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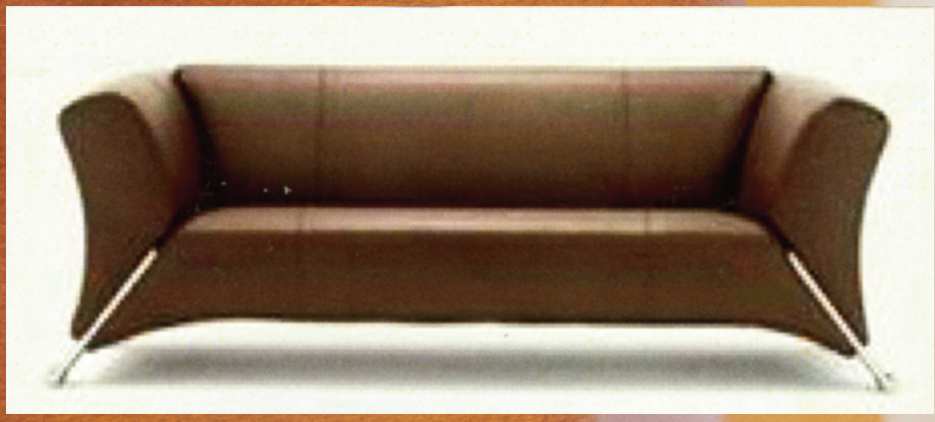

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Production of Carboxymethyl Starches from Oxidized Starches and Determination of Their Tanning Characteristics

by

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Abstract

Hydrogen peroxide and sodium metaperiodate oxidation of starch and their possible utilization in tanning/retanning were examined in our previous studies. In the present part, accordingly with our previous findings, hydrogen peroxide and sodium metaperiodate oxidation products having appropriate molecular weight/size were selected and additionally carboxymethylated. The yields of the processes (carboxymethyl starches) were characterized comprehensively and the effect of carboxymethylation process on structures and tanning abilities were tried to be identified. The characterization results revealed that the carboxymethyl groups were successfully included into the structure and the water solubility of oxidized starches (especially periodate oxidized ones) increased by carboxymethylation process. From the evaluation of the tanning results and considering its properties i.e. gentle tanning effect with less astringency and correspondingly a relatively soft leather handle and smooth grain, it is concluded that dialdehyde carboxymethyl starch (CMS 1:0.7) can be utilized as yet another good alternative sustainable green tanning/retanning agent from starch.

Introduction

The bio-based tanning/retanning agents which are able to replace mineral tanning agents have been the focus of interest in leather industry due to environmental regulations and coming into prominence of ecological chemical processes. In line with this purpose, leather chemists and manufacturers head towards production of bio-based tanning/retanning agents from inexpensive, ubiquitous, sustainable, biodegradable biopolymers. When viewed from this aspect, starch is an important raw material for producing sustainable green tanning/retanning agents. For this reason, we focused on different modification techniques of native corn starch for possible utilization in leather making as a tanning agent. In our previous studies,[1,2] native corn starch was oxidized by hydrogen peroxide (H_2O_2) and sodium metaperiodate ($NaIO_4$) oxidation methods in different molar ratios with the aim of reducing its molecular weight/size to penetrate between skin fiber matrix and introducing reactive groups to react and establish stable bonds with the active groups of collagen.

The obtained results encouraged us to investigate different modification methods and in this part of our study, we decided to focus on the carboxymethylation process. Carboxymethyl starch (CMS) has unique properties due to including a negatively charged functional group (CH_2COO^-), this modification provides decreased gelatinization temperature, increased solubility and improved storage stability, in addition soluble starch in cold water can be produced depending on the degree of substitution (DS).[3] Besides, negatively charged CH_2COO^- groups can make additional bonds with the active groups of collagen. However, considering the promising results of our earlier studies and necessity of appropriate molecular weight/size, we have decided to apply carboxymethylation process on previously oxidized starch samples. For this purpose, oxidized starch samples, having appropriate molecular weight to penetrate within fiber structure of collagen were chosen (1:7, 1:9, 1:11 molar ratios from H_2O_2 oxidation, 1:0.3, 1:0.5, 1:0.7 molar ratios from $NaIO_4$ oxidation) to be used in carboxymethylation processes and the effect of carboxymethylation process on chemical structures, features and tanning abilities of oxidized starches were investigated.

Materials

The picked H_2O_2 and $NaIO_4$ oxidized starch samples pursuant to our earlier studies[1,2] were used in carboxymethylation processes. Monochloroacetic acid ($C_2H_3ClO_2$, 99%), sodium hydroxide (NaOH, 98-100.5%) ethanol (C_2H_6O, ≥99.8%) were supplied from Sigma Aldrich and the remaining chemicals/solvents used in experiments were in analytical grade. In tanning experiments, pickled goat skins were used.

Methods

Carboxymethylation of H_2O_2 and $NaIO_4$ Oxidized Starch Samples: In our former studies,[1,2] native corn starch was oxidized by H_2O_2 and $NaIO_4$ oxidation methods and the samples (1:7, 1:9, 1:11 molar ratios from H_2O_2 oxidation, 1:0.3, 1:0.5, 1:0.7 molar ratios from $NaIO_4$ oxidation) which were thought to be more favorable for tanning were picked and used in tanning trials. In present part of our study, these picked samples were synthesized pursuant to our earlier studies and subjected to a further modification by carboxymethylation process. Carboxymethylation was performed

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(a) **(b)**

Figure 1. Carboxymethylated starches; **(a)** dried in an oven after carboxymethylation, **(b)** milled after drying.

in a similar way described by Hebeish et al.[4] with slight changes (molar ratios, reaction temperature, shaking and drying conditions). Firstly, 8.1 g oxidized starch sample, 9.45 g monochloroacetic acid, 10 mL of 5 N NaOH solution and 45 mL distilled water were added in a 100 mL flask (which corresponds to 1:2:1 molar ratios of Starch:Monochloroacetic acid:NaOH respectively). The mixture was continuously stirred to obtain a homogenous mixture. After that, the flask was transferred to a water bath at 50°C and the reaction was maintained for 1 h with continuous shaking at low constant speed. The products were precipitated in ethanol and washed for decontaminating from alkali then dried in a hot-air oven at 50°C for 48 h until constant weight (Figure 1). The product yields were determined by using the method described in our previous studies[1,2] and the yields were calculated according to the following formula 1. Finally, dried samples were milled to obtain the products in powder form.

$$\text{Yield \%} = \frac{\text{Obtained oxidized starch (g)}}{\text{Amount of native starch used (g)}} \times 100 \qquad (1)$$

Determination of degrees of substitutions: The degree of substitutions (DS) of carboxymethylated starches (CMSs) were determined according to the titrimetric method which was described by Jiang et al.[5] The DS was determined by following formula 2.

$$DS = \frac{n_{NaOH} \times M_o}{m_c - n_{NaOH} \times M_R} \times 100\% \qquad m_c = m_p - \left[\frac{mp \times F}{100}\right] \qquad (2)$$

M_o = the molar mass of anhydroglucose unit (162 g/mol)

M_R = the molar mass of carboxymethyl residue (58 g/mol)

n_{NaOH} = the quantity of sodium hydroxide used (mol)

m_p = the weight of polymer taken (g)

m_c = the corrected weight of polymer (g)

F = the moisture (%)

Determination of water solubility of CMSs: The method given by Singh and Singh[6] was used to determine water solubility of carboxymethylated starches with minor modifications[1,2] and it was calculated by following formula 3.

$$\text{Water solubility \%} = \frac{\text{Supernatant solid weight (g)} \times 2}{\text{Sample weight (g)}} \times 100 \qquad (3)$$

Structure characterizations: The changes in the structures of native and carboxymethylated starches by modification were tried to be identified by FT-IR and ^{1}H-NMR analyses. The FT-IR spectra were recorded in the range of 4000-650 cm^{-1} by Perkin Elmer Spectrum 100 FT-IR spectrometer. The ^{1}H-NMR spectra were gained on a MERCURYplus-AS 400 MHz spectrometer (Ege University, NMR Satellite Laboratory, Izmir/Turkey). DMSO-d6 was used as solvent and the concentration was 20 mg/mL.

Tanning trials: Pelt pieces, 20x20 cm in size, from croupon areas of pickled goat skins were used in preliminary tanning trials. Since carboxymethylated derivatives of oxidized starch samples from $NaIO_4$ oxidation and H_2O_2 oxidation bear different reactive groups (–COH & covalent bonds; –OH, –CO, and –COOH groups & hydrogen bonds and salt bridges) and therefore require different process parameters in order to achieve proper penetration and establish bonds with relevant functional groups of collagen, different tanning recipes were used for each product (Table I and Table II).

After assessment of the preliminary tanning trials' results; CMS sample having the best tanning effect was determined and a whole pelt was tanned with selected CMS according to similar recipe given in Table II with the changes: introduction of dialdehyde starch in 2 portions and running the drum for 120 min. after each introduction, raising the pH up to 7.5-7.8 at the end of tanning process, introduction of a replacement syntan (3%) and an amphoteric polymer (4%) before fatliquoring process and application of a fatliquoring process consisting of natural+synthetic fatliquor combination (4%), synthetic fatliquor (3%), sulfone synthetic fatliquor (2%), polymeric fatliquor (2%) and phosphoester based fatliquor (1%).

Table I

Tanning recipe for carboxymethylated H_2O_2 oxidized starches (CMS 1:7, CMS 1:9, CMS 1:11)

PROCESS	AMOUNT (%)	PRODUCT	TEMP. (°C)	TIME (min.)	pH
Depickle	150	Water 7 °Be NaCl	28-30	10	
	1	HCOONa		45	
	X	$NaHCO_3$		120	5.0
Washing & Draining					
Tanning	100	Water 3 °Be	30	10	
	20	Starch sample		180 (left in bath overnight statically)	
	x	HCOOH		60	3.5
Washing & Draining					
Neutralization	100	Water	40		
	2	Neutralizing syntan		45	5.0-5.5
Fatliquoring	5	Natural+synthetic fatliquor combination	45	60	
	3	Sulfone synthetic fatliquor			
	2	Phosphoester based fatliquor			
Fixation	X	HCOOH		60	3.8-4.0
Washing					

Table II

Tanning recipe for carboxymethylated periodate oxidized starches (DCMSs) (CMS 1.0.3, CMS 1:0.5, CMS 1:0.7)

PROCESS	AMOUNT (%)	PRODUCT	TEMP. (°C)	TIME (min.)	pH
Depickle	150	Water 7 °Be' NaCl	28-30	10	
	1	HCOONa		45	
	x	$NaHCO_3$		120	5.5
Draining					
Tanning	100	Water	30		
	20	Starch sample		180	
	0.25	$NaHCO_3$		30 (left in bath overnight statically)	
	0.25	$NaHCO_3$		30	
	0.25	$NaHCO_3$		30	
	x	$NaHCO_3$		60	7.0-7.5
Washing & Draining					
Fatliquoring	100	Water	45		
	5	Natural+synthetic fatliquor		60	
	3	Sulfone synthetic fatliquor			
	2	Phosphoester based fatliquor			
Fixation	x	HCOOH		60	3.8-4.0
Washing					

Determination of tanning effects: The tanning abilities of carboxymethylated starch samples were evaluated by investigating hydrothermal stability,[7] filling[1,2] and fiber isolation characteristics (Hitachi TM-1000 table top scanning electron microscope (SEM) at 400 magnifications) of tanned leathers.

Physical characteristics of the leathers gained by carboxymethylated starch tanning were investigated by measuring the tensile strengths and percentages of elongation[8] and tear loads[9] of the samples. However, the whole leather which was tanned with selected CMS (having the best tanning effect) was additionally tested in terms of distension and strength of surface test.[10] Shimadzu AG-IS Tensile Tester and Trapezium-2 software program was used for all physical tests.

After performing the tests mentioned above, additionally leachability test was applied to the leather samples tanned with carboxymethylated derivatives of oxidized starches in preliminary tanning trials. Leather pieces, sized 2x5 cm, were placed in 250 mL flasks and agitated on a shaker for 24 hours at 120 rpm at room temperature with 100 mL of distilled water. Then, hydrothermal stability[7] tests are repeated in order to investigate irreversible binding abilities of the carboxymethylated derivatives of oxidized starches.

Results and Discussion

The yields of carboxymethylated starches (CMSs)

The yields of CMSs are shown in Figure 2. From the results, it was seen that the yields of carboxymethylated derivatives of oxidized starches reduced more or less with increasing amounts of oxidants previously used in reactions. Introduction of carboxymethyl groups into the oxidized starch structure and increasing water solubility came along with them was thought to be the reason for the decline in yields. Recalling that carboxyl and dialdehyde groups were introduced by peroxide and periodate oxidation reactions[1,2] respectively before carboxymethylation and considering the fact that while the carboxyl groups redound to water solubility, dialdehyde groups do not have a significant contribution on it, the yields of carboxymethylated derivatives of oxidized starches with H_2O_2 (CMS 1:7, CMS 1:9, CMS 1:11) were found to be lower than $NaIO_4$ oxidized ones (CMS 1:0.3, CMS 1:0.5, CMS 1:0.7) as expectedly.

Degree of substitutions (DS)

Substitution degrees of CMSs are shown in Figure 3. From the results, it was seen that the number of carboxymethyl groups introduced into the structure of CMS 1:7, CMS 1:9 and CMS 1:11 samples were reduced in conjunction with increasing oxidation degree, contrary to dialdehyde carboxymethyl starches (DCMSs) (CMS 1:0.3, CMS 1:0.5, CMS 1:0.7). It is known that the hydroxyl groups in C-2 and C-3 of anhydrous glucose units are selectively oxidized to aldehyde groups in periodate oxidation while the hydroxyl groups in starch molecules are oxidized to carbonyl and carboxyl groups, primarily at C-2, C-3 and C-6 in peroxide oxidation. For this reason, there are more un-substituted groups in periodate oxidized starch samples. It was thought that the unsubstituted hydroxyl groups in C-6 of anhydrous glucose units in starch structure, which have the strongest reactivity, replaced with carboxymethyl groups by carboxymethylation process and resulted in higher substitution degrees. Accordingly, Hebeish et al.[4] remarked that the carbonyl and carboxyl groups included in starch molecule decrease the efficiency of carboxymethylation.

Although higher degrees of substitution were reported in literature, it was seen that in these studies isopropanol, methanol, ethanol or their mixture with a small amount of water was selected and used as solvent for carboxymethylation processes.[11-15] Pursuant to literature, in the present study these alcohols and their mixtures were tried to be used as solvent to carry out carboxymethylation process. But, oxidized starch samples became sticky immediately after mixing with them. Thus, water was decided to be used as solvent for reaction. As a matter of fact, there are a few studies in

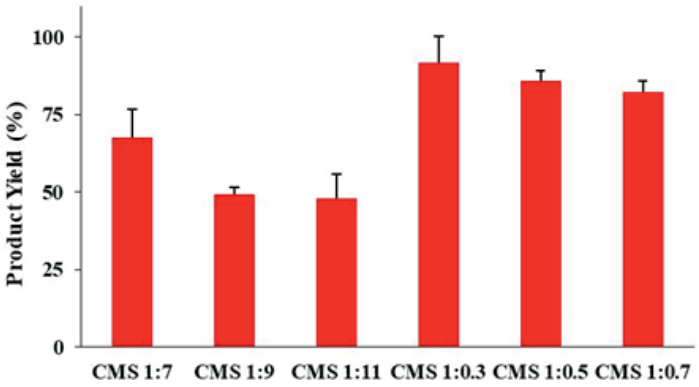

Figure 2. The yields of CMSs.

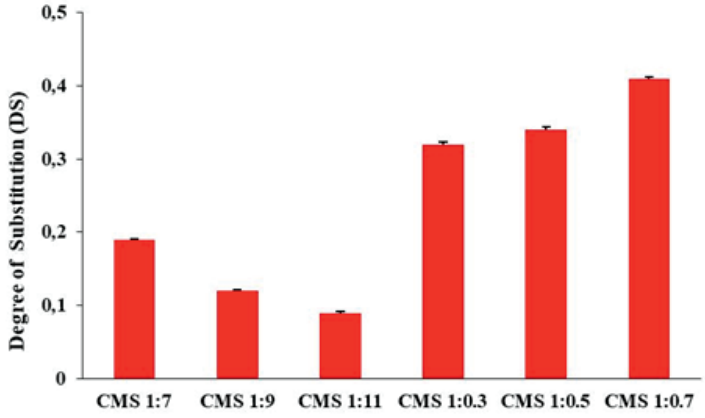

Figure 3. The degree of substitutions of CMSs.

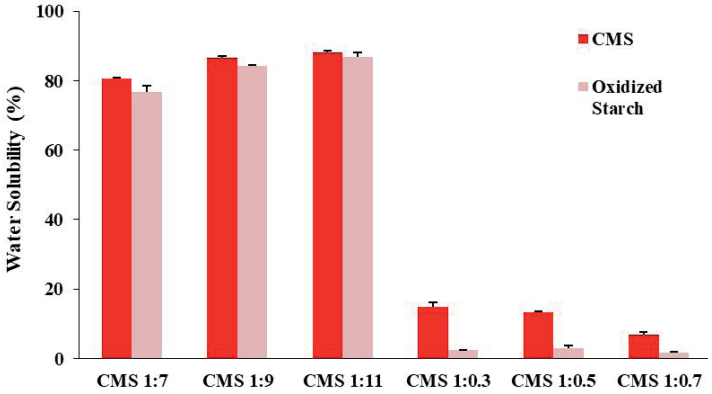

Figure 4. The water solubilities of CMSs.

literature on carboxymethylation of oxidized starches, and similar with our findings, lower degrees of substitution were obtained in carboxymethylation processes when the water was used as solvent.[4,16]

Water solubility of CMSs

The effect of carboxymethylation process on water solubility of oxidized starches is shown in Figure 4. From the evaluation of the data regarding the solubility of CMS 1:7, CMS 1:9, CMS 1:11 samples, it was noticed that the solubility of oxidized starches increased slightly by carboxymethylation process. In first place, the water solubility of H_2O_2 oxidized starches[1] was found to be between 76.9 - 86.9%, 1.5-4.8% of additional increment arose from carboxymethylation process.

