas de curtido al cromo de alto agotamiento. Sin embargo, una solución biotecnológica eficaz para reducir la contaminación de cromo que sea rentable, comercialmente adaptable del proceso de alto agotamiento del curtido al cromo no ha sido explorado. En la presente investigación, se ha intentado asistir al proceso de curtido al cromo mediante una enzima, la proteasa ácida. El efecto en diferentes condiciones de tratamiento enzimático sobre el agotamiento del curtido al cromo se han estudiado y optimizado. Tres diferentes controles de los sistemas de curtido al cromo han sido elegidos con y sin flota en el proceso de curtido. A fin de contar con un estudio comparativo, curtido al cromo con una oferta de 6% y 8% de sulfato básico de cromo(BCS) en los procesos experimentales también se han realizado. Se ha observado que la penetración y la distribución de cromo es mucho mejor en los procesos de curtido al cromo asistidos por enzimas a pH 4,5 en comparación con otros sistemas. No hay deposición de cromo sobre la superficie de los cueros experimentales, a pesar que el curtido al cromo ha alcanzado valores de pH superiores a 4,5. Esto ha sido analizado mediante análisis microscópico de barrido electrónico. Un agotamiento superior a 95% se ha logrado en un sistema de curtido al cromo asistido por la enzima optimizada. La cantidad de cromo presente en estos cueros ha sido relativamente superior en comparación con cueros convencionalmente curtidos al cromo. Las características táctiles y físicas evaluadas han estado a la misma altura que los cueros convencionales.

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Manuscript received August 7, 2007, accepted for publication July 11, 2008

INTRODUCTION

The leather industry has creatively implemented pollution prevention techniques that improved efficiency and increased profits while at the same time minimized environmental impacts. This has been possible through many ways such as reducing material inputs, re-engineering processes by reuse, improved management practices and employing substitution of toxic chemicals. The present ubiquitous status of chromium as a tanning agent is being challenged owing to its toxic nature.[1-4] In the present scenario, the need to reduce chromium in the effluent is becoming increasingly important. Hence over the years, several chrome management technologies have emerged.[5-14] In process control measures have emerged as one of the effective methods of preventing chrome pollution. While improvements in basic chromium sulfate for increased uptake, near zero waste concepts[15] etc. have been introduced. The most significant challenge for the tanning industry arises from the need to dispose off solid wastes like sludge containing high amounts of chromium.[16] While technologies are available for the recovery of protein hydrolysates and chromium from shaving and buffing dusts[17] as well as use of these wastes for the manufacture of basic chromium sulfate,[18] the need to dispose of sludge from effluents treatment plants without the danger of conversion of Cr(III) to Cr(VI) is a matter of concern.[19] It has become essential to develop process technologies that not only lowers productivity but also increases production complexity when implemented. Organic tanning could at best occupy 50% share in tanning of the future.[20] However, development of such technologies does not enable production of leathers with matching properties of conventional chrome tanning.[21] The unique position of chromium based on its electronic configuration and aqueous chemistry still remains to be confronted. Hence, the present day technologies for chromium management in tanneries require end-of-pipe treatment systems to meet the stipulation norms for discharge.[22] Eventually, recycling or near zero discharge of chromium in tanning calls for stringent regulations in processing.

The growth of biotechnology has resulted in significant improvements in the production and application of byproducts in various leather-processing steps.[23] Enzymes have found uses in various pretanning processes of leather manufacture such as soaking, unhairing, bating and degreasing. Proteolytic enzymes have also been used during dyeing process to increase the exhaustion of dyes in post tanning processes.[24] However; information on the use of enzymes in tanning process is scanty.[25] The use of enzymes options in beam house as well as post tanning process has been well established. Hence, there exists enormous scope for application of the same in tanning processes. Hence, in the present work an attempt has been made to investigate the application of enzymes in chrome tanning process. This can also pave way for the leather manufacturer to produce high quality leathers with reduced pollution load. In this work, a bio-catalytic enzyme assisted chrome tanning process with fiber opening and splitting has been established on partially pickled pelts using acid protease at its optimum pH conditions. The increase in exhaustion of chromium, extent of opening up and splitting up of fiber bundles, stratigraphic chromium distribution analysis, layer wise distribution of chromium, softness and surface color measurements due to enzyme treatment have been assessed. Influence of enzyme treatment on strength properties of leathers have been tested through physical testing measurements, while the bulk properties have been evaluated manually.

