A Novel Approach Towards Preservation of Skins

by

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Abstract

Wet salting, the conventional method of curing is followed by most of the tanners because of its practical advantages; employs approximately 40–50% sodium chloride on raw material and is subsequently removed during the soaking operation. It contributes to the total solids content of the effluent, accounting for nearly 40% of the total solids load. Hence an attempt has been made to completely replace the salt with polyethylene glycol(PEG) to address the pollution problems without compromising much of the practical advantages of salt. The molecular weight and percentage offer of PEG has been standardized based on the dehydration and rehydration profile of the preserved skin matrix. The PEG based preservation has been found to be more effective than the conventional method by the determination of hydroxyproline(HP) release in soak liquors during various time periods of storage. The fiber orientation of the crust leather has been demonstrated through scanning electron microscopy analysis. The pollutant loads of the developed preservation method using PEG was found to be 71, 34, 99, 93% less in terms of biochemical oxygen demand(BOD), chemical oxygen demand(COD), chloride(Cl^-), total dissolved solids(TDS), respectively, when compared to the conventional method of preservation. Further, the physical and organoleptic properties of the crust leathers were found to be on par with control crust leathers. Hence, the developed preservation method seems to be a techno-economically viable alternative for salt-based preservation.

Resumen

La salazón en húmedo, el método convencional de conservación es seguido por la mayoría de los curtidores, debido a sus ventajas prácticas, emplea a aproximadamente 40 a 50% de cloruro de sodio en el cuero crudo y posteriormente se retira durante la operación de remojo. Contribuye con el contenido de sólidos totales del efluente, cercano al 40% de la carga total de sólidos. Por lo tanto se ha tratado de sustituir por completo la sal con polietilenglicol (PEG) para abordar los problemas de contaminación, sin comprometer la mayor parte de las ventajas prácticas de la sal. El peso molecular y el porcentaje de oferta de PEG ha sido estandarizado basado en el perfil de la deshidratación y rehidratación de la matriz de la piel conservada. La conservación basada en PEG se ha encontrado para ser más eficaz que el método convencional mediante la determinación de hidroxiprolina (HP) liberada en el licor de remojo en distintos periodos de tiempo de almacenamiento. La orientación de las fibras sobre el cuero semi-terminado se ha demostrado a través de análisis de microscopía de barrido electrónico. Las cargas de contaminantes del método de conservación desarrollado con PEG se encontró 71, 34, 99, 93% menos en términos de demanda bioquímica de oxígeno (DBO), demanda química de oxígeno (DQO), cloruros (Cl-), sólidos disueltos totales (SDT), respectivamente, en comparación con el método convencional de conservación. Además, las propiedades físicas y organolépticas de los cueros semi-terminados se encontraron a la par con los cueros semi-terminados de control. Por lo tanto, el método de conservación desarrollado parece ser una alternativa técnico-económicamente viable a la preservación a base de sal.

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INTRODUCTION

The presence of approximately two-third water and one-third protein in raw hides and skins coupled with favorable conditions makes it a soft target for microbial attacks.[1] So the leather making protein should be preserved between the times it is removed from the animal until it can be processed into leather. This temporary preservation can be done in two ways, by either killing the microorganism or creating unfavorable conditions for the microorganisms to thrive. The former technique is called bactericidal while the latter is called bacteriostatic. However, the bactericidal method employs chemicals that are also harmful for humans and hence, the bacteriostatic based preservation method is most suitable for temporary preservation of raw hides or skins.

The salt based curing is one of the bacteriostatic methods of preservation practiced widely around the globe because of its many advantages. The principle lies in the fact that the reduction of moisture content results in unfavorable condition for any microorganism to thrive and the extent of curing has no irreversible changes in the quality of raw material and finished products. This method employs approximately 40% w/w sodium chloride on the raw weight of hide/skin. Almost 75% of the salt used gets discharged into the effluent stream during soaking, which contributes 40% of total solids content in the tannery effluent.[2] The higher salinity in the tannery effluent leads to an increase in the operating and maintenance cost of the treatment plants. Moreover the ground water pollution near the vicinity of tannery sectors forced tanners to opt for reducing/ avoiding the usage of salts for preservation.[3] Less salt based preservation methods with and without anti microbial agents have been explored.[4-7] However, these methods do not reduce the TS significantly. Salt free methods such as chilling and irradiation based preservation methods have been developed.[8,9] These methods are expensive and difficult to adopt in a tannery. So an alternative techno economically viable preservation method that completely replaces salt needs to be developed.