On the other hand, it was observed that the increase in water solubility of dialdehyde carboxymethyl starches (DCMSs) was more noticeable. The water solubility values of DCMSs were 3.8-6.2 times higher comparing with their oxidized forms[2] (between 1.8-2.9%). The increment in water solubility values of DCMSs was attributed to introduction of more CH_2COO^- groups into the structure. However, the water solubility of DCMSs decreased by increasing molar ratio of $NaIO_4$, previously used for oxidation. The reason might be that higher degrees of oxidation with $NaIO_4$ resulted with introduction of higher number of aldehyde groups, correspondingly formation of more cross-links and finally at this stage resisted access of water molecules into the structure.

Characterizations of CMSs

Structures of carboxymethylated derivatives of H_2O_2 and $NaIO_4$ oxidized starches were confirmed by FT-IR as shown in Figure 5 and Figure 6. The comparative study between the FT-IR spectra of native starch and CMSs shown the new peaks occurred by carboxymethylation process. Comparing with the native starch (Figure 5), the new peak at 1730.79 cm^{-1} which expands gradually according to the degree of oxidation belongs to C=O stretching vibrations. Although this peak was also previously seen in the FT-IR spectra of oxidized starches with H_2O_2,[1] it was noticed that it was more pronounced in carboxymethylated derivatives. This is an evidence for including additional carboxyl groups into the structure because the protonated carboxylic groups (-COOH) similarly give the C=O band at 1730.79 cm^{-1}.[17,18]

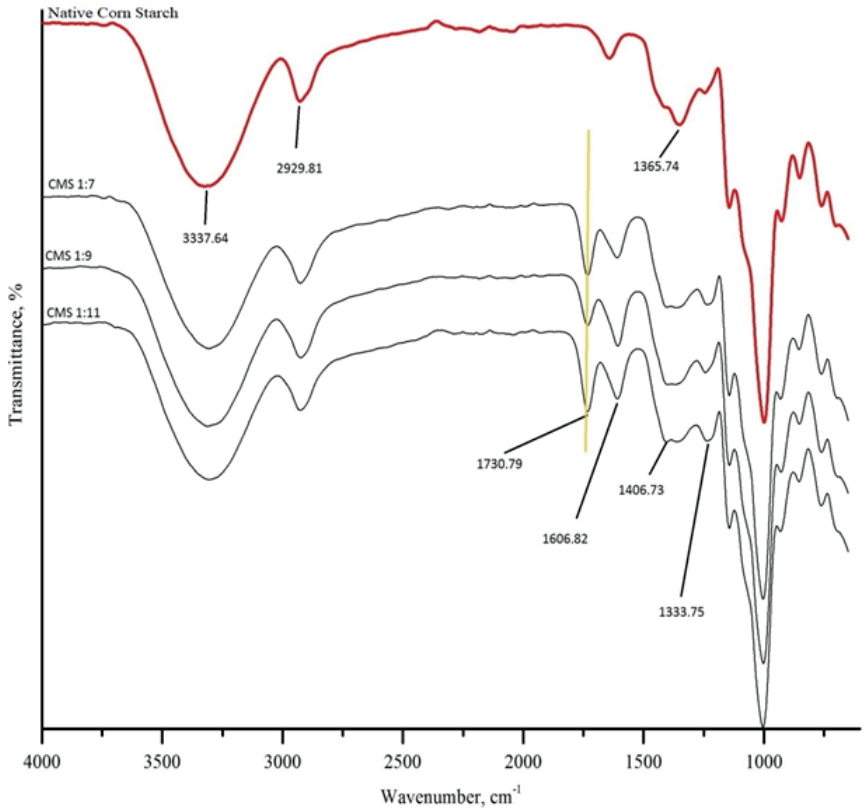

Figure 5. The FTIR spectra of native and carboxymethylated derivatives of H_2O_2 oxidized starches.

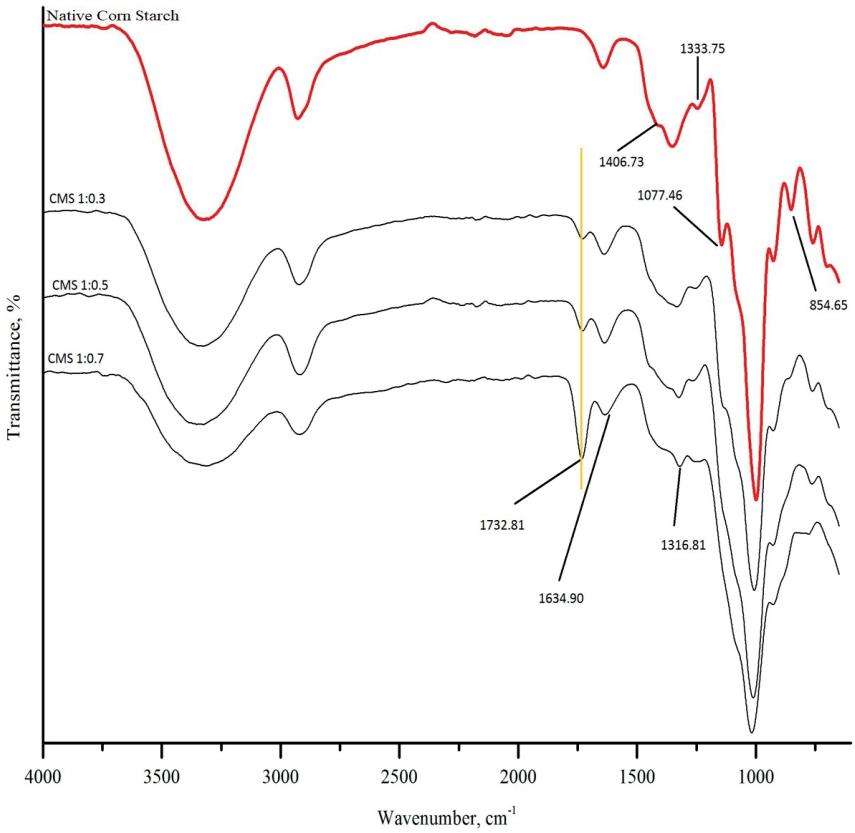

Figure 6. The FT-IR spectra of native and carboxymethylated derivatives of $NaIO_4$ oxidized starches.

In addition, it was observed that the peaks at 1606.82, 1406.73 and 1333.75 cm^{-1} which are attributed to characteristic carboxylate (-COO-) absorption peaks of carboxymethyl starch[5,18] became more prominent and OH absorption peak of native starch at 1365.74 cm^{-1} gradually decreased with carboxymethylation.[19] The obtained FT-IR spectra proved that the carboxymethylation process applied to hydrogen peroxide oxidized starches was successfully carried out.

Figure 6 shows the FT-IR spectra of native starch and DCMSs. It was noticed that DCMS samples had a new peak at 1732.81 cm^{-1} and this peak gradually increased with increasing oxidation degree. In the FT-IR spectra of periodate oxidized starches,[2] this peak occurred with a lower intensity at 1726.94 cm^{-1}, but the increasing intensity of this peak after carboxymethylation shows that additional carboxyl groups were included into the structure of periodate oxidized starches. As a matter of fact, protonated carboxylic groups (-COOH) also give a C=O band in this area, as mentioned before. In addition, it was observed that the peaks at 1077.46 cm^{-1} and 854.65 cm^{-1}, which show the C-O bond stretching of the C-OH group in the glucose chain of native starch and the skeletal stretching vibration of the starch respectively, disappeared with carboxymethylation.

The structural changes in chemical structure of oxidized starches by carboxymethylation were also verified by ^{1}H-NMR (Figure 7 and 8). The ^{1}H-NMR spectrum of 1:9 H_2O_2 oxidized starch[1] was also presented as a reference in order to clearly discern the changes in the structure of oxidized starches by carboxymethylation. Comparing with the spectra of oxidized form (Figure 7), it was seen that the new signals between 3.8-4.5 ppm occurred in ^{1}H-NMR spectra of carboxymethylated derivatives of H_2O_2 oxidized starches and it was revealed that carboxymethylation process was effective on oxidized starches. It was noticed that the OH-6 proton signal in the spectrum of oxidized starch gradually disappeared by increasing oxidation degree in the spectra of carboxymethylated derivatives, which shows that carboxymethylation preferentially occurs on C-6. Cízová et al.[20] remarked that the peak at 4.3 ppm attributed to the protons of the substituted carboxymethyl. However, the signal at 1.8 ppm which is attributed to the protons of the CH_2 group next to the carbonyl or carboxyl group in oxidized starches disappeared by carboxymethylation, it also confirms that the carboxymethyl groups are mostly substituted with the group in C-6. On the other hand, the occurring alterations in OH-2 and OH-3 signals in the spectrum of oxidized starch after carboxymethylation revealed that substitution actualize also in these groups.

The ^{1}H-NMR spectra of DCMSs are shown in Figure 8. The new signals, distinctively from their oxidized forms,[2] are indicated by the blue arrow in the ^{1}H-NMR spectra of the carboxymethylated derivatives. The signals at 3.8 and 4.2 ppm were attributed to methylene protons in C-CH_2-O-R (R:CH_2COONa) group and carboxymethyl protons in O-6, respectively. However, it was seen that the signals of the aldehyde groups included in starch structure

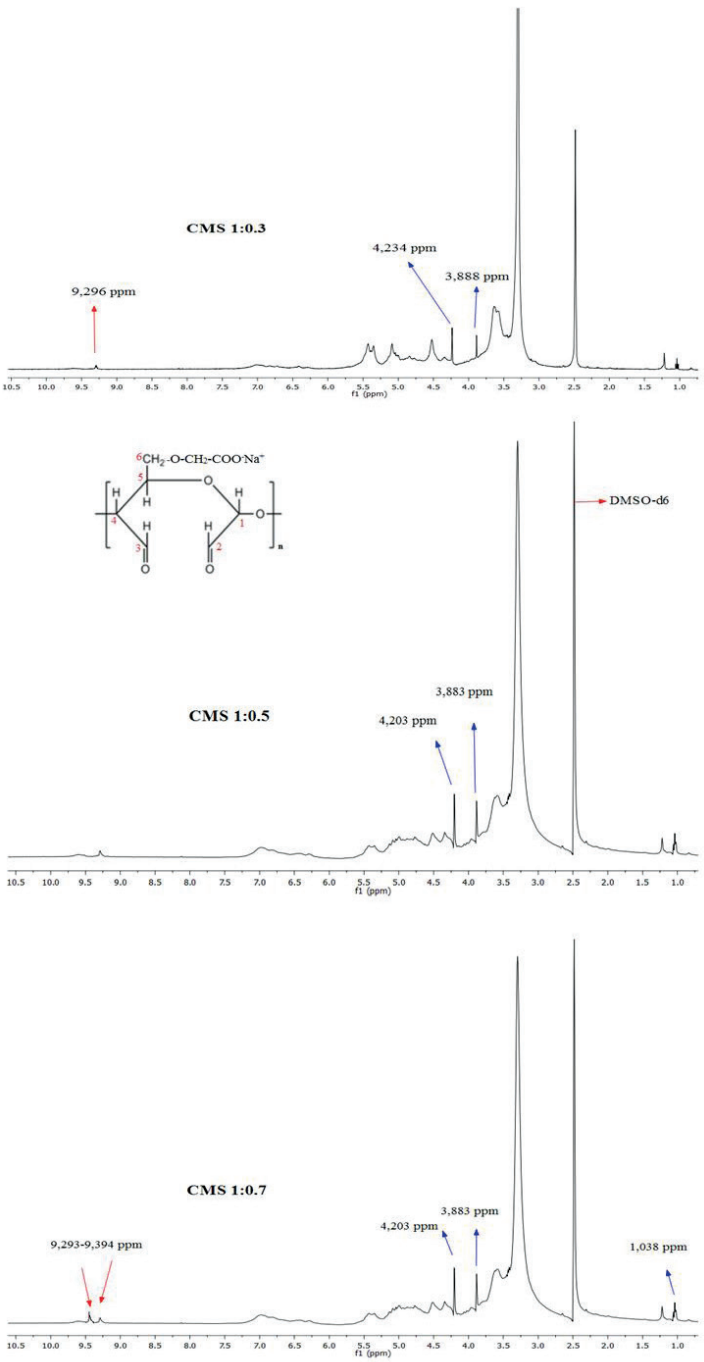

Figure 7. NMR spectra of carboxymethylated derivatives of H_2O_2 oxidized starches.

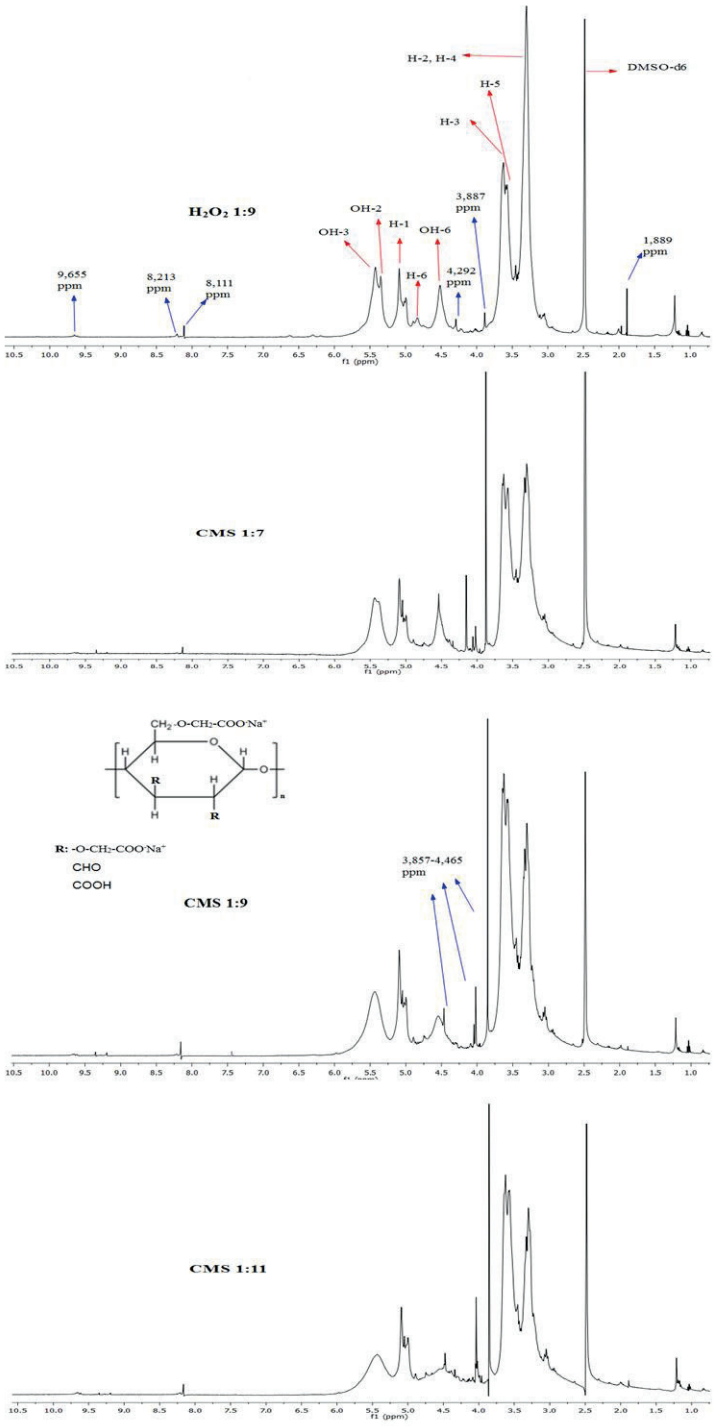

Figure 8. NMR spectra of carboxymethylated derivatives of $NaIO_4$ oxidized starches.

after $NaIO_4$ oxidation did not change markedly. Thus, it was thought that the carboxymethyl group included in the structure by carboxymethylation mostly replaced with the hydroxyl group in C-6 and it was confirmed with the signal seen at 4.2 ppm.

Tanning effects of CMSs

The data related to tanning abilities of carboxymethylated derivatives of oxidized starches were given in Table III. Considering the filling properties of CMSs, it was determined that the filling coefficients of DCMSs were slightly better than carboxymethylated derivatives of peroxide oxidized starches and the highest filling effect was obtained with CMS 1:0.7 among all samples. However, it was noticed that the filling coefficient values significantly regressed by carboxymethylation especially in $NaIO_4$ oxidized starch samples comparing with our previous study.[2]

Table III

Tanning properties of CMSs

	Modification Methods	Sample Name	Filling coefficient (%)	Shrinkage temp. Ts (°C)	Ts (°C) after leaching
Tanning with pelt pieces	H_2O_2 Oxidation (1:7 molar ratio) + Carboxymethylation	CMS 1:7	4.2(±0.4)	52.5(±0.7)	53.0
	H_2O_2 Oxidation (1:9 molar ratio) + Carboxymethylation	CMS 1:9	4.9(±0.3)	54.0(±0.5)	54.5
	H_2O_2 Oxidation (1:11 molar ratio) + Carboxymethylation	CMS 1:11	10.5(±0.6)	54.5(±0.6)	55.0
	$NaIO_4$ Oxidation (1:0.3 molar ratio) + Carboxymethylation	CMS 1:0.3	4.7(±0.5)	65.5(±0.8)	62.5
	$NaIO_4$ Oxidation (1:0.5 molar ratio) + Carboxymethylation	CMS 1:0.5	5.5(±0.6)	66.0(±0.8)	63.5
	$NaIO_4$ Oxidation (1:0:7 molar ratio) + Carboxymethylation	CMS 1:0.7	14.0(±0.3)	67.0(±0.5)	64.5
Tanned whole pelt	$NaIO_4$ Oxidation (1:0:7 molar ratio) + Carboxymethylation	CMS 1:0.7	-	71.0(±0.4)	-

From the evaluation of hydrothermal stabilities gained to the leathers, it was noticed that the shrinkage temperatures of leathers increased little by little in conjunction with increasing previously applied oxidation molar ratio. However, it is clearly seen that the shrinkage temperatures of leathers tanned with DCMS samples are higher than carboxymethylated derivatives of peroxide oxidized starches, as expected. The underlying reason is that the incorporated aldehyde groups into the structure of DCMSs by $NaIO_4$ oxidation are linked to collagen by more robust covalent bonds, while the carbonyl/carboxyl groups and carboxymethyl groups, which are incorporated into the starch structure by peroxide oxidation and carboxymethylation processes respectively, are essentially linked to collagen by feebler ionic and/or hydrogen bonds (Figure 9). Although the highest shrinkage temperature was obtained by CMS 1:0.7, between 9 and 23.5°C increase was achieved in tanned samples comparing with the intact pelt's hydrothermal stability (43.5°C). The preliminary tanning trials' results showed that CMS 1:0.7 sample has the best tanning effect thus a whole pelt was also tanned with this CMS sample. From the tanning of the whole pelt with CMS 1:0.7 sample, the shrinkage temperature of the leather was found to be 71°C, which means that 27.5°C of increase at shrinkage temperature was achieved comparing with the intact pelt.

