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Application of Acid Protease for Eco-friendly Pre-treatment of Goat Skin to Improve Antimicrobial Finish Using Herbal Natural Extracts

by
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Abstract

Due to its moisture retention capacity and huge surface area, leather is highly prone to microbial proliferation and biodeterioration; hence, leather products desired have an antimicrobial finish. In this study, acid protease enzyme pre-treatment of goat skin was utilized as an eco-friendly substitute for conventional wet-chemical processing. The treatment can impart the desired surface properties to improve the antimicrobial finish with natural extracts obtained from leaves of *Azadirachta indica* (Neem Tree), *Ocimum sanctum* (Holy Basil, Tulsi), and *Camellia sinensis* (Green Tea). The procedure was optimized for different process parameters, including enzyme concentration, pH, material to liquor ratio (MLR), treatment time, and temperature. The effect of the treatment on bulk and surface properties of the skin was characterized by weight loss analysis, X-Ray Diffraction (XRD), Thermal Gravimetric Analysis (TGA), and Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR), X-Ray Photoelectron Spectroscopy (XPS), Water contact angle measurement, Scanning Electron Microscopy (SEM) respectively. The effect of the enzymatic treatment on organoleptic properties and the mechanical strength of the skin was also studied. The enzymatic treatment resulted in weight loss, and removal of non-collagen components, thus opening the fibrous collagen matrix of the skin. Hence, the skin treated with acid protease enzyme provides better affinity and accessibility for phytoactive compounds from the natural extracts and better attachment by electrostatic attachment due to an increase in surface functional groups after the enzymatic treatment compared to untreated skin. The effectiveness of the antimicrobial finish was measured as a zone of inhibition and with a modified Hohenstein test against test microorganisms *E. coli and S. aureus. Azadirachta indica* (Neem Tree) extract showed the highest inhibitory activity (97%) against *E. coli*, while the *Ocimum sanctum* (Holy Basil, Tulsi) extract exhibited the highest inhibitory activity (95%) against *S. aureus*.

Introduction

Leather/leather products have become a valued commodity in the global market. Also, the leather/leather product manufacturing industries have emerged as a prime economic sector in developing countries.[1] Despite having a prominent role in economic development worldwide, the leather production industries continually face challenges. Due to environmental pollution, the leather/leather product manufacturing industries are under strict criticism by societies and pollution control authorities.[2] Leather produced from flayed animal skins by a series of operations/treatments briefly classified under beam hose/pre-tanning, tanning, and post-tanning and finishing. In the conventional process of leather manufacturing, a substantial quantity of water and various chemicals are required at each stage of sequential operations.

Additionally, a massive quantity of chemicals is required to impart/enhance the desirable qualities of the final product. Thus, conventional leather production processes expel massive amounts of pollutants, including hazardous chemicals, into the environment. The conventional wet-chemical treatment generates wastewater that requires extensive treatment and is a source of environmental pollution even after treatment and disgorges toxic gases like ammonia and hydrogen sulfide.[2–4]

As a result of the emergence of awareness in society, strict legislation, and global concern about the environmental impacts caused by leather industries, the industries are under constant pressure to opt for alternatives, cleaner and greener processing which can limit the usage of hazardous/other chemicals in processing. The utilization of enzymes in various pre-tanning operations and incorporation of enzymes in post-tanning operations to impart required properties to the processed leather is gaining considerable importance as an alternative eco-friendly approach.[4–6] Various enzymes such as collagenase, keratinase, protease, and lipase are being explored and utilized for leather processing.[5] These enzymes are reported to soften the collagen fiber matrix, augment the characteristics of the leather, and significantly can lower environmental pollution by reducing the use of hazardous and polluting chemicals. Enzyme-based skin processing has emerged as a promising technology for leather production by incorporating enzymes at various stages in leather processing.[5] However, for successful applications of enzymes in leather processing, stringent control of process parameters is obligatory to acquire substantial results,[2–4,7] and therefore researchers are working to gain a critical understanding of the application of enzymatic treatment in leather processing.[8]

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Microbial deterioration of skin/leather is another critical aspect affecting its aesthetic properties, life expectancy, and utilization of leather products.[9] Skin/leather, made up of collagen protein, is highly susceptible to the growth of microorganisms due to its large surface area and moisture retention capacity.[10,11] Consumer awareness toward personal hygiene and health risk associated with dreadful microorganisms and decrease in life expectancy due to microbial deterioration also seeks to impart antimicrobial properties/finish to leather/leather products. For imparting antimicrobial finish, various synthetic organic chemical agents are under use; however, many of them are now banned due to ecological toxicity and growing environmental concern.[9,12] Recently, the application of natural antimicrobial agents to impart antimicrobial properties to leather/leather products has attracted significant attention from researchers worldwide as they are environment-friendly, skin-friendly, safe, non-toxic, and exhibit broad-spectrum activity.[13,14]

The finishing of skin/leather is challenging because of variations in the three-dimensional matrix of collagen fiber weave in skin/leather. Particularly, absorption of antimicrobial agents by skin/leather varied because of differences in affinity and accessibility and the presence of non-collagenous materials in the skin. The conventional wet-chemical pre-treatment to impart desired finish is not environmentally friendly; also, the bulk properties of skin/leather may be affected during the conventional wet-chemical pre-treatment. Thus, there is a quest for appropriate eco-friendly methods to modify the collagen weave matrix of the skin and remove non-collagenous material without affecting its bulk properties.[15] The use of acid protease enzyme as an alternative to the wet-chemical treatment is investigated in this study. Enzymatic treatment of skin/leather can remove/reduces interfibrillar cementing substances like sulfated glucosamine glycans, uronic acid, hexosamines, and other glycoproteins from skin/leather[16] and results in fiber opening, the opening of collagen bundle weave[17] and ultimately results in better absorption and affinity for a finishing agent.[2,18,19] Among the enzymes used for skin/leather processing, the protease is widely investigated for its isolation, screening, production optimization, purification, characterization, and applications,[20] particularly for substitution of chemical dehairing,[8,15,20] waste treatment.[21] Apart from this, the application of collagenolytic protease (collagenase) and acid protease as eco-friendly pre-treatment was reported for improved dyeing of leather due to better absorption and diffusion of dyes in the leather matrix due to well-opened collagen fiber matrix.[22,23]

In this study, acid protease enzyme pre-treatment of goat skin was investigated as an environment-friendly alternative to conventional wet-chemical processing to improve antimicrobial finish with natural extracts. The pre-tanning stage goat skin was treated with acid protease at variable process parameters for optimization of enzymatic treatment, and the enzymatic treatment-induced changes in bulk and surface properties were studied using various characterization techniques. Subsequently, the skin was treated with the natural extracts obtained from leaves of *Ocimum sanctum* (Tulsi, Holy Basil), *Azadirachta indica* (Neem Tree), and *Camellia sinensis* (Green tea) to impart antimicrobial activity, which is tested against test microorganisms *E. coli and S. aureus* with the zone of inhibition and modified Hohenstein test.

Experimental Work

2.1 Material:

Full-grain goat skin with all pre-tanning treatments was used in this study for enzymatic treatment and subsequent antimicrobial finishing. The goat skin was purchased from a tannery in Agra city, Uttar Pradesh, India. The skin has an average thickness of 1mm, cut into pieces of appropriate size for experimental study. The acid protease enzyme (Activity~ 4000 units/mg, Product code RM6186, Source: *Aspergillus spp.*), citric acid, and agar powder used in this study were purchased from HiMedia Laboratories, Mumbai, India. Luria Broth used in this study was purchased from SRL chemicals, India. The enzyme 'acid protease' used in the study is a commercial product of Himedia Company. The company provided the enzyme activity and stability data; the enzyme's shelf life is three years upon storage in the recommended storage condition. After preliminary experiments, the optimum conditions were determined to be 60 minutes, pH 4.5, MLR 1:20, temperature 50°C, and enzyme concentration 1%w/v. Leaves of *Azadirachta indica* (Neem Tree) and *Ocimum sanctum* (Holy Basil, Tulsi) were collected from the campus garden of Manipal University Jaipur, Rajasthan. Leaves of *Camellia sinensis* (Green Tea) were purchased from a local vendor. The leaves were washed with distilled water to remove dust and impurities, then air dried, converted to powder using a domestic mixture grinder, sieved (U.S. Standard Screen Size No. 35) and subsequently used to obtain the antimicrobial extract.

2.2 Enzymatic Treatment of Goat Skin Samples with Acid Protease:

The goat skin was cut into pieces of 5cm x 5cm and provided enzymatic treatment with the 1% w/v acid protease enzyme solution prepared in phosphate buffer at pH of 4.5,[22] material to liquor ratio (MLR) of 1:20, at 50°C for 60 minutes. The effect of time on the acid protease treatment of goat skin was studied by varying treatment times from 40 min to 70 min. Further, the effect of the acid protease enzyme concentration was studied by conducting experiments with above mention condition with enzyme concentration in the range of 0.5 to 2% w/v prepared in phosphate buffer. The material-to-liquor ratio varied in the range of 1:10 to 1:40. The temperature of the enzymatic treatment was optimized by providing treatment at different temperatures of 30°C to 60°C with other similar experimental conditions mentioned above. For the effect of pH on acid protease enzyme treatment of the goat skin, the pH of enzyme solution was set in the range of 4 to 5.5 using phosphate buffer and goat skin treated with enzyme concentration of 1% w/v, MLR of 1:20 at 50°C for 60 minutes. In all the experiments, enzymatic treatment was stopped by removing goat skin pieces from the acid protease solution and dipping them in hot water at 70°C for 5 seconds. After that, enzyme-treated skin pieces were washed thoroughly with doubled distilled water and dry in ambient conditions, followed by drying in a hot air

oven at 70°C for 24 hours to remove moisture. All the experiments were performed three times, and from obtained results, the average value and standard deviation were calculated for quantitative analysis. For comparison, control samples were prepared for all the above experimental conditions by dipping the goat skin pieces in a phosphate buffer solution without the acid protease enzyme.

2.3 Characterization:

The goat skin's surface and bulk properties, the effect of the enzymatic treatment on the surface, and the bulk properties of the goat skin were studied using various characterization techniques. Changes in the bulk properties of the goat skin due to the enzymatic treatment were studied by weight loss % measurement, analysis of physical properties by TG and DTG analysis, XRD analysis, SEM, analysis of Organoleptic properties, and analysis of mechanical properties such as tensile strength, % elongation at break, and tear strength. Effect of the enzyme treatment on non-collagen components of the goat skin studied by estimation before and after. Changes in the surface chemical composition of the goat skin by the enzymatic treatment studied by ATR-FTIR and XPS analysis of the grain surface of the goat skin and resultant improvement in wettability studied by water contact angle measurement at the grain surface. A detailed description of the characterization experiments is provided in the supplementary data.

3 Preparation of antimicrobial extract and antimicrobial finishing of Goat skins:

Natural antimicrobial extracts were prepared from the dry powder of leaves of *Azadirachta indica* (Neem Tree), *Ocimum sanctum* (Holy Basil, Tulsi), and *Camellia sinensis* (Green Tea) and phytochemical analysis of the prepared extracts was carried out. Identification of active constituents of the extracts was carried out using UV-visible spectroscopy and High-performance Liquid Chromatography (HPLC). The natural extracts were used for the antimicrobial finishing of the goat skin using citric acid as a binding agent. The antimicrobial finish and effect of the enzymatic treatment on the antimicrobial finish were studied using the Agar diffusion test (AATCC Test 90-2011; Agar plate method) and modified Hohenstein test (JIS L 1902). *Staphylococcus aureus* (MTCC9542) (Gram-positive organism) and *Escherichia coli* (ATCC 25922) (Gram-negative organism) were utilized as test microorganisms. The details of the experimental condition for the preparation of antimicrobial extracts, characterization, and identification of active constituents and obtained results, along with experimental details of antimicrobial finish and testing of antimicrobial finish, are provided as supplementary data.

4 Results and Discussion

4.1 Weight loss percentage:

The weight loss observed after acid protease treatment of goat skin samples may be due to breaking proteins into amino acids. In this study, the measurement of weight loss percentage was utilized to study the effect of different treatment parameters on the performance of the acid protease enzyme.

The effect of treatment time on acid protease resultant weight loss is presented in Figure 1(a). From Figure 1 a, it can be inferred

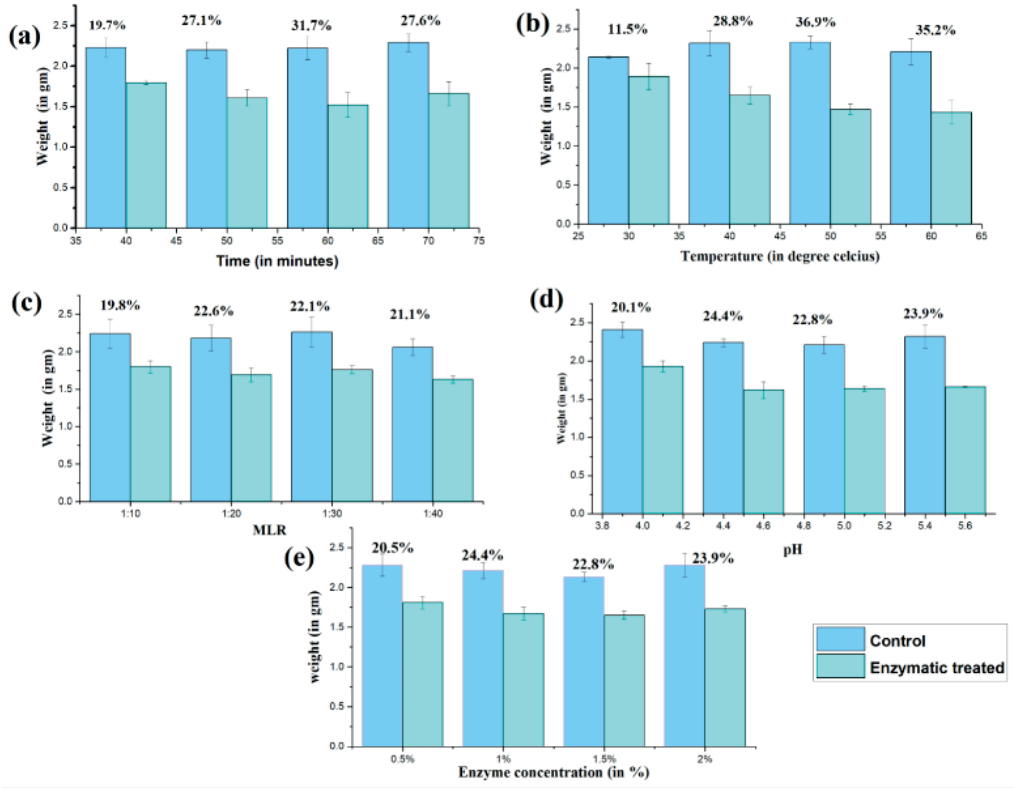

Figure 1. Weight of control and enzymatic treated goat skin for different treatment variables
a) Treatment time **b)** Temperature **c)** Material to liquor ratio **d)** pH **e)** Concentration of the enzyme.

that the maximum weight loss (31.7%) occurred for 60 minutes of treatment time. The acid protease treatment of goat skin samples beyond 60 minutes does not increase weight loss; the substrate may become one of the limiting factors by becoming depleted over time, resulting in no further interaction between the enzyme and substrate. These factors contribute to the constant weight of the goat skin beyond 60 minutes.[24–27] Figure 1(b) presents the effect of temperature on acid protease treatment resulting in the weight loss percentage of goat skin. Weight loss percentage increase with the increase in treatment temperature from 30°C to 50°C; there is a rise in weight loss percentage from 11.5% at 30°C to a maximum of 36.9% at 50°C because the increase in treatment temperature increases inter and intramolecular kinetic energy, resulting in more collision of enzymes and substrate, thereby enhancing the reaction rate. When the treatment temperature increased beyond 50°C, a slight decrease in weight loss percentage was observed. This decrease may be caused by the high internal energy of enzyme molecules that result from vibration, which stresses the amino acid bonds at the reactive site and changes the enzyme protein's secondary, tertiary, and quaternary structures. These weak connections are broken by high temperatures, which eventually causes the enzyme's activity to decline.[24,28] Weight loss % increased when the material to liquor ratio increased from 1:10 to1:20, but as the ratio increased further, the weight loss percentage decreased. This increase is due to the enzyme's lower diffusion rate when the MLR (Material to liquid ratio) increases (Figure 1(c)).[24,29] As presented in Figure 1(d), the optimum performance of the enzyme treatment was observed at pH 4.5, providing a maximum of 27.8% weight loss. At a pH other than 4.5, a decrease in the enzyme performance is observed because as the pH deviates from its optimum range, the amino acid composition of the reactive site changes so that the substrate can no longer react with the enzyme.[28] The impact of enzyme concentration on weight loss percentage was investigated at different concentrations ranging from 0.5% to 2% (w/v). As shown in Figure 1 (e), the weight loss percentage increased with an increase in the enzyme concentration from 0.5 % to 1%. Maximum 24.4% weight loss occurred at 1% w/v enzyme concentration. An increase in the enzyme concentration further than 1% did not increase weight loss, which might be due to the unavailability of substrate for the increased enzyme concentration.[24,28]

4.2 ATR-FTIR analysis:

ATR-FTIR spectrum of the upper surface of the goat skin obtained before and after the enzymatic treatment (Enzyme concentration: 1% w/v, pH: 4.5, MLR: 1:20, Temperature: 50°C, Treatment time: 60 minutes) to study changes in the chemical composition of the skin sample due to the enzyme treatment. Figure 2 shows the ATR-FTIR spectrum of untreated and enzymatic-treated goat skin. The skins are mainly composed of collagen, a fibrous protein with a triple helix structure arranged in microfibril, macro-fibril, and fibril bundles that are tightly woven to form a three-dimensional matrix of skin/leather. The acquired spectra matched the collagen IR spectrum, and the corresponding peaks were identified.

In the IR spectrum of proteins, the peptide group, a structural repeat unit of protein, provides nine characteristic peaks known as amide A, amide B, and amide I to VII. In the obtained IR spectrum, the most intense absorption peak in the 1616 cm^{-1} is identified as Amide I, which primarily gives rise due to the stretching vibration of C=O and, to an extent, C-N groups. The peak in the range of 1510 cm^{-1} and 1580 cm^{-1} is identified as Amide-II, which gives rise due to N-H bending coupled with C-N stretching vibration. Amide-I and amide II are complex peaks modulated by the protein's secondary and tertiary structure; hence, collagen's backbone configuration and bonding determine the exact peak position of Amide I and Amide II 1.[30] Peaks obtained at 1321 cm^{-1}, 1232 cm^{-1}, and 1196 cm^{-1} are due to the C-N stretching and N-H bonding vibration from amide linkage as well as wagging vibration of CH_2 group in glycine backbone and prolines and assigned as Amide-III.[31] The peak appeared at 1450 cm^{-1} arising from typical amide vibration assigned to C-N stretch and N-H wagging. The peak at 1196 cm^{-1} and 1111 cm^{-1} is assigned to C-O-C, and the peak at 1031 cm^{-1} is assigned to C-O stretching vibration.[1] The absorption peaks at 2852 cm^{-1} and 2921 cm^{-1} are assigned to the aliphatic C-H, CH_2, CH_3 stretching of the aliphatic side chain of amino acid. The peak at 3075 cm^{-1} and 3300 cm^{-1} overlapped with a broad peak due to the OH group identified as

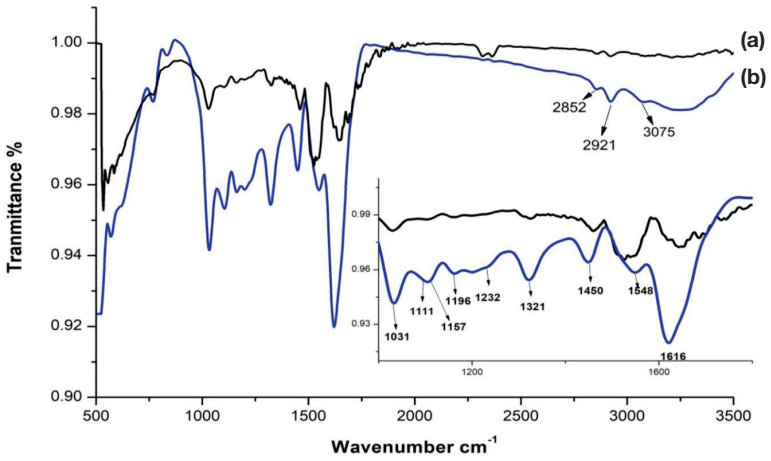

Figure 2. FTIR analysis of a) untreated b) enzymatically treated goat skin

Amide B and Amide A, respectively. These peaks are derived from a fermi resonance between the first inference of amide II and N-H stretching vibration.[1]

As seen in Figure 2, no changes in the overall spectrum of skin treated with the enzymatic treatment were observed compared to the IR spectrum of the untreated skin. Enzymatic treatments profoundly affect the peak positions and result in the development of some new peaks without affecting the overall spectrum. The enzymatic treatment increased the intensity of peaks in general; the intensity of peaks within 1196-1111cm^{-1} increased in the spectrum of the enzyme-treated skin.[1,32] These indicate enzymatically induced changes in the chemical composition of the grain surface of the skin. The enzymatic treatment did not result in changes in the position of major amide peaks, but after the enzymatic treatment, the intensity of Amide III and Amide II increased and the peaks broadened to some degree. In the case of the enzyme-treated skin, the peak position of amide I in the spectrum shifted a little to 1616cm^{-1}, along with a shape change and significant increase in intensity. As amide bands (particularly amide I) are comprised of various bands dependent on secondary conformations of collagen, the changes noted in the IR spectrum of the enzymatically treated skin imply a slight modification of collagen due to the enzyme treatment and removal of denatured collagen. The difference between the peak position of Amide I and Amide II indicates the presence of denatured collagen if the difference is higher than 100cm^{-1}. However, in both, the spectrum difference between the peak position is less than 100cm^{-1} and lower in the case of enzymatically treated skin compared to untreated skin. So it can be concluded that the enzymatic treatment had not affected the triple helix conformation of collagen but removed the denatured protein from the skin.[1,30,33] Apart from this, the enzyme-treated skin decreased intensity, and a shift in the position of amide B was observed. The alkyl C–H stretching bands at 2921cm^{-1} and 2852cm^{-1} remain unaffected; the Amide A and Amide B peaks became intense.