EXPERIMENTAL

Wet salted goatskins of good quality have been chosen as raw material. Since this work involves study of the extent of diffusion and distribution of tanning agent, more compact goatskins of uniform thickness have been selected as raw material. All chemicals used for leather processing are of technical grade except for formic and sulfuric acid, which are of laboratory grade; while the chemicals used for the analysis of leather and spent liquors are of analytical grade. In the present study, use of technical grade acid protease enzyme has been obtained from Biotan Chemicals, Chennai, India.

Experimental Trials

Wet salted Goatskins have been pickled and the effect of proteolytic enzyme treatment before chrome tanning has been studied employing the process described in Table I. Various experimental trials have been carried out at different conditions of enzyme treatment before tanning as given in Table I and subsequent to the enzyme treatment, tanning process with BCS has been followed.

Effect of concentration of enzymes:

Sixteen halves of eight partially pickled goat skins at pH 4.5 have been treated with acid protease enzyme at six different concentrations *viz.,* 0.1, 0.2, 0.3, 0.4 and 0.5% and the duration of treatment has been 30 min. All the pelts have been tanned with 8% of BCS.

Effect of pH:

Ten halves of 5 pickled goat skins have been treated with 0.10% acid protease for 30 minutes. The enzyme treatment has been carried out at five different pH conditions viz., 3, 4, 4.5, 5 and 6 adjusted prior to enzyme treatment. All the pelts have been tanned with 8% of BCS.

Effect of time:

Twelve halves of 6 pickled goat skins have been treated with 0.10% acid protease at varied running times of 15', 30', 45', 60', 75', and 90'. All the pelts have been tanned with 8% BCS.

The pH of all the experimental leathers has been adjusted to 4.5 and piled for 24 hours. The exhaust liquor and the wet blue leather have been analyzed for Cr_2O_3 content. Next day, hydrothermal stability of leathers has been measured using a shrinkage tester. [26]

TABLE I
Control and Experimental Chrome Tanning Process

PROCESS	%	CHEMICALS	DURATION (min)	REMARKS
MATERIAL: Bated Goatskins from a similar lot				
CONTROL TANNING PROCESSES				
Control 1	100	Water		
	10	Sodium Chloride	30	
	1.0	Sulphuric acid	3X15 +30	pH 2.8, drain 50% liquor
	8	BCS	120	
	0.1-0.5	Sodium formate	30	
	0.1-0.5	Sodium bicarbonate	3X15 + 60	pH adjusted to 3.8-4.0
Control 2	100	Water		
	5	Sodium Chloride	30	pH 4.5, drain 100% liquor
	0.5	Sulphuric acid	3X15 +30	
	8	BCS	120	
	100	Water	60	
	0.1-0.5	Sodium formate	30	
	0.1-0.5	Sodium bicarbonate	3X15 + 60	pH 3.8-4.0
Control 3	100	Water		
	5	Sodium Chloride	30	
	0.5	Sulphuric acid	3X15 +30	pH 4.5, drain 100% liquor
	6	BCS	120	
	100	Water	60	
	0.1-0.5	Sodium formate	30	
	0.1-0.5	Sodium bicarbonate	3X15 + 60	pH 3.8-4.0
EXPERIMENTAL TANNING PROCESSES				
Experiment 1 & Experiment 2	100	Water		
	5	Sodium Chloride	30	
	0.5	Sulphuric acid	3X15+30	pH 4.5 Drain
ENZYME TREATMENT (Experimental 1 & 2 trial groups)				
Trial A – Treatment at varying concentration (0.1, 0.2, 0.3, 0.4,0.5%) at pH 4.5, 30 min Trial B – Treatment at varying pH conditions (3, 4, 4.5, 5 and 6) at 0.10% enzyme for 30 min Trial C– Treatment at varying time (15,30,45,60,75 & 90 min) at 0.10% enzyme, pH 4.5 **Optimized enzyme treatment at 0.10% enzyme, pH 4.5 for 30 min**				
Experiment 1	8	BCS	120	
Experiment 2	6	BCS	120	
Experiment 1 & Experiment 2	100	Water	60	
	0.1	Sodium formate	30	
	0.1	Sodium bicarbonate	3X15 + 60	pH adjusted to 3.8-4.0
All control and experimental leathers were then sammed, split and shaved to uniform thickness (1.0-1.1 mm)				

Comparison of Optimized Experimental and Control Trials

Matched pair comparison of control and experimental trials at optimized conditions of enzyme treatment has been carried out using twenty six partial pickled goat skins. Three control processes have been adopted. In control 1, six goat skins pickled to pH 2.8 has been subjected to chrome tanning process using 50% pickle liquor and 8% BCS. In control 2, ten goat skins partial pickled to pH 4.5 have been subjected to dry chrome tanning process with 8% BCS without any enzyme treatment. In control 3, ten goat skins partial pickled to pH 4.5 have been subjected to dry chrome tanning process with 6% BCS as mentioned in Table I without any enzyme treatment. The right halves of all the respective control leathers (twenty six right half skins) have been equally divided for optimized experimental 1 and 2 processes. Two optimized enzyme assisted experimental processes have been carried out with, 0.10% enzyme, 30 minutes enzyme treatment at pH 4.5; one process using 8% BCS and the other using 6% BCS as given in Table I. The wet blue leathers have been piled for 24 hrs. Next day, hydrothermal stability of all the leathers has been measured.