Polyethylene glycol is an inert, flexible, water-soluble polymer of ethylene oxide having specific interaction with biological systems. It has a capacity to create very high osmotic pressures which helps as a precipitant for protein crystallization.[10,11] The widening use of PEG, and its neutral charge behavior gives positive insight to replace salt for curing. Moreover the little tendency to denature or to specifically interact with proteins even when present in high concentrations and at elevated temperatures provides a possible choice to use it as a curing agent without any further chemical modification of the virgin macromolecule.[12-14] In the present work, salt is completely replaced with polyethylene glycol for preservation of goat skins to achieve greener leather processing. Different molecular weights of PEG are used in the preliminary trials and the best one is selected based on the dehydration and rehydration studies. Then the percentage offer of PEG of selected molecular weight was optimized. The effectiveness of the preservation of goat skin was assessed by determining the hydroxyproline release as well as the bacterial count in the soak liquor at various period of storage. Scanning electron microscopy analysis has been carried out for the wet salted and PEG preserved crust leather. Bulk trials were carried out and the soak liquor was collected and analyzed for salinity, oxygen demand and chloride. Physical testing and hand evaluation were carried on crust leathers.

EXPERIMENTAL

Materials

Raw goatskins with weight range of 1–2 kg were obtained from Perambur Slaughter House, Chennai, India. Skins were removed from the animal and transported through an ice packed container within 2 hour. PEG of different molecular weight and salt used for preliminary preservation trials were of laboratory grade. The chemicals used for bulk trial studies were of commercial grade. The chemicals used for the analysis of spent liquors were of analytical grade.

Selection of Molecular Weight of PEG for Preservation

Five raw goatskins were sided, trimmed and washed immediately. The washed skins were allowed to drain for 15 minutes and made into half sides by equally cutting across the backbone. Ten sides were numbered and weighed individually. Different molecular weights of PEG200, 300, 400, 600 and 1000 were applied on the fleshed side of each side at an offer of 5% (w/w of each side). Other five sides were used for control preservation trials. Two sides were preserved by conventional method of drying at room temperature (34±2°C) and other three sides were treated with sodium chloride 40% w/w for control preservation trials. Moisture content of the skin samples was determined every one hour until constant moisture content is reached and checked after every 24 hours. The preserved sides were stored at room temperature (34±2°C).

Rehydration Studies

Rehydration assay for the preserved samples were carried out by immersing the control and experimental samples in water. Approximately, 10 g preserved sample was put in 100 ml distilled water at room temperature (34±2°C) in a 250 mL beaker. At predetermined sampling periods, the samples were removed from the beaker and gently blotted on the tissue paper to remove surface water and then weighed by an electronic balance with an accuracy of ±0.0001 g. The preserved goat skins were evaluated for rehydration characteristics in respect of rehydration ratio, from the weight before and after the rehydration.

Optimization of Amount of PEG Offer
Five raw goat skins were trimmed, washed and made into ten half sides by cutting along the backbone. Each side was numbered and weighed. Concentration of PEG of the selected molecular weight was varied as 1, 2, 3, 4 and 5% w/w of each half side. Other five sides were used for control preservation trials as described earlier. Moisture content of the skin samples was determined until constant moisture is retained and checked after every 24 hours. The preserved sides were stored at room temperature (34±2°C).

Bulk Preservation Studies
Ten raw goatskins were trimmed, washed and made into half sides by cutting along the backbone. Each side was weighed. Ten left sides and ten right sides were used for control and experimental trials, respectively. Experimental and control trials were performed using optimized offer of PEG. Ambient drying and sodium chloride (40% w/w) method of curing is conducted on two and three sides, respectively. Then the sides were kept undisturbed for 24 hours at room temperature (34±2°C). Control sides were manually desalted and soaked in a pit employing three changes of 300% (w/w) water for a total period of 8 hour. Experimental goat sides were directly taken for soaking employing the above method for a period of 5 hours. Soaked control and experimental goat sides were separately converted into shoe upper employing conventional post soaking operations.