4.3 XPS analysis:

XPS analysis of grain surface of untreated and enzyme-treated skin was carried out to study changes in chemical composition due to the enzymatic treatment (Enzyme concentration: 1% w/v, pH: 4.5, MLR: 1:20, Temperature: 50°C, Treatment time: 60 minutes). Figure 3 presents the XPS survey scan for the untreated and the

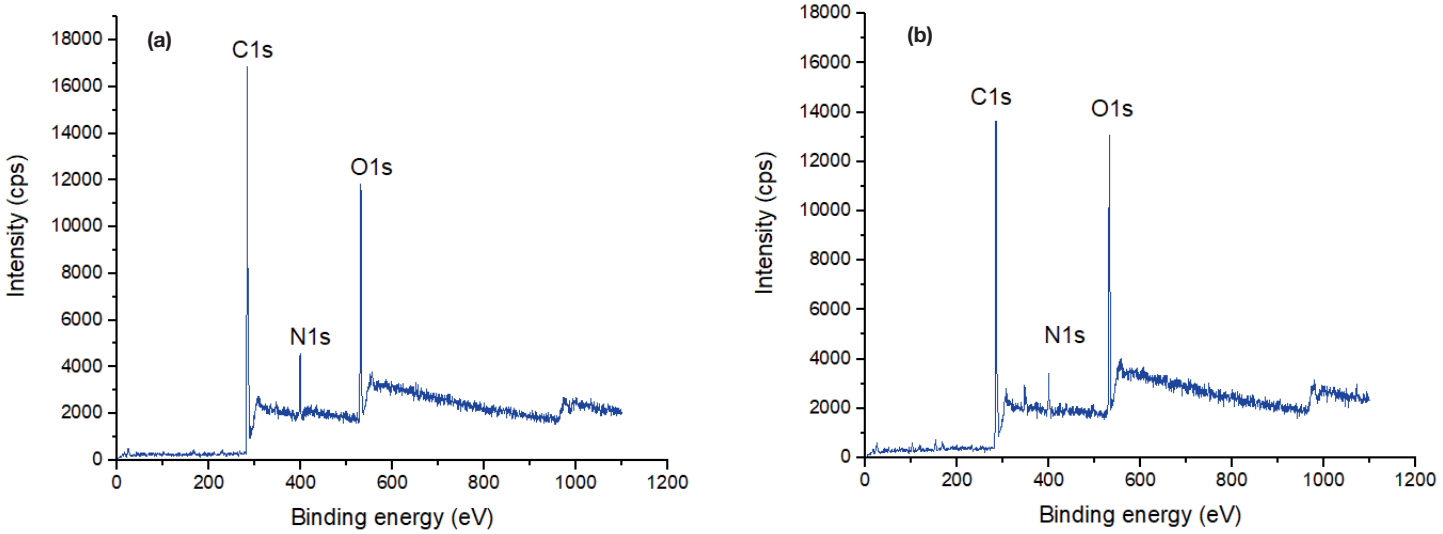

Figure 3. XPS survey scan spectra of **a)** untreated and **b)** enzymatically treated goat skin

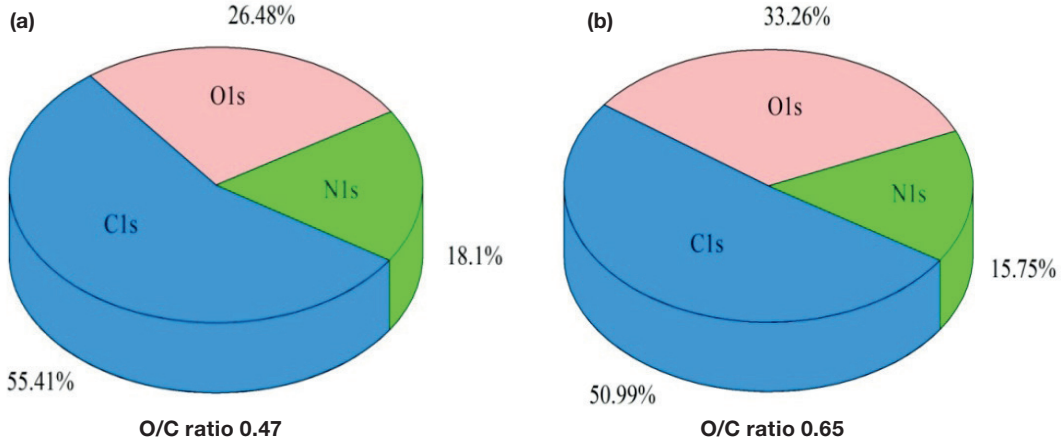

Figure 4. Elemental composition of **a)** untreated **b)** enzymatically treated goat skin

enzyme-treated goat skin. As revealed in Figures 3 and 4, the elemental composition of the grain surface of the goat skin changed significantly after the enzyme treatment. XPS survey analysis revealed that the grain surface of the enzymatically treated goat skin surface has higher oxygen content than the grain surface of untreated goat skins, which was further quantified from the high-resolution survey. The O/C atomic ratio of untreated goat skin grain surface was found to be 0.477, which increased to 0.65 after treatment with the acid protease enzyme. The enzyme treatment increased oxygen concentration from 26.8% for untread skin to 33.2%, with an increase of 6.78% relative to untreated goat skin. This considerable upsurge in the atomic O/C ratio shows that new oxygen-containing groups are being exposed to the grain surface after the enzymatic treatment. The enzymatic treatment decreased nitrogen concentration from 18.1% for untreated skin to 15.75% for the enzyme-treated skin, and carbon content also changed. These overall transitions in surface chemistry can result from breaking peptide bonds and loss of amino acids due to the acid protease treatment. The enzyme treatment can lower the carbon content by forming certain volatile products and also help to expose the group containing oxygen.[2,4]

4.4 TG and DTG analysis:

The impact of the enzyme treatment on thermal stability and thermal degradation of the goat skin was studied by TG and DTG analysis, and the acquired TG and DTG thermograms are shown in Figure 5. As revealed in Figure 5, non-isothermal degradation of goat skin occurred in three successive stages. In the initial stage, both untreated and the enzyme-treated (Enzyme concentration: 1% w/v, pH: 4.5, MLR: 1:20, Temperature: 50°C, Treatment time: 60 minutes) skin showed similar and smooth weight loss (10%) profiles, which attributed to the loss of bound and unbound water. During this stage, moisture present in untreated and the enzyme-treated skin sample entirely lost; this stage was endothermic.[34,35] The successive stages were exothermic, involving thermo-oxidation and decomposition of the goat skin samples. The skin samples' thermic oxidation and decomposition started at 320°C and continued up to 800°C. Untreated goat skin showed a melting phase with a sharp peak at 336°C, while the enzyme-treated skin sample showed a melting phase with a sharp peak at 369°C. The results indicated that the enzyme-treated goat skin exhibited higher thermal stability and sharped weight loss than those observed for untreated skin samples 35. The obtained TGA data were converted to a DTG curve for better comprehension. As seen in Figure 5, the first peak in the DTG curve corresponds to the loss of water content from the samples, and the second peak is related to denaturing of the goat skin protein structure due to the decomposition of the peptide bonds and thermal degradation subsequently. Sharper and higher amplitude peaks obtained for the enzyme-treated skin compared to untreated skin indicate that structural alternation occurred due to the enzymatic treatment, further characterized by XRD analysis.[33]

4.5 XRD analysis:

XRD analysis was conducted to study the effects of the enzymatic treatment on goat skin's structural properties and crystalline structure. Figure 6 shows the XRD pattern of untreated goat skin and the enzyme-treated (Enzyme concentration: 1% w/v, pH: 4.5, MLR: 1:20, Temperature: 50°C, Treatment time: 60 minutes) goat skin. As seen from Figure 6, for both untreated and enzyme-treated goat skin, one high amplitude peak at 2θ value of about 20.8° and small peaks at 2θ value of about 7.1°, 30.1°, 40.2° were obtained; further details about peaks are given in Table I. The obtained XRD patterns indicate the amorphous nature of the material of which skin is composed. The sharp peak at a 2θ value of about 7.1° corresponds to the crystalline component of the skin. Whereas at 2θ value of about 20.8°, a broad peak due to the amorphous component of goat skin is observed.[37] The broad peak observed at 2θ value of about 20.8° assigned to reflections by the weave of collagen fibers which cause the diffuse peaks. The intensity of this peak represents the dimensional structure regularity of collagen and crosslinking degree.[38] A decrease in intensity of peak observed at 2θ value of about 20.8° indicates that compared to untreated goat skin, the enzyme-treated goat skin has less compact and tightened weaving

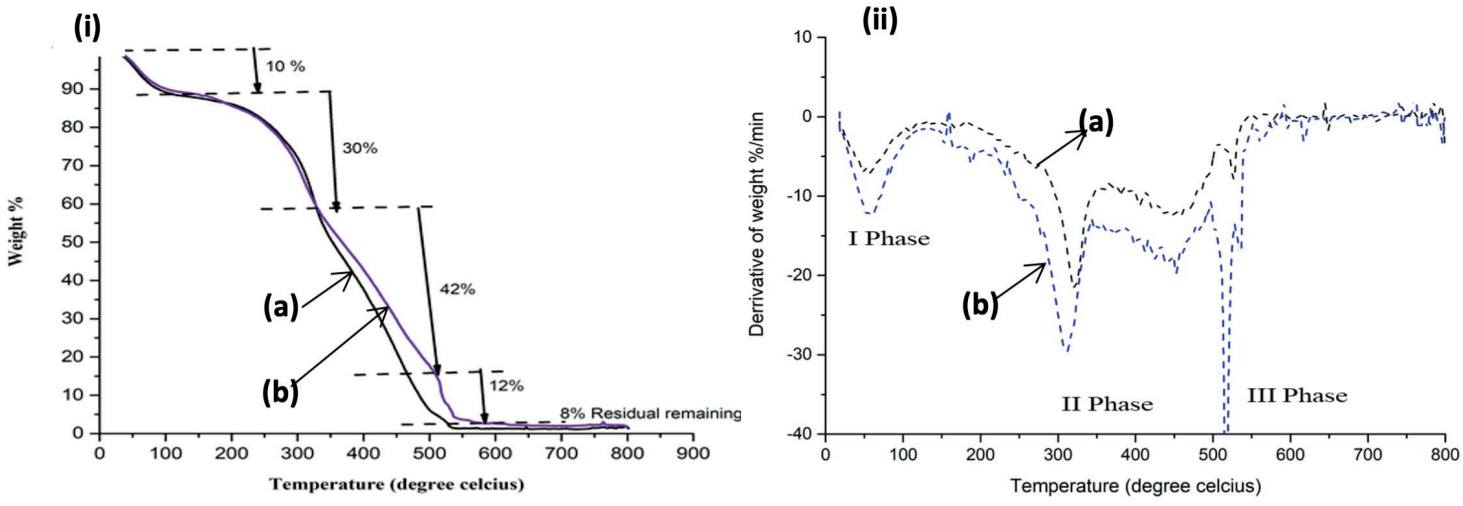

Figure 5. **i)** TGA graph **ii)** DTG curve of **a)** untreated and **b)** enzymatically treated goat skin

Table I

XRD parameter of untreated and enzymatic treated goat skin

Particular	2θ value	FWHM	d spacing (nm)	Crystallinity index
Untreated goats skin	7.1°	8.3159	12.440	73%
	20.8°	10.2353	4.267137	
	30.1°	3.6717	2.96655	
	40.2°	31.73	2.241462	
Enzymatic-treated goat skin	7.1°	8.7615	12.440	65.73%
	20.8°	10.3001	4.267137	
	30.1°	5.74588	2.96655	
	40.2°	29.303	2.241462	

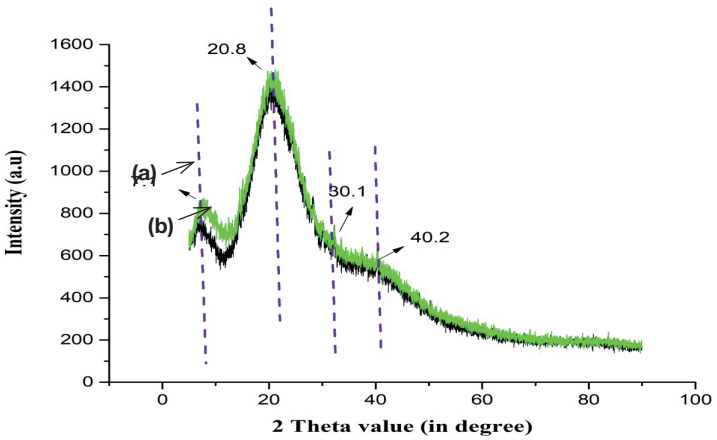

Figure 6. XRD analysis of **a)** untreated **b)** enzymatically treated goat skin

of collagen fibrils at grain surface as a consequence of the enzyme treatment.[36] The peak position of α-helix (2θ value of around 10°) and β-sheets (2θ value of around 20°) remains unaffected even after the acid protease treatment.[37,39] Thus, the enzymatic treatment with acid protease modified the structural properties of the goat skin; still, the bulk structure of the skin remained unaffected. The crystallinity index of untreated skin was about 73%, which was reduced to 65.73% in the case of enzyme-treated skin. Thus, it is worth noticing from the XRD pattern that the enzymatic treatment increased the amorphous part without affecting the bulk structure of the goat skin.[37] This amorphous portion helps increase the hydrophilicity of the skin, which ultimately affects its absorption capacity for natural antimicrobial extracts.[31,37]

4.6 SEM analysis:

Figure 7 shows the morphology of the grain surface of untreated and enzyme-treated (Enzyme concentration: 1% w/v, pH: 4.5, MLR:

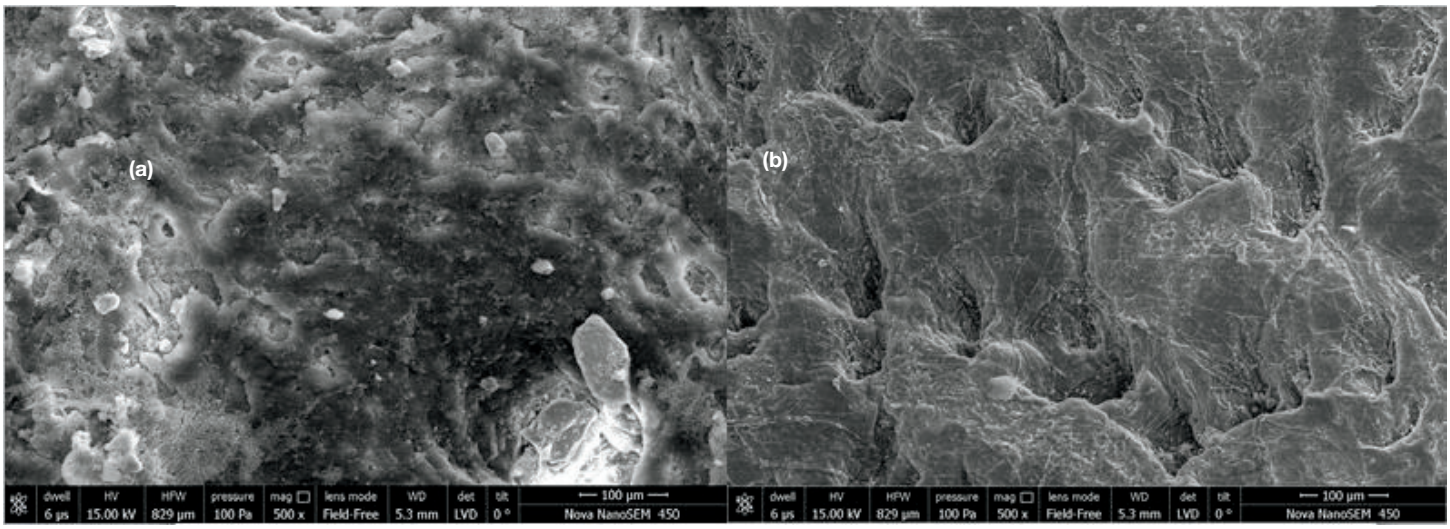

Figure 7. Morphological observations of grain surface of **a)** untreated and **b)** enzymatically treated goat skin

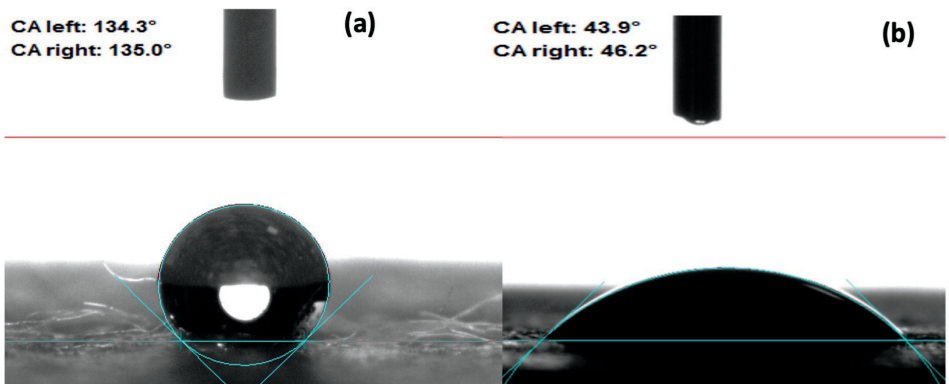

Figure 8. The water contact angle at the grain surface of **a)** untreated and **b)** enzymatically treated goat skin

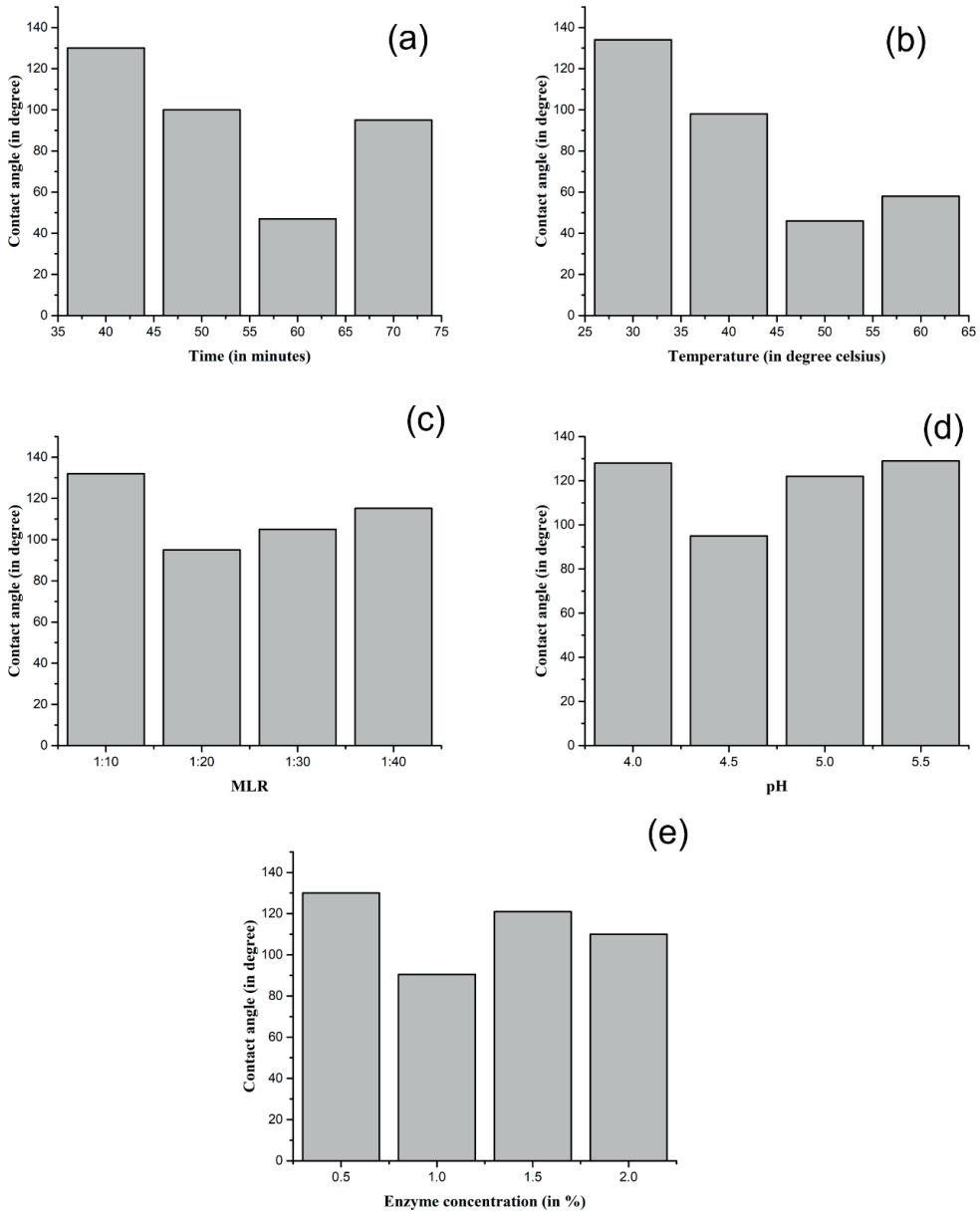

Figure 9. Changes in water contact angle at grain surface of the enzyme-treated goat skin for different treatment variables **a)** Treatment time **b)** Temperature **c)** Material to liquor ratio **d)** pH **e)** Concentration of the enzyme.

1:20, Temperature: 50°C, Treatment time: 60 minutes) goat skin. It revealed from the SEM image that the untreated skin's grain surface has a non-uniform structure with many asperities. The acid protease treatment resulted in the detachment of interfibrillar structure, opening of some fibers at the macrolevel, removal of non-collagenous substances, and modification of micropores. Thus, acid protease treatment caused the loosening of the grain surface; as revealed in Figure 7, the grain surface of goat skin became cleaner, uniform, and more homogeneous.[1]

4.7 Water contact angle measurement:

The untreated goat skin presented a hydrophobic character; when the water contact angle was measured at the grain surface of the untreated skin, the water contact angle value of 135°±.0.5 (Figure 8) obtained. After the enzymatic treatment, the wettability of the goat skin increase.