The leathers have been sammed and shaved to 1.0-1.1 mm thickness. Common post tanning operations have been carried out for all the wet blue leathers using a standard recipe followed for processing upper leathers. The leathers have been hooked for drying, conditioned, staked, trimmed and buffed. The dyed crust leathers have been compared for color, fastness, strength, and organoleptic properties and subjected to SEM analysis.

Stratigraphic Chrome Distribution and Total Chrome Content Analysis

Samples from the official butt portion[27] of all the optimized experimental and control wet blue skins have been split into three uniform layers using a splitting machine and analyzed for layer wise chrome content. A known weight of the sample has been digested and the amount of chromium estimated as per standard procedure.[28] Samples have been initially analyzed for moisture content[28] and chrome content has been expressed on dry weight basis. Total chromium content of the leathers has been obtained using similar procedures without splitting the leather into layers.

Scanning Electron Microscopic Analysis of Leather Samples

Samples from selected experimental 2 and control 3 skins have been cut from the official sampling position[28] after chrome tanning process. Samples have been first washed in water. Subsequently the samples have been fixed by soaking them with buffered formalin for 18 hrs. Samples have been then dehydrated gradually using acetone and methanol as per standard procedure.[29] Excess solvent from the samples has been removed by placing them between filter papers. Samples have been then cut into specimens with uniform thickness. The specimens have been then coated with gold using a Polaron SC500 sputter coater. A Leica Cambridge Stereoscan 440 scanning electron microscope has been used for the analysis.

Physical Testing and Hand (Tactile) Evaluation of Leathers

Samples for various physical tests from all the experimental and control crust leathers have been obtained as per IULTCS method.[30-32] Specimens have been conditioned at 80 ± 4°F and 65 ± 2% R.H over a period of 48 hrs. Physical properties such as tensile strength, percent elongation at break, tear strength and grain crack strength have been examined. Experimental and control wet blue and the crust leathers have been assessed for softness, fullness, grain tightness (break), color uniformity and general appearance by hand and visual examination. Experienced tanners rated the leathers on a scale of 0-10 points for each functional property, where higher points indicate better property.

Determination of Color Difference of Crust Leathers

The control and optimized experimental crust leathers processed in this study have been subjected to the reflectance measurements using a Milton Roy color mate HDS instrument. Color measurement (L, a, b, h and C) have been recorded and the total color difference (ΔE) and hue difference (ΔH) have been calculated using the following equations:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad \textbf{(1)}$$

$$\Delta H = \sqrt{\Delta E^2 - \Delta L^2 - \Delta C^2} \quad \textbf{(2)}$$

Where ΔE = overall color difference; ΔL = Lightness difference; Δa and Δb = difference of a and b values, where 'a' represents the red and green axis and 'b' represents the yellow and blue axis; ΔH, hue difference, ΔC, chromaticity difference.

RESULTS AND DISCUSSION

Enzyme Assisted Chrome Tanning Process: Optimization of Process Parameters

Process parameters such as concentration, pH, and duration of enzyme treatment have been varied and the optimum process parameters for effective high exhaust chrome tanning process have been arrived at. Preliminary trials have been performed to select the amount of enzyme as well as the time required for optimum opening up and splitting up of fiber bundles.

Optimization of concentration of enzymes: The percentage exhaustion of chromium and the shrinkage temperature of the leathers tanned at different concentrations of acid protease treatment are given in Table II. Above 0.1% offer of enzyme, there has been no significant increase in the shrinkage temperature and fixation of chromium. Acid protease can result in mild degradation of pelts and reduce the strength of the leathers at higher concentration. Therefore,

TABLE II

Exhaustion of BCS of and Shrinkage Temperature of the Leathers Tanned with Enzyme Treatment