Determination of Hydroxyproline
Approximately 25 g of control and experimental preserved samples were taken at various time periods up to 12 weeks of storage. The samples were soaked with 900% (w/w) water for 8 hours in a water shaker and the spent liquor was collected. Hydroxyproline was determined using the method of Woessner[15], after acid hydrolysis of the sample. The amount of hydroxyproline(HP) was calculated by multiplying concentration(mg/L) with volume of spent liquor(L) per kg of raw skins(dry weight basis).

Determination of Bacterial Count
Skin specimens weighed about 5 g were cut from the samples at different stages of preservation and soaked separately in bottles each containing 50 ml of sterile water. The soak solution was prepared by shaking the bottles in an orbital shaker at 200 rpm for 30 min. Soak liquor of 1 ml was diluted to 10 ml with sterile water and was kept for shaking to get a uniform suspension of bacteria. An aliquot of 0.1 ml of the resulting diluted solution was taken in sterile petriplates and molten nutrient agar was poured and shaken gently to get a uniform distribution of the bacteria. The plates were incubated at 37°C for 24 hours.[16] The bacterial population was determined and expressed as CFU per gram of skin.

Spent Soak Liquor Analysis
Spent liquor from soaking process was collected from control and experimental processes. The spent liquor was analyzed for chloride(Cl^-), BOD, COD and salinity as per the standard procedures.[17] Effluent loads were calculated by multiplying concentration(mg/L) by volume of spent soak liquor(L) from the soaking process per ton of preserved raw skins.

Composite Liquor Analysis
Composite liquor from conventional and experimental leather processed were collected from all unit operations up to post tanning and analyzed for BOD, COD, Cl^- and salinity (TDS) as per the standard procedure.[17] Emission loads were calculated by multiplying the concentration (mg/L) with volume of effluent (L) per ton of raw skins processed.

Input and Output Analysis
Input–output analysis for the raw materials, water and chemicals was carried out for control and experimental processes. The amount of salt removed during desalting was estimated for control preserved goat sides. The spent liquor from control soaking processes was analyzed for sodium chloride as per the standard procedure.[17] PEG was not analyzed in the spent soak liquor from experimental process since it is difficult to analyze in a mixed form.

Scanning Electron Microscopy Analysis
The control and experimental crust sides were cut from the official sampling position. Then the samples with uniform thickness were directly taken for analysis without any pre-treatment. Quanta 200 series scanning electron microscope was used for the analysis. The micrographs for the cross section were obtained by operating the SEM at low vacuum and an accelerating voltage of 12–15 kV at 200 magnification level.

Physical Testing and Hand Evaluation of Leathers
Samples for various physical tests from experimental and control crust leathers were obtained as per IUP method.[18] Specimens were conditioned at 27±2°C and 65±2% relative humidity (R.H.) over a period of 48 hours. Physical properties such as tensile strength, % elongation at break, tear strength and grain crack strength were examined as per the standard procedures.[19-21] Experimental and control crust leathers were assessed for softness, fullness, grain smoothness, grain tightness (break) and general appearance by hand and visual examination. The leathers were rated on a scale of 0–10 points for each functional property where higher points indicate better property.

RESULTS AND DISCUSSION

Selection of Molecular Weight of PEG for Preservation
Different molecular weight of PEG was employed to select the suitable molecular weight for preservation of skin matrix. The selection of molecular weight of PEG is based on the

dehydration and rehydration studies. The dehydration and rehydration profile of the control and experimental samples are given in Fig.1 and 2. From the dehydration graph it is clear that PEG of different molecular weights namely 200, 300, 400, 600 and 1000 reduces the moisture content to below 20% but only PEG200 and 1000 follows a similar dehydration pattern to that of wet salted skins. The reduction of moisture content to around 12% (final) by PEG200, 300, 400, 600 may be attributed to the fact that molecular size of these polymers are very low and hence provide through and through removal of moisture content and also the fluid nature of these polymers helps in uniform distribution during application. From the rehydration graph, it is evident that the rehydration rates are comparable to that of the wet salted skins for all the molecular weight of PEG. The required moisture content of the soaked skins were obtained for all the molecular weight in 5 hours similar to that of the wet salted skins. Since PEG200 reduces the moisture content to the required amount (30%) in just 8 hours, well below the time taken by sodium chloride and also matched the rehydration rate, PEG200 was taken for further studies.