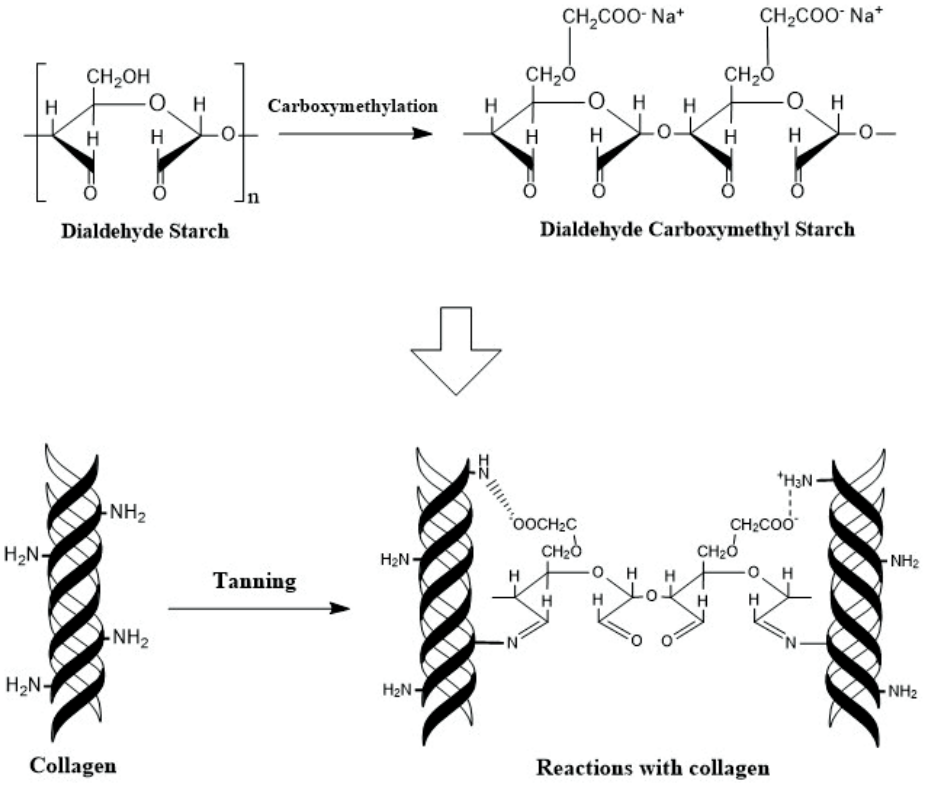

Figure 9. Schematic illustration for the reaction of collagen with DCMS.

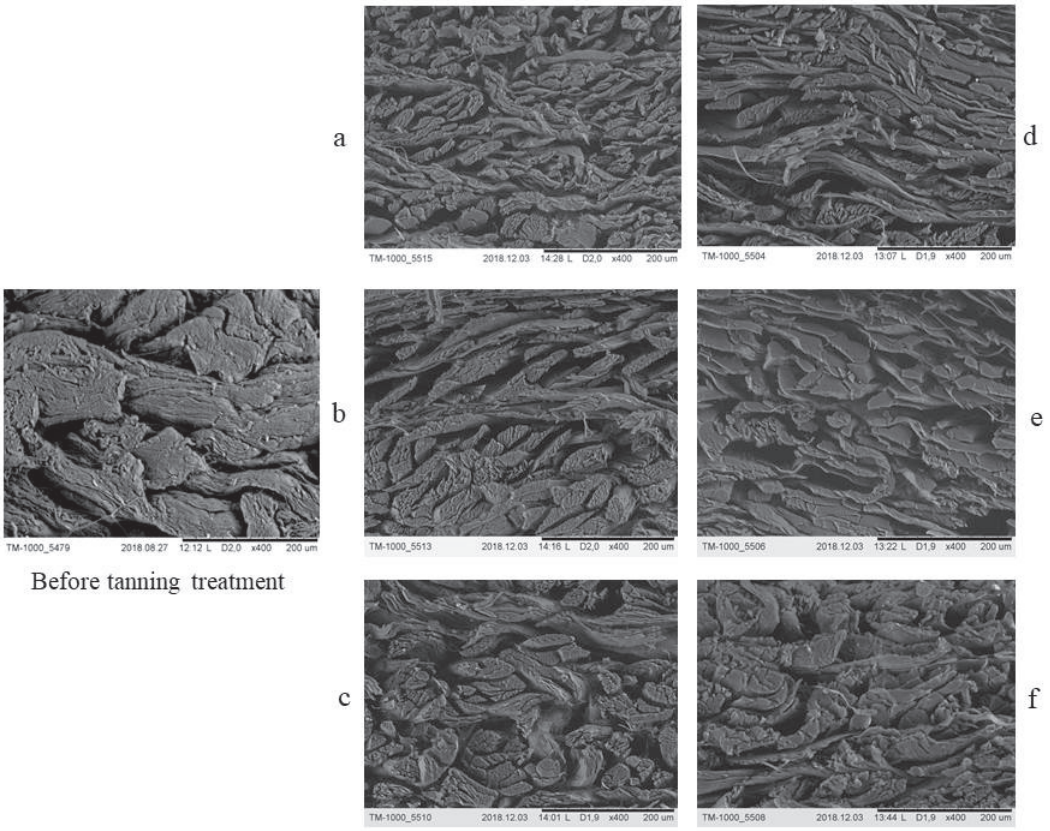

Figure 10. SEM images of pickled goat skin, carboxymethylated derivatives of H_2O_2 oxidized starches **(a, b, c)** and carboxymethylated derivatives of $NaIO_4$ oxidized starches **(d, e, f)**.

Additionally leachability test was applied to the leather samples obtained from preliminary tanning trials. From the comparison of the shrinkage temperature (T_s) values of the leathers before and after leaching, it was determined that while there was no considerable change in the shrinkage temperatures of the leathers tanned with the H_2O_2 oxidized and carboxymethylated samples, 2-3°C of decreases were detected in shrinkage temperatures of the leathers tanned with $NaIO_4$ oxidized and carboxymethylated samples (Table III). Considering the shrinkage temperature changes occurred after leaching, it was concluded that modified starch samples established stable bonds with the functional groups of collagen to a certain extent.

However, comparing the tanning characteristics of 1:0.7 periodate oxidized starch[2] (dialdehyde starch) with CMS 1:0.7 it was observed that the astringency of dialdehyde starch reduced and it gained gentler tanning effect by carboxymethylation. For this reason, although slightly lower shrinkage temperature (71°C) was obtained with carboxymethylated derivatives of periodate oxidized starch compared to its oxidized form (73°C) it is concluded that DCMS is more favorable to be used as a tanning agent in leather production considering its properties i.e gentle tanning effect with less astringency and as a result of this a leather relatively soft handle and smooth grain.

The SEM graphics of pickled goat skin and CMS tanned leathers at a magnification of 400× are shown in Figure 10. The SEM investigations showed that isolation of fibril bundles was performed for all CMS tanned leather samples which indicate the tanning effect.

The physical test results of pieces tanned with CMSs and the whole leather tanned with CMS 1:0.7 were given in Table IV. Comparing with the recommended values for goat leathers (20 N/mm^2 for tensile strength, 40 N/mm for tear strength) by UNIDO,[21] excluding the tensile strength values of the leathers which were tanned with CMS 1:0.3 and CMS 1:0.5 samples, all leather samples meet the recommended values.

Table IV

Physical properties of tanned pelt pieces and whole leather

	Sample	Tensile Strength (N/mm^2)	Elongation (%)	Tear Strength	
				Max. Force (N)	Thickness (mm)
Tanned pelt pieces	CMS 1:7	23.1(±1.3)	37.2(±1.7)	105.5(±6.3)	0.8(±0.05)
	CMS 1:9	29.9(±1.6)	42.0(±3.5)	138.2(±7.4)	0.9(±0.01)
	CMS 1:11	25.8(±2.6)	49.9(±1.0)	124.3(±7.3)	0.9(±0.07)
	CMS 1:0.3	9.9(±1.1)	18.2(±2.9)	116.8(±3.6)	1.0(±0.2)
	CMS 1:0.5	10.8(±0.2)	22.0(±1.0)	126.8(±5.49)	0.8(±0.01)
	CMS 1:0.7	20.7(±3.1)	59.6(±1.8)	94.4(±5.1)	1.0(±0.05)
Tanned whole pelt	CMS 1:0.7	20.88(±3.5)	81.48(±6.2)	69(±1.7)	0.75(±0.02)

Table V

Cracking and bursting values of tanned whole leather with CMS 1:0.7

Cracking		Bursting	
Cracking load (Kgf)	Distension at crack (mm)	Bursting load (Kgf)	Distension at burst (mm)
30(±1.3)	15.61(±1.1)	35.3(±2.2)	16.89(±1.8)

Table VI

Organoleptical properties of tanned leathers with CMSs

	CMS Sample	Color	Handle	Grain smoothness
Tanning with pelt pieces	CMS 1:7	Beige light c.	Slightly Firm	Slightly rough
	CMS 1.9	Beige light c.	Slightly Firm	Slightly rough
	CMS 1:11	Beige light c.	Slightly Firm	Slightly rough
	CMS 1:0.3	Beige c.	Very Firm	Rough
	CMS 1:0.5	Beige c.	Firm	Slightly Rough
	CMS 1:0.7	Beige c.	Firm	Smooth
Tanning with whole pelt	CMS 1:0.7	Beige c.	Firm	Very Smooth

Additionally, the whole leather which was tanned with CMS 1:0.7 was also tested in terms of distension and strength of surface. The cracking and bursting values (Table V) meets the recommended value (min. 7mm) for shoe upper leathers by UNIDO.[21]

From the evaluation of the tanned leather by organoleptically (Table VI), it was observed that the leathers tanned with CMSs were beige colored and has firm and compact structure similar with synthetic/vegetable tanned leather.

Conclusion

In our earlier studies[1,2] it was revealed that native corn starch can be oxidized by H_2O_2 and $NaIO_4$ and oxidation products having appropriate molecular weight/size and bearing functional groups that can establish stable bonds could be utilized as tanning and/or retanning agent in leather processing. In the present part, H_2O_2 and $NaIO_4$ oxidation products of starch were additionally carboxymethylated. Besides comprehensive characterization of

the CMSs, the effect of carboxymethylation process on structure and tanning ability tried to be identified. From the preliminary tanning trials with pieces of pelts, CMS 1:0.7 was chosen as the sample having the best tanning ability and a whole pelt was also tanned with it. Then, the tanned whole leather was evaluated physically and organoleptically. The characterization results demonstrated successful preparation of CMSs from oxidized starches by H_2O_2 and $NaIO_4$ oxidation methods. Compared with oxidized forms, it was determined that the carboxymethyl groups were successfully included into the structure and the water solubility of oxidized starches (especially $NaIO_4$ oxidized ones) increased by carboxymethylation process. From the evaluation of the tanning results and considering its properties i.e. gentle tanning effect with less astringency and correspondingly a relatively soft leather handle and smooth grain, it is concluded that 1:0.7 DCMS can be utilized as yet another good alternative sustainable green tanning/retanning agent from starch.

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Valorisation of Tannery Waste and Animal By-Product for Acoustics Applications

by

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Abstract

Disposal of chromium-containing solid wastes generated from the leather industry poses a major threat to tanners worldwide. Herein, we propose a strategy to utilize chrome shaving waste for sound absorption application by blending it with natural fiber, wool. The composites were prepared at various ratios with different thickness by compression molding method and subjected to characterizations like scanning electron microscope, porosity measurements, and tensile strength analysis. The sound absorption behavior of the composites was evaluated using the two-microphone impedance tube method. The results indicate that the composites with higher thickness show better sound absorption at higher frequencies when compared to the natural wool and composites with lesser thicknesses. Thus, this material can be used as a sound-absorbing material thereby paving the alternative use of leather waste utilization.

Introduction

Leather making process results in the generation of solid and liquid wastes. From about 100 kg rawhide/skin processed, nearly 75 kg of solid waste is generated with only 25 kg of raw material being converted into leather.[1] Solid waste mainly includes shavings, trimmings, animal hair and fleshing.[2,3] Shavings are one of the chromium-containing tannery solid wastes, generated during the shaving process to obtain an even leather surface.[4] Currently, majority of the chrome shavings waste is landfilled and the once the disposal exceeds the total capacity of the landfilling site, finding a new site becomes difficult. Landfilling also leads to the leaching out of chromium into the soil and ground water. Although chromium(III) has been reported to be an essential nutrient, exposure to high levels via inhalation, ingestion, or dermal contact may cause some adverse health effects.[5] It also causes an environmental threat due to the possible oxidation of Cr (III) to Cr (VI) and Cr(VI) is a well known carcinogen. Thus, unsafe disposal of shaving waste causes environmental impact due to its toxicity and carcinogenic effects.[6] The utilization of chrome shaving solid waste for alternative applications using viable technologies has been researched.[7-9] In the present study, we intend to utilize chrome shaving waste to reduce another type of pollution namely, noise.

Noise pollution is consistently increasing due to urbanization, implementation of industry, machines and vehicles. Noise pollution has a considerable impact on humans and animals.[10] The human hearing sound level ranges from a maximum of 20,000 Hz and a minimum level of 20 Hz.[11] Hearing loss, sleeping disturbance, high blood pressure, headaches, psychological problems, etc., are the adverse effects of noise pollution, which can be controlled by acoustic materials that are capable of absorbing incoming sounds. Hence, the need for acoustic materials is in high demand. Currently, natural and synthetic materials with fibrous and porous nature are being used to make acoustic materials.[12-14] Synthetic fibrous materials like glass fibers and mineral wool are extensively used in sound absorption due to their high surface area and high acoustical performance. However, their usage causes several health effects on humans like skin irritation and lung inflammation. Natural fibers like wool can be used as alternatives for synthetic fibers. Chrome shavings also have fibrous nature with porosity, which enables their usage as sound-absorbing materials.[15-17] In our earlier study, we had explored the use of natural fibers incorporated chrome shavings composite in low and mid frequencies.[18] The solid wastes from tanneries, show disposal difficulties due to the presence of the heavy metal chromium. In this present study, we have prepared sound absorbing materials from chrome shavings by blending with suitable synthetic and natural polymers via compression molding technique. Polypropylene (PP However, those composites did not show better noise absorption properties in the high-frequency range viz., above 2000 Hertz.

In the current study, we intend to explore the sound-absorbing behavior of composites made up of chrome shavings for use in the high frequencies range by combining with another natural fiber, wool. Wool, which is known to have acoustic properties, has been blended with chrome shavings and polystyrene at different ratios and the composite has been prepared using compression molding at two different thicknesses. The sound absorption studies of the prepared composite have been performed through the two-microphone impedance method. The morphological studies, pore size measurements, and tensile strength analysis have been performed for the prepared composites.

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Table I

Preparation of acoustic composite materials of varying ratios

Blend composition (100%) (W:Cr)	Wool (g)	Chrome shaving (g)	Polystyrene (g)
100	10	–	5
100	–	10	5
70:30	7	3	5
30:70	3	7	5

Materials and Methods

Materials

Chrome shavings and sheep wool were collected from the tannery division of Central Leather Research Institute, Chennai, India. The diameter of the wool is 20 µm and the length of wool is approximately 2 mm to 5 mm. Polystyrene was purchased from Fevicol 998FW. Polystyrene was used as a binder.

Methods

Preparation of Acoustic Composites

The acoustic materials were prepared with two different thicknesses (2 mm and 5 mm) and by varying ratios of wool and chrome shaving (Table I). The wool and chrome shaving were manually blended for 5 mins with polystyrene to enhance the binding capacity and then compressed in compression molding at room temperature under 50 bar pressure. The whole process was performed under dry conditions. Fixed thickness molding dies were used for the preparation of 2 mm and 5 mm thickness panels. The total weight of the blend composition has been maintained as 10 g for all acoustic panels. The density of each panel was found to be 2 Kg/m^3.

Characterization of Acoustic Materials

The thickness of the acoustic materials was measured using a screw gauge. The sound absorption studies of varying thickness and ratios of wool and chrome shaving acoustic materials were analysed using the two-microphone impedance tube method (BRUEL AND KJAER, Denmark (2716C)). The experiment was performed in triplicates and the average value was taken. The morphology of the acoustic panels was analysed using Scanning Electron Microscope (SEM) (Phenom-World). The pore size of the acoustic panels was characterized using Porous materials incorporation advanced automated humid air porometer HCFP 1100. Instron universal tensile tester machine (AE. Instron, 3369 / J7257.A) was used to study the tensile strength of the acoustic panels according to the ASTM standard (D882-12).