Process Parameters		BCS offered %	Exhaustion %	Chrome- Effluent as Cr (ppm)	Shrinkage Temperature Ts (°C)
Control 1 – C1		8	65±1.12	4200±28	> 115°C
Control 2 – C2		8	78±1.68	2640±22	> 115°C
Control 3 – C3		6	82±1.56	2160±24	> 115°C
Concentration# (pH 4.5 and 30')	0.1%	8	95±1.89	720±12	> 115°C
	0.2%	8	96±1.16	480±13	> 115°C
	0.3%	8	97±0.98	360±18	> 115°C
	0.4%	8	98±0.28	240±23	> 115°C
	0.5%	8	98±0.42	240±168	> 115°C
pH* (0.1% enzyme offer, 30')	3	8	88±2.15	1440±16	> 115°C
	3.5	8	90±1.46	1200±22	> 115°C
	4	8	93±1.38	840±16	> 115°C
	4.5	8	95±0.68	720±14	> 115°C
	5	8	93±0.56	840±26	> 115°C
	6	8	91±0.96	1080±36	> 115°C
Duration^ (0.1% enzyme offer, pH 4.5)	15'	8	91±1.24	1080±42	> 115°C
	30'	8	95±0.59	720±16	> 115°C
	45'	8	96±0.32	480±11	> 115°C
	60'	8	97±0.56	360±12	> 115°C
	75'	8	97±0.98	360±14	> 115°C
	90'	8	97±1.12	360±8	> 115°C
Experimental 1 – E1 Optimized Conditions (0.1% enzyme offer, 8% BCS, pH 4.5 & 30')		8	95±0.62	720±15	> 115°C
Experimental 2 – E2 Optimized Conditions (0.1% enzyme offer, 6% BCS, pH 4.5 & 30')		6	98±0.26	240±9	> 115°C

0.1% acid protease appeared to be sufficient for maximum uptake of chromium and has been taken as optimum concentration. The shrinkage temperature of the leathers has been found to be > 115°C and exhaustion of chromium at this concentration has been found to be 95% at 8% chromium offer.

Optimization of pH of enzyme treatment: The percentage exhaustion of chromium at different pH conditions of acid protease treatment are given in Table II. From the table it can be seen that the maximum exhaustion of chromium has been attained when enzyme treatment had been carried out at pH 4.5. However, further increase in chromium uptake has been not observed at higher pH values. Opening up of the fiber

structure is expected to be enhanced at maximum activity of the enzyme. Hence, pH 4.5 has been considered as the optimum pH condition for enzyme assisted chrome tanning process. Furthermore, surface fixation of chromium has not been observed at pH 4.5, as dry tanning process has been adopted, which could have decreased the rate of formation of cationic aqueous chromium complexes. However, the anionic sulphated chromium complexes present initially would have penetrated and distributed uniformly throughout the leather. Moreover, significant increase in chromium exhaustion at higher pH values i.e. at pH 5.0 and pH 6.0 has not been observed. The risk of surface fixation of chromium increases when the starting pH of chrome tanning is high in the absence of masking agents. Hence, pH 4.5 has been taken as the optimum condition. Acid protease treatment at optimum pH condition i.e. pH 4.5 opens up and splits the fiber structure thereby increasing the availability of carboxyl groups in the collagen matrix. Therefore, pH 4.5 at 0.1% offer of acid protease has been taken as optimum condition for better exhaustion of chromium.

Optimization of time: The percentage exhaustion of chromium obtained for different duration of acid protease treatment is given in Table II. It is evident from the table that the uptake of chromium increases gradually with time. It requires 30 minutes to bring about higher exhaustion of chromium in the tanning bath at pH 4.5. Running time greater than 30 minutes had not resulted in significant increase in chrome exhaustion of enzyme assisted leathers. In addition, increased time duration of enzyme treatment before tanning may possibly result in damaging the pelts. Furthermore, the treatment of acid protease for 30 minutes resulted in maximum chromium uptake of 95%. Hence, pH 4.5 at 0.10% offer of acid protease for 30 minutes has be en taken as optimized conditions for better exhaustion of chromium.

Chrome Uptake

Three control processes have been adopted. Conventional chrome tanning process has been taken as control 1. Dry tanning had to be followed for experimental leathers as optimized pH for enzyme treatment has been 4.5. In addition, 6% and 8% BCS has been used for the experimental trials. Hence, control 2 and 3 processes have also been introduced for better comparison of chrome exhaustion values. The percentage uptake of chromium and the shrinkage temperature values for all the control and experimental trials are given in Table II. It has been observed that the uptake of chromium has been significantly increased on employing enzyme prior to chromium tanning. This has been evident from the lower amount of chromium present in the spent chrome liquor as compared to the conventional process. Conventional chrome tanning process exhibited chromium uptake of 65% at a chromium offer of 8% at pH 2.8 (control 1). However, chromium uptake at 8% offer of BCS has been 78% at pH 4.5 (control 2). 82% exhaustion of chromium resulted at an offer of 6% BCS at pH 4.5 (control 3). Use of 6% BCS and higher pH conditions (4.5) resulted in an increase of chromium exhaustion in control 3 compared to conventional 8% BCS offer.