Optimization of Amount of PEG200 for Preservation

Various percentage offer of PEG200 were employed to optimize the amount required to preserve the goat skins. Since selection of PEG200 was done at 5% offer on weight basis to that of skin, this study is to assess the minimum offer of PEG200 on weight basis, that provide the optimal and effective preservation. The dehydration and rehydration profile of the

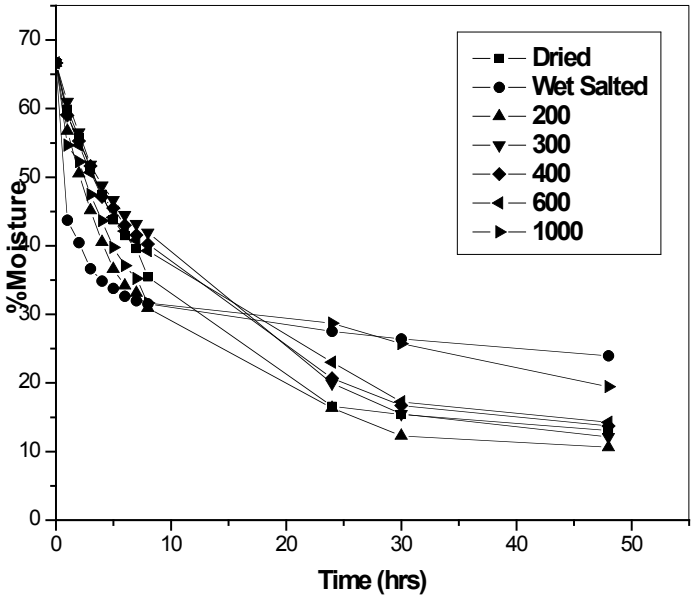

Figure 1. Dehydration profile of preserved skins

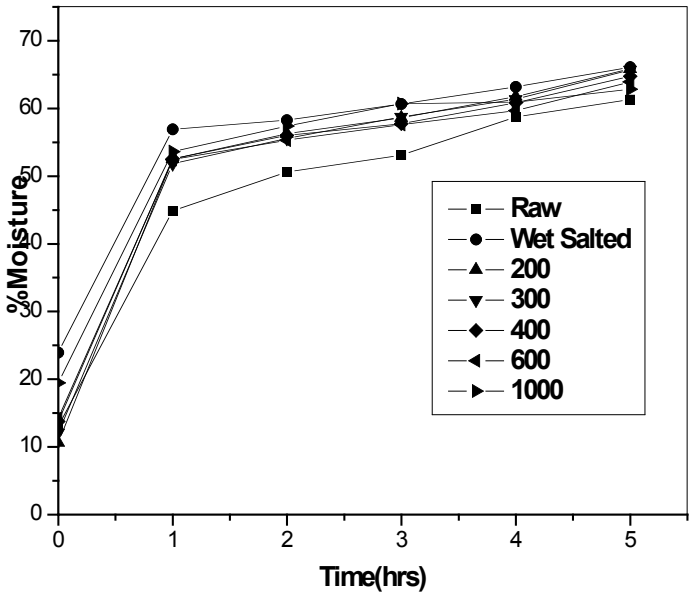

Figure 2. Rehydration profile of preserved skins

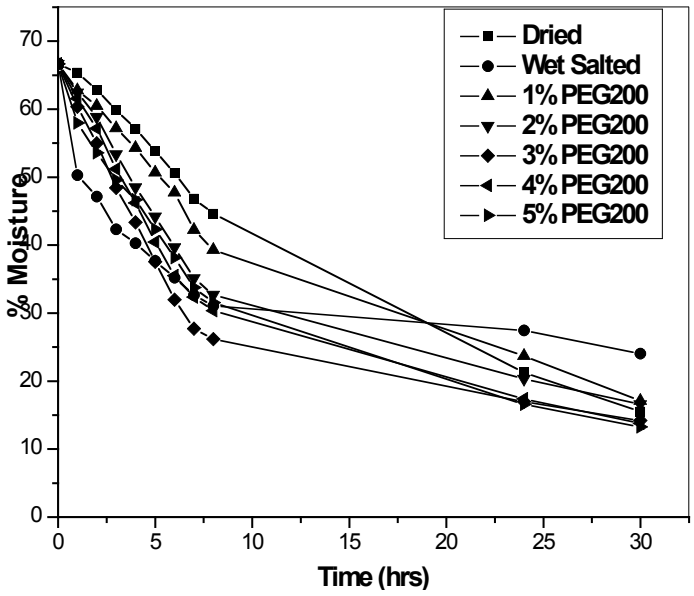

Figure 3. Dehydration profile for various % offer of PEG200

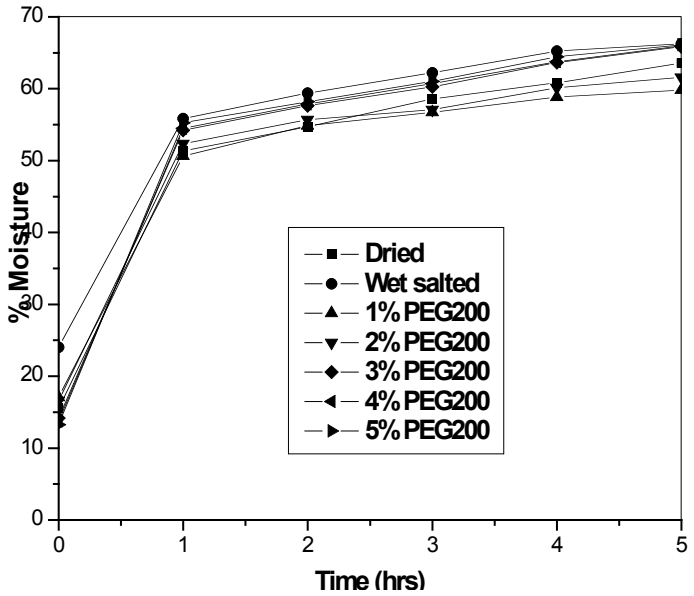

Figure 4. Rehydration profile for various % offer of PEG200

skins preserved by offer variation of 1, 2, 3, 4 and 5% of PEG200 is given in Fig. 3 and 4. From the graphs it is clear that the offer of PEG200 above 2% exhibited similar dehydration and rehydration rate compared to that of wet salted skins. During dehydration the required moisture content of 30% was obtained in 8 hours for an offer of 3% and above, whereas it took more than 8 hours for 1% and 2% offer. Also the rehydration rates of 1% and 2% offer were found to be insufficient. Hence, an offer of PEG200 at 3% has been optimized for bulk trials.

Efficiency of Preservation

Estimation of Hydroxyproline

Effectiveness of preservation process was assessed through the determination of loss of leather making protein during the storage of preserved skin samples for 12 weeks. It is known that estimation of hydroxyproline in spent soak liquor is used as a potential marker to identify the degradation of leather making protein. Hence, the estimation of hydroxyproline in the spent soak liquors was carried out for preserved skin samples at various storage periods and the results are given in Fig. 5.