Results and Discussions

Morphology

The morphology of the acoustic panels is shown in figure 2 (a-d). It is observed that the chrome shaving acoustic panels showed porous and fibrous structures with agglomerated bundles, whereas neat fiber structures were observed for the wool acoustic panel. The wool and chrome shaving composite panel shows mixed morphology of both wool and chrome shavings and chrome shavings showed fibrous structures with lesser fiber diameter when compared to wool.

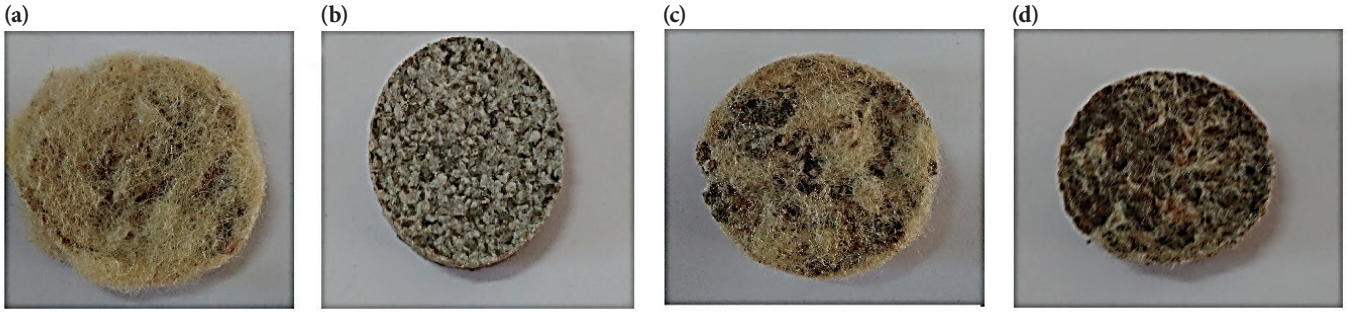

Figure 1. Acoustic panels with 5 mm thickness **a)** Wool **b)** Chrome shaving **c)** wool and chrome shaving (70:30) and **d)** wool and chrome shaving (30:70)

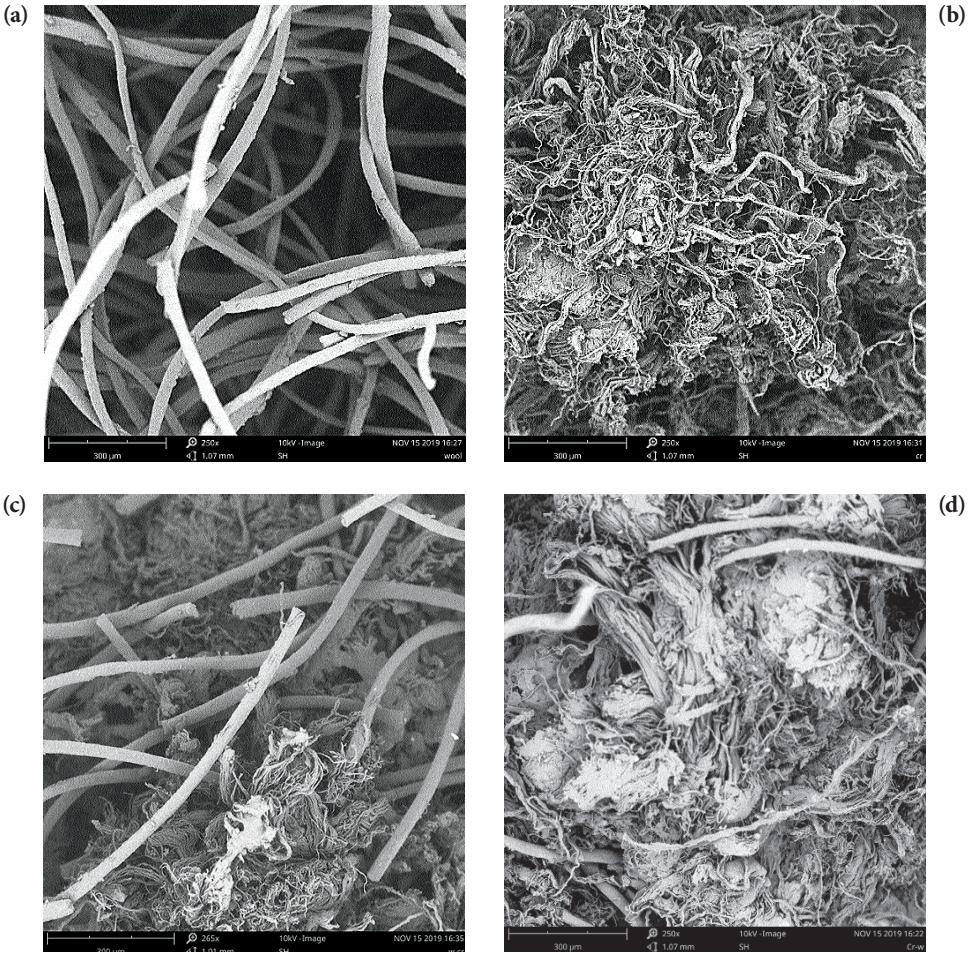

Figure 2. SEM images of acoustic panels **a)** Wool **b)** Chrome shaving **c)** Wool and Chrome shaving (70:30) and **d)** Wool and Chrome shaving (30:70)

Porosity

Figure 3 shows the bar chart for the pore size of the composite acoustic panels for varying thickness. It is observed that the porosity increases with the increase in chrome shaving content for both 2 mm and 5 mm thickness samples. At the same time porosity increases with increasing thickness of the composite acoustic panels. Enhanced porosity leads to enhanced sound absorption property, which is evidenced from the sound absorption studies. The average pore sizes of wool – chrome shaving (70:30) composite panel for 2 mm and 5 mm were found to be 45 and 60 µm, respectively and for wool – chrome shaving (30:70) composite panel for 2 mm and 5 mm were found to be 48 and 79 µm, respectively.

Tensile Strength

The tensile strength for the acoustic composite panels is shown in Figure 4. It is observed that the tensile strength of chrome shaving is higher than the wool. It can also be found that increasing the chrome shavings content in the composite increases the tensile strength. The tensile strength did not show any significant difference with different thicknesses. Since wool is a single fiber with a limited thickness may have a lesser tensile strength when compared to the

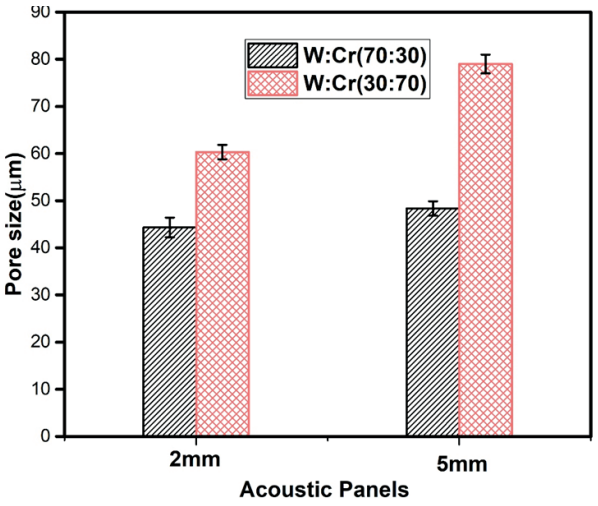

Figure 3. Pore size of varying thickness of wool and chrome shaving composite acoustic panels

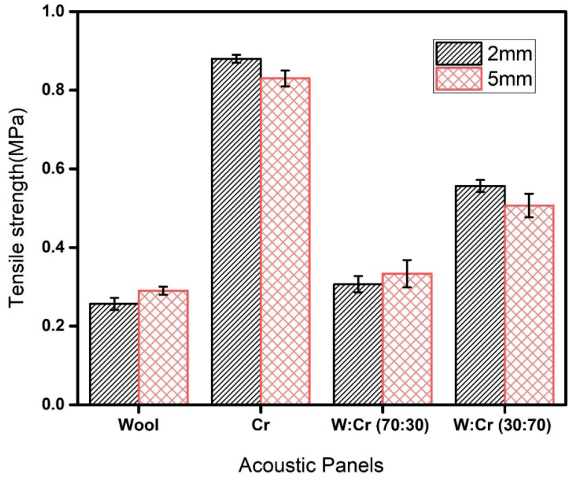

Figure 4. Tensile strength of wool (W), chrome shaving (Cr) and wool: chrome (W:Cr) shaving composite acoustic panels

chrome shavings where the collagen fibers are crosslinked by the tanning process, which might have been responsible for the higher tensile strength.

Sound Absorption Coefficient Studies

The sound absorption coefficient of the acoustic panels has been analysed through the two-microphone impedance tube method.[19] The acoustic panels were tested with the sound frequency level ranging from 16 Hz to 6300 Hz by taking some standard frequency points at 1600 Hz, 2000 Hz, 2500 Hz, 3150 Hz, 4000 Hz, 5000 Hz and 6300 Hz. Figure 5 shows the values of the sound absorption coefficients obtained. It is observed that at 2 mm thickness the sound-absorbing nature of both the composites is higher than the wool at all the frequencies. At the higher frequencies, the composites showed better sound absorption behavior when compared to wool. Thickness plays a major role in enhancing the absorption coefficient. It was found that the sound absorption is better for 5 mm panel than 2 mm for the same ratio (ex. W:Cr 30:70). In a 2 mm panel, after 2500 Hz a slight decrease and saturation was observed, which may be due to less thickness and porosity as against 5 mm panel.

The composite W: Cr (30:70) showed enhanced sound absorption in the higher frequencies. The combined fibrous nature of both the wool and chrome shavings might have increased the sound absorption behavior. Increasing the content of chrome shavings in the composite might have contributed to the presence of more amounts of fibers, which results in enhanced the sound absorption behavior.

Conclusion

The experimental results indicate the presence of highly fibrous structures with porosity in the composites resulting in good tensile strength. The higher percentage of chrome shavings containing composites show increased sound absorption when compared to the wool at higher frequencies. Thus, this material can be used as a sound-absorbing panel by eliminating the use of synthetic fibers and also this technology can evolve as a proper method of utilization for leather solid waste.

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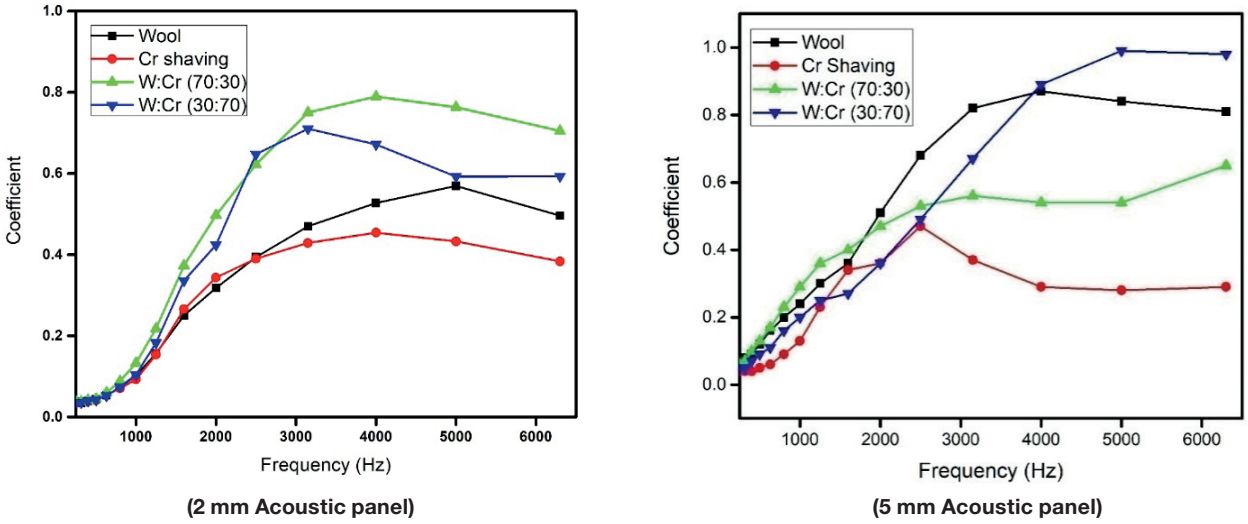

Figure 5. Sound absorption co-efficient studies for the acoustic panels of different thickness

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Biochemical and Physical Changes in Goatskin during Bacterial Putrefaction

by

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Abstract

The quality of the raw animal skin decides the final quality of leather. Preservation processes of raw animal skins until leather making predominantly uses salting as a popular method owing to the bacteriostatic effect provided by salt. The detrimental impact caused by the usage of salt from the leather processing is well established. This necessitates the quest for developing an economical, efficacious and environment-friendly preservation system. The present work investigates the effects on the physical and chemical characteristics of the animal skin caused by the putrefactive bacteria with respect to time. Physical changes were studied using visual examination, SEM analysis, and histological staining techniques where the structural deterioration was evidently established. Changes in biochemical aspects were studied by observing degradation in proteoglycan levels and collagen from the goat skin taken at various time intervals. Furthermore, the microorganisms that were responsible for the degradation of various skin components were isolated from the skin over the period of 36 hours from flaying. The occurrence of collagen-degrading organisms within 6 hours of initiation of putrefaction and increased number of proteolytic and collagenolytic bacteria at the end of 36-hours observation were indicative of tremendous skin spoilage leading to deteriorated quality of raw material.

Introduction

Leather manufacturing is one of the industrial activities globally widespread, which involves the use of animal hides and skins as raw material. Animal skins are non-edible by-products of the meat industry which, when accumulated, can cause serious pollution issue. Animal skin is a heterogeneous fibrous mass, which becomes a biological substrate for the microorganisms to proliferate when left unattended. Skin, which is the raw material for manufacturing of leather, is susceptible to growth of bacteria, mold and other microorganisms due to the fact that it is a rich source of protein.[1] The prime constituents of skin are proteins, fats and minerals. Fresh skin contains 65% water, 33% proteins, and rest is fats and minerals.[2] The inherent moisture and atmospheric physiological conditions are very much suitable for microorganisms to grow at a rapid rate. In order to arrest the growth of putrefactive microorganisms, the water content of the skin is principally decreased using several curing techniques.[3] Curing techniques that are based on decreasing the moisture content are drying, salting, dry-salting, chilling and freezing.[4,5] Methods that use common salt for curing are predominant in tanning industry. Although salt is beneficial in terms of preservation cost and does not lead to any quality loss in finished leathers, it has been identified as a serious environmental pollutant. The usage of salt increases the pollution load of the tannery effluent leading to higher levels of TDS (total dissolved solids) and chloride content.[6] The TDS remains high even in the treated effluent which comes out of the common effluent treatment plant (CETP).[7] This type of pollution problem needs to be addressed. Many alternatives to salt based preservation system have been researched over the past few decades yet salt still remains the most cost-effective option when preservation is concerned.[8-12]

Putrefactive activities by microorganisms' initiate just after skin/hide is flayed from the carcass.[13] Thereafter, a process called autolysis governs the onset of degradation, followed by microbial putrefaction. Autolysis is a process involving self-degradation initiated by lysozymes and an enzyme called cathepsin, that provides a nutrient-rich environment for other microorganisms to invade.[14] The process of putrefaction is a collective effort by several varieties of microorganisms. These microorganisms involved in the process of putrefaction proliferate by sourcing carbon and energy from the animal skin. Microorganisms breaks down larger molecules of proteins and other substrate components with the help of extracellular enzymes.[15] Once the core matrix protein collagen gets degraded by the collagenases, other proteolytic enzymes begin to further break down the fragments, collapsing the fibrous structure of the substrate. Several aerobic and anaerobic microorganisms that were involved in the degradation of fibrous protein collagen have been investigated so far. Clostridial hydrolytic enzymes from some *Clostridium* species have been known for their ability to degrade the extracellular matrix components of skin such as collagen, hyaluronan etc.[16] Certain anaerobic bacteria such as *Clostridium perfringens*, *C. histolyticum* and *C. capitovale* were found to cause considerable damage to hides.[17] There is evidence that states a correlation between the rawhide bacterial collagenolytic activity and leather decay.[18] And so, the time delay that could be exempted for curing based on the inception of the collagenolysis must be explored. By such means, the final leather quality could be preserved.

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The leather is made mainly of interweaving collagen fibers (or fiber bundles), and the physical properties of the leather are the sum of the physical properties of the fibers and their interweaving that makes up the fiber structure of the leather.[19] During putrefaction, there occur several changes in the physical and chemical features in the fiber structure of the skin, which eventually degrades the final value of the leather. Therefore, it is possible to attribute the faulty leather to the physicochemical changes taken place in the fiber structure due to putrefaction or delayed/ underrated curing.

Detailed understanding of the putrefaction and its effect on skins is important from the point of view of protecting the skins from the microorganisms. The degradative microbial population causes damaging impacts on the morphological quality of the raw material. This has been established previously by several biochemical research efforts which studied the effect of putrefaction on unpreserved and salted goatskins kept at ambient conditions for several days. The current study is aimed to elucidate and consolidate the effects caused by putrefactive microorganisms on the physicochemical characteristics of the animal skin, within a short time span of 36 hours. Putrefactive bacteria were isolated from the putrefying skin within time intervals for a set time duration and characterized for their proteolytic, lipolytic and collagenolytic behavior. The physical changes in the skin were observed by visual examination, histological staining and using Scanning Electron Microscope (SEM). The chemical changes brought about by putrefaction was determined by performing elemental analysis, estimating proteoglycan loss and collagen loss from the putrefied skin.

Materials and Methods

Materials

All microbiological media were procured from Hi-Media laboratories. Other chemicals and reagents were of laboratory or analytical grade procured from M/s Merck. Fresh Indian goatskins were procured from local slaughterhouse located in Chennai, India.