The increase in wettability/hydrophilicity of the goat skin after the enzymatic treatment and the effect of different process variables on wettability were studied using water contact angle measurement at the grain surface presented in Figure 9. For optimum enzymatic treatment conditions (Enzyme concentration: 1% w/v, pH: 4.5, MLR: 1:20, Temperature: 50°C, Treatment time: 60 minutes), the water contact angle at the grain surface of the goat skin was reduced from 135°± 0.5 to 45.6°±1 as illustrated in Figure 8. The improvement in wettability after the surface treatment was attributed to the removal of cementing substances between the collagen, impurities, modification in surface properties, and removal of nanoscale roughness at the grain surface.

4.8 Effect of the enzymatic treatment on mechanical strength and organoleptic properties:

The effect of the enzymatic treatment (Enzyme concentration: 1% w/v, pH: 4.5, MLR: 1:20, Temperature: 50°C, Treatment time: 60 minutes) was analyzed on the mechanical strength of the skin as tensile strength, elongation at break, and tear strength. The results of the mechanical strength analysis are reported in Table II. The results reveal that the enzymatically treated goat skin has slightly better mechanical properties than untreated. Due to enzymatic treatment, the surface of the goat skin becomes more hydrophilic. It was observed from SEM analysis that the enzyme-treated goat skin has well defined porous and opened fiber structure. Due to the collagen's open structure and impurities' removal, the goat skin's tensile strength has slightly increased.[2,6] The results for organoleptic properties are presented in Figure 10. In analyzing organoleptic properties by visual and hand assessment, the enzyme-treated skin was found to have comparable results with that of untreated skin. The enzyme treatment modified the skin's

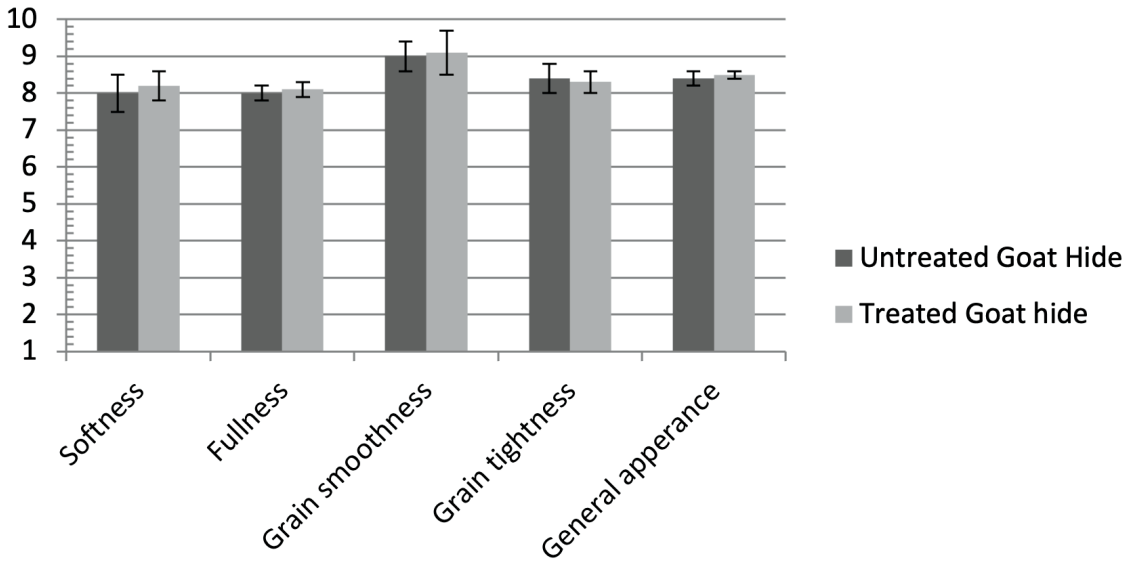

Figure 10. Assessment of organoleptic properties of untreated and enzyme-treated goat skin; the presented result is an average value of 3 determinations: Scale of 1-10, Poor-1, Best- 10

Table II

Results of mechanical strength analysis for untreated and enzyme-treated goat skin.

Experiment	Tensile strength (Kg/cm²)	% Elongation at break	Tear strength (Kg/cm)
Untreated Skin	190	78	60
Enzyme treated skin	200	81	75

Table III

Quantitative analysis of non-collagen skin components of the skin.

Non-collagen components	Control	Enzyme treated skin
Sulfated GAG (µg/g)	480	785
Uronic acid (µg/g)	2457	1000
Hexosamines (µg/g)	3485	1100

grain surface by opening the collagen matrix and removing impurities and asperities, providing better organoleptic properties such as softness, smooth grain surface, fullness, and appearance, as shown in Figure 10.[18,19]

4.9 Effect of the Enzymatic Treatment on Non-collagen Components of the Goat Skin:

Quantitative estimation of non-collagen components of untreated and enzyme-treated skin (Enzyme concentration: 1% w/v, pH: 4.5, MLR: 1:20, Temperature: 50°C, Treatment time: 60 minutes) was performed to analyze the effect of the enzymatic treatment. Reduction in interfibrillar constituents (Uronic acid, Hexosamines) was observed after the enzymatic treatment, whereas sulfated glucose amine glycan increased after the enzymatic treatment. The reduction in non-collagen components was significant in the enzymatic process due to the site-specific action of the enzyme; the results are presented in Table III. In skin/leather's triple matrix structure, collagen fiber is more densely packed due to these interfibrillar substances. These non-collagenous substances hinder diffusion and affect goat skin's adsorption/uptake of chemicals/finishing agents, which requires penetration during processing/finishing. The degree of opening of the collagen matrix of skin/leather is directly related to the extent of removal of interfibrillar substances. So, eliminating these substances is an inevitable step for producing better quality skin.[2,40]

4.10 Identification of active constituents of the natural extracts:

4.11 Antimicrobial activity:

To impart antimicrobial finish, untreated and enzyme-treated (Enzyme concentration: 1% w/v, pH: 4.5, MLR: 1:20, Temperature:

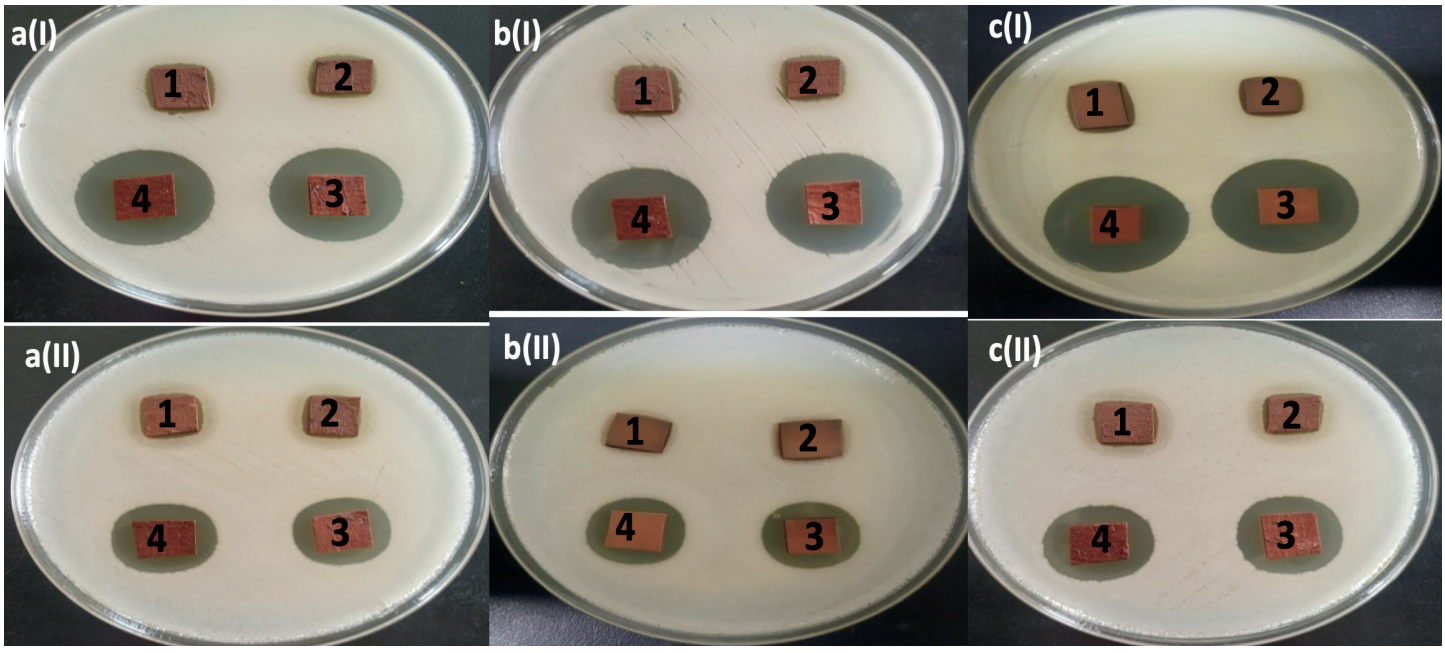

Figure 11. Zone of inhibition obtained due to antimicrobial finish with 5% w/v extract of leaves of a *Azadirachta indica* against *Escherichia coli* **a(i)** and *Staphylococcus aureus* **a(ii)**; b *Ocimum sanctum* against *Escherichia coli* **b(i)** and *Staphylococcus aureus* **b(ii)**; c *Camellia sinensis* against *Escherichia coli* **c(i)** and *Staphylococcus aureus* **c(ii)**. 1- Pieces of untreated skin, 2- Pieces of skin with the enzymatic treatment, 3- Pieces of untreated skin finished with the natural extracts, and 4- Pieces of enzyme-treated skin finished with the natural extracts, respectively.

Table IV

Zone of inhibition (cm) obtained for untreated skin finished with the natural extracts (UT+EX) and the enzyme-treated skin finished with the natural extracts (ET+EX)

Antimicrobial finish with	Zone of inhibition obtained against test microorganism			
	E. coli		*S. aureus*	
	UT+EX	ET+EX	UT+EX	ET+EX
5% w/v leaves extract of *Azadirachta indica* (Neem Tree)	2.53±0.05	2.70±0.1	1.9±0.15	2.36±0.15
5% w/v leaves extract of *Ocimum sanctum* (Holy Basil, Tulsi)	2.4±0.1	2.7±0.1	2.03±0.02	2.23±0.04
5% w/v leaves extract of *Camellia sinensis* (Green Tea)	2.3±0.4	3.03±0.05	2.03±0.4	2.33±0.1

Table V

Percentage reduction of bacteria obtained by modified Hohenstein test for the enzyme-treated and untreated skin samples finished with the 5% w/v natural extracts; UT = Untreated skin, ET = skin with the enzymatic treatment

Test Microorganism	*Ocimum sanctum* extract (Holy Basil)		*Camellia sinensis* extract (Green tea)		*Azadirachta indica* extract (Neem)	
	UT	ET	UT	ET	UT	ET
E. coli	85%±0.05	95%±0.06	88%±0.04	96%±0.03	86%±0.04	97%±0.08
S. aureus	82%±0.02	95%±0.01	81%±0.03	87%±0.05	83%±0.03	92%±0.03

50°C, Treatment time: 60 minutes) goat skin was treated with 5% w/v of leaves extract of *Camellia sinensis* (Green Tea), *Azadirachta indica* (Neem Tree) and *Ocimum sanctum* (Holy Basil, Tulsi). Zone of inhibition obtained against test microorganisms *Escherichia coli* and *Staphylococcus aureus* due to the antimicrobial finish presented in Figure 11. The obtained zone of inhibition was measured, and the details are presented in Table IV.

As seen from Figure 11, untreated and enzyme-treated skin without antimicrobial finish, which was kept as control, showed robust growth of the test microorganisms without an inhibition zone around them. This growth of test microorganisms indicates that untreated and enzyme-treated skin does not exhibit antibacterial activity. However, when untreated goat skin and the enzyme-treated goat skin provided an antimicrobial finish with the natural extracts, a remarkable zone of inhibition was obtained for all three natural extracts against both the test microorganism.

As reported in Table IV, the enzymatically treated and the natural extracts coated goat skin have a high zone of inhibition than untreated and extracts coated goat skin against both the bacteria. Quantitative analysis of antimicrobial finish is evaluated as percentage reduction of bacteria using a modified Hohenstein test, and obtained results are presented in Table V. The results obtained using the modified Hohenstein test agree with the zone of inhibition results. As presented in Table V, a higher value for the percentage reduction of bacteria was obtained in the case of the enzyme-treated skin finished with natural extracts compared to untreated skin finished with natural extracts. This improvement in the antimicrobial finish could be because enzymatic scouring eliminates the non-collagenous substances, resulting in an open skin structure and increasing the antimicrobial agent absorption.

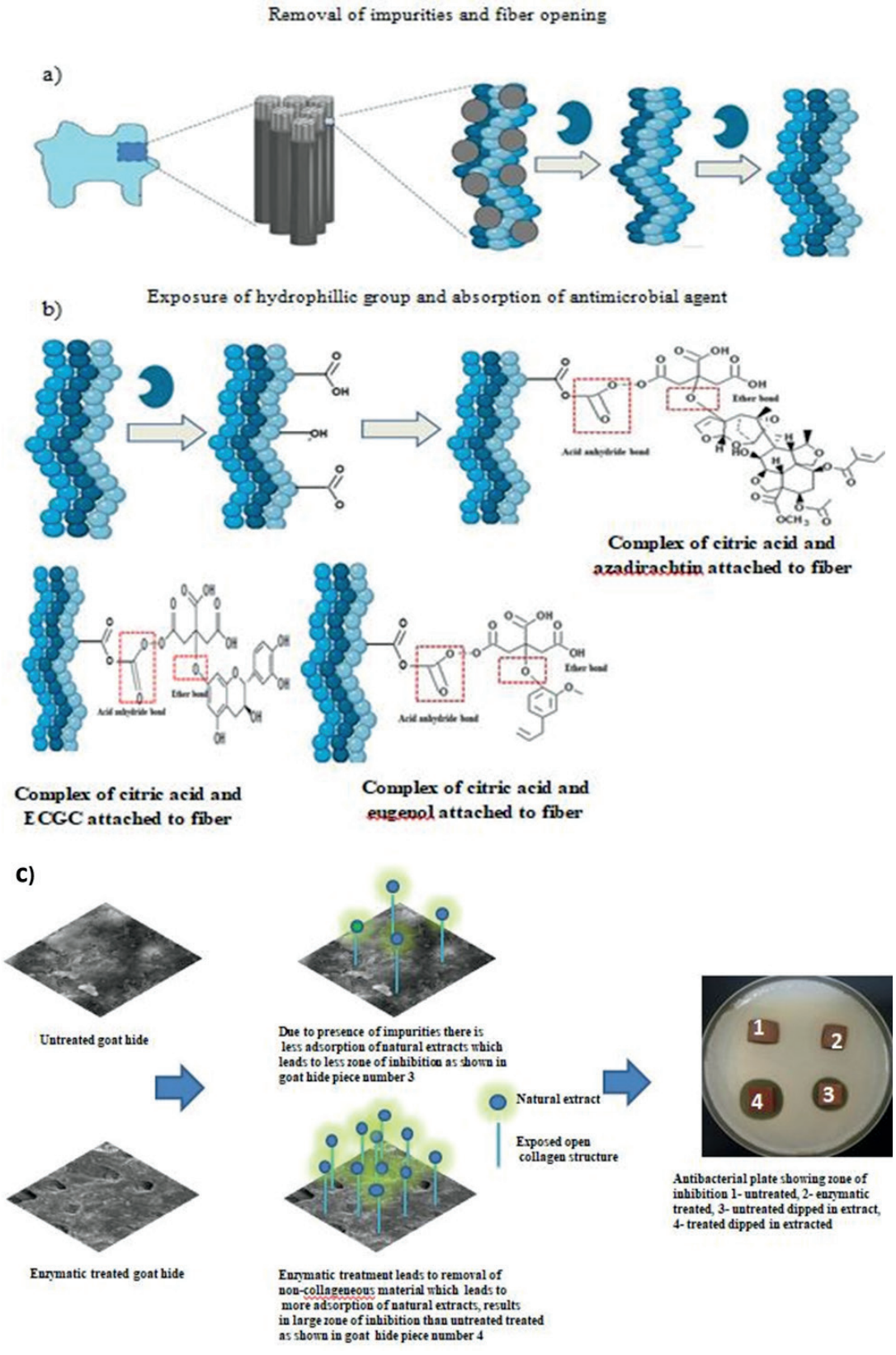

Figure 12. Overall scheme of interaction of treated and untreated goat skin with natural extract

Many scientific studies have alleged that natural plant extract comprises different antimicrobial agents such as flavonoids, terpenoids, polyphenols, tannins, etc.[41] The key ingredients which are responsible for the antimicrobial activity of *Ocimum sanctum*, *Azadirachta indica*, and *Camellia sinensis* leave extracts are eugenol, azadirachtin, and catechin, respectively.[42–47] These antimicrobial agents may significantly alter the lipid layers of bacterial cells or produce morphological changes that inevitably alter the cell membrane's porosity and cause cell degranulation.[47,48]

Skin/leather is a material with variations within the three-dimensional matrix of collagen fibrous weave, which provide a unique nature and governs penetration/uptake of an antimicrobial agent or any finishing agent utilized to impart specific properties. Due to the skin/leather matrix variation, antimicrobial agent penetrates to lesser or greater depth and in varying proportion. Thus, skin/leather provides different affinities and accessibility for antimicrobial agent/finishing agents, and affinity very much depends on the structure and state of skin/leather. The interfibrillar cementing substances pack the collagen fibers, make them hydrophobic and prevent the diffusion of various agents that need to impart new quality to the leather. This study observed a significant decrease in non-collagen components uronic acid and hexosamine with a slight increase in sulfated glucosamine glycan (GAG) after the enzymatic treatment with an acid protease, as reported in Table III. This decrease in non-collagen components compared to control skin after the enzymatic treatment results in the opening of collagen fiber bundles and modification in the three-dimensional matrix of collagen fibrous weave, thus improving wettability and organoleptic properties of the enzyme-treated skin as reported under section 4.7 and 4.8 respectively.

This opening could further accelerate the penetration of the acid protease enzyme through the collagen matrix to act upon anchoring the interfibrillar component. The protease enzymes are known for hydrolyzing the interfibrillar non-collagenous skin components and loosening the collagen weave matrix. Different studies reported similar results with a different class of protease enzyme.[16,49] As revealed from the SEM analysis, the grain surface of skin treated with the acid protease enzyme appeared smoother, uniform, and more homogeneous with observed pores for hair follicles compared to untreated goat skin. The chemical composition of the grain surface of the skin changed after the acid protease treatment without much effect on the bulk properties of the skin. The changes in the chemical composition of the skin after the acid protease treatment studied by ATR-FTIR analysis and XPS indicate an increase in oxygen-containing functional groups at the grain surface as reported in sections 4.2 and 4.3; the XPS study showed an increase in O/C ratio after the enzymatic treatment.

Further, in the XRD analysis, an increase in amorphous properties was observed after the enzymatic treatment of goat skin. These changes in surface chemical composition and decrease in the crystalline fraction of the skin due to the enzymatic treatment provides better affinity and accessibility for the antimicrobial agent present in the natural antimicrobial extracts. The schematic diagram for the interaction of the natural antimicrobial extracts with untreated and enzyme-treated goat skin is presented in Figure 12. As presented in Figure 12, the enzymatic treatment with acid protease enzyme increase the affinity and accessibility of active antimicrobial components of the natural extracts at a time, provides more functional sites for interaction of active components present in these natural extracts, and better attachments due to electrostatic attractions and hence better antimicrobial property.

It can be inferred that the acid protease treatment of goat skin has improved wettability due to the removal of non-collagen components and matrix opening, which eventually led to better absorption of the natural extracts and, thus, enhanced antimicrobial activity. The experiments' results thus allude to the potential of enzymes for environmental-friendly skin processing/leather production. Using enzymes and natural antimicrobial extracts for skin/leather processing would be a privilege for the leather industry workers and the environment, with the ability to replace hazardous chemicals used for skin preservation/tanning operations and provide antimicrobial properties.

5 Conclusions

In this study, goat skin at the pre-tanning stage was treated with the acid protease enzyme; treatment variables such as treatment time, temperature, enzyme concentration, pH, and MLR were studied to optimize the treatment. It was observed that the enzymatic treatment has a profound effect on the goat skin's physical and chemical structure, resulting in improved organoleptic parameters without affecting mechanical strength. The acid protease treatment of goat skin resulted in the removal of non-collagen components from the skin without much damage to the collagen fibrous matrix structure of the skin as conformed with ATR-FTIR spectroscopy, TG/DTA analysis, XRD and SEM characterization, and mechanical strength analysis. The enzymatic treatment also resulted in significant improvement in wettability as observed by measurement of the water contact angle at the grain surface, which reduced to 45.6°±1 after the enzymatic treatment

from 135°± 0.5 for untreated skin. Because of these modifications in the chemical and physical properties of the enzyme tread goat skin, the enzyme-treated skin has better affinity and uptake for the natural antimicrobial extracts obtained from leaves of *Camellia sinensis* (Green Tea), *Azadirachta indica* (Neem Tree) and *Ocimum sanctum* (Holy Basil, Tulsi) and thus better antimicrobial finish which tested against test microorganism *Escherichia coli* and *Staphylococcus aureus*. The acid protease treatment can be utilized as an environmentally friendly alternative to conventional wet chemical treatment to improve antimicrobial finish; the use of enzymatic treatment combined with natural extracts renders an overall environment-friendly antimicrobial leather/leather product.

Acknowledgments

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Competing Interests

The authors declare that they have no conflict of interests.