The skin sample preserved by drying shows a significant degradation compared to sodium chloride and PEG200 based preservation. The hydroxyproline content at the end of 12 weeks was found to be 0.384 g/kg for dried skins. This is primarily due to the improper removal of moisture during the drying process, which results in the degradation of inner layer during storage. The hydroxyproline content of skin samples preserved by sodium chloride is 0.066 g/kg which is higher compared to 0.052 g/kg by PEG200 based preservation. This difference in hydroxyproline content between dried and PEG200 is primarily due to through and through removal of moisture content.

Determination of Bacterial Count

The curing efficacy of a curing agent depends mainly on the growth inhibitory activity of proteolytic bacterial species on the skin protein. The skin degradation can be indirectly measured by the presence of proteolytic bacteria present in the preserved goat skin during the incubation period. Fig. 6 shows the bacterial population of the dried, wet salted and PEG200 treated skin at different intervals. The PEG200 treated skins showed relatively lower bacterial count in comparison to the wet salted and dried skins. From the Fig. 6 it is clear that the log phase of bacterial growth for PEG200 treated skins is much less compared to that of the wet salted and dried skins.

Input and Output Analysis

The input and output of raw materials, chemicals and water were analyzed for both conventional and experimental preservation and soaking processes. The observed input and output values have been calculated for processing one ton of raw skins and are given in Table I. The conventional preservation method employs 400 kg salt to displace 270 kg water. However, the experimental preservation employs only 30 kg PEG to displace 450 kg of water. The experimental process provides a reduction in chemical input by 92.5%. The excess amount of salt used during preservation is removed before soaking through desalting operation. The desalting operation removes nearly 124 kg salt, which is a solid waste that causes a disposal problem.