Isolation and characterization of putrefactive microorganisms

Fresh goatskin was allowed to putrefy under ambient tannery conditions of temperature ranging between 27°C and 30°C and humidity ranging between 60 and 80%. The goatskin was visually examined for every 6 h and skin samples of size 2.5 cm x 2.5 cm were cut using a sterile surgical blade from the butt and belly regions of that goatskin. Sampling was done every six hours until 36 hours. The samples were labelled as S0, S1, S2, S3, S4, S5 and S6 corresponding to each 6-hour sample respectively (Fig.1). The samples were collected and transferred to a sterile centrifuge tube (50 mL) containing 10 mL of sterilized distilled water right after collection and shaken using a vortex mixer for 15 min. The skin extracts of all samples were serially diluted using sterile physiological saline (0.85% w/v). The suitably diluted suspension of each sample was then plated out using spread plate method on nutrient agar and incubated at 37°C for 24 – 48 h.

Sample Code	Time (h)	Sample Code	Time (h)
S0	0	S1	0
S1	6	S2	6
S2	12	S3	18
S3	18	S4	24
S4	24	S5	30
S5	30	S6	36
S6	36		

Figure 1. Schematic for sample collection from putrefied goatskin

The plates from several dilutions were observed for growth of the bacteria. The bacterial isolates were screened and selected based on its repetitive appearance on the plates from the subsequent dilutions of each sample.

The isolated strains of bacteria from each sample were Gram stained using Gram staining procedure and further tested on various selective and differential media. The isolates were morphologically characterized and screened for their proteolytic, lipolytic and collagenolytic activity on skim milk agar, tributyrin agar and gelatin liquefaction media respectively.

Studying the physical changes in the skin fiber network during putrefaction

Fresh goatskin was procured and left to putrefy for observation. Samples from the butt section of the skin were collected right from the initiation of experiment taken as 0^{th} h and labelled as S1. Further sampling was done at every 6 h and labelled as S2 up to S6 (Fig.1). The samples were examined for the effects of microbial action on the internal fiber network using histological staining and Scanning Electron Microscopy (SEM) analysis.

Histological staining of skin samples

Samples were thoroughly washed with distilled water and cut into small pieces prior to fixation and sectioning process. The samples were fixed in formalin solution prepared by adding 0.9 g sodium chloride in 100 mL of 10% formaldehyde solution.[20] After fixation, the samples were progressively dehydrated using 70% ethanol, 80% ethanol, 95% ethanol, and 100% ethanol with two changes for every one hour. Xylene was used to clear the alcohol, three changes for

every one hour. Xylene cleared samples were treated with paraffin wax (56-58°C), two changes, one and a half an hour each. Samples were embedded in paraffin blocks and trimmed to be cut using microtome. Sections of 4 μm were obtained and stained using various selective and differential stains – Masson's Trichrome stain, Sudan Black B stain, Safranin O stain and Hematoxylin & Eosin stain to examine the degradation in the levels of collagen, lipids, proteoglycans and the overall fiber network of putrefied skin respectively.

Scanning Electron Microscope technique to study physical changes

Washed skin samples were gradually dehydrated using absolute ethanol ranging from 10-100% v/v concentration. Dehydrated samples were then mounted on an aluminum specimen stub, and coated with a thin layer of gold by Edwards E306 sputter coater. The samples were viewed microscopically using Leica Cambridge Stereoscan 440 Scanning Electron Microscope to examine the degradation caused by bacterial putrefaction.

Studying chemical changes in the animal skin during putrefaction

Fresh goatskin was left to putrefy under ambient tannery conditions and sampling was done as mentioned above. The skin samples were cut in order to determine the elemental degradation and collagen degradation. The extracts from the same skin samples were used to estimate the proteoglycan loss caused due to putrefaction. The experiments were done in triplicates and the standard deviation was calculated to measure the dispersion of data.

Elemental analysis of skin samples

Skin samples, after removal of hair, were weighed to determine the moisture content. A known amount of sample was taken in a previously weighed porcelain dish and kept in a hot air oven at 105°C for about 5 hours. After drying, the china dish was cooled in a desiccator and weighed. The percentage of moisture content[21] was calculated using equation 1. Same samples were then lyophilized and analyzed for carbon, hydrogen, nitrogen and sulfur content (CHNS) using Elementar analyser (Vario Micro Cube – Elementar, Japan). For CHNS analysis, lyophilized samples were weighed (3-5 mg) and taken in a silver tin capsule, fed into a combustion zone, which was then combusted in a reactor at 1150°C.

$$\% \text{ Moisture} = \frac{(\text{Inital weight (g)} - \text{Final weight (g)})}{\text{Initial weight (g)}} \times 100 \qquad (1)$$

Determination of degradation in proteoglycan levels

The skin extracts of the putrefied skin samples were estimated for the presence of proteoglycan. The determination of proteoglycan was done using Periodic – Schiff's assay.[22] Briefly, 10 μL of 50% periodic acid was mixed with 10 mL of 7% acetic acid to prepare periodic acid mixture. To 1 mL of skin extract, 100 μL of freshly prepared periodic acid solution was added, mixed and incubated at 37°C for 120 min. For full-color development, 100 μL of decolorized Schiff's reagent was added, mixed and allowed to stand at room temperature for 30 minutes. Water was taken as blank. Samples were tested in duplicates. The absorbance of blank and sample was read at 555 nm using UV-Visible spectrophotometer (Jasco V-660 Spectrophotometer). The concentration of proteoglycan was calculated by taking mucin as a standard, calibrated at a range from 0 to 100 μg.

Determination of degradation in collagen levels

The extent of collagen degradation of putrefied skin samples was estimated by determining the hydroxyproline content, a key amino acid released during break-down of the collagen.[23] Putrefied skin samples were washed with physiological saline, unhaired and cut into small pieces weighing about 10 milligrams. Skin pieces were hydrolyzed with 5 mL of 6N hydrochloric acid at 110°C for overnight in sealed test tubes. After hydrolysis, the samples were evaporated to dryness at 80°C for 3-4 hours, the residue was dissolved in water and made up to known volume and used for hydroxyproline estimation. Hydroxyproline oxidation was initiated by adding 1 mL of Chloramine-T to each sample test tube. The contents of the tubes were mixed by shaking for few minutes and allowed to stand for 20 min at room temperature. To each test tube, 1 mL of perchloric acid was added and the contents were mixed and allowed to stand for 5 min. Finally, 1 mL of 20% p-Dimethylaminobenzaldehyde (PDAB) solution was added and the mixture was shaken. The tubes were placed in a 60°C water bath for 20 min and cooled for 5 minutes. The color developed was read using a spectrophotometer (Jasco V-660 Spectrophotometer) at 557 nm and the concentration of hydroxyproline was calculated using hydroxyproline standard ranging from 1-10 μg/mL. Collagen content was then calculated using equation 2

$$\text{Collagen} = 7.46 \times \text{hydroxyproline (μg/mL)} \qquad (2)$$

Results and Discussions

Observations during putrefaction

Goatskins left in ambient tannery conditions were observed visually during different time intervals, considering the initiation of observation to be 0^{th} hour and so on up to 36 hours. Visual observation at each time interval was done by carefully examining the flesh and grain side of the putrefying skin. During the first 3 hours from the initiation of visual examination, there was no perceptible odor. The following hour, a putrid odor that emanated from the skin was vividly observed. This obnoxious odor indicated the initiation of putrefaction. After 8 hours from the initial observation time, the flesh side of the skin was observed to be covered with a slimy coating on its surface. Around this hour, the skin's physical appearance and rigidity of the skin had started deteriorating. Around 10 hours, the loosening of hair follicles from within the surface of skin had started drastically, indicating the progress of putrefaction. Such loosening of hair is called hair slip and it is known as the first sign of putrefaction.[24] After 12 hours, complete hair loosening was observed.

The putrid odor at 12 hours was found to be strong. At 18 hours, the skin sample was found to be slippery due to release of protein degradation products. The structural integrity of the skin was found to be very poor. After 24 hours, the adipose (flesh) layer was found to be degraded significantly and loosened. The skin components such as hair, flesh and the grain surface had been worsened noticeably. Around 36 hours, the characteristic putrid odor was highly objectionable and the oozing fluids from the skin invited maggots (Fig. 1), marking the end of visual observation activity.

Characterization of putrefactive microorganisms

Animal skin is an outstanding organic substrate for microorganisms to grow. The present study aimed at isolating different kinds of microorganism that thrived between the time interval window of 0 to 36 hours observation period, on the surface of the putrefying skin. Varied microorganisms including few fungal species were observed to be grown on the nutrient agar plates. Amongst others, only the bacterial species were isolated at every 6 hours from 0 hours of the observation window until the 36th hour. The microbial isolates were characterized by their morphology, Gram stain response, proteolytic activity, lipolytic activity and collagenolytic activity. The invasion profile of bacterial isolates with time in Fig. 3 reveals that the onset of degradation of collagen and other protein has begun since the initial six hours. Presence of proteolytic and lipolytic bacteria was witnessed from the samples collected at zero hour of observation. This observation necessitates the need for a preservation technique that could curb the microbial growth within hours of flaying of skin.[25] At the outset, the organisms that could thrive on the globular proteins might have started growing. After 12 hours, the proteolytic bacteria increase in number feeding onto the degradation products of the globular proteins from the goatskin. After 18 hours, which marks the 4th stage of the observation window, lipolytic bacteria were found to be in abundance, which was found to be utilizing the lipid from the flesh side of the skin as their substrate for growth. During visual examination of the putrefied goatskin, after 24 hours the adipose layer was found to be heavily degraded. This could be explained owing to the action of lipolytic bacteria that degraded the lipid portions of the flesh side. Breakdown of inter-fibrillary globular proteins within the initial 24 hours, provided access to the lipid pockets for the lipolytic bacteria. At around 30 – 36 hours time scale, bacteria with multiple hydrolytic behavior (i.e. isolates exhibiting proteolytic, collagenolytic behavior) predominated the bacterial population (Table I). These consortia of bacteria synergistically degraded the collagen remaining in the skin. At this stage, collagen had deteriorated extensively, releasing several byproducts that caused the foul odor and invited the maggots. The microbiological aspect of the current study marked a certain set of organisms, which exhibited proteolytic activity without any collagenolytic activity identified at nine hours and thereafter might be primarily responsible for the degradation of globular proteins and proteoglycans. Similarly, the lipolytic organisms identified after nine hours might have been primarily responsible for degradation of lipids and adipose tissue. The bacterial profile corroborates well with the visual observation of the completely putrefied skin.

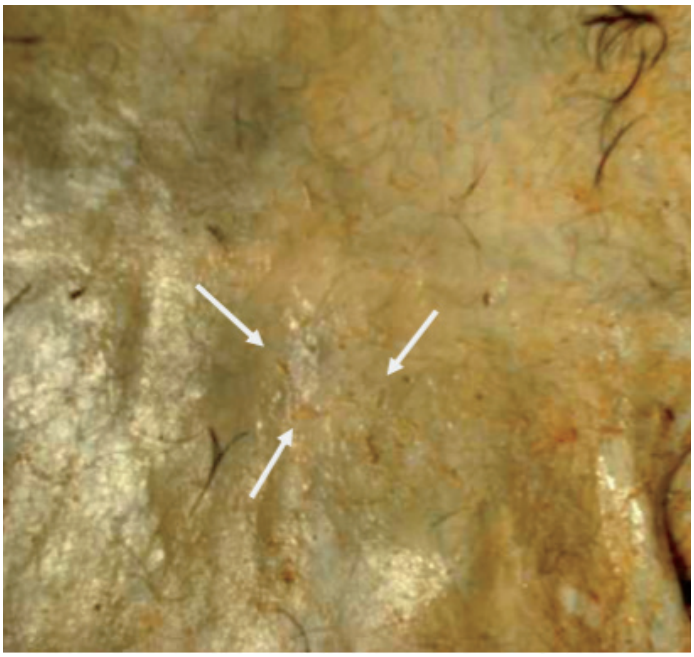

Figure 2. Flesh side of putrefied goatskin after 36 h (*Arrows indicating invasion of maggots*)

Similar results were observed when some of the leather making raw materials were assessed for bacterial abundance. Fresh hides prior to leather processing were found to be inhabited by a variety of microorganisms such as *Bacillus cereus*, *B. megaterium*, *B. sphaericus*, *B. subtilis*, *Kurthia variabilis*, *Micrococcus roseus* and *Staphylococcus aureus* that were proteolytic and collagenolytic in nature.[26] In a study conducted by Kayalvizhi *et al.* (2008),[27] almost 60% and 34% of total bacterial population isolated from fresh goat skins were found to be proteolytic and lipolytic, respectively. Unpreserved hide which was putrefied by the detrimental effects of several coccus-shaped species and rod-shaped species bacteria such as *Proteus vulgaris, B. subtilis* were also investigated.[28] The diversity of microorganisms that have degradative effects on raw cowhides and goatskins were studied for developing plant-based preservative formulation.[29] *Staphylococcus spp.*, *Micrococcus spp.*, *Corynebacterium spp.*, *Bacillus spp.*, *Escherichia coli* and *Pseudomonas spp.* were the predominant microorganisms isolated from Sudanese raw cattle hides and sheep skins that were obtained in unpreserved conditions in a tannery for leather processing.[30]

Physical changes observed during putrefaction - Histological staining of skin sections

Physical deterioration of the internal components of animal skin with time is one of the evident guides of microbial putrefaction. The skin samples collected were cut into sections and stained using four selective stains, to study the microscopic changes occurred in each of the key skin components such as collagen, proteoglycans, and lipids and the overall fiber network. The stained micrographs of the samples revealed the progress of degradation that occurred with time.

Table I

Characterization of bacterial isolates from putrefied goatskin

Isolate ID	Appearance on agar plate	Gram Reaction	Collagenolytic Activity[a]	Proteolytic Activity[b]	Lipolytic activity[c]
S0: 0th hour sample					
0.1	Dry flat white dots	Gram positive	_	+	_
0.2	Moist slimy smeared growth	Gram positive	_	+	+
0.3	Layered pale yellow growth	Gram positive	_	+	+
S1: 6th hour sample					
1.1	Creamy white shiny growth	Gram positive	_	_	+
1.2	Creamy yellowish small dots	Gram positive	+	_	_
1.3	Creamy flat moist dots	Gram positive	+	+	_
1.4	Dark creamy lustrous growth	Gram positive	_	+	_
1.5	Very small pale yellow dots	Gram positive	+	_	_
S2: 12th hour sample					
2.1	Swarming layered growth	Gram negative	+	+	_
2.2	Yellow dry growth	Gram positive	_	_	+
2.3	Very small creamy transparent dots	Gram positive	_	_	+
2.4	Orange lustrous viscous growth	Gram negative	_	+	_
S3: 18th hour sample					
3.1	Reddish orange viscous growth	Gram negative	+	+	_
3.2	Swarming streamlined growth	Gram negative	+	+	_
3.3	Bright yellow moist small dots	Gram positive	_	_	+
S4: 24th hour sample					
4.1	Swarming streamlined growth	Gram negative	+	+	_
4.2	Swarming streamlined growth	Gram negative	_	_	+
4.3	Filamentous growth	Gram positive	_	+	_
4.4	Transparent milky white growth	Gram negative	_	_	+
4.5	Creamish moist flat growth	Gram positive	+	+	_
S5: 30th hour sample					
5.1	Swarming growth	Gram negative	+	+	_
5.2	pale cream small transparent growth	Gram positive	+	+	+
5.3	transparent creamy slime like growth	Gram negative	+	+	_
S6: 36th hour sample					
6.1	Transparent creamy flower small dots	Gram negative	+	+	+
6.2	Creamish orange moist viscous growth	Gram negative	+	+	_

Masson's Trichrome stain for collagen

The Masson's Trichrome stain, that selectively stained collagen blue, revealed the collagen's degradation levels inside the matrix (Fig. 4). The connective tissue is stained blue, nuclei are stained purple and the cytoplasm is stained pink. The samples were viewed at two levels of magnification – 10x (Fig. 4 a-f) and 40x (Fig. 4 a'- f'). The collagen fiber bundle at the initial stage at 0th hours was found to be intact and tightly interwoven. It is evident from micrographs that at 6 hours, the collagen fiber bundle had started splitting. This could be due to the degradation of reticulin sheath that was responsible for the structural integrity of the collagen bundles. Also, the voids that appeared with brown rings indicated the details of hair follicles (Fig. 4b and Fig. 4b'). The samples from 18 hours showed that the collagen fibers were split up further. The voids pertaining to hair follicles were then seen to be stretched wide and empty, proving to be evidence of hair slip phenomenon as observed during the visual examination. At 24 hours, the structural integrity of collagen fibers was found to be impaired tremendously to the extent that the fibers were split up almost completely (Fig. 4d'). At 30 hours, the disintegration and degradation of collagen were witnessed clearly

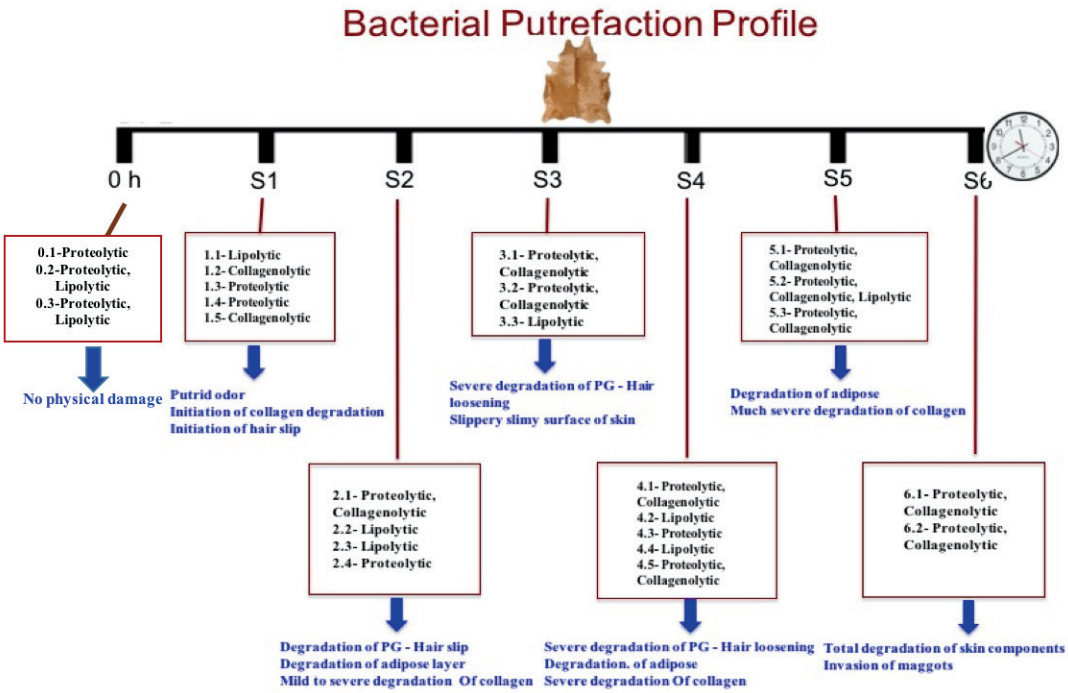

Figure 3. Putrefactive bacterial profile of putrefied skin samples collected over a period of 36 hours

by the disrupted fiber arrangement. The samples from 36th hour show that the fiber network was dilapidated and lost all the cellular network tightness leading to cellular atrophy. These results fall well in agreement with the visual observations. The degradation effects observed here could be linked to the results obtained while studying the bacterial invasion on the skin.