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Effect of Acid Swelling and Its Impact on the Properties of Cow Industrial Glove Leathers

by

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Abstract

The development of commercially successful salt-free chrome tanning technology is the need of global leather sector to reduce the total dissolved solids in wastewater. Though some attempts have been made to develop salt-free chrome tanning technology, drawbacks such as slow diffusion of chromium, unevenness in softness and other organoleptic properties are the major concern. The generation of localized acid swelling might be a reason for the above drawbacks and no scientific literature available for the same. Hence, it is important to analyze the effect of acid swelling and its impact on the diffusion of chromium, ageing characteristics of wet-blue leather and physical properties of crust leather. The results show that the acid swelling delays the diffusion of chromium in tanning process and also enhances the growth marks on wet-blue leather. The ageing study reveals that the wet-blue obtained from acid swollen system dehydrates faster than the conventional salt based chromium tanning system. In addition, the wet-blue leather from acid swollen system is prone to fungal attack. The industrial glove leather obtained from acid swollen system has reduced strength characteristics viz., 15% reduction in tensile strength, 17% reduction in tear strength and 20% reduction in grain crack/bursting strength. Further, the degree of heterogeneity in softness is high for crust leather obtained from acid swollen system and also more looseness with internal emptiness. Color value measurements reveal that the crust leather obtained from the conventional tanning system is lighter in shade than the acid-swollen system. The results will be useful to design commercially viable salt-free or low-salt tanning systems as well as to tackle the inadvertent industrial scenario where the tanners are looking for solutions for accidental acid swelling and subsequent salvage of the leathers for possible recovery and applications.

Introduction

Sodium chloride is commonly used in the pickling process to avoid the osmotic swelling of collagen fiber during the acid treatment. More specifically, sodium chloride acts as an electrolyte that eliminates the Donnan potential and subsequently prevents the swelling of the collagen matrix.[1] Though it found a prominent place in leather making, the drawbacks such as discharge of high total dissolved solids (TDS) load in wastewater[2] and the requirement of a sophisticated end-of-pipe treatment system motivate leather manufacturers to develop salt-free tanning systems. Considerable attempts have been made to develop salt-free tanning systems such as (a) pickling with non-swelling acid and (b) pre-treatment using weak organic acid.

i. ***Pickling with Non-Swelling Acid*** – In this method, aromatic sulfonic acids such as naphthol 3-6-disulphonic acid, p-hydroxy diphenyl sulphonic acid, blend of naphthalene and naphthol sulphonic acids, and polyacrylic acid have been used in pickling process up to the level of 2% offer without salt to adjust the pH to 2.8-3.0. Subsequently, tanning is done using basic chromium sulfate (BCS) followed by regular basification. Further, the variation in swelling as the function of chemical compounds and its influence on pickling/tanning baths and also the mechanical properties of leather have been studied in detail.[3-4]

ii. ***Pre-treatment with Weak Organic Acid*** – In this method, the delimed pelt is pre-treated with weak organic acids like acetic acid, formic acid, and its mixture to adjust the pH up to 5.0-5.5 without salt. Subsequently, the pelts are treated with BCS without basification.[5-8]

Though the salt-free tanning systems are environmentally sustainable the following limitations have been observed based on our research experience and also feedback obtained from the tanners.

1. Achievement of complete penetration of chromium is difficult for thicker hides (complete penetration is normally achieved during ageing).
2. Requirement of longer neutralization than for conventional wet-blue.
3. Need for modification in the post-tanning process recipe.
4. Unevenness in softness.

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Hence, it is important to understand the causative factors for the above-mentioned limitations in order to develop a commercially successful salt-free tanning system.

Further, the authors hypothesized that the nucleation of localized acid swelling during the addition of BCS could be a possible reason. In addition, there are situations in leather industries where occasionally the tanners are faced with the problem of acid swelling inadvertently and there is no scientific literature available currently to salvage the swollen pelts. Hence, a study on acid swelling and its effect on the physical properties of final leather may help to understand the above problems. Herein, we carried out conventional pickling-basification based chrome tanning process with and without the addition of salt to understand the effect of swelling on the physico-chemical properties of the tanned leathers. After tanning, the leathers were post-tanned to prepare industrial glove leathers and their morphology, physical and organoleptic properties were analyzed.

Materials and methods

Wet-salted cow hides were conventionally processed and taken as raw material for chromium tanning. All the chemicals used for leather processing were of commercial grade and analytical grade chemicals used for analysis.

Chromium Tanning and Post-Tanning Process

The delimed pelts were cut along the backbone and the left (With Acid Swelling -WAS) halves were subjected to acid swelling (pH: 3.0) without the addition of sodium chloride whereas right halves (Without Acid Swelling-WOAS) were subjected to a conventional pickling process (Sodium chloride + Sulfuric Acid) followed by chromium tanning. The detailed process recipe is given in Table I. Further, the obtained wet-blue leathers were subjected to mechanical operations and converted into industrial glove leather as per the post-tanning process recipe given in Table II.

Analysis of Thermal Stability

Hydrothermal stability of the pelts and wet-blue leather was measured using SATRA shrinkage tester (SATRA STD 114).

Ageing Characteristics of Wet-Blue Leather

The wet-blue leathers obtained from both experimental/control processes were piled at room temperature (35°± 1°C) and stored in an airtight cover. The leathers were continuously monitored for changes in color, fungal growth and, drying nature.

Microscopic Analysis

Celestron handheld microscope and the Phenom tabletop scanning electron microscope were used to analyze the surface/cross-sectional morphology of the leathers.

Table I

Process recipe for tanning the delimed cow pelts

WAS (Left halves)		WOAS (Right halves)	
Acid Swelling		***Pickling***	
Water	100%	Water	100%
Salt	0%	Salt	10%
Sulfuric acid Water	1.2% 10% 4 × 10min + 120 min pH: 3.0	Sulfuric acid Water	1.2% 10% 4 × 10min + 120 min pH: 3.0
Tanning			
BCS	6% 2 × 30 min + 180 min	BCS	6% 2 × 30 min + 180 min
Basification			
Sodium formate	1% - 30 min	Sodium formate	1% - 30 min
Sodium bicarbonate Water	1% 10% 3 × 20 min + 180 min pH: 4.0 Drain/Pile	Sodium bicarbonate Water	1% 10% 3 × 20 min + 180 min pH: 4.0 Drain/Pile

Table II

Process recipe for the manufacture of cow industrial glove leather from chrome tanned cow wet blue (Thickness: 1.0-1.1 mm)

Process/Chemicals	% Offer	Time	Remarks
Wetting back			
Water	100		
Wetting agent	0.1	30 min	Drain
Acid bating			
Water	100		
Sodium formate	1	30 min	pH: 4.5-4.8
Acid bate	0.5	4 hrs	
Bleaching			
Sulfuric acid + Water	0.5 + 10	2 × 10 min + 30 min	
Potassium permanganate	0.5	60 min	
Oxalic acid	0.5		
Sodium bisulfite	0.5	60 min	Drain/Wash
Rechroming			
Water	100		
BCS	1.5	60 min	
Sodium formate + Sodium bicarbonate	0.25 + 0.5	pH: 4.0	O/N, Drain/Wash
Neutralization			
Water	100		
Sodium bicarbonate	1		
Ammonium bicarbonate	1	pH: 6.0-6.5	Drain/Wash
Fatliquoring			
TiO_2	1.5	30 min	
Synthetic fatliquor	15		
Sulfited fatliquor	5	150 min	
TiO_2	1	30	
Fixing			
Formic acid + Water	1.5 + 5	2 × 10 min + 60 min	Drain/Wash, drying and staking followed by dry milling (3 hrs). Final staking

Note: Process recipe collected from commercial industrial glove leather manufacturing unit in Kolkata, West Bengal, India.

Physical Strength Characteristics and Organoleptic Properties

Physical strength characteristics (tensile strength,[9] tear strength[10] and bursting strength[11]) of the crust leathers processed from experimental/control wet-blue were analyzed as per the standard methods. All the specimens were conditioned at 25°± 1°C and 65% RH for 24 hr[12] before the test performance. Further, a group of leather experts evaluated the final crust leathers and provided their remarks on different organoleptic properties such as softness, stretchiness, grain smoothness, uniformity, internal softness, looseness and touch.

Color Value Measurements

Color measurement parameters viz., L, a, b, were recorded using a Lambda 35 instrument for grain side of experimental and control crust leathers. "L" represents lightness, "a" value represents redness and greenness, "b" value represents yellowness and blueness. The wet-blue leathers were subjected to a controlled dehydration process before the measurement.[13]

Table III

Changes in weight of cow pelts and leathers

Process	WAS (kg)	WOAS (kg)
Deliming	3.4	3.6
Acid swelling/Pickling	4	3.0
After chrome tanning	3.2	3.2
After sammying	2.4	2.3

WAS – With Acid Swelling ; WOAS – Without Acid Swelling

Results and Discussion

Effect of Acid Swelling on Weight and Dimensional Properties

Collagen fiber, the leather-making protein, is considered as a charged material and the change of pH leads to an alteration of its charge. The alkaline pH leads to negative charge potential due to ionization of carboxylic group of amino acid sidechain whereas it becomes positive under acidic condition due to the protonation of amino group. Besides, the non-diffusible ionic nature of collagen matrix tends to produce an osmotic pressure gradient in an aqueous medium leading to osmotic swelling under acid/alkali conditions. Generally, the osmotic swelling ruptures the matrix structure and changes the dimensional properties. Further, the excess amount of water will be absorbed by the collagen matrix to equilibrate the osmotic potential.[1,14] The change in weight of cow hides subjected to acid swelling/conventional picking and subsequent tanning process is given in Table III.

It is evident from Table III that the acid swelling increases the delimed pelt weight up to 17% whereas 17% weight reduction was observed for conventional pickling and this may be due to the dehydration effect of sodium chloride used in pickling process. It is also worth noticing that the addition of tanning salt into the acid swollen pelt reduces the weight (-20%). The ionicity induced by neutral salt present in BCS and the formation of different charged chromium complexes may reduce the osmotic potential thereby reversing the swelling state in WAS system. In the case of conventional process, the weight has increased after the tanning (+6%) and it may be due to the absorption of chromium/neutral salts. It is also evident from Table III that after sammying, the weight reduction of acid swollen wet-blue is 3% lesser than the conventional wet-blue and it may be due to the high water holding capacity of the acid swollen wet-blue. The effect of acid swelling on pelt thickness at various processing stages is shown in Table IV. Because of the heterogeneity, the thickness was measured at various places and averaged.

It is evident from Table IV that the acid swelling increases the thickness and reduces the area. Whereas in conventional pickling, the thickness was reduced[14] and marginal increase in area was observed. The photographic images of the acid swollen and conventional pickled pelt are shown in Figure 1.

Effect of Acid Swelling on Chromium Diffusion

The diffusion of chromium at different time intervals of chrome tanning has been monitored for both WAS and WOAS systems. The micrographic images taken at different time intervals are shown in Figure 2. It is evident from the figure that the diffusion of chromium in WAS system is much slower than in the conventional salt-based tanning system. In the case of WOAS system, complete diffusion was achieved within 4 hrs whereas it is incomplete in the case of WAS system even after 24 hrs. The reason for delayed chromium diffusion in WAS system is unknown and a separate study needs to be conducted in order to understand the phenomena. However, the following factors may have contributed to the delayed chromium diffusion in WAS system.

Table IV

Changes in thickness and area of cow pelts and leathers

Process	WAS		WOAS	
	Thickness (mm)	Area (cm^2)	Thickness (mm)	Area (cm^2)
Deliming	1.63 ± 0.4	10531.3	1.55 ± 0.4	11121.2
Acid swelling/Pickling	2.52 ± 0.3	9684.1	1.36 ± 0.3	11513.5
After chrome tanning	2.20 ± 0.5	9080.3	2.05 ± 0.4	9880.8

WAS – With Acid Swelling ; WOAS – Without Acid Swelling

Figure 1. Photographic image of **(a)** acid swollen pelt and **(b)** conventional pickled pelt

a) Acid swelling may cause differential strain at different layers of collagen matrix and could have resulted in the loss of interconnectivity between pores.

b) Increase in fiber diameter may predominantly reduce the porosity contributed by macro pores. The study carried out with plant-based fibers revealed that the porosity/ permeability of the swollen fiber is reduced than the normal fibers.[15]

c) The removal of hyaluronic acid from the matrix is essential because its viscous nature inhibits the penetration of process chemicals. The hyaluronic acid carried forward after the beam house process is generally removed by neutral salt present in the pickling process by collapsing its gel structure. Due to the absence of neutral salt in WAS system, the carry forwarded hyaluronic acid may be transformed into a gel-like structure and act as a barrier for chromium diffusion. It is also reported that the presence of residual hyaluronic acid after the beam house processes inhibits the diffusion of chromium.[16]

Analysis of Hydrothermal Shrinkage Temperature

The hydrothermal shrinkage temperature of the cow matrix taken from different processing stages is given in Table V. Further, it is reported that the shrinkage temperature of the acid swollen pelt is below 45°C in the absence of salt.[17] As expected, the hydrothermal stability of acid-swollen pelt is lower (-27%) than the pickled pelt.

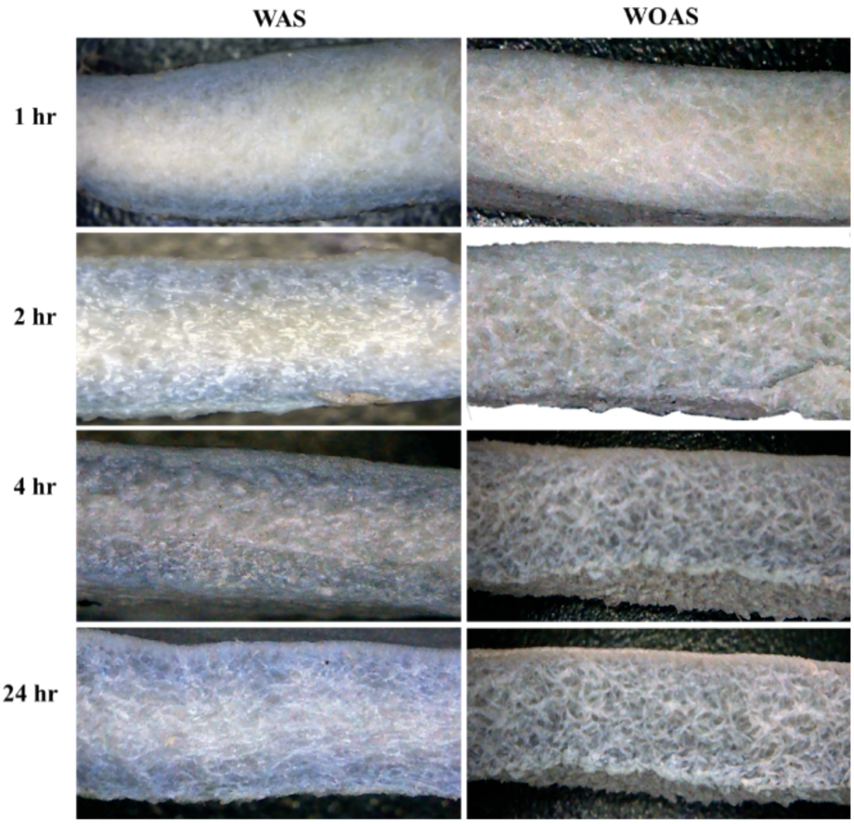

Figure 2. Micrographs taken at different time interval of chromium tanning

Table V

Hydrothermal shrinkage temperature at different processing stages

Processing Stage	WAS (°C)	WOAS (°C)
Deliming	59 ± 1	59 ± 1
Acid swelling/*Pickling	40 ± 2	55 ± 1
Chrome tanning (after basification)	98 ± 1	101 ± 1
After 48 hrs ageing	100 ± 1	105 ± 1

* Saline water (10% NaCl solution) used for shrinkage temperature measurement of pickled pelt

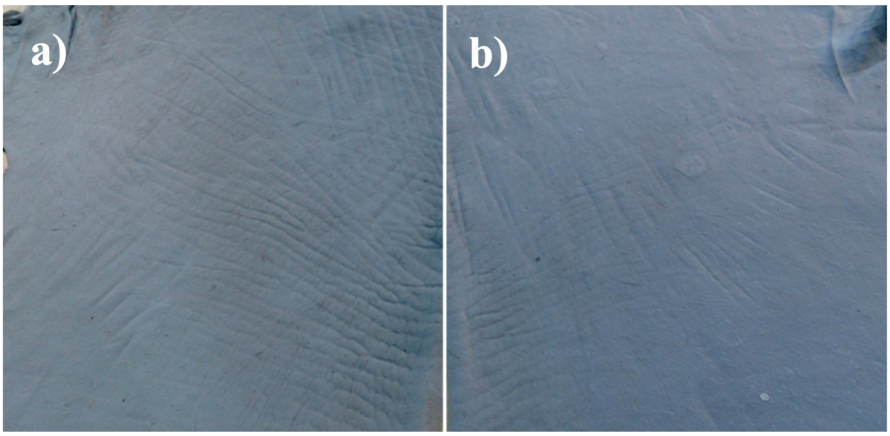

Figure 3. Photographic image of **(a)** WAS wet-blue and **(b)** WOAS wet-blue

Further, the shrinkage temperature of the wet-blue leather obtained from WAS is 98°C whereas it is 101°C for WOAS system. It may be due to the synergetic effect of improper chromium diffusion and change in inter-molecular interaction due to the acid swelling. However, shrinkage temperature is marginally improved after the ageing (48 hrs) process. Nevertheless, it is not comparable to the level of wet-blue obtained from the WOAS system.

Characteristics of Wet-Blue

The wet-blue leather obtained from WAS system is rubbery in nature. In addition, the color of chrome tanned leather is green with pronounced growth marks (Figure 3a). In the case of the WOAS system, grain pattern is uniform in nature (Figure 3b).

The wet-blue leathers obtained from both WAS/WOAS systems were stored at room temperature and continuously monitored. It is evident from Figure 4a that the fungal growth was observed on WAS-wet-blue within 7 days whereas no such growth noted in WOAS system. Besides, the wet-blue of WAS system loses its moisture content faster than the conventional wet-blue. After 30 days, the moisture content of the WAS wet-blue was 42% w/w whereas it was 60% w/w for WOAS wet-blue. The reason for faster fungal growth formation and drying of WAS wet-blue may be due to the lower salinity concentration.

It is demonstrated that the salinity inhibits the growth of fungal formation and the inhibition potential is directly proportional to the salinity concentration.[18] The presence of salinity limits the hyphae and mycelium growth of fungi. Further, the presence of salt decreases the vapor pressure of water and thereby avoids faster dehydration.[19] In addition, commercial sodium chloride contains some amount of potassium chloride which is hygroscopic in nature.

Hence, the decrease in vapor pressure of water and the presence of hygroscopic salt in wet-blue leather may prevent the faster dehydration of wet-blue leather.

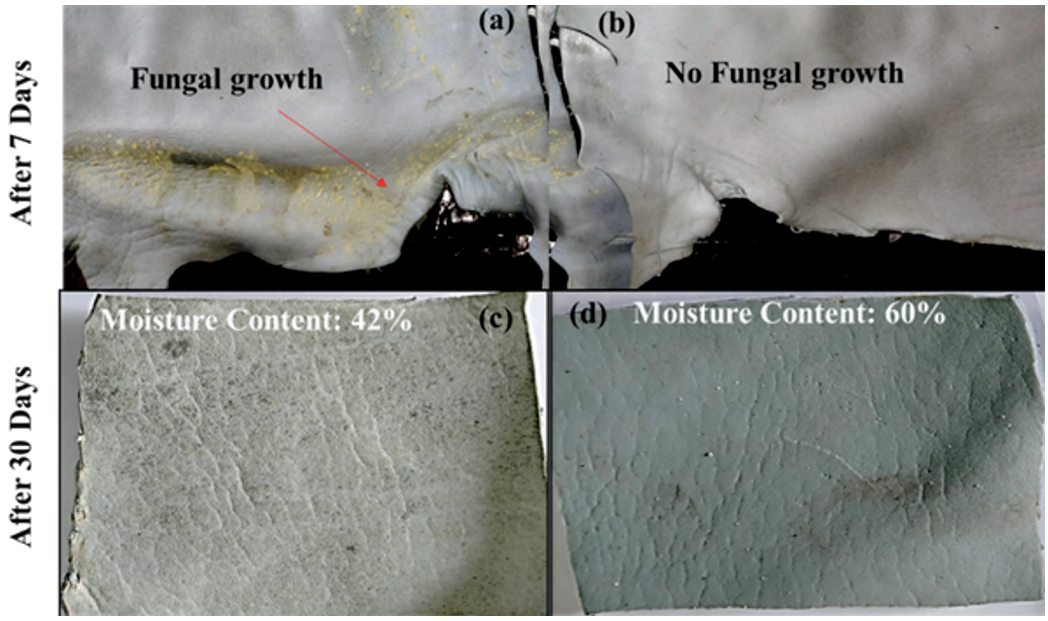

Figure 4. Wet-blue leather obtained from WAS system **(a and c)**, and WOAS system **(b and d)** after ageing for 7 and 30 days

Evaluation of Crust Leather – Industrial glove

The wet-blue leathers obtained from the WAS/WOAS system were processed into industrial glove leather and subsequently, the properties of matched pair crust leathers were analyzed.

In order to avoid the interference of collagen stabilization by synthetic tanning agents employed in the post-tanning process, the industrial glove leather process (without any synthetic tanning agents) was adopted. The process recipe was collected from a commercial industrial glove leather manufacturing unit in Kolkata, West Bengal, India. The physical strength characteristics of crust leather obtained from WAS/WOAS system are given in Table VI. It is evident from Table VI that all the physical strength characteristics of WOAS crust leather are superior to that of WAS system. Acid swelling reduces the tensile and tear strength of the final leather by about 15% and 17%, respectively. However, negligible difference was observed for % elongation. Further, about 20% reduction in both grain crack and bursting strength is observed. On the other hand, the grain crack and bursting distance are high for WOAS-crust leather. The results clearly indicate that the fiber bundle of the grain layer is well lubricated/dispersed and stronger in nature for WOAS crust leather.