The enzyme assisted chrome tanning process exhibited chromium uptake of 95% with 8% BCS and 98% uptake with 6% BCS offer at pH 4.5. An increase of 33% chromium uptake has been observed for optimized experiment 2 (6% BCS offer, 0.1% enzyme at 4.5 pH) as compared to conventional chrome tanning process (control 1). However, an increase in chrome exhaustion of 16% has been observed when compared with control 3 (6% BCS offer without enzyme treatment). At 8% offer of BCS, there has been an increase of 17% chromium exhaustion in optimized experiment (8% BCS offer, 0.1% enzyme at 4.5 pH) when compared to control 2(8% BCS offer without enzyme treatment. There has been also significant reduction in the emission of chromium discharged in all the experimental tanning trials.

Chrome Distribution: Stratigraphic and Total Chromium Content in Control and Experimental leathers

The variation in the compactness of a skin/hide matrix is known. It is important to look at the layer wise chromium distribution for assessing the extent of opening up and splitting of fiber bundles. The distribution of chromium in the butt (official sampling position) has been analyzed in three strata of cross section, viz. grain, middle and flesh. The average values of stratigraphic chromium distribution are presented in the Table III. The distribution of chromium in all the three layers has been almost uniform for all the experimental trials compared to control trials. This substantiates that the extent of opening up of fiber bundles using enzyme is almost the same along the entire cross section. The distribution of chromium for enzyme assisted chrome tanning at pH 4.5 and 8% BCS offer has been 34, 31 and 35% and at 6% BCS offer 33.5, 32.5 and 34% in the grain, middle and flesh layers, respectively. However, previous theories [25-28] on chrome tanning suggest that at higher pH values, chromium fixes only to the surface and does not penetrate into the inner layers and the remaining chromium precipitates as $Cr(OH)_3$. Nevertheless in present study, it has been observed that about 31-32% of Cr_2O_3 has penetrated in to the middle layer as dry tanning process had been followed at higher (4.5) pH condition. This hypothesis demonstrates that at higher pH value of 4.5, chromium distribution can be uniform and comparable to conventionally tanned leather when dry tanning has been adopted, (control 2). As seen in Table III, the amount of chromium present in all the three layers has been higher for enzyme assisted chrome tanning than control trials. Hence, enzyme treatment leads to increased fixation of chromium along with uniform distribution through out the fiber matrix.

TABLE III
Layer Wise Distribution of Chromium and Chemical Characteristics of Control and Experimental Wet Blue Leathers

Trial	Layer wise distribution of chromium % Cr_2O_3 content		
	Grain	Middle	Flesh
Control 1	3.02±0.12	2.93±0.09	3.04±0.11
Control 2	3.11±0.08	3.07±0.06	3.15±0.08
Control 3	3.52±0.16	3.56±0.14	3.62±0.16
Experiment 1	4.96±0.14	4.53±0.11	5.01±0.08
Experiment 2	4.27±0.08	4.14±0.11	4.31±0.18

TABLE IV
Strength Properties of Control and Optimized Experimental Crust Leathers

Sample	Tensile Strength	Extension at break	Tear Strength	Grain Crack Resistance	
	(Kg/cm²)	%	(Kg/cm)	Load (Kg)	Distension (mm)
Control 1	254±10	75±6	83±6	30±3	9.0±0.2
Control 2	243±15	73±4	87±7	31±2	9.3±0.2
Control 3	229±18	70±6	82±3	29±4	9.1±0.2
Experiment 1	245±12	82±5	93±3	38±3	10.8±0.5
Experiment 2	240±8	80±6	91±3	36±3	10.5±0.5

TABLE V
Visual Assessment Data for Control and Optimized Experimental Leathers after Tanning

Parameters	Control 1	Control 2	Control 3	Experiment 1	Experiment 2
Ts (°C)	>115	>115	>115	>115	>115
Grain Smoothness	7.5±0.5	8.0±0.5	8.0±0.5	8.0±0.5	8.5±0.5
Fullness	6±0.5	6.5±0.5	6.5±0.5	6.5±1	6.5±1
Fluffiness	7±1	7±1	7±1	8±0.5	8±0.5
Color of leather	7±1	7±1	7.5±1	8±0.5	8±0.5
Wrinkles	Nil	Nil	Nil	Nil	Nil
General Appearance	7±1	7.5±1	7.5±1	8.5±0.5	8.5±0.5

TABLE VI
Total Colour Difference Values of Control and Optimized Experimental Crust Leathers