Safranin O stain for Proteoglycans

Proteoglycans, that form an important integral part of the skin's fiber network, were observed using Safranin O stain (Fig. 5 a-f). The micrographs indicated the physical changes in proteoglycans due to putrefaction. Proteoglycans are known to be present near the hair bulb and are responsible for holding the hair intact with the skin.[31] It is established that the presence of mucoid component such as proteoglycans play a very important role in tissue cohesion, stability and provide protection to the collagen.[32] Degradation of proteoglycan is associated with consequent loosening of hair and change in the cohesion of collagen fiber structure. This, in turn, brings about hair slip. The pictures clearly indicate the presence of proteoglycans around hair bulb during the initial hours of observation. The

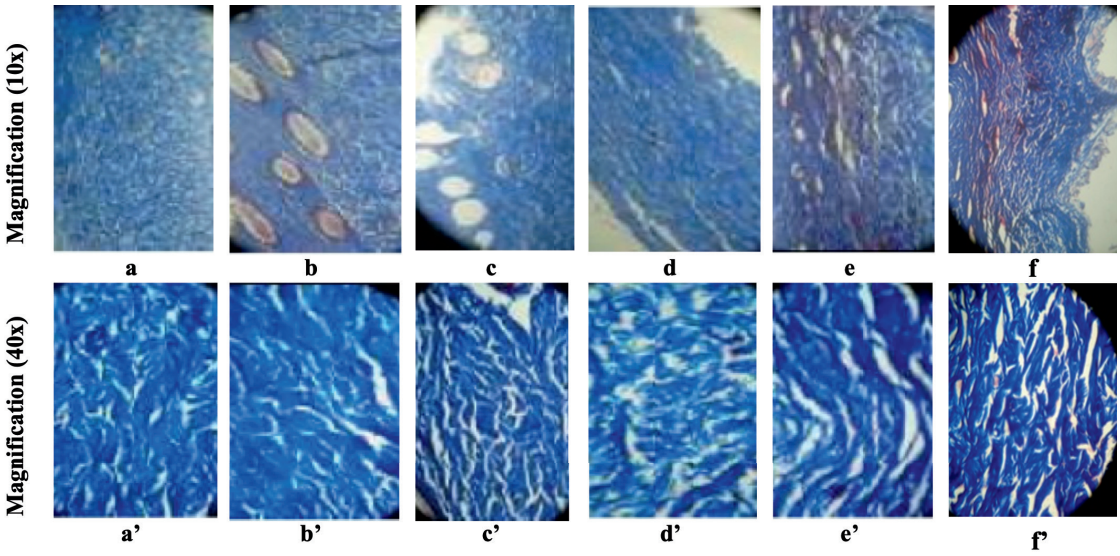

Figure 4. Masson's Trichrome Stained sections of putrefied goatskin for studying Collagen degradation of putrefied skin samples: **a, a'** – skin section showing intact and tightly interwoven fibers at 0th h; **b, b'** – details of the hair follicles visible during 6th h; **c, c'** – initiation of collagen fiber splitting at 18th h; **d, d'** – enhanced fiber splitting at 24 h; **e, e'** – disruption of fiber arrangement at 30th h; **f, f'** – major dilapidation of fiber arrangement causing cellular atrophy at 36th h

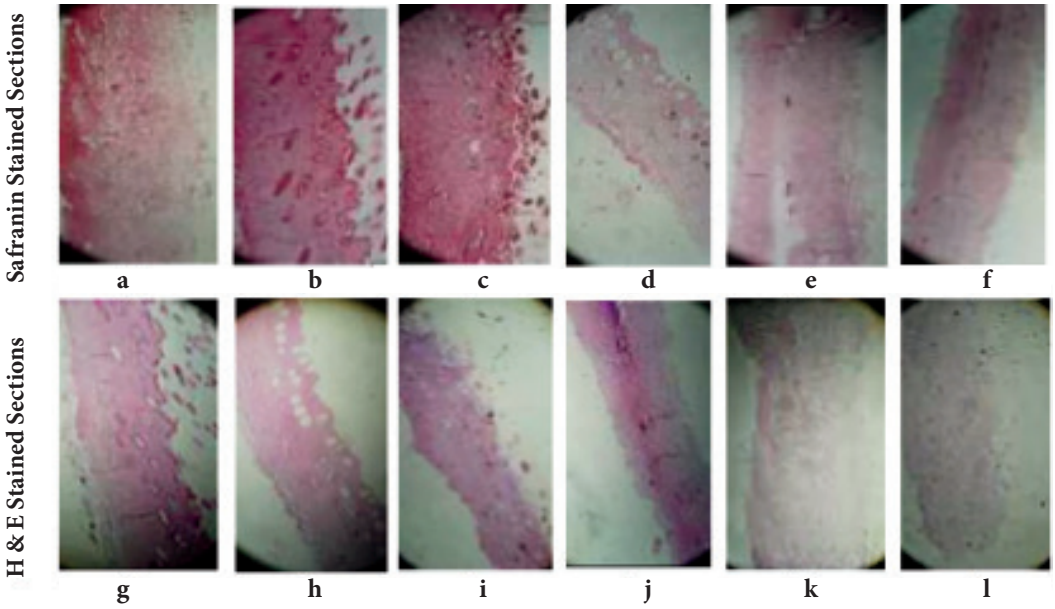

Figure 5. Safranin O stained sections from putrefied goatskin:
a) skin section dense red color indicating the presence of proteoglycan at 0th hour; **b)** details of the elliptical voids indicating hair follicles visible during 6th hour; **c)** elliptical voids being present at 18th hour; **d)** empty rings indicating lost hair follicles at 24 hours; **e)** loss of proteoglycan at 30th hour; **f)** very minimal levels of proteoglycan 36th hour

Hematoxylin and Eosin stained sections for studying changes in overall fiber network:
g) – i) entire fibrous matrix with elliptical hair follicle voids being seen until 18th hour;
j) – l) significant material loss visualized from 24 hours to 36 hours

elliptical dark red circles found in the micrographs from the samples of 6 hours and 18 hours are indicative of the presence of hair follicles. The reduction in the intensity of dark red color over the time indicated distinctly that the proteoglycan was degraded gradually with the progression of time. The empty rings visualized from the 24 hour sample implied that proteoglycans were immensely broken down which led to complete removal of hair from the surface. It was found that at around 30 – 36 hours, the presence of proteoglycan was very minimal. This was in accordance with the visual observations where hair slip and exudations of protein degradation products was observed at the similar time frame of observation.

Hematoxylin and Eosin stain for fiber network

Overall fiber network was visualized using Hematoxylin and Eosin stain that stained the collagen, elastin and erythrocytes (Fig. 5 g-l). It can be seen that the density of overall matrix that could be stained, had started decreasing from 18 hours and significant loss of skin network was witnessed at 30 hours (Fig. 5 k). The results from Hematoxylin and Eosin staining further confirmed the results from other staining results. Degradation of epidermis, dermis and hair follicle were observed to be falling in a similar trend when degradation of goatskins was histologically analyzed.[33] Distortion of grain and corium layers made of fibrous proteins of raw buffalo hide carried by enzymatic degradation was detected by Hematoxylin and Eosin staining method.[25] In a recent study involving sheep skins and cattle hides, histological examination of wet blue leathers made from putrefied raw material showed internally damaged tissue structures.[30]

Sudan Black B stain for lipid

The levels of lipid being physically transformed were visualized microscopically with Sudan Black B stain, that stained lipids blue-black (Fig. 6 a-f). Lipids are an important component that gives the skin handle and suppleness. Presence of lipids inside the network and in the adipose layer was found to be prominent till 18 hours. After 18 hours, the lipids started disappearing due to the action of lipolytic microorganisms, as evident from the discussions from the previous

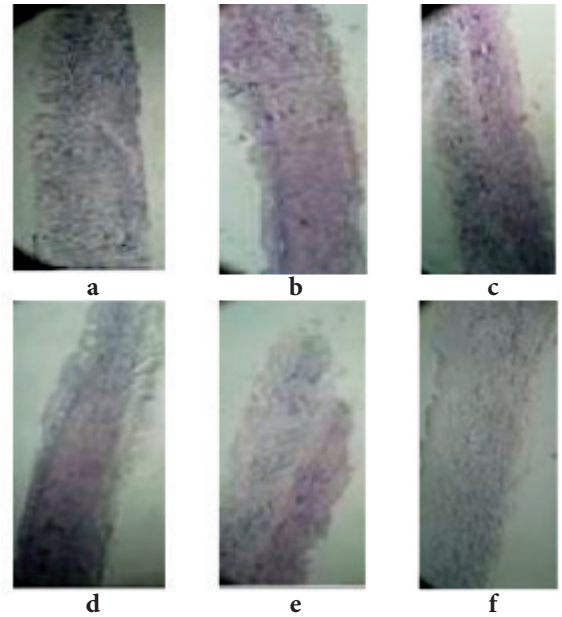

Figure 6. Sudan Black B stained sections of putrefied goatskin: **a) – c)** presence of lipids prominent till 0 to 18th hour; **d)** disrupted lipid layers at both sides of the skin viewed at 24 hours; **e) – f)** very little lipid presence at 36th hour

section. At 36 hours, there was very little evidence of presence of lipid in the skin sections. From the earlier results on investigations of microorganisms, it was found that the lipolytic bacteria were present from 9 hours of putrefaction and continued until 36 hours. Therefore, it could be expected that the degradation of lipids and the adipose layer had commenced from 9 hours and progressed further until the end. This trend was established from the gradual decrease in the intensity of pigmentation indicating the lack of lipid.

Scanning Electron Microscopy (SEM) study of putrefied skin samples

The structural details of the putrefied skin samples were pictured using scanning electron microscope in order to further ascertain the physical changes occurring in the fiber network of skin. The samples were viewed at a magnification of 3000× (Fig. 7 a-f). The micrographs revealed that the fiber network remains stable during the initial hour (Fig. 7a and Fig. 7b). Separation of fiber network had started occurring at around 18 hours (Fig. 7c). At 30 hours, significant level of degradation of fibers was witnessed. This was in congruence with the results from H & E stained samples. At 36 hours, the fiber network was found to be completely shattered. Similar nature of damaged fiber network was observed when the leathers made from unpreserved or insufficiently preserved raw materials were viewed using SEM, which revealed changes in structure and loss of protein leading to poor quality leather.[34]

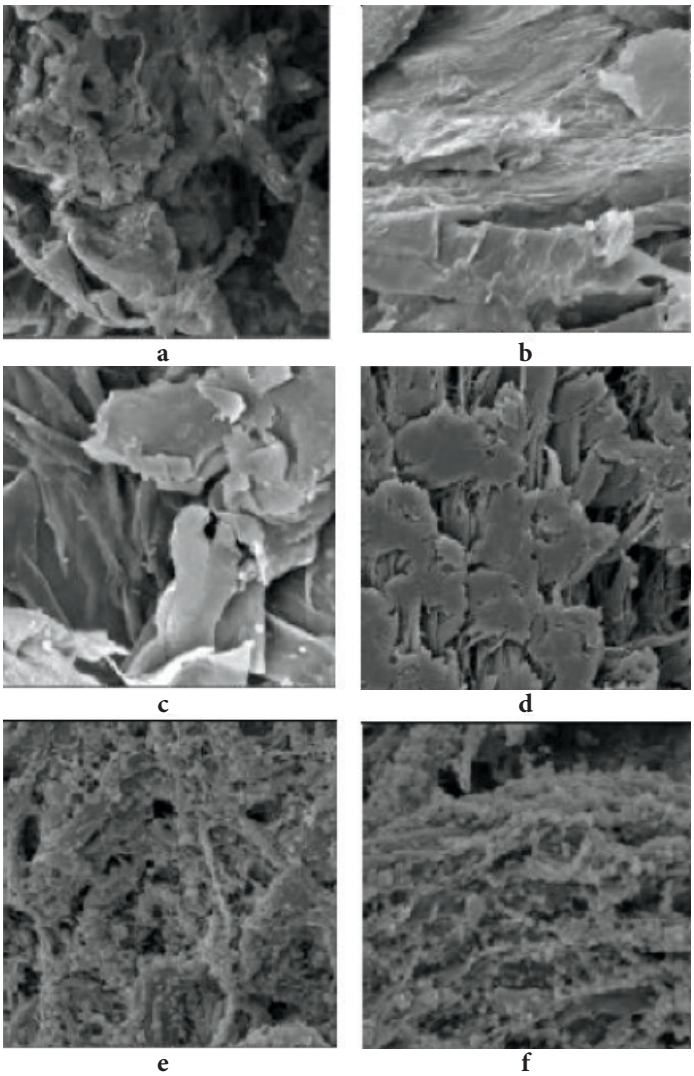

Figure 7. SEM micrographs of putrefied skin samples collected over the period of 36 hours: **a)** interwoven fiber network at initial period at 0th hour; **b)** 6th hour sample showing the breakage of fibers; **c)** fiber network getting separated at 18th hour; **d)** voids in the fiber network and fiber bundle seen at 24th hour; **e) – f)** completely shredded fibrous network working its way towards degradation viewed at around 30 – 36 hours

Chemical Changes Observed During Putrefaction

Elemental analysis of skin samples

The key changes in chemical composition of putrefied skin samples was monitored by estimating moisture content and other elemental components. The moisture content of each skin sample was analyzed. The moisture content was found to be sustained throughout the putrefaction. Moisture is the key growth factor required for the microorganisms to grow. The elemental composition (C, H, N and S) of the skin samples during the course of putrefaction was also estimated (Table II). It can be seen from the results that the total elemental composition did not change much within this time scale of observation. A declining trend could be perceived in the nitrogen levels, if the observation of degradation was extended for days. Evidently, Preethi et al. (2006)[33] observed that the total protein content of the degraded goatskins was found to be decreased over the period of 10 days when monitored in unpreserved condition.

Proteoglycan estimation based on Periodic acid – Schiff's assay

The proteoglycan released from the skin was estimated with respect to time. Fig. 8 gives the results of proteoglycan estimation. It is evident from the results that the proteoglycans release was not significant until 6 hours but increased rapidly within 18 hours. This exponentially increased release could be explained by correlating with the hair slip phenomenon that had started at around 10 hours and progressed to 12 hours leading to complete hair loss. After 18 hours, it was noted that the protein degradation products had made

Table II

Elemental analysis of skin samples

Sample ID	Elemental Analysis (%)						Moisture (%)
	N	C	H	S	C/N ratio	C/H ratio	
S1	16.38	51.89	7.54	0.41	3.16	6.87	72.61 ± 1.6
S2	15.30	45.03	6.68	0.48	2.94	6.73	69.88 ± 0.87
S3	15.70	46.42	7.09	0.44	2.95	6.54	70.21 ± 1.2
S4	15.15	46.09	7.23	0.40	2.98	6.36	67.22 ± 1.4
S5	15.89	47.84	7.24	0.48	3.01	6.60	70.15 ± 0.98
S6	15.48	45.24	7.11	0.36	2.92	6.35	69.12 ± 1.1

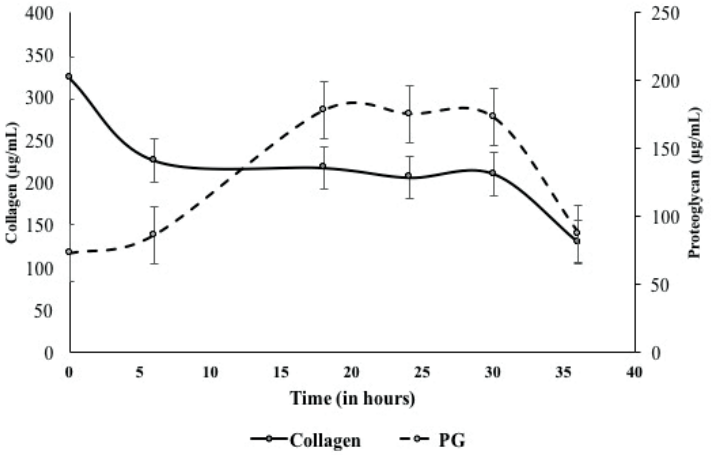

Figure 8. Collagen from putrefied skins and proteoglycan released from putrefied skin samples with respect to time (in hours)

the skin appear slippery and slimy. Such effect is validated by the results from proteoglycan estimation, as there occurs a levelled off proteoglycan release between 18 hours to 30 hours. Hair loosening was the result of the loss of proteoglycans that held the hair bulb. The observations from histological studies of proteoglycan were found to be in agreement with the proteoglycan release.