Table VI

Physical properties of crust leathers

Characteristics	WAS	WOAS	% Variation w.r.t. WOAS
Average Thickness (mm)	0.9 ± 0.1	1.0 ± 0.1	-10.0
Tensile strength (MPa)	12.8 ± 1.6	15.1 ± 1.5	-15.2
Elongation (%)	50.5 ± 5.6	50.1 ± 4.7	-0.1
Tear Strength (N/mm)	73 ± 7.7	88.4 ± 10.0	-17.4
Grain crack load (kgf)	23 ± 1.3	29 ± 0.3	-20.7
Grain crack distance (mm)	8 ± 0.7	8.7 ± 1.7	-8
Bursting load (kgf)	26 ± 2	33 ± 2	-21
Bursting distance (mm)	9.6 ± 0.5	10.7 ± 1.1	-10.2
Softness (mm) – Neck	4.5 ± 0.4	5.1 ± 0.6	-11.8
Softness (mm) – OSP	5.0 ± 0.3	5.5 ± 0.3	-9

OSP – Official Sampling Position; All the specimens were taken from OSP

Table VII

Organoleptic properties of crust leathers

Properties	WAS	WOAS
Softness	Softness is lower than the WOAS-crust leather. The degree of heterogeneity in softness is high	Good softness with low heterogeneity
Stretchiness	Stretchiness is on par with the WOAS crust leather (both along and across the backbone) at OSP. However, the neck region is hard and tight	Good stretchiness
Grain smoothness	Grain is not smoother as like WOAS crust	Good smoothness with less heterogeneity
Uniformity	Non-uniform and patchy grain	Uniform grain
Internal softness	Internally empty	Good internal softness
Looseness	More looseness	No looseness with good body
Touch	Papery touch	Leathery touch

Note: The methodology for the analysis of organoleptic properties is described in Materials and Methods section

In addition, acid swelling reduces the softness of the final leather and a detailed investigation is required to understand the phenomena. The organoleptic properties of crust leathers have been analyzed and compared in Table VII.

Morphology Analysis

Generally, acid swelling leads to a reduction in fiber length and also induces differential strain at various layers. Subsequently, the induced strain may alter the surface morphology of the final leather. It is evident from Figure 5 that the neck portion of the WAS-crust leather is non-uniform in nature and also the enhanced growth mark is observed, whereas it is uniform in WOAS-crust leather. However, the grain pattern of WAS and WAOS crust leathers is uniform at OSP. It is also evident from Figure 6 that the uneven patches have been observed on neck portions of WAS crust leathers, whereas uniform coloration is seen in other places. No such issues were found in WOAS crust leather. The cross-sectional morphology of the crust leathers (WAS/WOAS) is visualized through scanning electron microscope and the same is shown in Figure 7.

It is evident from Figure 7 that the fiber bundles of WAS-crust leather (Neck/OSP) are more highly separated than the WOAS leather. Whereas the fiber bundles are thicker in WOAS system but

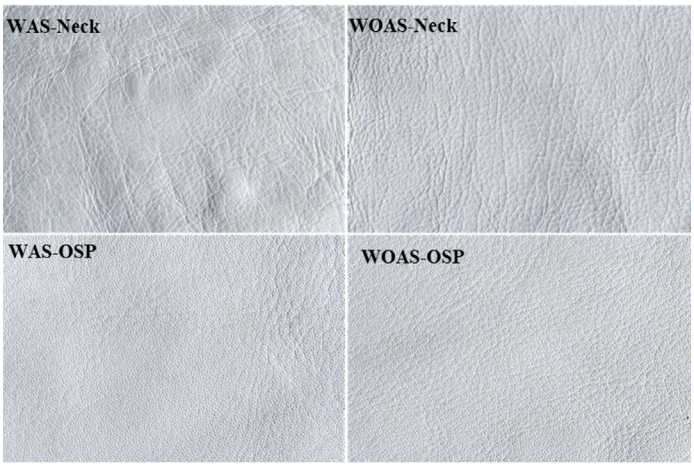

Figure 5. Photographic images of crust leathers

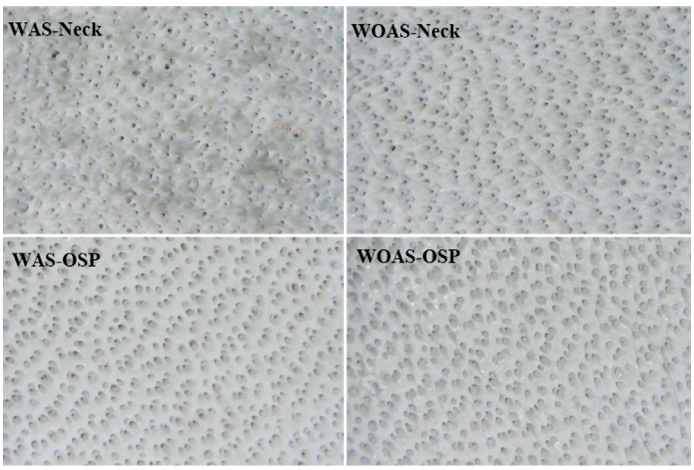

Figure 6. Microscopic images of crust leathers

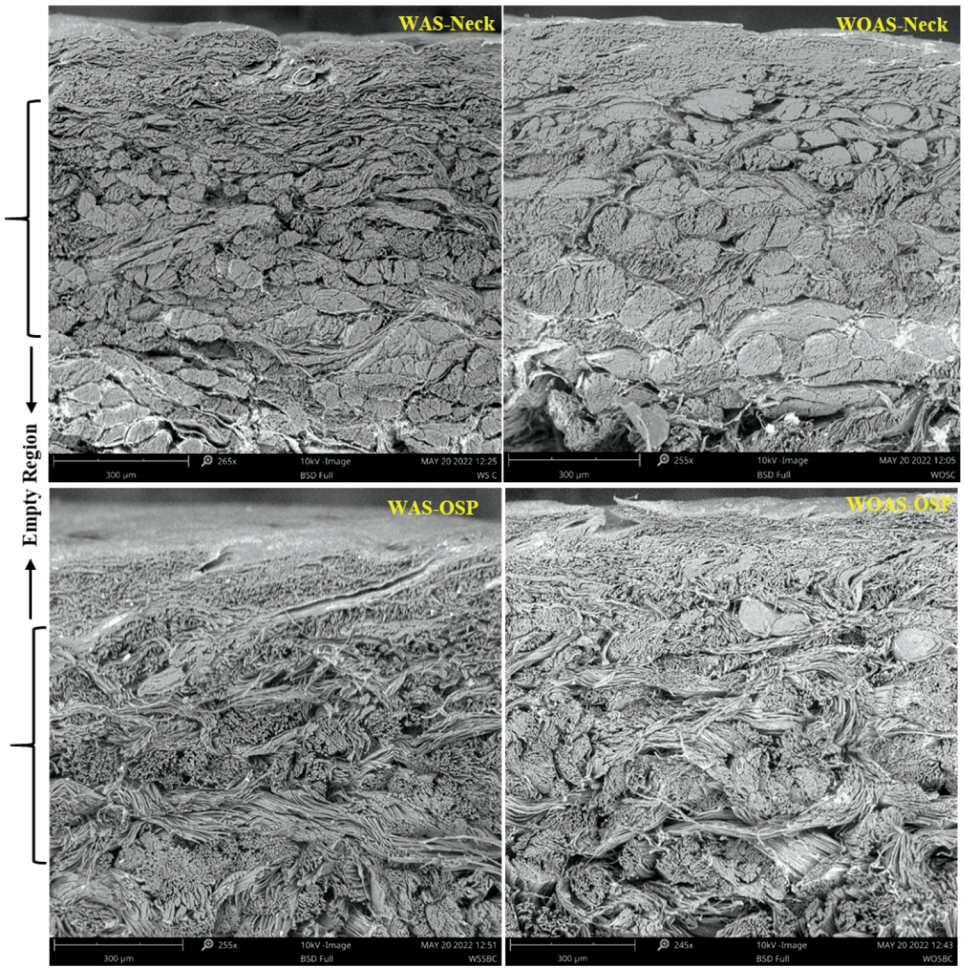

Figure 7. Scanning electron micrographs showing the cross-sectional view of crust leathers

not cemented in nature (cemented fibers make the leather harder). The extensive sub-division of fiber bundles may be the reason for the production of empty/loose leather in WAS system.

Color value measurements

The color coordinates of the wet-blue/crust leathers obtained from both WAS and WOAS systems have been measured and given in Table VIII. The lightness value of both wet-blue and crust leather obtained from WOAS system is higher than the WAS leather. This clearly indicates that the leather obtained from WOAS system is lighter blue (after chrome tanning) and whiter (after crusting process) than the WAS leather. Similarly, the high a* value indicates that WAS wet-blue leather is slightly greener than WOAS leather.

Table VIII

Color value measurement of wet-blue and crust leather

Name	L	a*	b*
WAS-WB	52.90	-6.40	3.37
WOAS-WB	59.97	-3.87	5.36
WAS-Crust	75.32	-4.22	1.87
WOAS-Crust	81.39	-3.43	1.83

L – Lightness (0-Black, 100-White); a* - (-ve value toward green/+ve value towards Red), b* - (-ve value toward blue/+ve value toward yellow)

Conclusions

It may be concluded from the present investigation that the acid swelling increases the delimed pelt weight by about 17% w/w and the addition of tanning salt reverses the swelling state to a maximum extent. Further, the diffusion of chromium is greatly affected by acid swelling and the time taken for chromium diffusion up to the cross-section is about 4 hrs in WOAS system whereas in the case of WAS system it is incomplete even after 24 hrs. The wet-blue leather obtained from WAS system has enhanced growth marks, dehydrates faster and is also more prone to fungal attack than the WOAS system. Acid swelling reduced the physical strength characteristics of the leathers and also makes the leather tight especially in neck area and empty (looseness) in other areas. Finally, the crust leather obtained from WAS system was slighter greener than the WAS system. The study provides avenues for commercial exploitation of salt-free chrome tanning systems.

Acknowledgement

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Preventing Enzymatic Damage to Hides by Timely Inhibition of Trypsin Activity with Soybean Flour during Bating Process

by

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Abstract

Enzymatic bating is important for producing clean, soft, and high-yield leather. However, it is prone to cause damage to hide collagen fibers and result in loose grain or damaged grain surface due to the longer enzymatic hydrolysis time of the grain layer than the middle layer caused by the slow mass transfer and the rapid hydrolysis reaction of the bating agent in hide. Considering trypsin is the most common bating agent, soybean flour (SF) that contains environmental friendly Kunitz trypsin inhibitor (KTI) was used after delimed hides were treated with trypsin for a certain time to opportunely inhibit its activity in the grain and avoid bating defects. The fluorescence micrographs of hides bated with fluorescently labelled trypsin and KTI showed that KTI could cover only the surface layers when it was added after bating for a certain time, whereas trypsin could penetrate the hide completely. When sufficient SF was added to the bating float at 2 h, the damage to hide collagen caused by trypsin was effectively reduced. In particular, the grain surface remained intact after bating for 8 h. The decrease in the strength of leather caused by bating was also reduced. In addition, the usage of SF scarcely affected the fiber dispersion of the middle layer, thereby ensuring the softness of leather. In summary, opportunely inhibiting the activity of trypsin in the grain could prevent hide damage and improve the quality of leather.

Introduction

Enzymatic bating is an indispensable process for leather making because it can remove non-collagenous proteins from hide and disperse hide collagen fibers, which is important for producing clean, soft, and high-yield leather.[1-4] As known, if the bating time is too short, hide collagen fibers cannot be well dispersed (viz. inadequate bating). However, if the bating time is too long, the hide grain may be damaged, thereby resulting in loose grain and even damaged surface (viz. overbating or bate runs). Extreme caution is required for tanners throughout the bating process to prevent enzymatic damage to hides, such as loose grain and damaged surface.[5,6] Unfortunately, the bating endpoint is difficult to be assessed because it unavoidably depends on subjective judgment. Therefore, an efficient method that can make the bating easy to operate and especially avoid enzymatic damage to hides is crucial for high-quality leathers and the economic benefit of tannery.

Identifying why enzymatic bating is prone to damage hide is vital for developing an easy bating method to prevent damage. The slow mass transfer and the rapid hydrolysis reaction of bating agent in the bating process are known to be the main reasons.[5] Trypsin is the most common bating agent.[2] It has high catalytic activity under a weak alkaline condition (e.g., bating pH 8–9);[7] a molecular weight of 23.3 kDa, which is much higher than those of other leather chemicals; and an isoelectric point of approximately 10.5.[8,9] The high molecular weight and the alkaline isoelectric point make trypsin penetrate the hide slowly and prone to hydrolyze the grain layer stronger than the middle layer of hide during the bating process (Figure 1).

Properly controlling the catalytic activity of trypsin and accelerating the penetration of trypsin into the hide have been proven to be effective in alleviating the problem caused by the stronger enzymatic hydrolysis of the grain.[5,10,11] For example, Wang et al. proposed an interesting method by using Kunitz trypsin inhibitor (KTI, isoelectric point of 4.5[12]) at the beginning of the bating process to reduce the hydrolysis time of the hide surface by trypsin and accelerate the penetration of trypsin into the hide.[11] KTI, which was first crystallized from soybean by Kunitz in 1945,[13] is a common and environmental friendly competitive inhibitor for trypsin.[14] KTI can prevent protein substrates from combining with trypsin through binding itself to the active site of trypsin.[15] Wang et al. found that when the mass ratio of trypsin to KTI was 5:2 (1:0.4), the trypsin activity was less than 37% within 5 min but it returned to more than 88% after 100 min. Inspired by this phenomenon, they used a trypsin–KTI complex to bate hide, prevented trypsin from rapidly hydrolyzing the hide surface, and reduced the grain damage.[11] However, inhibiting the trypsin activity with KTI at the beginning of bating results in a weak bating performance, because although the trypsin activity could be mostly restored after a certain bating time, some trypsin molecules already lost their activities in the bating float over time. Therefore, continuing to develop preventive methods to avoid bating defects is necessary.

As known, bating defects are mainly caused by the longer enzymatic hydrolysis time of the grain layer than the middle layer. Hence, decreasing the hydrolysis time of the grain is undoubtedly able to prevent bating defects. If a sufficient inhibitor (e.g., KTI) is added

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after trypsin penetrates the hides completely, the activity of trypsin on the surface could be adequately inhibited. As a result, the excessive hydrolysis of the hide surface could be avoided, and the bating performance, especially the dispersion of collagen fibers in the middle layer, could not be affected (Figure 2).

Crops like soybeans that have a high yield and low cost contain the environmental friendly trypsin inhibitor KTI.[16,17] Therefore, in this study, soybean flour (SF) was used to opportunely inhibit the activity of trypsin in the grain and avoid bating defects. The SF amount for bating was first optimized by analyzing the inhibitory effects of KTI and SF on the trypsin activity. Subsequently, the mass transfer behaviors of trypsin and KTI in hides were observed to determine the adding time of SF. Finally, SF was used after bating with trypsin for 2 h, and the performance of bating system was analyzed according to the hide damage, the dispersion of hide collagen fibers, and the leather properties.

Experimental

Materials

Trypsin (the proteolytic activity at pH 8.5 and 32°C was 270,000 U/g; trypsin activity is defined as how much tyrosine is released per minute by trypsin under given conditions)[11] and KTI were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Soybean was ground into SF by using a blender (7011HS, Waring Commercial, USA). Fluorescein isothiocyanate-labeled trypsin (FITC-trypsin) was prepared as described in the authors' previous study.[19] Rhodamine B isothiocyanate-labeled KTI (RBITC-KTI) was prepared using the same method for preparing FITC-trypsin, except that FITC and trypsin were replaced with RBITC and KTI, respectively (Figure 3). Delimed cattle hides (pH 8–9) were prepared by deliming conventional limed hides (2.2 mm in thickness) with 3.0% ammonium sulfate and 100% water at 32°C for 90 min and used for bating trials. Analytical-grade chemicals

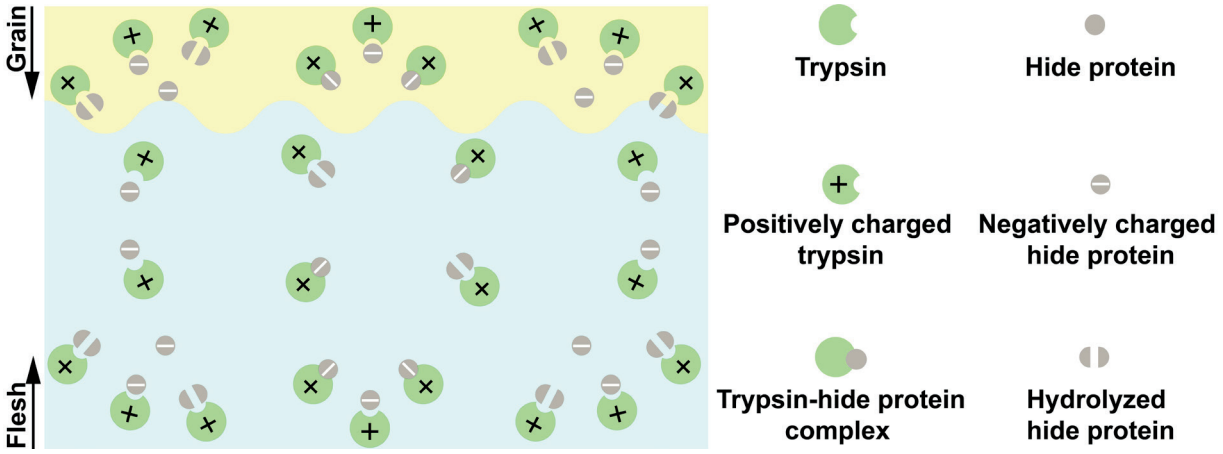

Figure 1. Schematic of traditional bating with trypsin. Hide protein (isoelectric point of 6–7[18]) is negatively charged, and trypsin (isoelectric point of 10.5[8,9]) is positively charged at the bating pH of 8–9.

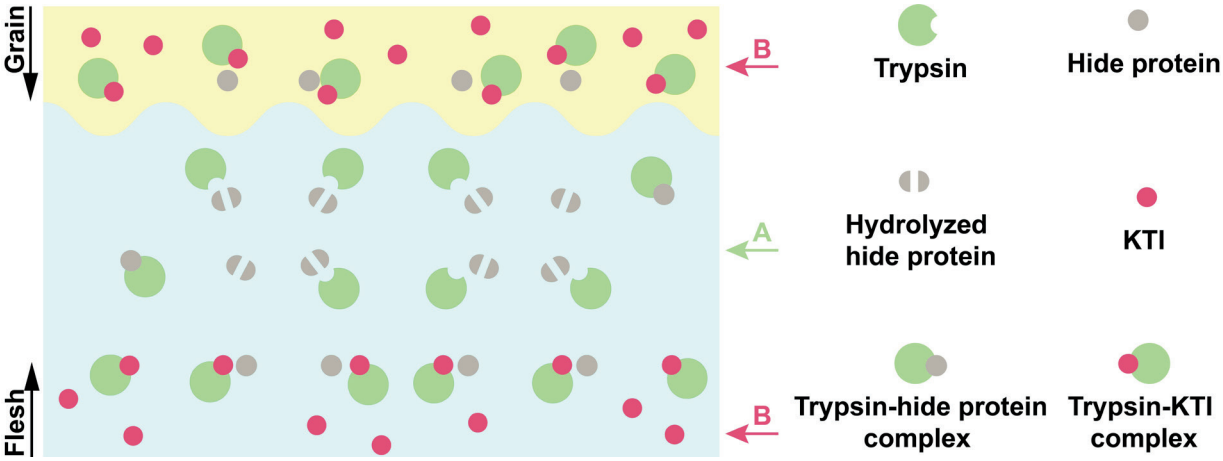

Figure 2. Schematic of trypsin activity inhibition on hide surface with KTI after trypsin penetrates hide in bating

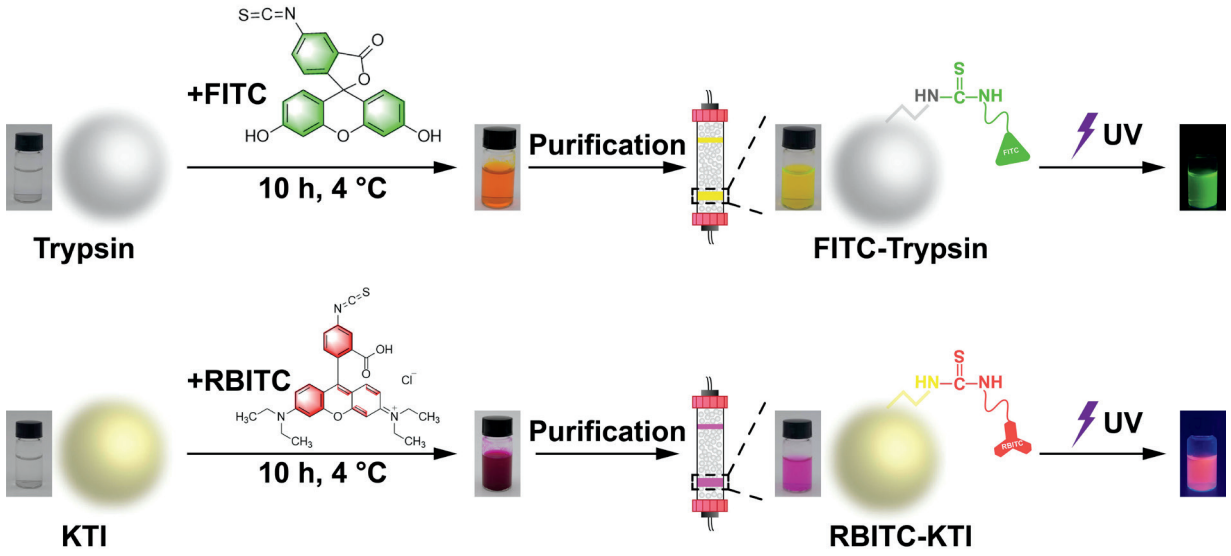

Figure 3. Schematic of the preparation of FITC-trypsin and RBITC-KTI.

were used for analyses, and commercial-grade chemicals were used for making leather.