Analysis of Soak Liquor

The spent soak liquors contain polluting matter and it contributes to high salinity in tannery wastewater. The emission loads of pollutants are given in Table II. Pollutant loads from spent soak liquor of experimental is lower compared to salt based preservation method. Generally, globular proteins

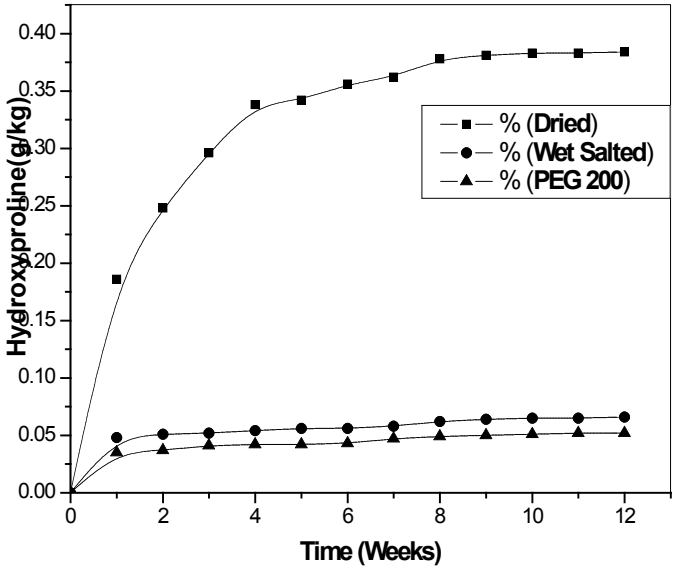

Figure 5. Effectiveness of preservation through hydroxyproline release

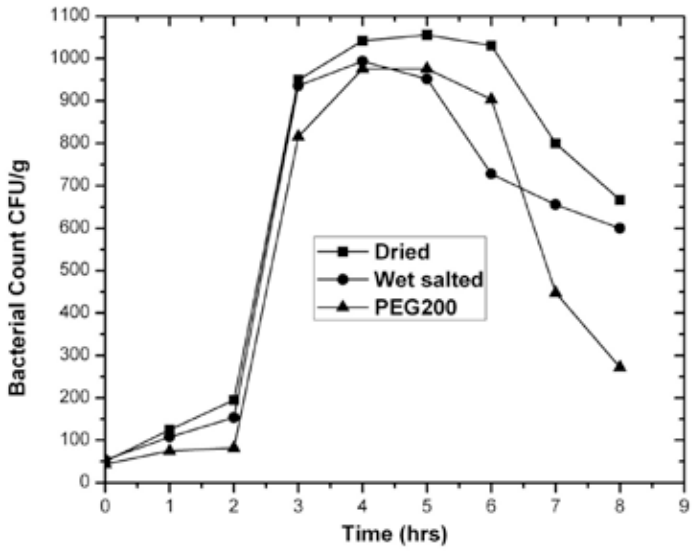

Figure 6. Effectiveness of preservation through bacterial count

TABLE I

Input-Output Analysis

Process	Chemicals/Raw Material	C		E	
		Input (kg)	Output (kg)	Input (kg)	Output (kg)
Preservation	Raw Goat Skins	1000	1150	1000	600
	Salt	400	—	—	—
	Water	—	270	—	450
	PEG 200	—	—	30	n.e
Desalting	Preserved Skins	1150	1030	—	—
	Salt	—	124	—	—
Soaking	Desalted Skins	1030	1050	600	1070
	Water	9000	8260	5400	4700
	Salt	—	264	—	—

n.e - not estimated

TABLE II

Emission Loads of Control and Experimental Spent Soak Liquors

Process	Emission loads (kg/metric ton of preserved skins)							
	BOD		COD		Cl^-		TDS	
	Wet salted	PEG200	Wet salted	PEG200	Wet salted	PEG	Wet salted	PEG
1st soak	2.88	1.23	12	8	99	0.4	180	9
2nd soak	2.52	0.61	9	7	44	0.5	78	6
3rd soak	2.32	0.44	8	4	5	0.3	10	4
Total	7.72	2.28	29	19	148	1.2	180	19