Parameters	ΔL	ΔC	ΔH	Δa	Δb	ΔE
Control 2	d=-4.387	S=-0.250	D=-0.201	MR=0.409	MY=0.162	7.613
Control 3	d=-5.312	S=-0.312	D=-0.216	MR=0.453	MY=0.245	8.113
Experiment 1	d=-7.214	S=-0.558	D=-0.498	MR=0.549	MY=1.058	14.098
Experiment 2	d=-6.863	S=-0.289	D=-0.249	MR=0.429	MY=0.215	13.821

Effect of Enzyme on the structure of leather matrix: Scanning Electron Microscopic Analysis

In this study, enzymes have been used for fiber opening and splitting processes. It is of paramount importance to investigate the grain surface since it is expected that the use of enzyme could result in grain damage. The scanning electron photomicrographs showing the grain surface of wet blue leathers tanned using conventional chrome tanning (control 2) and enzyme assisted chrome tanning (experiment 2) at a magnification of 100 are shown in Figures 1a and 1b, respectively. Both the samples exhibit a clear grain surface without any macro pores, which indicates that there has been no surface deposition of chromium at higher pH (pH 4.5). Scanning electron photomicrographs showing the cross section of the wet blue leathers tanned using conventional chrome tanning and enzyme assisted chrome tanning at a magnification of 1000 are depicted in Figures 2a and 2b, respectively. The fiber bundles of the experimental samples seem to be well opened up and split with the individual fibers clearly visible in the fiber bundle. This supports that the condition that the splitting and opening of fiber bundles arises from the biocatalyic opening up and cleavage during enzyme treatment and in principle exhibits an increased uptake of chromium, softness and color in the final leather.

Performance of Leathers

Characterization of Strength Properties

It is imperative to analyze the strength characteristics upon treatment with enzymes as one may expect opening up and splitting of fiber structure influencing the strength properties of the leathers. The various strength characteristics of the experimental crust leathers treated at optimized conditions of enzyme treatment along with control leathers are given in Table IV. It has been observed that the strength characteristics of the experimental leathers are not affected due to enzyme treatment. The values of various strength properties of experimental leather are found to be comparable to that of the control leathers.

Bulk Properties by Tactile (Hand) Evaluation Method

Visual assessment has been carried out by comparing the left control leathers of the matched pair leathers with enzyme assisted experimental leathers. The statistical difference between the values of the three control trials did not shown any major difference; hence all the control values have been grouped together to a single control value. It is well known that the results of hand evaluation method are subjective, which varies from person to person. Yet it could be taken as reliable, if carried out by experienced persons. The hand evaluation method has been carried out for both wet blue and crust leathers and given in Table V. It has been observed that the experimental leathers have good bulk properties as compared to control. Chrome patches and grain characteristics like swelling, case hardening have not been observed from any of the samples. No variation in the color of wet blue has also been observed, but the grain smoothness has been good for all the leathers. As shown in Figure 3, fullness, fluffiness, color uniformity and general appearance showed better values for the entire enzyme assisted leathers. This may be due to greater fixation of post tanning chemicals as the amount of chromium has been high and better softness may possibly be due to enzyme treatment.

Effect of Enzyme Assisted Tanning Method on Dyeing Characteristics

Variation in color and color difference of the control 1 and enzyme assisted chrome tanned leathers is presented in Table VI. From the ΔL and ΔC values shown in the table, it has been observed that there has been 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 chrome content of the leathers as observed in Table III. Enzyme assisted chrome tanned leathers exhibited maximum increase in color intensity and darkness values. With enhanced fibre opening and splitting, increase in chrome content of leathers, dye uptake and other post tannin chemicals facilitates better darkness values and color intensity. However, there has been not much of hue difference in the enzyme treated leathers as compared to control. The optimized experimental 2 samples showed an evidence of total color difference (ΔE) of 13.821 compared to control leather, which substantiates the overall color difference as significant and visible. The results of the instrumental color values are found to be similar to that observed by visual assessment.

Plausible Mechanism of Enzyme Assisted Chrome Tanning

The enzyme treatment before chrome tanning has illustrated the way to bio-catalytic opening up of and cleavage of fiber bundles. This has, in principle exhibited an increase in uptake of chromium, softness and color in the final leather. In conventional chrome tanning, pickling process reduces the pH of the hide matrix to 2.8, wherein the side chain carboxyl groups of the aspartic and glutamic acids are in the unionized form, thereby providing proper penetration, followed by fixation of chromium. Sodium chloride is added to avoid the acid swelling of collagen at an effective ionic strength of 0.9-1.1 M as collagen is known to swell significantly at pH 2.8.[33-35] In dry float chrome tanning (as followed in control and enzyme assisted processes), pickling has been carried out at pH 4.5 and the swelling of bated pelt has been reduced . Dry tanning has been followed by addition of water after the penetration of chromium and hence swelling has been not observed. It has been reported by Palop and Marsal[36] that the minimum salt concentration required to suppress the swelling in pickling is 5% on pelt weight with an effective ionic strength of 0.53 M (equivalent to 1.03 g/mL or 5°Bé).[37] The ionic strength from the