Assay of trypsin activity in the presence of KTI and SF

The effects of KTI and SF on the trypsin activity were investigated by the method reported in our previous study with some modifications.[11] A trypsin, three trypsin–KTI complex (trypsin was incubated with KTI for 30 min), seven trypsin–SF mixture (trypsin was incubated with SF for 30 min) solutions (pH 8.5) were prepared. The concentration of trypsin in all the solutions was 0.05 g/L. The concentrations of KTI in the three trypsin–KTI complex solutions were 0.022, 0.043, and 0.086 g/L separately. The concentrations of SF in the seven trypsin–SF mixture solutions were 0.43, 0.86, 1.72, 2.58, 3.44, 5.16, and 6.88 g/L separately. Trypsin, trypsin–KTI complex, and trypsin–SF mixture solutions (1 mL each) were separately incubated with casein solution (1 mL, 20 g/L, pH 8.5) at 32°C for 10 and 120 min. After the incubation was performed, trichloroacetic acid (2 mL, 0.4 mol/L) was used to terminate the enzymatic reaction. The mixture was settled for 10 min and filtered. The filtrate (1 mL) was reacted with sodium carbonate (5 mL, 0.4 mol/L) and Folin-Ciocalteu's phenol reagent (1 mL). The color reaction was performed at 40°C for 20 min. The absorbance of the filtrate was measured at 680 nm with a microplate reader (Multiskan Go, Thermo Fisher Scientific, USA) after cooling to room temperature. A blank test was conducted by adding trichloroacetic acid before the casein solution was added. Equation (1) was used to calculate the relative proteolytic activities of the trypsin–KTI complex and trypsin–SF mixture.

$$\text{relative proteolytic activity} = \frac{A_1}{A_2} \times 100\% \quad (1)$$

where A_1 is the increment in the absorbance of the trypsin–KTI complex or trypsin–SF mixture solution compared with the blank, and A_2 is the increment in the absorbance of trypsin solution compared with the blank.

Analyses of trypsin and KTI penetrations during bating

The delimed hides were bated with 0.1% FITC-trypsin and 100% water at 32°C. After bating was performed for 2.0 h, 0.17% RBITC-KTI was added into the float. The above percentages were based on the weight of delimed hide. The hides were sampled after bating for 0.5, 1.0, 2.0, 3.0, 4.0, and 8.0 h, and sample sections were prepared on a freezing microtome (CM1950, Leica, Germany) at a thickness of 20 μm. The sections were observed using a fluorescence microscope (DMi8, Leica, Germany) with a FITC/RHOD filter cube for FITC-trypsin /RBITC-KTI localization. The fluorescence micrographs of the hides were analyzed by LAS X software to obtain brightness induced by FITC-trypsin/RBITC-KTI. The penetration rate of FITC-trypsin/RBITC-KTI in hide was calculated using Equation (2) as follows:

$$\text{penetration rate} = \frac{T_1}{T_2} \times 100\% \quad (2)$$

where T_1 is the thickness of hide with brightness (mm), and T_2 is the total thickness of hide (mm).

Bating trials by using trypsin and KTI/SF

Three groups of delimed hides (Nos.1–3) were bated with 0.1% trypsin and 100% water at 32°C for 2.0 h. KTI (0.17%) and SF (5.16%) were then added to the Nos.2 and 3 groups. The above percentages were based on the weight of limed hide. The three groups of hides were subsequently bated for another 6.0 h at 32°C. The bated hides (Nos.1 and 3) were then pickled, chrome tanned and post-tanned to obtain crust leathers (Figure 4).

After bating for 2.0, 3.0, 4.0, and 8.0 h, the bate-liquors and the hides were sampled. The hydroxyproline (Hyp) concentration of bate-liquor was measured to evaluate the damage to hide collagen. The grain surface of hide was observed using field-emission scanning electron microscopy (FESEM, Nova NanoSEM 450, FEI, USA). The

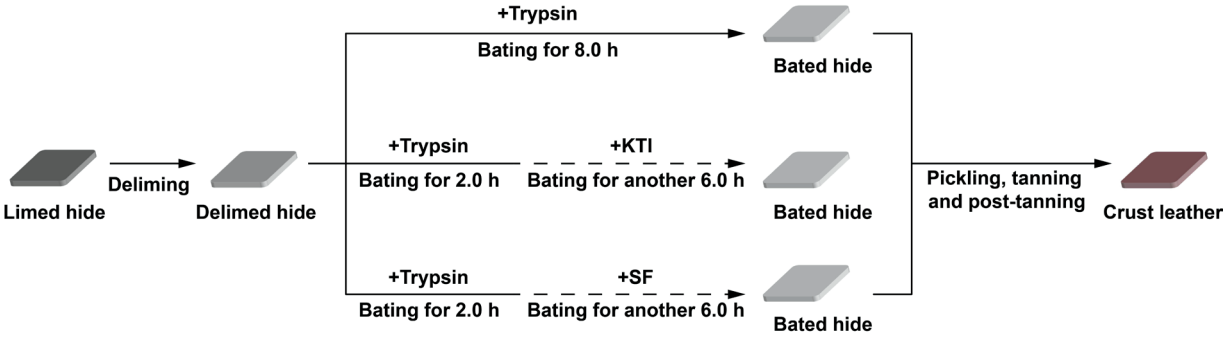

Figure 4. Schematic of bating trials.

hide sample was fixed in 10% neutrally buffered formalin for 48 h, and then the fixed sample was dehydrated, infiltrated, embedded in paraffin, and cut into 4 μm-thick sections by using a pathology slicer (RM2016, Leica, Germany). The sections were stained with hematoxylin and eosin (HE) to observe the dispersion of collagen fibers by using a brightfield microscope (DMi8, Leica, Germany).

The crust leathers were sampled, and their grain surfaces and middle layers were observed using a stereo microscope (M205C, Leica, Germany) and FESEM, respectively. Moreover, the physical properties, such as softness,[20] tensile strength,[21] tear strength[22] of the crust leathers were measured after conditioning at 20°C and 65% relative humidity for 24 h.[23]

Results and discussion

Effects of KTI and SF on trypsin activity

In this work, we aimed to achieve an opportune inhibition of trypsin on the hide surface to prevent excessive grain hydrolysis without weakening the bating performance. For this purpose, the inhibitory effects of KTI and SF on trypsin activity were first investigated. When the molar ratio of trypsin:KTI was 1:0.5, the relative trypsin activity was approximately 41% over 10 min and restored to more than 87% in 120 min; when the trypsin:KTI ratio was 1:1, the relative activity remained lower than 3% after reacting for 10 min and lower than 20% at 120 min; when the trypsin:KTI ratio increased to 1:2, the activity was still lower than 3% after reacting for 10 min and remained lower than 12% at 120 min [Figure 5(a)]. The results indicated that the trypsin:KTI molar ratio of 1:2 was sufficient to inhibit the trypsin activity.

In accordance with the content of KTI in SF, the effect of SF concentration from 0.43 g/L to 6.88 g/L on trypsin activity was studied to obtain the optimal SF amount for subsequent experiments. Figure 5(b) shows that the inhibitory effect of SF on trypsin activity increased with increasing SF concentration. When the concentration of SF was 2.58 g/L, the relative trypsin activity was lower than 5% after reacting for 10 min and returned to lower than 11% in 120 min. When the concentration of SF was 6.88 g/L, the relative activity was nearly 0% after reacting for 10 min and remained lower than 4% at 120 min. Considering the inhibitory effect and economic benefits, the SF concentration of 2.58 g/L was found to be more suitable. Therefore, the mass ratio of trypsin to SF of 1:51.6 was selected for the following bating trials.

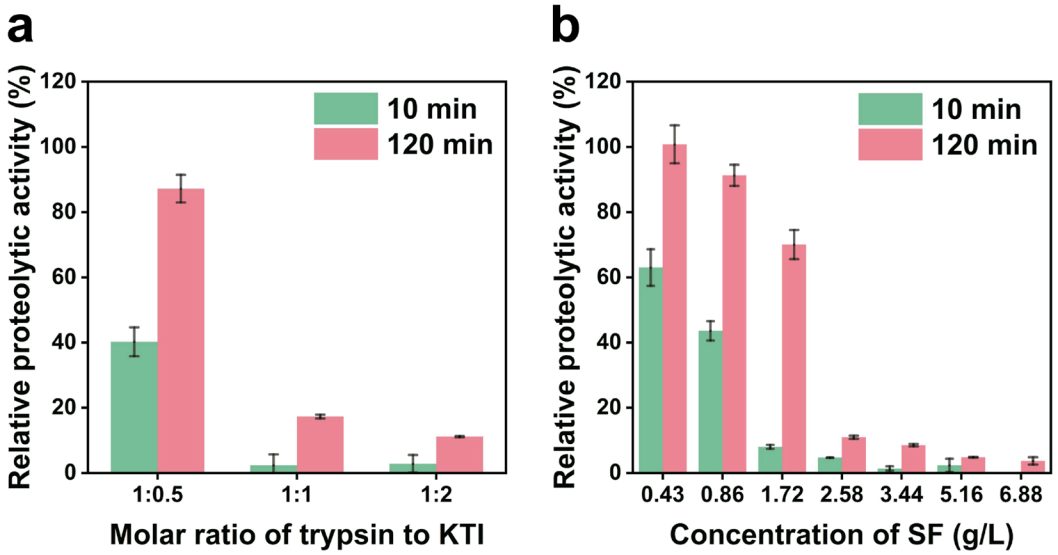

Figure 5. Effects of KTI (a) and SF (b) on trypsin activity.

Penetrations of trypsin and KTI in hide

The penetration behaviors of trypsin and KTI in hides are shown in Figure 6. The fluorescence micrographs in Figure 6(a) show that FITC-trypsin (green) gradually penetrated the hide over time. The penetration rate of FITC-trypsin was 54.88% after bating for 2.0 h [Figure 6(b)], indicating that considerable trypsin existed in the grain within 2.0 h. Hence, RBITC-KTI was added to the bating float at 2.0 h. The penetration rate of FITC-trypsin only increased to 59.86% after bating for 3.0 h, indicating that trypsin penetrated the hide slowly and hydrolyzed the grain for a longer time than the hide's middle layer during the bating process. RBITC-KTI (red) also gradually penetrated the hide over time. Interestingly, after bating for 4.0 h (viz. after adding KTI for 2.0 h), the penetration rates of FITC-trypsin and RBITC-KTI were 100% and 60.16%, respectively. The green and red fluorescence micrographs were merged. The merged image of hide bated for 4.0 h shows that the upper and lower surface layers are yellow due to green and red overlay, while the middle layer is green. The phenomenon indicated that RBITC-KTI was in the grain, whereas FITC-trypsin was in the whole hide. These results illustrated that adding KTI after bating for a certain time could make the inhibitor cover only the surface layers, thereby preventing grain damage without affecting the enzymatic hydrolysis of the hide's middle layer. After bating for 8.0 h, FITC-trypsin and RBITC-KTI completely penetrated the hide, implying that KTI may even inhibit the trypsin activity in the whole hide at the end of bating.

Effects of KTI and SF on hide damage

This research aimed to avoid enzymatic damage to hide by opportunely adding SF containing KTI to inhibit the trypsin activity. Therefore, the Hyp concentrations of bate-liquors and the morphology of bated grain surfaces were analyzed to evaluate the SF prevention effect. As shown in Figure 7(a), the Hyp concentration of bate-liquor increased over time, and the increase followed the order: control (trypsin) > KTI (trypsin + KTI) > SF (trypsin + SF). The results revealed that KTI and SF effectively reduced the damage to hide collagen caused by trypsin. In addition, the SF group had less collagen damage than the KTI group, which may be attributed to the fact that SF has other proteins besides KTI.[16] The proteins in SF could also be hydrolyzed by trypsin, thereby reducing the hydrolysis of hide protein and the damage to hide collagen. The SEM images of bated grain surfaces are shown in Figure 7(b). It is obvious that the hair pores were gradually damaged over time when only using trypsin for bating. Here, it should be mentioned that the trypsin activity used in this study was much higher than that usually used in tannery, so that the hide damage could inevitably occur in a short time. When the bated hides were treated with KTI and SF after bating for 2.0 h, the hair pores became more intact than those bated with only trypsin. In particular, the grain surfaces remained intact after bating for 8.0 h. These phenomena proved that SF greatly reduced the enzymatic damage to the grain because SF containing sufficient KTI effectively inhibited the activity of trypsin in the grain.

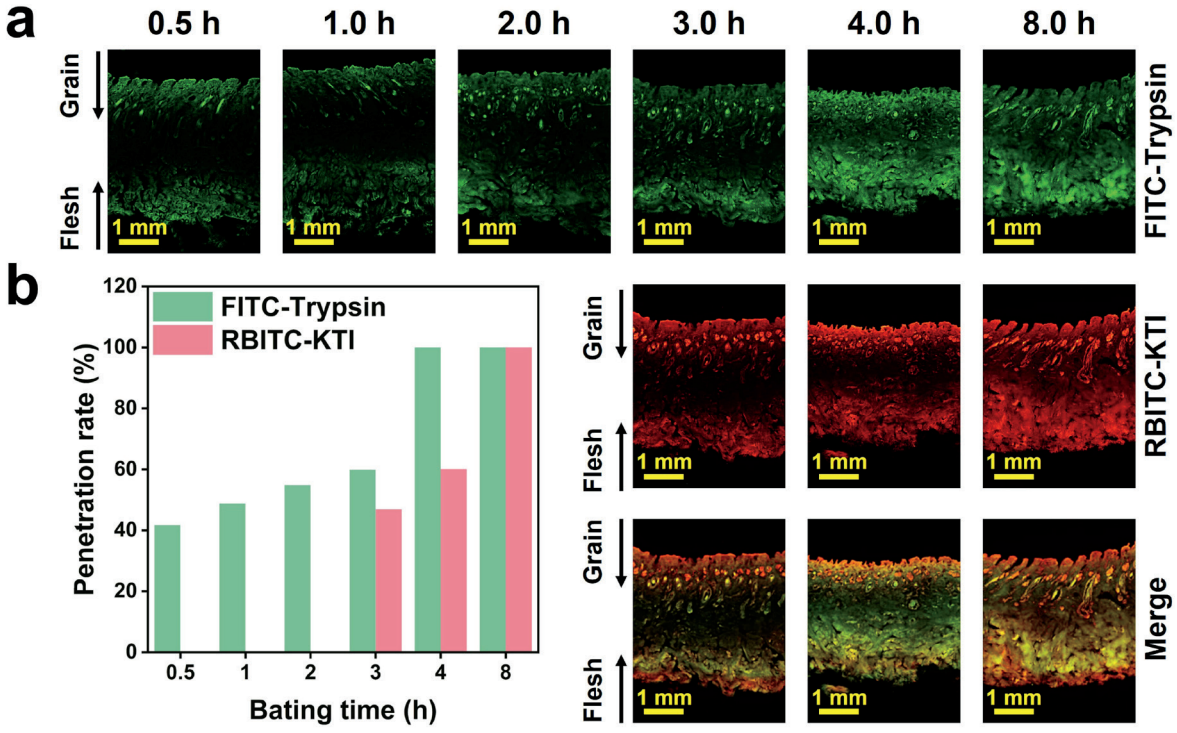

Figure 6. (a) Fluorescence micrographs of longitudinal sections from hides bated with FITC-trypsin and RBITC-KTI (scale bars, 1 mm) and (b) penetration rates of FITC-trypsin and RBITC-KTI in bated hides.

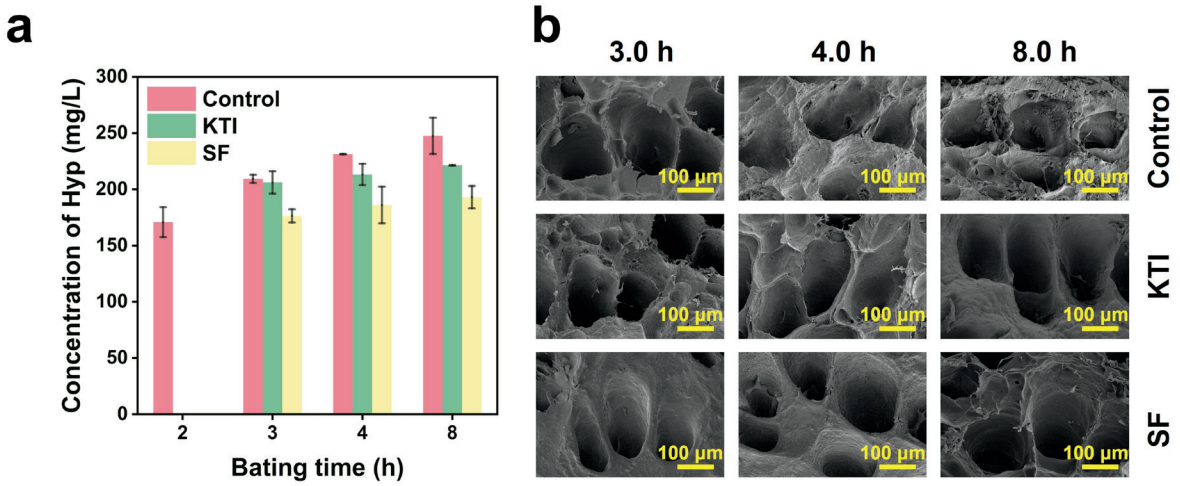

Figure 7. (a) Concentration of Hyp in bate-liquor and (b) SEM images of grain surfaces of bated hides.

Effects of KTI and SF on fiber dispersion

As mentioned earlier, an important purpose of adding SF after bating with trypsin for a certain time was to inhibit the activity of trypsin in the grain without weakening the bating performance. Therefore, in this section, the dispersion of collagen fibers in hide, especially in the middle layer, was analyzed to determine whether SF affected the bating performance. The collagen fibers were stained with HE and observed using a microscope. It was found that the collagen fibers in the middle layer were similar in the control, KTI, and SF bating groups (Figure 8). The results of fiber dispersion indicated that the method proposed in this study could ensure no attenuation of the bating performance.

Effect of SF on leather properties

The effect of SF on leather quality was further evaluated by comparing the surface morphology, the fiber dispersion, and the physical properties of the SF- and control crust leathers. The stereomicroscopic images of grain surfaces in Figure 9(a) showed that the hair pores were more intact and clearer in the SF group than in the control group (bating with only trypsin). The middle layers of the two leathers presented similar fiber dispersion degree, as shown in Figure 9(b). These results indicated that opportunely inhibiting the activity of trypsin in the grain protected the hide surface without weakening the bating performance.

The data in Table I showed that the softness of the two crust leathers increased as the bating time prolonged, and the increments in the softness of the control and SF groups were similar, which proved that opportunely inhibiting the activity of trypsin in the grain did not weaken the bating performance, including the fiber dispersion of the middle layer. In addition, the tensile and tear strengths of

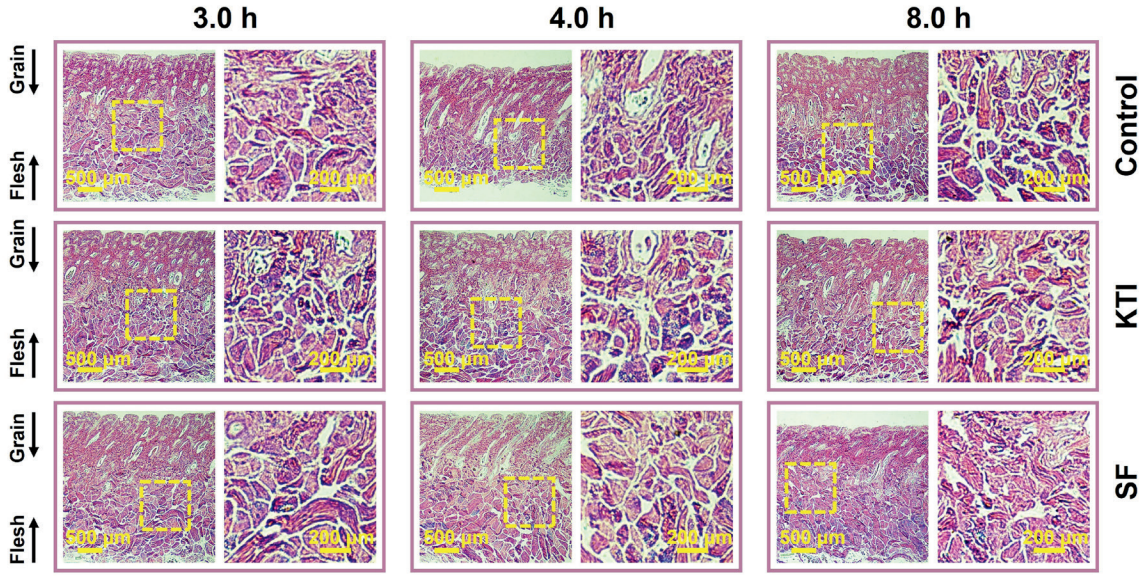

Figure 8. Microscopic images of HE-stained longitudinal sections of bated hides.

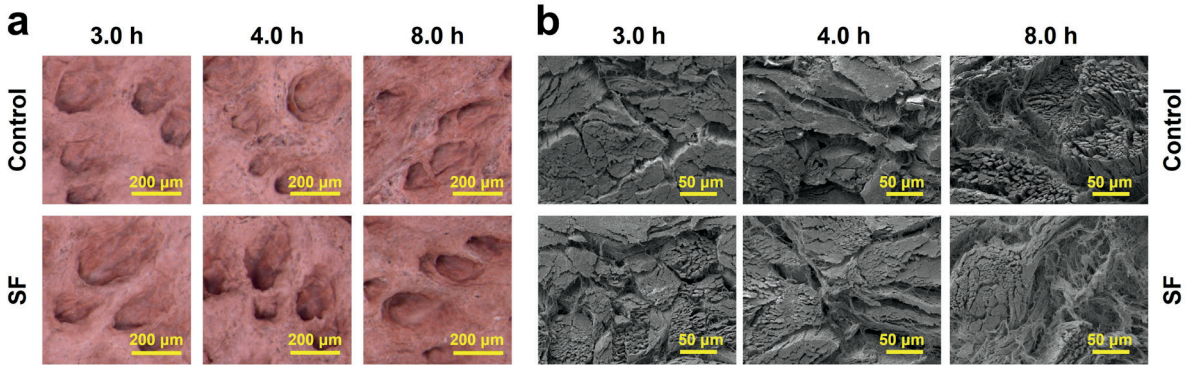

Figure 9. (a) Stereo microscopic images of grain surfaces of crust leathers and (b) SEM images of middle layers of crust leathers.

Table I

Physical properties of crust leathers

Sample	Bating time (h)	Softness (mm)	Tensile strength (N/mm^2)	Tear Strength (N/mm)
Trypsin	2.0	7.73 ± 0.43	11.40 ± 0.61	54.62 ± 1.10
Control (Trypsin)	3.0	8.52 ± 0.11	9.06 ± 0.10	39.49 ± 1.07
	4.0	8.55 ± 0.15	6.66 ± 1.42	32.11 ± 4.95
	8.0	8.76 ± 0.18	5.14 ± 0.09	28.52 ± 3.04
SF (Trypsin+SF)	3.0	8.80 ± 0.08	8.10 ± 0.82	42.07 ± 2.68
	4.0	8.73 ± 0.25	8.01 ± 0.12	32.96 ± 0.06
	8.0	8.86 ± 0.16	7.57 ± 0.43	39.54 ± 4.33

Values are means ± standard deviations of six determinations.

the crust leathers decreased with increasing bating time. However, it was interesting that the decrement in the strengths of the SF-crust leather was less than that of the control leather. These results also proved that adding SF during the bating process reduced the damage to hide collagen, especially the damage to the grain, which is important to the leather's strengths.