Hydroxyproline estimation of skin samples

Hydroxyproline is an amino acid very specific to the collagen protein, which becomes the marker component for this assay. The hydroxyproline released from the putrefied skin was estimated with respect to time. The content of hydroxyproline is the direct measure of level of collagen present in the sample. Hydroxyproline amounts were found to be gradually decreasing with respect to time from 6 hours and dropped down to a very low collagen content until the 36th hour of the observation (Fig. 8). These results are in congruence with the data from previous experiments pertaining to microorganisms, staining, electron microscopy and elemental analysis. Initiation of loss of hydroxyproline from the very early stage (within 6 hours) of putrefaction scale, establishes that the damage of the leather making protein starts within a few hours after flaying. This necessitates the need for the right kind of preservation to be done at the right time.

Insights into the cascade of events leading to a phenomenon called putrefaction

Microbial deterioration of an organic substrate such as raw animal skin takes place in several stages as seen in this study with help of several experiments. The initiation of putrefaction started right after flaying of the skin from the carcass by autolysis. The atmospheric temperature, humidity and the inherent moisture of the goatskin provided a conducive condition for several microorganisms to utilize the skin for its growth. The synergistic action of bacteria possessing multiple hydrolytic characteristics disintegrated each of the skin components starting from the easily accessible surface material. This detrimental activity by putrefactive bacteria caused the closely knit fiber network to collapse leading to indications such as hair slip and excretions of degradation products. The biodegradation also released gases such as ammonia from the skin that caused putrid odor indicating the degradation of the protein. The proteoglycan portion that held the hair bulb degraded by the proteolytic enzymes led to loosening of the hair along with the disruption of collagen stability. Since the collagen's protective sheath such as reticulin, elastin and other cementing mucoidal proteoglycan were destroyed by the action of proteolytic enzymes, the collagen became vulnerable to the action of bacterial collagenases that cleaved it into several fragments. The lipid portion also gets broken down releasing several slimy by-products that made the skin slippery and lacking physical solidarity. The complex integral components mineralized into simpler compounds such as amino acids, further inviting several other organisms such as maggots that resulted in the total deterioration of the raw material. The value of the raw material downgraded every hour after the flaying operation. Monitoring the effects of putrefaction with respect to time would provide a lead for devising a fool-proof and environment-friendly preservation technology. These results pave way for exploring the salt-free preservation alternatives that check microbial infestation with few hours of hide/skin flaying.

Conclusions

Goat skins were allowed to putrefy in ambient tannery conditions which indicated many physical as well as biochemical changes during spoilage. Physical observations helped in identifying the initiation of putrefaction that started at around six hours from flaying. Histological staining studies and scanning electron microscope studies were evident in indicating the significant level of collagen degradation that started occurring at the initial stage. The presence of collagenolytic bacteria from the six hours of initiation further confirmed that the collagen degradation started early. Therefore, on the grounds of conservation of quality of raw material, it can be concluded that the preservation should be started much earlier than six hours of flaying. However, it needs to be kept in mind that this phenomenon is dependent on the ambient temperature and other physiological conditions. In conclusion, this work provides evidence to understand the putrefaction processes with respect to time, which would aid in better comprehension of the factors that are involved in skin spoilage and would help in devising methods to avoid damage occurring due to bacterial putrefaction.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Development of Improved Liming Process based on Automated pH Monitoring and Control System

by

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Abstract

The control of pH of a process plays an important role in many chemical or biological reactions. The monitoring and control of pH of processes like wastewater treatment, manufacturing food and leather making facilitate to reduce pollution and improve the quality of the final product. The focus of this study is to optimize the usage of lime and recycling of spent liquor through continuous monitoring system of pH to achieve near zero residue from liming operation. But the challenges are nonlinear behaviour of the system and frequent fouling of pH sensors. The system developed monitors the pH values and controls the cycling time and the addition of lime as per the user set profile. The real time data of pH values in the process is logged on to the PC for further analysis. The efficacy of the system developed was validated at lab level and can be easily scaled up for implementation in industries. The results showed that the effluent from leather making can be minimized by adopting automated pH monitoring and control systems.

Introduction

Typically, treatment of animal skin or hides with Calcium hydroxide –($Ca(OH)_2$) is called liming. Liming is an important process in leather processing where soaked hides/skins are treated with a Calcium Hydroxide ($Ca(OH)_2$) solution along with Sodium Sulfide (Na_2S) or other unhairing agents in pit/drum/paddle methods. It is a slow process and depending upon the type of leather, the processing time varies from a few hours to a few days. During this process,alkali soluble proteins - interfibrillary proteins and keratinous matter are removed in addition to opening up the fiber bundles of skin. After unhairing the hides/skins are treated with $Ca(OH)_2$ solution again in the reliming process to achieve the required degree of fiber opening and taken for flesh removal. This process is also time consuming and requires large quantities of alkalis.

A detailed literature survey on liming showed different chemicals and different methods to speed up liming process have been attempted by many researchers. A very rapid liming and subsequent tanning process without effluent by dipping in sodium sulfide solution and followed by treatment with 10% sodium peroxide solution for 1 hour has been attempted earlier.[1,2] A lime free unhairing system has been reported by using 3% caustic soda, 1% salt, ,1-2% sodium sulfhydrate.[3]A continuous automatic beamhouse processing was reported by rapid soaking of brine cured hides and unhairing using 4-6% sodium sulfide solution for 10 minutes.[4] Other reports include rapid oxidative unhairing using calcium peroxide to avoid using sodium sulfide in the unhairing process.[5] Attempts were made to apply the beam-house chemicals with optimal requirement to minimize pollution.[6,7] A modified method where the unhairing-liming liquids were reused several times after being recharged by reduced quantities of chemicals.[8] Some reported other methods such as oxidative unhairing of skin using hydrogen peroxide, amine,[9] oxidative unhairing,[10] sulfide free unhairing using ozone,[11] ionic liquids,[12] and phase transfer catalyst[13]as alternative improved liming methods.

The monitoring and control of the pH in the liming process is needed because the degree of swelling of skin or hide is high at higher concentrations of lime solution. The hair is also loosened at higher concentrations, making it suitable for further processing.

Automation of processes in different industries such as food processing are pursued in order to ensure product safety and quality.[14] In garment manufacturing automation has been reported to reduce the labor cost and improve the quality of the finished products.[15] In fertilizer industry the soil nutrient sensor detects and quantifies the amount of nitrogen, potassium and phosphorous in the soil and the automation system helps in recommending the right amount of fertilizers to be added to the soil.[16] Programmable logic controller (PLC) based dosing systems in leather making reduces wastage of chemicals and help in reducing pollution load.[17]

The main objective of this research work is to develop a process technology to minimize the usage of lime and the reduction of lime sludge and effluents from the liming process through suitable instrumentation technology.

This research work describes an automated liming process. It also includes development of an instrumentation system along with

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its application software for measurement, data logging, analysis, control and generation of reports. Testing of developed automated pH monitoring and control system in the liming process under various conditions in real time have been carried out and presented in this paper.

Experimental

Materials and Methods

The automated pH monitoring and control system for liming operation was installed and studied at Central Leather Research Institute, India. A model of automated pH monitoring and control system is provided in Figure 1. This consists of lime saturation tank, magnetic stirrer, liming tank, liquid transfer pump and pH electrodes for continuous monitoring of pH of the solution. Lime, sodium sulfide used were of industrial grade supplied by commercial manufacturers, India. The experiments were carried out with goat skins weighing approximately 1 kg per piece. The requirements of lime and water have been optimized through preliminary experiments. The process details are given in Table I. Studies have been carried for 6 batches using 3 skins per batch.

Lime at 0.375% and 2.0% sodium sulfide added and stirred to 250% water in the lime saturation tank to attain pH 12. Subsequently, computer monitoring and control was initiated. Then through the solenoid valve the transfer of fluid took place from the saturation tank to the reaction tank where soaked goat skin was processed for the removal of hair for 4 hours duration. After 4 hours duration, the spent liquor was transferred to the saturation tank and the next instalment of lime 0.375% was added. The stirrer starts through application software and the lime dissolves and pH raises close to 12 within 10 minutes. Then the transfer of liquid to reaction tank

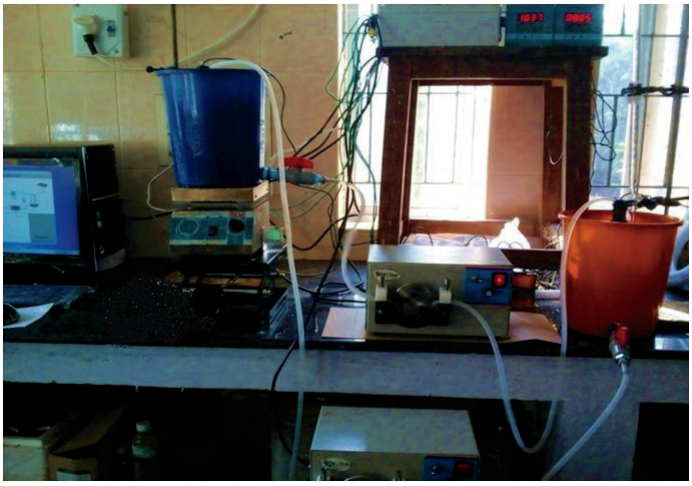

Figure 1. Automated pH Monitoring and Control System

and comes in contact with the skin for liming. Then, the cycle was repeated for six times(six instalments of lime addition) and monitored for quality parameters of the skins. Then the skin was unhaired, weighed and reliming was done for the duration of 24 hours. The experimental and conventionally limed skins were taken for further processing into crust leather as per the process details given in Annexure.

Description of Instrumentation System

Based on the requirements of the automated liming process, the prototype system has been developed with two tanks - one for holding saturated lime solution with a stirrer and one reaction tank for liming and reliming of the skin. The instrumentation system consists of acrylic tanks, pump, pH sensors, stirrer which operates on the power supply of 230 V AC 50Hz. Two pH measurement systems with industrial grade sensors and transmitters were installed in

Table I

Chemical usage of Liming Process

Chemicals	Automated Liming (based on skin wt)		Conventional Liming (based on skin wt)	
	Liming Process	Reliming Process	Liming Process	Reliming Process
Quantity of Water (%)	250	250	250	250
Quantity of Lime (%) (per installment)	0.375	0.375	10	5
Quantity of sodium sulfide (%)	2.0	Nil	2	Nil
Total amount of lime used for 6 batches (%)	2.25	2.25	Nil	Nil
Total amount of lime used (%)	2.25	2.25	10	5

both the tanks. Since this process deals with high caustic pH values (10-12 pH), the transmitters should work in the desired range with an accuracy of 0.01% full scale display and communication output either 4-20 mA or digital output like RS485 based MODBUS communication. The pH measurement is dependent on the temperature of the process; hence, auto temperature compensating pH transmitters were used.

Sensing pH Electrodes (Industrial grade pH electrode, SIMS instrumentation, India) were selected with suitable construction for continuous operation in caustic pH values and to facilitate accurate measurement. Pumps were selected with variable speed to control the transfer of lime saturated liquor with high level of particulate matter.

The control system was developed with desk top PC and Data Acquisition Cards to acquire the pH inputs and generate control signals to the solenoid valves and pumps through Solid State Relays. The system was an open-source integrated development that supports the functions and utilities to develop all types of real-time automation and control applications with appropriate drivers for the data acquisition boards/cards.

Figure 2 shows the functional block diagram of the automated liming process. The instrumentation system consists of two pH meters, PC with data acquisition (DAQ) module, with an output signal through solid state relay (SSR) for actuating stirrer, solenoid valve and peristaltic pump. The pH meter records pH values in real time from both the tanks to obtain pH of the solution. The DAQ is programmed to log inputs from the two pH meters and analyzes the status and time. Based on the decision-making algorithm digital outputs generated through the DAQ card is applied to the SSR to the actuators.

The specification of the DAQ board is given below:

USB-4711A is a multifunctional module, which has 16-ch Analog Input, 2-ch Analog Output, 16-ch Digital I/O and counter channel which is able to output a constant frequency square wave.

Device Features

a) Analog Input

- 16-channel Single-Ended A/D Input for USB4711/A, 8-channel Differential-Ended A/D Input for USB4711A
- 12-bit A/D conversion
- Sampling rate for USB4711 from 1 Hz to 100 kHz
- Input Range: +/-0.625V, +/-1.25V, +/-2.5V, +/-5V and +/-10 V
- Automatic Channel/Gain Scanning

b) Analog Output

- 2-channel D/A Output
- Output Range with internal reference: +/-10V, +/-5V, 0~10V, 0~5V

c) Digital Input and Output

- ˜8-channel Digital Input

d) Counter

- USB4711: one 32 bit counter with max to 1k input rate

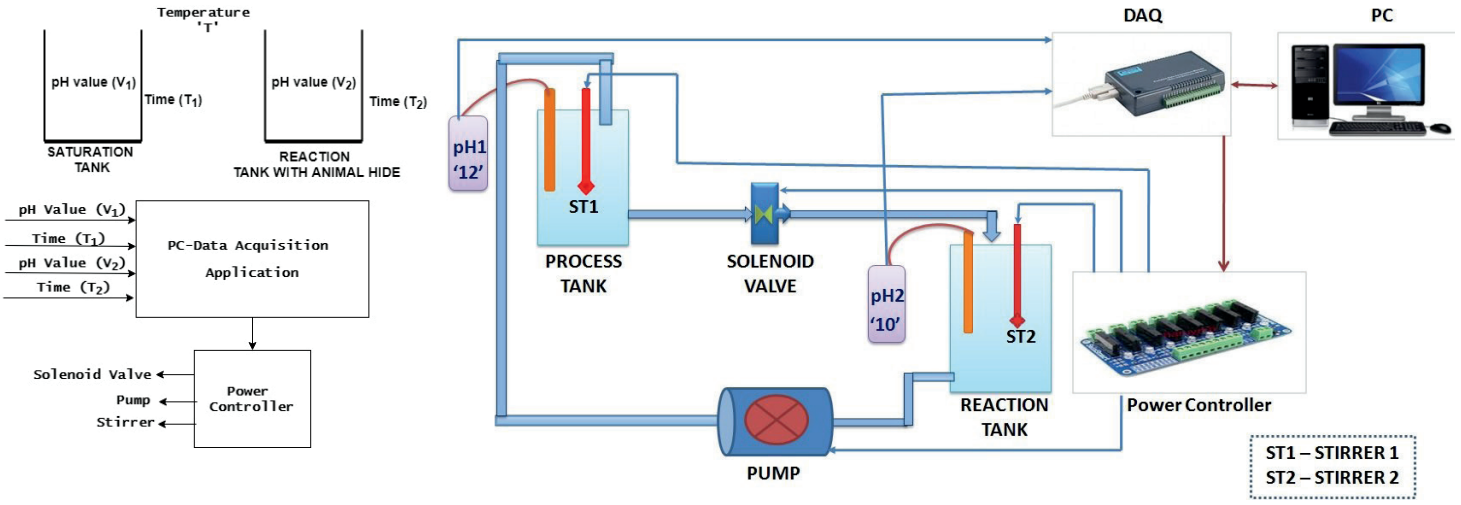

Figure 2. Functional Block Diagram of Automated Liming Process

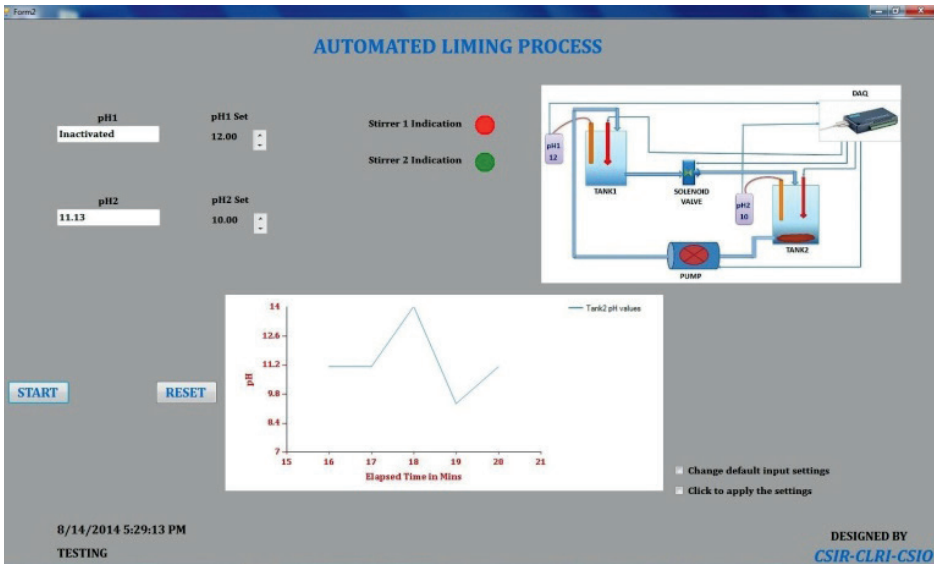

Figure 3. Window of Application Software

Application Software Description

Automated liming process application software was developed using Visual Studio IDE and compatible DAQ drivers. The application comprises of two windows, Form 1 deals with security and process details. Form 2 window comprises the automated liming process as shown in Figure 3. As per the storage interval entered, the data will be logged once for the specified interval in an Excel file.

The Form 2 window displays the real-time analog pH values against the programmable pH set-points, the status of the stirrers, solenoid valve and a peristaltic pump. The real time pH values of the elapsed time in the process are plotted and displayed continuously. This plot along with the current date and time and the project name, as indicated in the Form 2 Window, will be stored in an Excel file for future analysis. Moreover, an indication will appear in the window during the instant the data is logged along with the location of the file regularly.