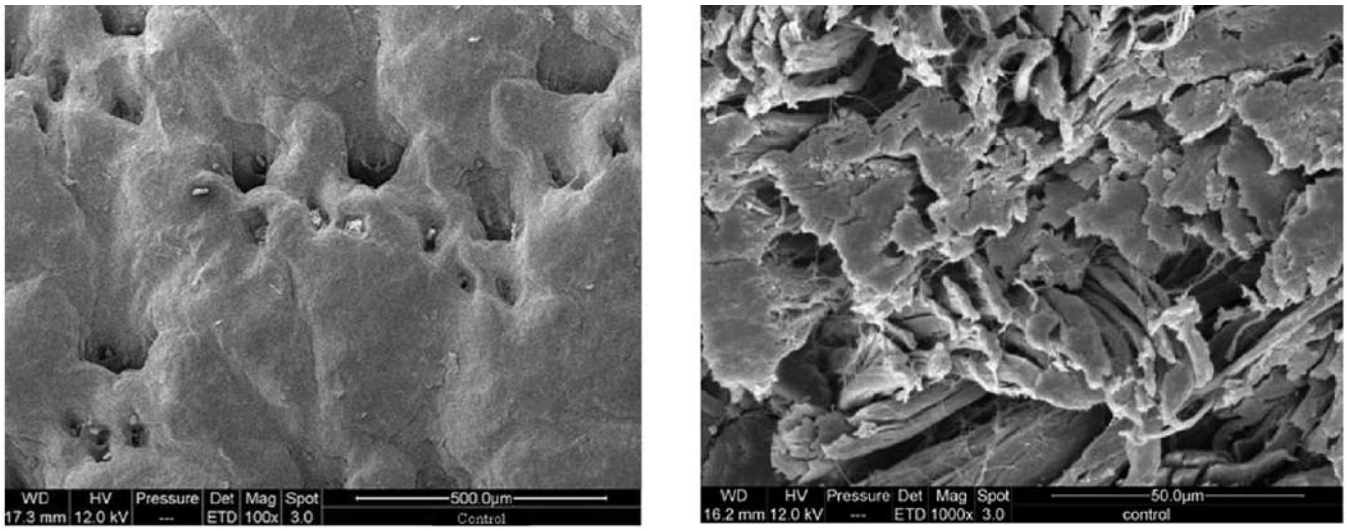

Figure 1: Scanning Electron Micrograph of Control-2 Crust Leather a) Grain Structure (X 100) b) Cross-section (X 1000)