Conclusion

The addition of sufficient inhibitor after bating for a certain time could effectively inhibit the activity of trypsin in the grain layer and not affect the enzymatic hydrolysis of the middle layer. As a result, the surface morphology (clearness and intactness) and the grain strength of the bated hide could be protected. The proposed bating method using SF to opportunely inhibit the trypsin activity exhibited simple, environmental friendly, and low-cost advantages and huge potential for producing high-quality leathers. New insights are provided into the development of bating methods that can avoid the enzymatic damage to hide and not affect the performance of bating system.

Acknowledgement

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The Kinetic Study on Potassium Persulfate Accelerated Fish Oil Oxidation-An Agreeing Conclusion on Chamois Tanning

by

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Abstract

The oxidation of fish oil is a type of chain reaction. The use of oxidizing agents enhances the rate of oxidation of the same. This study predominantly focuses on the role of potassium persulfate as an accelerating agent in fish oil oxidation, its kinetics, and application in rapid fish oil tanning. The use of potassium persulfate (1%) completes the fish oil oxidation within 4 days, confirmed by its kinetic studies. Chamois leathers made using potassium persulfate (1%) exhibited excellent water absorption capacity (454%). The physical parameters such as tensile strength, shrinkage temperature, surface morphology, and organoleptic properties of the experimental leather exhibit better results than control leathers.

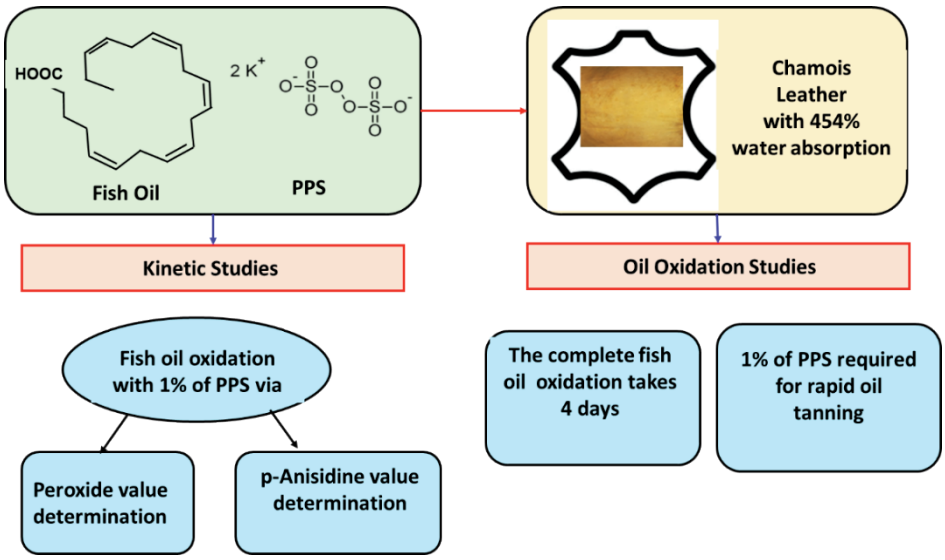
1. Introduction

The high degree of unsaturation is the foundation for the oil to be vulnerable to oxidation and other chemical reactions associated with reactions of double bonds, especially addition reactions.[1] Oil oxidation can be achieved in many ways, such as auto, thermally, photochemical, or catalytically.[2] In the case of monounsaturated fatty acids, autoxidation is performed at elevated temperatures, whereas polyunsaturated fatty acids experience instantaneous oxidation at room temperature.

The oxidation of highly unsaturated fish oil follows a free radical chain reaction where the primary oxidized products, such as allyl hydro peroxides and hydro peroxide, are prone to the formation of secondary oxidized products. These oxidized products, such as saturated and unsaturated aldehydes, short-chain ketones, alcohols, acids, esters, ethers, and hydrocarbons, can be utilized in versatile applications. The kinetic study of fish oil oxidation is determined by the Peroxide (PV) and Anisidine values (AV), which define primary and secondary oxidation products of the oil oxidation, respectively.[3-5]

Oil oxidation plays an important role in the tanning operation of leather processing, where skin protein converts into stable cross-linked

Graphical Abstract- Kinetic study of PPS accelerated fish oil tanning



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material and alters the configuration of collagen fibers. Oil tanning is one form of tanning of leather that gives highly absorbent, porous, low density, and heightened flexible leather, known as chamois leather.[6] A variety of oils have been explored for oil tanning, such as linseed oil[7], jatropha oil[8] rubber seed oil[9], oil from tannery fleshings[10], and epoxidized oil.[11,12] The most favored oil for making chamois leather is fish oil due to its high degree of unsaturation. The oil tanning takes approx. 10-12 days, depending upon ambient conditions.

Generally, antioxidants are used to prevent the oil from oxidation for various industrial uses.[13] The use of oxidizing agents to accelerate the oxidation of oil is utilized in the oil tanning process of chamois leather manufacture.

Few accelerators such as hydrogen peroxide,[14,15,16] sodium percarbonate,[17]ozone,[18] benzoyl peroxide,[6,19] benzenecarboperoxoic acid,[20] and Potassium persulfate[21] are reported with respect to the best of their activity, reaction conditions and mode of tanning.

This study investigates the influence of various percentages of Potassium persulfate (PPS) on fish oil oxidation rate and duration of the process. The current research primarily concentrates on reducing the oxidation duration from 10 days to 4 days to comprehend kinetically fish oil oxidation through peroxide and p- Anisidine values.

2. Materials and Methods

2.1 Materials

Fish oil was sourced locally in Chennai. PPS was procured from S.D. Fine Chem Ltd, Mumbai. Sheepskins were obtained from a nearby slaughterhouse. Other chemicals used for kinetic studies are obtained commercially and of analytical grade.

- p-Anisidine value measurements: p-Anisidine reagent, Acetic acid (glacial) 100%, and Iso-octane.
- Peroxide value measurements: Cyclo-hexane, 96% ethanol, 30% ammonium thiocyanate solution, 3.7% HCL, and Fe^{2+} solution.

Table I

Oil Tanning Process

Process	Chemical	Percentage (%)	Time	Remarks
Soaking	Water	300	One day	
	Preservatives	0.25		
	Wetting agents	0.50		
Unhairing and Liming	Water	20	Two days	
	Lime	10		
	Sodium sulfide (60%)	3		
Reliming	Water	300		
	Lime	10		
Fleshing				
Deliming	Water	100		
	Ammonium chloride	2	40 min	Check de-liming using phenolphthalein
	Alkaline bate	0.5	30 min	Drain
Washing	Water	200	10 min	Wash and drain
Partial pickling	Water	80		
	Salt	8	30 min	
	Formic Acid	0.5	30 min	In 1:10 dilution with water
	Sulfuric Acid	0.2		In three feeds with 1:10 dilution with water, adjust pH to 4
Depickling	Sodium bicarbonate	1		
Glutaraldehyde tanning	Glutaraldehyde	1	90 min	Drain, pile for overnight
	Soda ash dissolved	2		
Next day				
	Fish oil	25		Mix using stirrer, make paste. add to drum along with skin
	Potassium persulfate (experiment)	0.25, 0.5, 0.75,1and 1.25		
	Sodium carbonate	0.5		

*1.25% of PPS started showing patches on the skin, therefore from 0.25 to 1% of PPS only explored for the experiment

2.2 Methods

2.2.1 Method of oil tanning

After glutaraldehyde tanning, fish oil, soda ash, and Potassium persulfate are the key chemicals required for oil tanning. The mixture of a solution of 20 ml of (25% w/w ratio of sheep skin) fish oil with various percentages (0.25, 0.5, 0.75,1, and 1.25%) of Potassium persulfate and sodium carbonate (0.5%) was applied on sheep skin. The tanning process was carried out in a rotating drum approximately for 2 h. After completion of the tanning, the yellow color leathers were treated with water, soda ash, and a wetting agent to eliminate the extra oil from the experimental leathers. Further dried leathers were subjected to staking, buffing, and milling. The leather tanned only with fish oil without PPS is considered to control leather.

2.2.2 Water absorption

Chamois leather is known for its water absorption capacity. The standard method determined the extent of water absorption.[22]

2.2.3 Scanning Electron Microscope (SEM) analysis

The morphology of the leather fibers after oil tanning can be studied by SEM analysis. The analysis was carried out using Phenom Pro desktop scanning electron microscope. All leather samples were cut into 0.5X0.5 mm and mounted on sample holding stubs using a double side adhesive tape. SEM analysis was carried out at magnifications of 500x.

2.2.4 Shrinkage temperature measurement

The performance of leather towards heat is expressed by shrinkage temperature. The shrinkage temperature measurements of oil-tanned leathers were carried out by standard method.[23]

2.2.5 Physical and organoleptic properties of the chamois leather

The strength and organoleptic properties of experimental leathers were conducted as per the standard norms.[24]

2.3 Determination of kinetic study of fish oil oxidation

To determine the chemical kinetics of fish oil oxidation with and without Potassium persulfate, the following sets of experiments were carried out. The first batch of experimentations corresponds to the determination of the peroxide and p-Anisidine values of fish oil (2g) without an oxidizing agent. The next set of experiments was carried out in the presence of 1% PPS.

2.3.1 Determination of Peroxide Value

Peroxide value calculates the peroxides retained in the oil and determines this value via the iodine liberated from potassium iodide. The lipid hydroperoxides (LOOH) present in the fish oil oxidizes Fe^{+} into Fe^{3+}, which then reacts with ammonium thiocyanate to form a pink ferric thiocyanate complex. The absorbance of this formed complex is measured by using UV- spectroscopy. The absorbance of the substance is proportional to the number of lipid hydroperoxides present in the oil[25] (AOAC, 965.33).

The above procedure is repeated for the next 10 days for both the fish oil sample with and without an oxidizing agent (1% PPS), and absorbance is recorded accordingly.

2.3.2 Determination of p-Anisidine values

The p-Anisidine value (AV) is determined according to the AOCS Official Method Cd 18-90.[26]

Peroxide value (PV) and p-Anisidine value (p-AV) are used to measure the level of peroxide/hydroperoxide and secondary by-products formed during the oil oxidation. The peroxide value (PV) and p-Anisidine value (p-AV) of the oil with and without 1% of PPS are studied and compared and determine the kinetics of the fish oil oxidation.

3. Results and Discussion

The impact of Potassium persulfate on the oxidation of fish oil and its application in oil tanning is briefly discussed concerning all its physical, chemical, and organoleptic properties.

3.1 Water absorption

The intrinsic property of chamois leather is its water absorption capacity. Table II indicates the results for water absorption of experimental and control leathers. The 1% potassium persulfate shows the highest water absorption (454%) than other percentages (0.25,0.50, and 0.75). Hence, it could be inferred that 1% of PPS is sufficient for the rapid oil tanning of fish oil.

Table II

Water Absorption capacity of chamois leathers

S No	Sample	Water Absorption (%)
1	Control	375 ±20
2	Potassium persulfate(1.00%)	454 ±20
3	Potassium persulfate(0.75%)	412 ±20
4	Potassium persulfate(0.50%)	404 ±20
5	Potassium persulfate(0.25%)	378 ±20

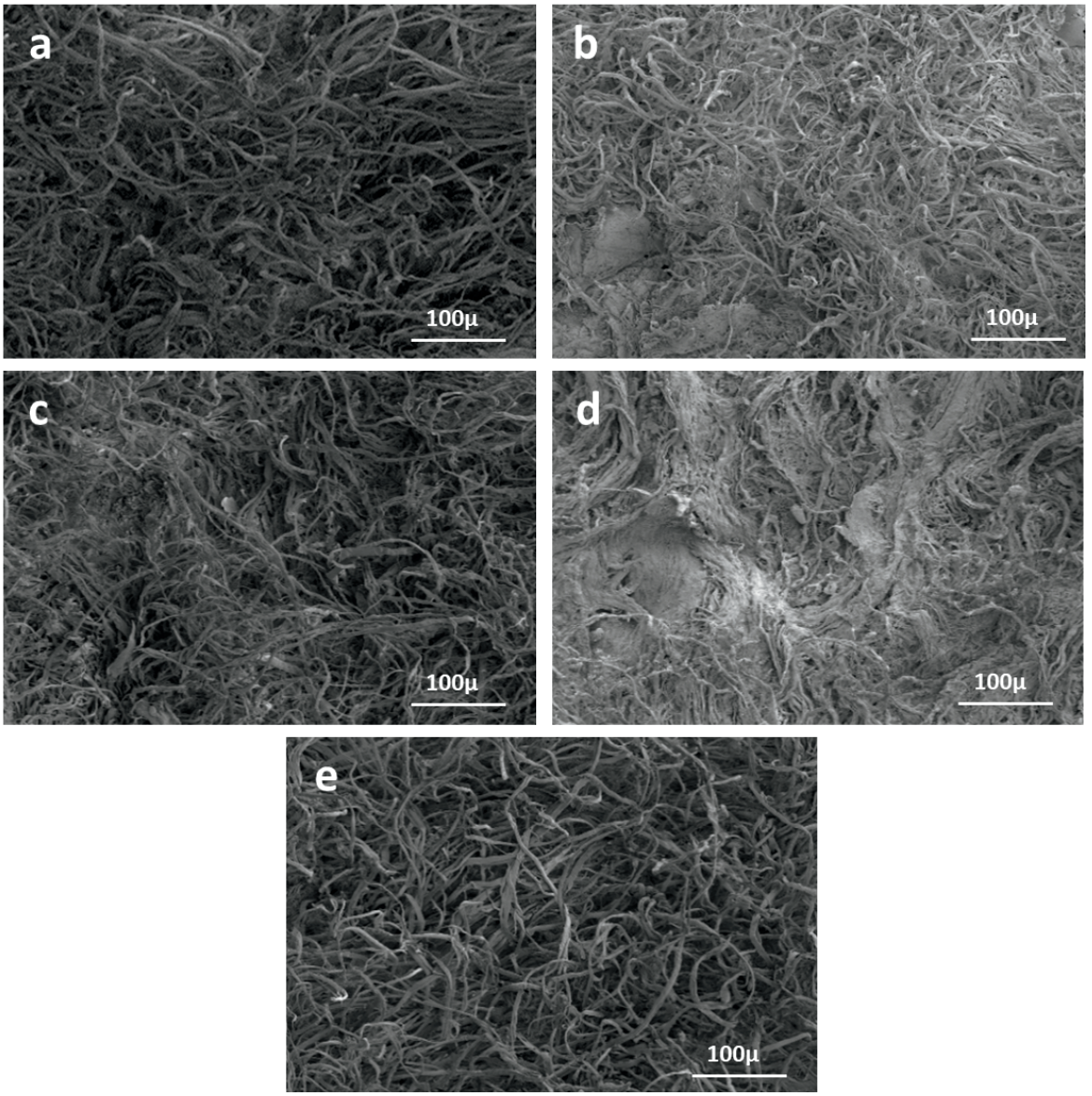

Figure 1. SEM images of chamois leather, **a)** Control, **b)** 0.25, **c)** 0.50, **d)** 0.75 and **e)** 1.0% PPS

Table III

Shrinkage Temperature measurement of chamois leather

S. No.	Sample	Shrinkage Temperature (°C)
1	Control	74±2
2	Potassium persulfate(1.00%)	78±2
3	Potassium persulfate(0.75%)	76±2
4	Potassium persulfate(0.50%)	75±2
5	Potassium persulfate(0.25%)	75±2

Table IV

Physical testing data of chamois leathers

S. No.	Sample	Tensile strength (N/mm²)
1	Control	12.05±2
2	Potassium persulfate(1.00%)	17.69±2
3	Potassium persulfate(0.75%)	14.57±2
4	Potassium persulfate(0.50%)	13.08±2
5	Potassium persulfate(0.25%)	12.77±2

3.2 Scanning Electron Microscopy analysis of chamois leathers

The Figures 1a-e shows the morphology of the fiber structure of the oil tanned leathers. Scanning electron microscopy images of control and experimental leathers are shown in Figures 1a and 1b-e respectively. The fiber compactness in control and experimental leathers with 1% of PPS are very much aligned. Therefore, it may be inferred that the addition of 1% of Potassium persulfate will not affect the morphology of leather fiber.

3.3 Shrinkage temperature measurement

Shrinkage temperature measurement of chamois leather gives information about the resistance of the leather due to hydrothermal shrinkage. Table 3 indicates the increase in the shrinkage temperature of experimental chamois leathers obtained from 0.50% to 1% of PPS. Moreover, experimental leathers with 0.25 and 0.50% of PPS show the same and slightly higher shrinkage temperature value (75 °C) than the control values (74°C).

Table V

PV values with/without oxidizing agent

Time in days	PV value without Oxidizing Agent (meq/kg)	PV value with Oxidizing Agent (meq/kg)
1	2.807 ±0.05	5.807 ±0.05
2	5.2508 ±0.05	10.638 ±0.05
3	9.771 ±0.05	17.588 ±0.05
4	12.008 ±0.05	13.323 ±0.05
5	13.343 ±0.05	9.4248 ±0.05
6	15.7856 ±0.05	
7	17.536 ±0.05	
8	14.359 ±0.05	
9	11.221 ±0.05	
10	9.845 ±0.05	

3.4 Physical testing data of chamois leathers

Experimental leathers were tested for strength and water absorption testing. Table 4, indicates that the tensile strength of the chamois leather increased with an increase in the concentration of the potassium persulfate, which completed the oxidation of the fish oil.

3.5 Organoleptic Properties

Chamois leather organoleptic properties were evaluated for softness, color, and odor. From Figure 2, observation can be drawn that

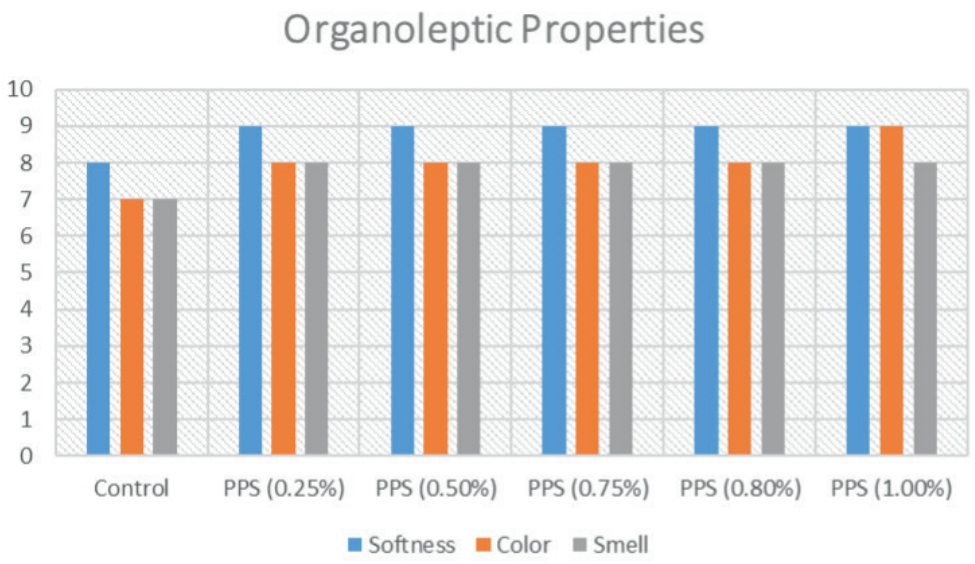

Figure 2. Organoleptic properties of chamois leathers

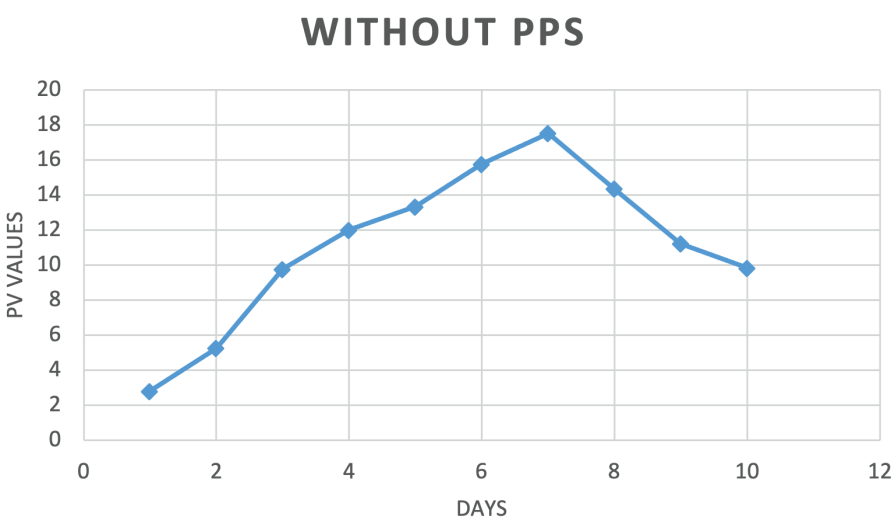

Figure 3. Peroxide values without oxidizing agent (PPS)

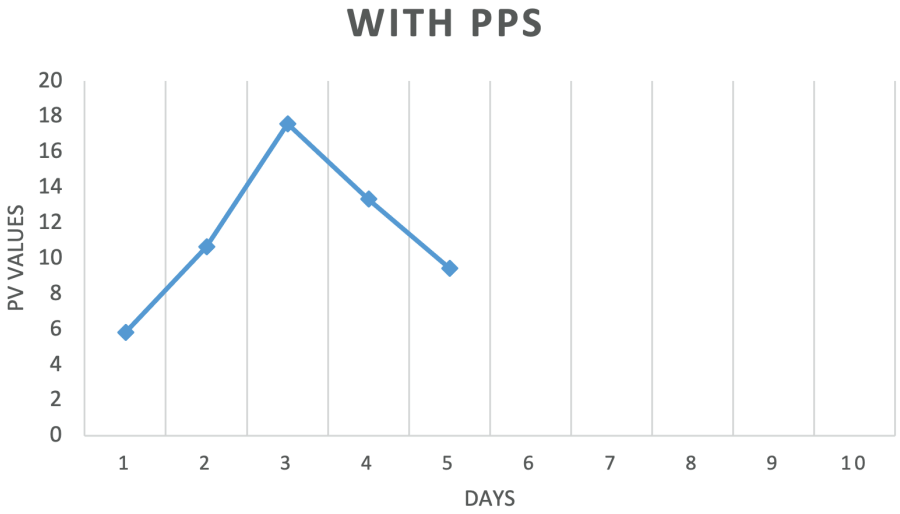

Figure 4. Peroxide values with oxidizing agent (1% PPS)

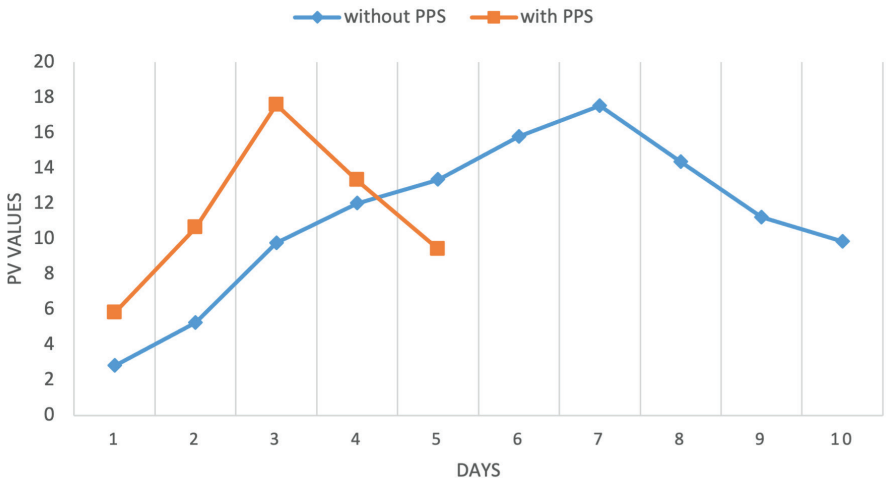

Figure 5. Comparison of peroxide values with and without PPS

the softness of chamois leathers improved with the increase in the percentage of PPS. Similarly, the color of the experimental chamois leathers with 1% potassium per persulfate showed lighter yellow than the golden yellow of the control leathers. Although chamois leathers are made using fish oil as the leading tanning agent, the odor is one of the essential qualities to assess.