Application Software

The various functions in the application software are explained in Figure 4. *Load and Initialize Process (Form 2 Window):* Form 2 is the process algorithm to control the pump, solenoid valve and stirrer based on the real time values of pH monitored in the two tanks and time.

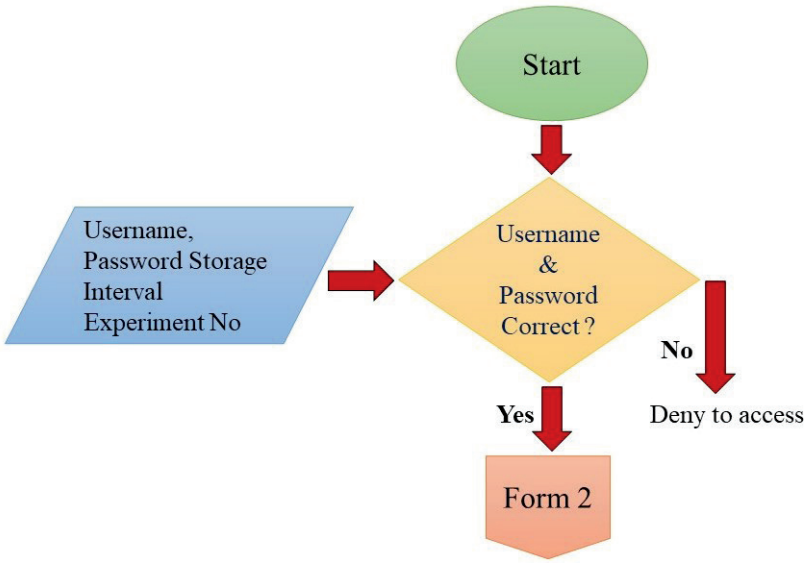

Figure 4. Functions of application software

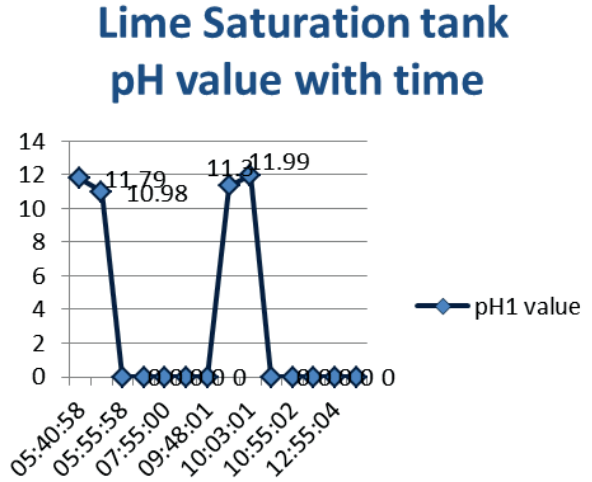

Figure 5. pH profile of saturation tank

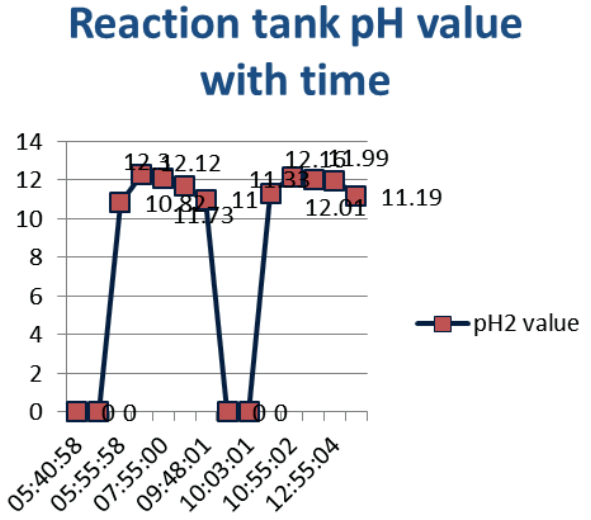

Figure 6. pH profile of reaction tank

Analyses of chemical, mechanical and organoleptic properties

The limed pelts were converted into crust leathers and conditioned at 20° ±2°C and 65 ± 2 % of relative humidity over a period of 48 hours before analysis. The chrome content and fat content and physical properties (shrinkage temperature, tear and tensile strength) were examined as per standard procedures (BIS 1971,[18] ASTM D6076,[19] IUP 2000a[20] and IUP 2000b[21]). The organoleptic properties were evaluated by a group of experienced leather technologists.

Results and Discussion

Addition of lime and sodium sulfide in case of liming or lime alone in case of reliming the pH raises close to 12 in saturation tank after 10 minutes stirring. Then the liquid is transferred to reaction tank through solenoid valve where the skins are placed and the reaction starts. The pH of the solution comes down close to 10 in 4 hours duration. Since the pH change is marginal after that, the liquid is transferred to saturation tank and the 0.375% lime is added, redissolved and sent again to reaction tank. This cycle continues for 6 times with fresh installment of 0.375% lime addition and liming / reliming process takes place. The pH profiles of saturation tank and reaction tank are given in Figures 5 & 6.

In conventional liming process 10% lime along with 2% sodium sulfide is used and during reliming 5% lime is used whereas in the case of experiment 2.25% lime is used for in unhairing and 2.25% lime is used during reliming process was used as shown in Fig 7. This enables to save up to 70% lime compared to conventional

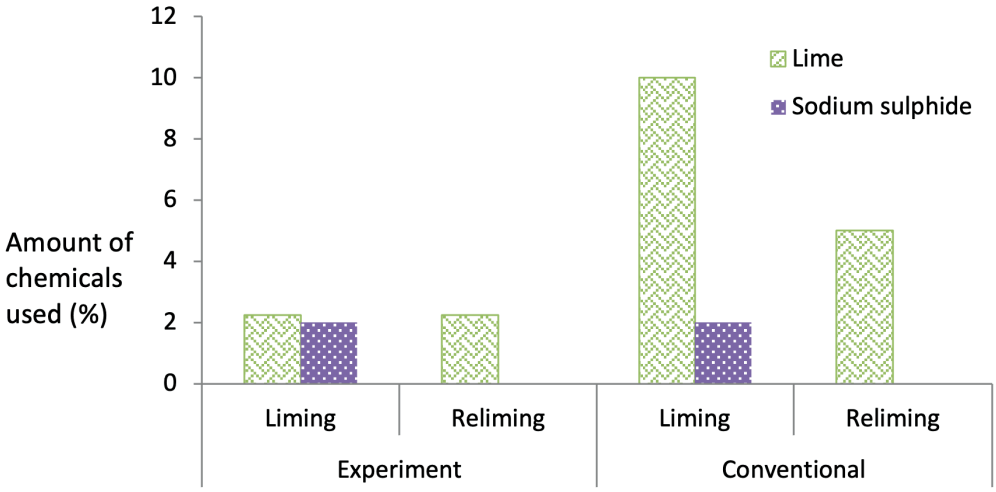

Figure 7. The amount of lime and sodium sulfide used in the process

Table II

Physical and chemical analysis of chrome tanned and crust leather

Process	Shrinkage Temperature °C	Chrome content % Cr_2O_3	Fat content %	Tensile Strength N/mm²	Tear Strength N
Conventional	103±0.5	3.2±0.1	6.2±0.2	23.1±1	86±2
Automated Liming	102±0.5	3.1±0.1	6.1±0.2	23.0±1	85±2

(Average value of three experiments)

Table III

Organoleptic properties of chrome tanned crust leather

	Softness	Smoothness	Grain characteristics	Overall appearance
Conventional	7±1	6±0.5	7±0.5	7±1
Automated Liming	6±1	7±0.5	7±0.5	7±1

0- poor, 10-Excelleant (Average value of three experiments)

process and 90% reduction in sludge generation. The study also proved theoretical alkali binding capacity of skin which is 2.15% lime.

The solubility of lime is 0.15% at room temperature and due to mutual solubilizing effect because of other constituents extricated from skin some quantity of lime will dissolve and get into effluent streams. Table II and Table III show chemical analysis, strength properties and organoleptic properties of leathers produced by both conventional and automated liming process.

The above results show properties of crust leathers produced from both conventional and automated liming processes have comparable characteristics.

Conclusion

This instrumentation system provides an efficient and automated pH monitoring and control system in the liming process. Several experiments have been carried out to ensure that the continuous monitoring and control of pH of liming process. Substantial reduction in lime input and consequent pollution reduction was observed in the experimental process. The technology is suitable for tanneries where pH monitoring and control will impact the material quality while providing significant environmental benefits.

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Annexure

Conventional process of goat skins

Process	Quantity	Product	Duration	Remarks
Soaking I	300 %	Water	30min	
Soaking II	300 %	Water	Left overnight	
	0.1 %	Preservative		
	0.5 %	Wetting agent		
Next day washing	200 %	Water		
Liming	250%	Water	Left overnight	This quantity is only for conventional liming process.
	10%	Lime		
	2%	Sodium sulfide		
Unhairing				Unhairing was done mechanically.
Reliming	250%	Water	Left overnight	This quantity is only for conventional liming process.
	5%	Lime		
Fleshing				The limed pelts were fleshed and taken for washing
Washing	200 %	Water	10 min	Conventional and experimental skins were processed together.
Deliming	150 %	Water	Run for 1 h Run for 45 Min.	Washed and drained pH 8-8.5
	1 %	Ammonium chloride		
	0.75 %	Bating agent		
Washing	100 %	Water	10 min	
Pickling	80 %	Water		pH 2.8-3
	8 %	Sodium chloride	Run for 15 min	
	1 %	Formic acid	3 x 10 min, Run for 10 min	
	0.75 %	Sulphuric acid	4 x 15 min, Run for 1 h	
Next day the pelts drummed for 30 min pH at cross section adjusted to 2.8- 3.0. Then 50% of pickle bath drained.				
Chrome tanning	8 %	Basic chromium sulfate (BCS)	2 x 30 min	Check for penetration in cross section
	1.0 %	Sodium formate	10 min	
	1 %	Sodium bicarbonate	3 x 20 min, run for 1 h	Check the pH to be 3.8 to 4. Drain the bath and pile overnight.
Next day sammyed and shaved in 1.0 mm. The shaved weight noted.				
Washing	200 %	Water		Drain
Neutralization	150 %	Water		pH 5-5.5
	0.5 %	Sodium formate	10 min	
	1.0 %	Sodium bicarbonate	3 x 15 min + 1 h	
Washing	100 %	Water	10 min	
Retanning and Fatliquoring	100 %	Water		Drain, rinse, pile over night
	10 %	Fatliquors (Semisynthetic fatliquor FB20 Balmer Lawrie, India; synthetic fatliquor SXE Balmer Lawrie, India)	1 h	
	6 %	Syntans (Phenolic based FBDI (BASF India), Renakotan FBN Melamine resin based syntan (Stahl,India),	45 min	
	1 %	Formic acid	3 x 5 min + 40 min	

Finally, leathers were dried and then conditioned for testing and evaluation.

Lifelines

Cigdem Kilicarislan Ozkan graduated from Ege University Department of Leather Engineering in 2008. The same year she started to M.Sc. She studied on extraction of vegetable tanning materials. She joined the staff of Leather Engineering Department as research assistant in 2010 and completed her PhD in 2018. Her research activities and fields of interests are tanning materials, extraction techniques, modification of biopolymers and leather technologies.

Hasan Ozgunay studied Leather Technology at the University of Ege (Turkey). After working in Leather Industry for one year, he joined the staff of Leather Engineering Department as research assistant in 1996. He obtained his M.Sc. in the same department. He studied on vegetable tanning materials and obtained his PhD in Leather Engineering. He is currently working as researcher / lecturer in the Leather Engineering Department. His research activities and fields of interests are tanning materials, leather processing technologies and cleaner leather processing methodologies.

Tesfay Gebryergs is student pursuing Master's degree in Leather Technology at Addis Ababa University, Ethiopia. He carried out this work in CSIR-CLRI as part of his Master's project under the Twinning program.

C. Sivaranjani is MSc Chemistry graduate working in CSIR-CLRI as Project assistant.

N. Nishad Fathima, see *JALCA* **98**, 263, 2003.

Vimudha Muralidharan received her master's degree in Leather technology from same university in 2016. She is currently pursuing her doctoral program in Leather technology under the supervision of Dr. Balaraman Madhan in Central Leather Research Institute, Chennai, India. Her research interests include leather processing, microbiology of leather, waste management and material science.

Renganath Rao R. is a Scientist in Leather Process Technology Department in Central Leather Research Institute, India. He received his M.S by research degree in Leather Technology from Anna University, Chennai, India. His research interests include enzyme technology in leather processing, biotechnology, sustainable material science and metal organic framework synthesis.

Balaraman Madhan is a Senior Principal Scientist in Central Leather Research Institute, India. He has been a visiting faculty to Anna University, Chennai, India. His research interests include cleaner leather processing, enzyme technology, value added products from solid wastes, and waste management.

Saravanan Palanivel is a Chief Scientist in Central Leather Research Institute, India. He has been a visiting faculty to Anna University, Chennai, India. His research interests include enzyme technology, zero emission research initiatives in leather technology. He also mentors and trains technologists on concepts of sustainability and business management.

Kota Srinivas completed his M.S (Electronics & Control) from BITS, Pilani and has 36 years of R&D experience in projects as Project member, Project leader and Principal investigator - Process control, Energy Management Systems, Bio Sensor based instrumentation for health. He is been teaching advanced instrumentation course for M.Tech Students of AcSIR from 2009 at SERC, Chennai. Has presented/ published more than 60 papers and 5 reports.

D. Krishnamoorthy completed his Diploma in Electrical & Communication from S.V. Polytechnic, Tirupati, A.P. India. He has obtained his bachelor degree in Engineering & Masters degree in software systems from BITS, Pilani, India under DLPD program. Started his carrier in CSIR-CSIO, Chennai Centre in the year 1983.In his 39 years of experience, He has actively participated in Repairing & Servicing (AMC) of Instruments at his early age of career, Research & Development of Embedded and PC based systems and installation of systems for various industries in mid years. Finally, served the industry in calibration & testing of instruments. CSIR-CSIO acquired NABL accreditation for the calibration activity for Electro –technical & mechanical sections as he was the Quality Manager.

V. Aparna received her undergraduate and postgraduate degrees in Electronics & Instrumentation Engineering. She has over 3 years of experience from reputed research organizations and has extensively published and presented research articles on Process Control & Instrumentation, Artificial Intelligence, Machine Learning, DCS & PLC Engineering, and Embedded Systems.

V. P. Anand holds Masters in Renewable Energy & Bachelors in Electrical and Electronics Engineering & is currently a scientist with CSIR CSIO. He has been actively engaged in instrumentation related to health monitoring of energy infrastructures for process and discrete industries for more than 5 years.

A. Saravana Raj, a post graduate chemical engineer with 10 years academic experience in engineering institution, is about to complete his doctoral research in the area of bioprocess and metabolic engineering from Anna University, Chennai. He has guided many student research projects in some key research areas such as process optimization, bioremediation and food nanoemulsions. He has 7 publications to his credit.

Mohammed Abu Javid. H. obtained his B.Tech degree in Leather technology from Anna University, Chennai. He has been actively engaged in different process control and development. He has actively working on environmental impact oriented leather process and technology. He has 7 research papers to his credit.

K. Iyappan obtained his Ph.D. Degree in Engineering from Anna University, Chennai, India. He has been actively engaged in Renewable energy generation & storage, environmental & eco-friendly process development, value added products, waste to energy and scale-up studies of green chemical for leather sectors for more than 30 years. He has developed number of leather process methodologies using renewable energy, by-product utilization and waste minimization. He has to his credit about 32 research papers and 5 patents.

N. Vedaraman, see JALCA 107, 435, 2012

V. John Sundar, see JALCA 107, 435, 2012

K. C. Velappan, see JALCA 110, 237, 2015

C. Muralidharan, see JALCA 110, 23, 2015

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Obituary

Guy Harold Moberg Sr., 102, of Hickory passed away Sunday, May 2, 2021 at Carolina Caring. Born Sunday, July 21st, 1918 in Cheboygan, MI, he was the son of the late Simon Edward Moberg and Minnie Abel Moberg.

A member of the Boy Scouts, he earned the Eagle Scout designation. In 1941 Guy married Phyllis Crane in Sault Ste Marie, MI. He served as Captain in the U.S. Army 595th Engineer Company during WWII and the Reserves after the war.

Guy graduated from Pratt Institute in 1949 with a Degree in Tanning Technology / Chemistry. He worked as a Tanner for Northwestern Tannery, Howes Leather Co., SB Foot, Brezner Tannery, Lowengard, Denison Hide (a division of Iowa Beef) and Woodbury Industries until retirement.

After retirement, Guy consulted for years in the Tanning Industry throughout the US and many countries throughout the world.

Guy joined The American Leather Chemists Association on May 12, 1949. He was given life status in the Association in 1989. During his years in the Association, he served on many committees including many years on the Education Committee, the Methods and Specifications Committee from 2002 to 2003, chair of the Rawstock Committee from August of 1974 to August of 1977, the Specification

Review Committee from 1988 to 2002, and the Officers Nominating Committee in 1974.

Guy is a lifelong member of Masonic Lodge #615and Veterans of Foreign Wars. He became an avid Ham Radio Operator in his retirement years, belonging to many groups of Ham Radio enthusiasts and specifically in Western NC.

Guy was preceded in death by his wife of 61 years, infant son Jonathan Moberg and his two sisters, June Nordgren and Fern Burt.

He is survived by his three children, Guy H Moberg Jr. of Fon Du Lac WI, Melanie Bohl of Racine WI, Mary Fyock (Jack) of Hickory, and four grandchildren, Kristian Moberg, Andrew (Andy) Bohl, Jonathan (Jon) Moberg, and Aron Moberg.

The family will hold a private service.

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