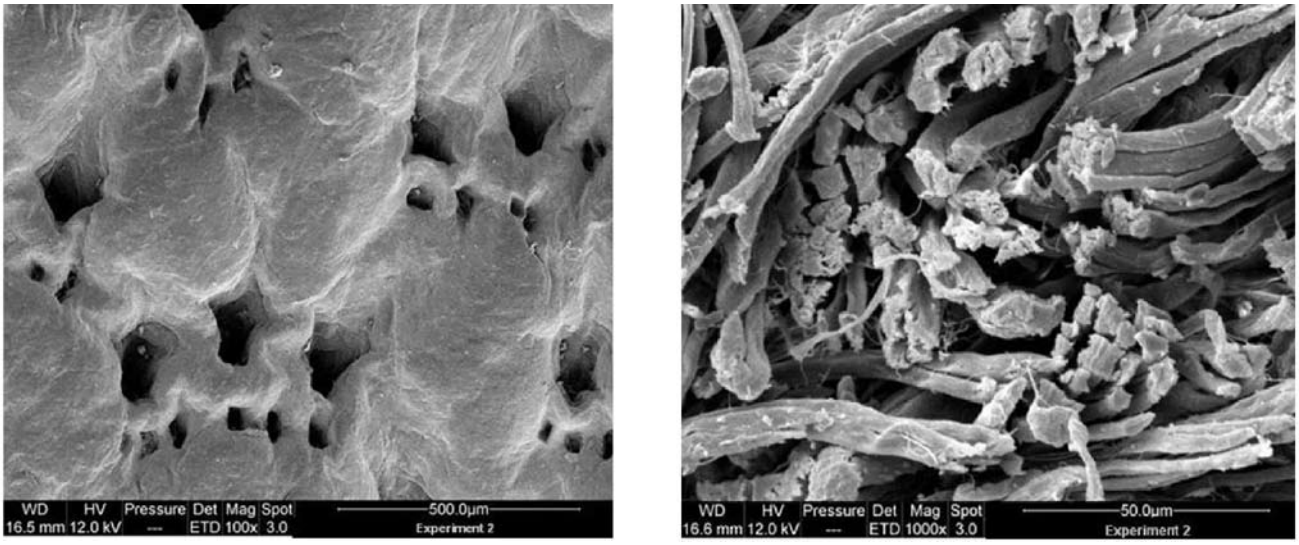

Figure 2: Scanning Electron Micrograph of Experimental-2 (Enzyme Treated) Crust leather a) Grain Structure (X 100) b) Cross-section (X 600)

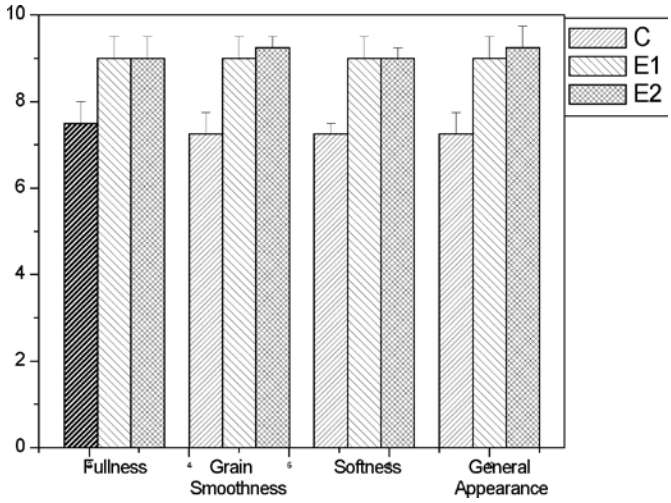

Figure 3: Graphical Representation of Organoleptic Properties of Control -1, 2, 3 (C), Experimental-1 (E1) and & Experimental-2 (E2) Crust Leathers

addition of BCS, which contains neutral salts of 30% sodium sulfate, without any salt content is 1.53 M (equivalent to 1.05 g/mL or 7°Bé).[37] Hence dry tanning at a pH 4.5 has not resulted to any swelling in the absence of sodium chloride.

Chromium (III) salts exchange with anionic species present in the skin matrix as it is well established that more than 80% of the chromium species are anionically charged in highly concentrated BCS solution in the dry tanning process.[38] Higher concentration of chromium in dry tanning results in better diffusion and uptake of chromium as it is well established that, the size of the chromium species present at this pH is low and hence rate of penetration of chromium species is higher.[38] At higher concentration, (dry tanning) chromium complexes remain as sulphated complexes and the hydrolysis reaction of chromium complexes is very slow. Hence, surface fixing of chromium species is less preferred at the beginning of this dry tannage. However, after through and through penetration of chromium, flooding with water results in hydrolysis and increases the rate of reaction and results in fixation. High chrome exhaustion obtained for control leathers 78-82% compared to normal conventional chrome tanned leathers is due to the high pH condition adopted which would have lead to the availability of more amount of ionized carboxyl groups for fixation.

It has also been shown that the amount of chromium uptake is 95-98% for enzyme assisted chrome tanned leathers. Proteolytic enzyme treatment on partial pickled pelts results in cleaning of fiber matrix, opening up and splitting of the fiber structure. Many reactive sites will be opened up and available for fixation which is responsible for enhanced uptake of chemicals in tanning process. The opened up fiber structure and higher pH has resulted in very high exhaustion of chromium.

CONCLUSIONS

In the present investigation, an attempt has been made to develop an enzyme assisted high exhaust chrome tanning process at pH of 4.5. The approach is based on the concept that the enzymes act as biocatalyst in opening up and splitting of the fibrous collagen matrix, which enhances the availability of carboxyl groups for interaction with chromium species. 0.1% offer of enzyme, at pH of 4.5 for 30 minutes has been found to be optimum with respect to the uptake of chromium. Enzyme pretreatment at these optimized conditions has resulted in good opening up and splitting of fiber structure resulting in an uptake of 98% chrome tanning agent. On visual observation, none of the experimental samples showed any physical deposition of chromium at higher tanning pH of 4.5. The enzyme assisted chrome tanning process resulted in leathers with uniform dyeing, intense and bright shades. The strength and bulk properties like fullness, grain smoothness, feel and general appearance have been improved by the introduction of enzymes in the chrome tanning process. The developed enzyme assisted process appears economically viable and commercially adaptable.

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