4. Kinetic studies

4.1 Determination of Peroxide Values:

Figures 3 and 4 show noticeable changes in the PV value for fish oil with and without PPS. The PV values are measured with a time interval of one day for the next ten days. The fish oil without PPS exhibits an incremental growth in PV values in the initial stage, achieving maximum by the 7th day. The PV values start decreasing from the 7th day till the 10th day. This increasing and then reducing graph is due to the non-availability of a driving force for additional oil oxidation.

However, in the case of fish oil with 1% of PPS, the initial first day gives a higher value of PV till the 3rd day, where it reaches the maximum value, compared with oil without PPS. The PV starts decreasing after 3rd day, indicating a less concentration of peroxides in the system.

However, in the case of fish oil with 1% of PPS, the initial first day gives a higher value of PV till 3rd day when it reaches the maximum value, compared with oil without PPS. The PV starts decreasing after 3rd day, indicating a less concentration of peroxides in the system. The 1% of PPS is a driving force for peroxide/hydroperoxide molecules to participate in the reaction with Fe ions actively. It demonstrates that 1% of PPS improves the rate of peroxide formation, enabling the long-term stable intermediate for higher PV values. It concluded that the sample without PPS would take up to 7 days to complete the reaction. In contrast, the sample with 1% of PPS takes 3 days for the same.

4.2 Determination of p-Anisidine Values:

P-Anisidine values are necessary to analyze secondary oxidation products of fish oil oxidation with and without 1% of PPS. The secondary oxidized products of fish oil without PPS were less in concentration from day 1 to day 5 and only increased from day 6 to day 9; after that, the concentration of secondary oxidized

Table VI

p-AV values with and without 1% of PPS

DAY	PV value without Oxidizing Agent (meq/kg)	PV value with Oxidizing Agent (meq/kg)
1	0.7092 ± 0.05	0.7121 ± 0.05
2	0.9571 ± 0.05	0.9612 ± 0.05
3	0.9889 ± 0.05	7.0465 ± 0.05
4	0.9999 ± 0.05	17.7754 ± 0.05
5	10.078 ± 0.05	17.19565 ± 0.05
6	11.5012 ± 0.05	17.0783 ± 0.05
7	13.7936 ± 0.05	17.0469 ± 0.05
8	16.0871 ± 0.05	
9	17.415 ± 0.05	
10	17.2245 ± 0.05	

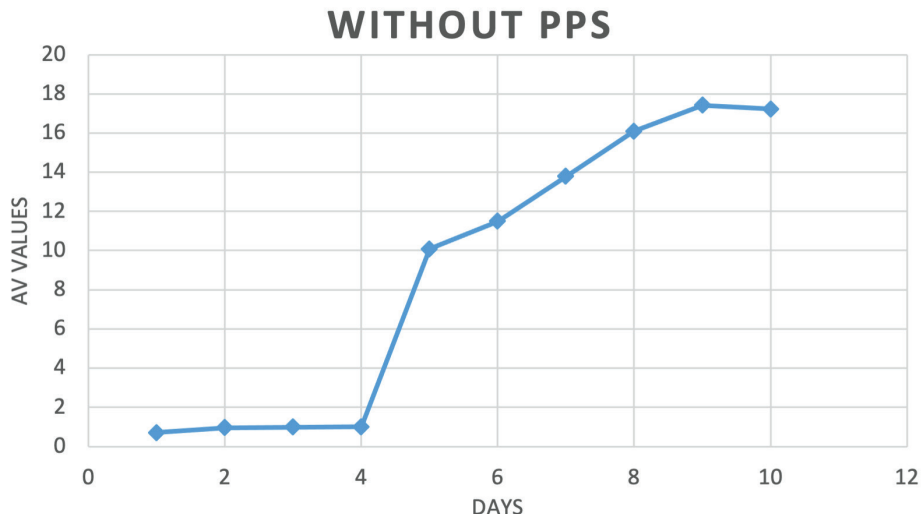

Figure 6. p-Anisidine values without oxidizing agent (PPS)

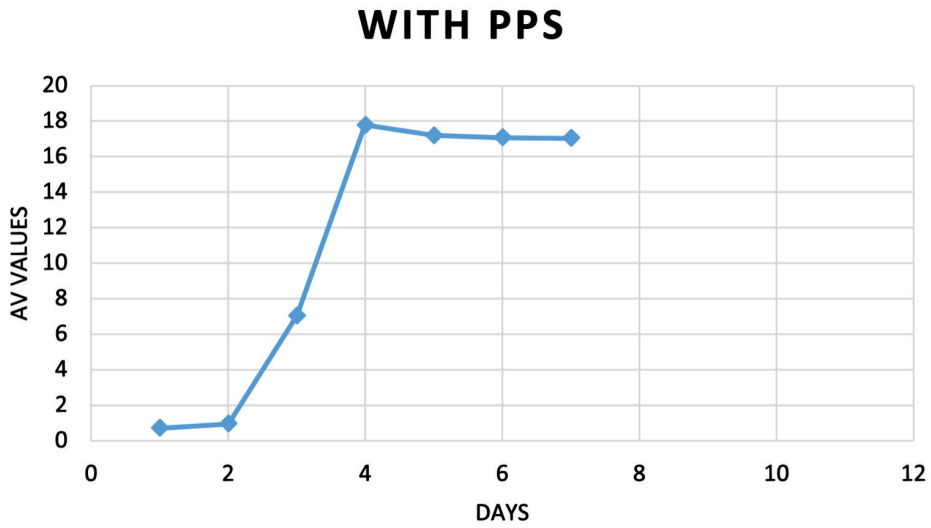

Figure 7. p-Anisidine values with oxidizing agent (1% of PPS)

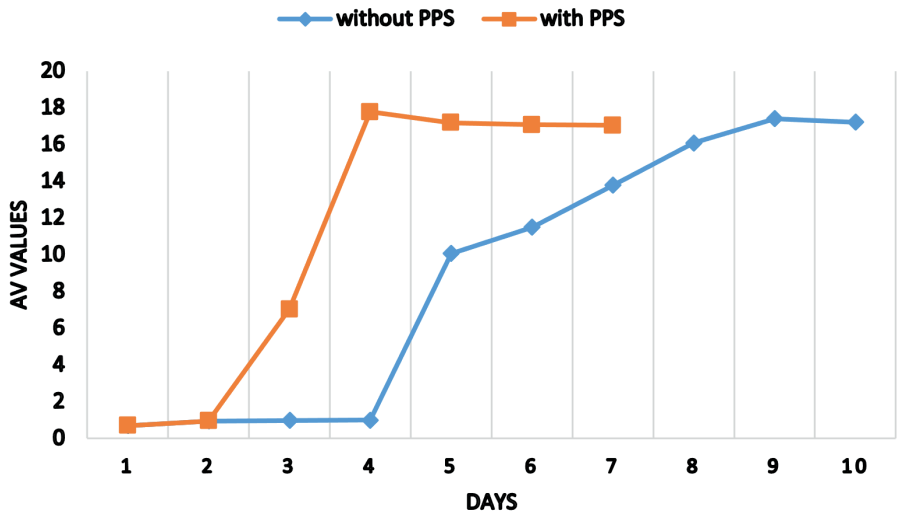

Figure 8. Comparison of p-Anisidine values with and without PPS

products started decreasing again. The graph produced is possibly due to the sluggish and steady production of secondary by-products. After attaining maxima, production begins declining.

Fish oil with 1% PPS shows high p-Anisidine values from day 2 up to day 4. It is probably due to the reaction of p- Anisidine reagent with the more secondary by-products (aldehyde, acetone, and their derivatives), which react with the amino groups of the p- Anisidine reagent.

5. Conclusions

In the present work, the kinetics of fish oil oxidation were studied, where 1% of PPS was used as an oxidizing agent. The study focused on the completion of an accelerated fish oil tanning process from 10 days to 4 days, with a remarkable increase in water absorption capacity by 454 %. The experimental leathers show better physical strength properties than control leathers. The conclusion may be drawn from the study that the use of 1% potassium per persulfate in chamois making reduces time and positively impacts the quality of final leathers.

6. Acknowledgments

The authors acknowledge Dr K J Sreeram, Director, CSIR-CLRI and Head, Department of Leather Technology, AC Tech, Anna University, for his support. Authors thank Dr J Raghava Rao, Retired Chief scientist, Dr B Madhan, Senior Principal Scientist, Dr GC Jayakumar, Senior Scientist, CSIR-CLRI for their continuous motivation. The authors thank CATERS for testing the samples. CSIR-CLRI communication number is 1787.

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Letter from the ALCA President

To: Members of the American Leather Chemists Association (ALCA)

Subject: ALCA Office and Administrative Operations

This letter is to inform our membership of recent changes to the Executive Secretary position.

Carol Adcock, our current Executive Secretary, will retire later this year (subject of an earlier announcement). In response, a Search Committee was organized to identify a replacement. Ultimately working with the Leather Hides Council of America (LHCA), the committee identified a path forward splitting resources approximately 60:40 between ALCA and LHCA as detailed in a MOU executed recently. There were many options and changes considered by the committee. In summary, resource sharing was selected as the best path forward to balance the position's evolving responsibilities, expressed needs and interests of both organizations, and the financial side, as the current economic cycle is impacting both organizations. In addition to the above, it was also decided to close the existing facility in Lubbock, Texas and transfer the mailing address to University of Cincinnati changing the position from office-based to remote work from home. It should be noted that the ALCA office was originally in the same building as the Leather Research Laboratory at the University of Cincinnati for over 50 years prior to moving to Texas.

We are happy to announce that Kristina Hall has accepted the position of Executive Secretary. Her contact details are listed below.

Kristina Hall, Executive Secretary
American Leather Chemists Association
University of Cincinnati
5997 Center Hill Ave. Building C
Cincinnati, OH 45224
513.290.2502
hallks@ucmail.uc.edu

While Carol is still working hard on conference details as is always the case this time of year, Kristina will gradually transition into her new role and conference attendees can meet her in person. Presently contacting Carol with current needs is unchanged, however, sometime after the conference a formal transition will take place. Details will be the subject of a separate notification.

At this time, I would like to thank the Search Committee for their fine work: Mike Bley, Sarah Drayna, Andreas Rhein, John Rodden, Steve Gilberg, and Steve Lange. I would also like to thank Stephen Sothmann of the LHCA for his support of the ALCA.

Sincerely,

Joseph Hoefler
ALCA President

Introducing the New ALCA Executive Secretary

Hello, my name is Kristina Hall, the new Executive Secretary for the ALCA. Although the leather industry is new to me, I bring with me years of experience in administration and organizational development.

I have what feels like two lifetimes of experience in the medical field of insurance billing and coding. In this ever-changing environment I have become flexible in meeting the needs of the department and individuals. This strengthened my organizational skills and made it possible for me to develop and implement the most effective policies and procedures.

I look forward to this new opportunity of moving with the ALCA and the industry into the future. During the convention, you will find me with Carol ingesting her wealth of information and knowledge of the ALCA, for a smooth transition. I look forward to personal introductions and getting to know you and the ALCA.

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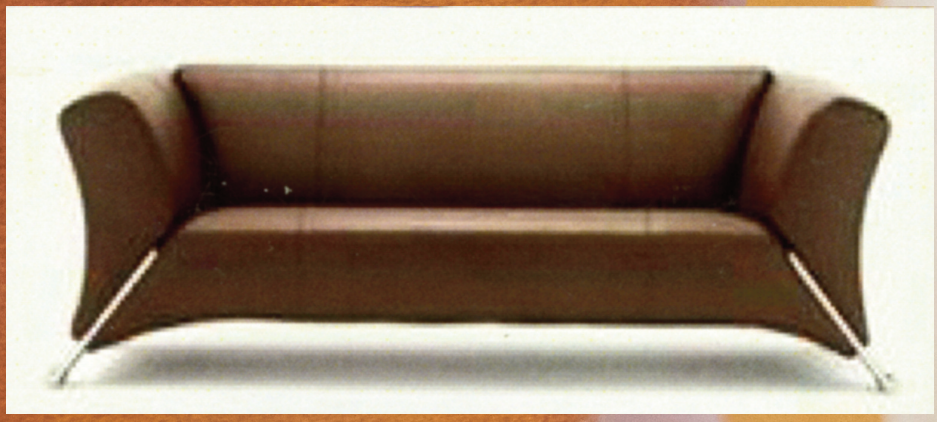

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Mona Vajpayee is a Research Scholar at Manipal University Jaipur, Rajasthan, India. She received her undergraduate and graduate degrees from Sardar Patel University Gujrat. She was awarded the Dayanand Saraswati and Meenakshi Subrahmanian gold medals in post-graduation. Her research interests include natural products and plasma surface modification, agriculture chemistry, natural fiber dyeing. She has written approximately five articles in reputable journals. She has also authored chapters for a book.

Mumal Singh received her Bachelor of Science degree from MDS University, Ajmer, in 2013. In the same year, she began her master's degree at IIS University's Department of Chemistry, which she completed in 2015. She is pursuing a doctorate in chemistry at Manipal University Jaipur. Her scholarly pursuits include plasma chemistry, nanotechnology, material characterization, natural dye and pigment chemistry, and the dying of natural textiles. She has contributed nearly five articles to international scholarly journals. She has also authored a book chapter through Springer. Additionally, she presented her results at over ten conferences and symposia.

Hemen Dave is working as an Assistant Professor at National Forensic Sciences University, Gandhinagar (An Institution of National Importance under the aegis of Ministry of Home, Government of India). He holds a Ph D Degree in Environmental Science and has more than 12 years of Research experience/teaching. The primary areas of his research work are Non-thermal plasma, eco-friendly processing of textile/leather, natural dyeing, natural products, adsorbent/advanced oxidation-based treatment of water/wastewater, and environmental forensics. He has published more than 20 research papers in journals of national/international repute, also written six book chapters. He is also having expertise in techno-legal consultation and environmental impact assessment.

Lalita Ledwani is working as a Professor of Chemistry and Dean, Faculty of Science at Manipal University Jaipur, Rajasthan, India. With over 20 years of post-Ph D experience in higher education, Dr. L Ledwani has been actively engaged in academic and research activities. Her areas of research interest are natural products, plasma surface modification polymers techniques, agriculture chemistry, green nano-materials, and wastewater treatment. etc. She is recipient of the SMC 2022 Bronze Medal Award by the Society for Materials Chemistry (SMC), Bhabha Atomic Research Centre (BARC), Mumbai; DST, Govt of Rajasthan 2022 Women Scientist Award; Research Grant Award 2015 from KWEF, Japan and also received financial support for over ten research projects from different funding agencies namely DST, Govt of India (05); DST, Govt. of Rajasthan (02); DBT, Govt of India (01) and UGC, Govt. of India (01). Dr. Ledwani has more than sixty research manuscripts published in reputed peer-reviewed journals and books, two patents, delivered around three dozen invited/expert/key note lectures besides over fifty-six research papers in conferences, and edited two books. She is guiding UG, PG, Ph.D and post-doctoral students. Four students have been awarded a Ph.D. degree under her supervision. Dr. Ledwani is associated with various national and international professional bodies as a fellow, life member, or member. Dr. Ledwani has also organized various national and international academic events in a lead role.

Murali Sathish - Working as a Scientist at CSIR- Central Leather Research Institute, Regional Centre, Kolkata, and his area of interest on leather science and technology and circular economy.

Palanisamy Thanikaivelan, see *JALCA* **112**, 356, 2017

Nayan Sarkar - Working as a Senior Technical Officer at CSIR-Central Leather Research Institute, Regional Centre, Kolkata, and his area of interest on leather science and technology.

Rathinam Aravindhan - Working as a Scientist at Leather Processing Department, CSIR- Central Leather Research Institute, Chennai, and his area of interest on Sustainable leather processing.

Jonnalagadda Raghava Rao - Retired Chief Scientist and currently working as Emeritus Scientist at Inorganic and Physical Chemistry Department, CSIR- Central Leather Research Institute, Chennai, and his area of interest on leather science and technology.

Tingyuan Chen received his Bachelor's degree in Light Chemical Engineering from Qilu University of Technology in 2020. Now he is pursuing his Master's degree in Leather Chemistry and Engineering at Sichuan University. His current research focuses on leather biotechnology.

Yunhang Zeng is currently a professor in National Engineering Laboratory for Clean Technology of Leather Manufacture, Sichuan University. She received her Ph.D. degree in Leather Chemistry and Engineering from Sichuan University in 2013. She joined Sichuan University as a lecturer from 2013 to 2016 and was an associate professor from 2016 to 2021. Her research focuses on clean leather production and leather biotechnology.

Bi Shi, see *JALCA* **99**, 220, 2004

Bindia Sahu, See JALCA, 117(6), Page no., 223-231

Jaya Prakash Alla, See JALCA, 116, Page no., 227-232

Akash Bhalla is a leather technologist with a Diploma in Leather Technology from Government Leather Institute, Kanpur. He has gained significant industrial experience through his work stints at Tata International in Dewas, Lanxess India Pvt Ltd, and Smit & Zoon India Pvt Ltd in Kanpur respectively. Currently, Akash is working as a Technical Assistant in the Leather Process Technology Department of CSIR-CLRI. His area of interest lies in the wet-end and cleaner leather processing techniques.

Diya Sharma is a **s**tudent of BTech (Fourth-year) chemical Engineering from National Institute of Technology, Tiruchirappalli, Tamil Nadu 620015, India

Yogesh Sekar is a **s**tudent of MSc Chemistry (Second year) from Institute of Chemical Technology, Mumbai 400019, India

Obituaries

Carl Roy Bagg died peacefully, on Friday, September 9, 2022 at the Oak Ridges Hospice in Port Perry, at age 86. He was the loved father of Paul Bagg and his wife Shelley of Uxbridge, Carla and her husband Steve Stamatis of Murrieta, California, Margo and her husband Alessandro Zara of Oshawa, and Brent Bagg and his wife Jennifer of Dorset, and proud grandfather of Jeremy, Lindsay, Samantha, Caitlin, Daniel, Ashley, Kristin, and Alexi and great grandfather of Jacob, Adriana, Anthony, Ethan, Aiden, Logan, and Ben.

Carl was born January 25, 1936 to the late Joseph Roy Bagg and Evelyn Janet (nee Sabine.) He graduated from Brock Collegiate Institute in 1952.

Carl was a retiree of Stahl Canada and Stahl U.S.A., employed in the leather and chemical industry for many years. He joined the ALCA in 1972 as an associate and become an active member on October 1, 1975. He was given retired status in the ALCA in 2004.

James E. Cartier, of Saco, passed away peacefully with his family by his side at the Maine Veterans' Home in Scarborough on November 17, 2022.

"Jimmy" was born on May 15, 1934 to Margaret (Hayes) and Charles Cartier. Jimmy graduated from St. Louis High School in 1953 and earned a B.S. in Analytical Chemistry from Boston College in 1957. He served in the U.S. Army from 1957-1959.

After working five years with Merck Chemical Inc. in Rahway, N.J., Jimmy and his family returned to Saco where he set up lab testing for Saco Tanning Corp. until it closed. During this time, he developed a patent, Chromefloc, for a waste water recovery process. Jimmy worked as a consultant with Jones and Beach Engineering in Stratham, N.H. until his retirement. Jimmy was civic minded and a man of faith. He was a charter member and past president of Saco Bay Rotary, and a Eucharistic minister at Most Holy Trinity.

Jimmy joined the ALCA on July 16, 1964. He was a member of the Effluent Committee in 1984. He became a retired member of the ALCA in 1997.

Jimmy is survived by his wife of 65 years Jeanne (Belair) Cartier and his two daughters, Jane and Judy.

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Wednesday, June 21
John Arthur Wilson Memorial Lecture
All Day Technical Sessions, Fun Run, Dinner
Boat Cruise

Thursday, June 22
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Social Hour, Awards Banquet

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