TABLE III

Physical Testing Data of Control (C) and Experimental (E) Crust Leathers

Sample	Tensile strength(Kg/cm^2)	Elongation at break (%)	Tear strength(Kg/cm)	Grain crack strength (average value)	
	Average value[a]	Average value[a]	Average value[a]	Load (Kg)	Distension(mm)
Wet salting	240±3	64±2	36±4	45±1	9.9±0.1
PEG200	264±5	67±4	46±4	38±2	10.2±0.3
BIS norms	200	40-65	30	20	7

[a] Average of mean of along and across backbone values

such as albumin and globulin are removed from the skins during the soaking of skins preserved using sodium chloride, due to its salt solubility nature. Hence, the presence of this matter increases the BOD and COD loads in the spent soak liquor of skins preserved by sodium chloride. In the case of PEG200 based preservation, the globular proteins are removed during the subsequent operations such as liming, reliming, bating and pickling. Salt based preservation contributes to nearly 148 and 268 kg of Cl^- and salinity, respectively for soaking one ton of preserved goat skins. This is primarily due to the removal of salt, which was used during the preservation process. The polymer based preservation contributes to 1.2 and 19 kg of Cl^- and salinity, respectively for soaking one ton of preserved goat skins. Hence, the PEG200 based preservation method reduces the BOD, COD, Cl^- and total dissolved solids(TDS) loads by 71, 34, 99 and 93%, respectively. The significant reduction in salinity and chloride loads helps in achieving cleaner and greener leather processing.

Composite Liquor Analysis

The composite liquors have been collected from control and experimental processes from soaking to post tanning and analyzed for their impact on environment. The calculated emission loads of the pollutants are given in Fig.7. There is no significant reduction in BOD and COD emission loads. However, the reductions in emission loads of Cl^- and total dissolved solids(TDS) are about 63 and 41% compared to conventional leather processing. It is evident that the developed preservation method reduces the salinity of water significantly, which is primarily due to the complete replacement of salt by PEG200.

Scanning Electron Microscopy Analysis

Fiber level change in the skin matrix due to preservation was assessed through scanning electron microscopy. Scanning electron micrographs of crust leather from wet salted and PEG200 based preservation showing the cross section at a magnification (200×) are given in Fig. 8a and b. The crust

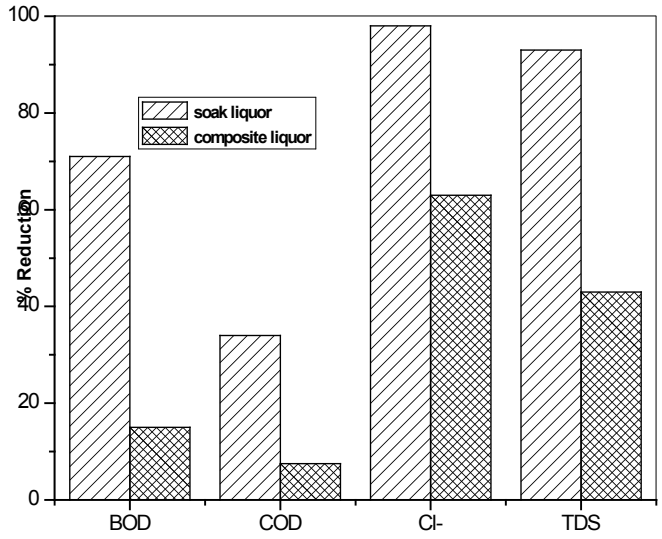

Figure 7. Reduction in pollution loads for soak and composite liquors

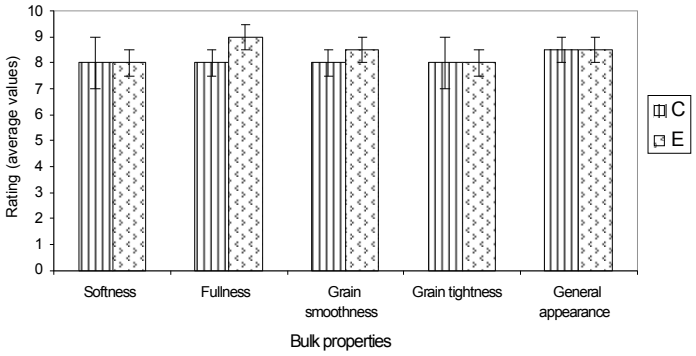

Figure 9. Organoleptic properties of control and experimental crust leathers

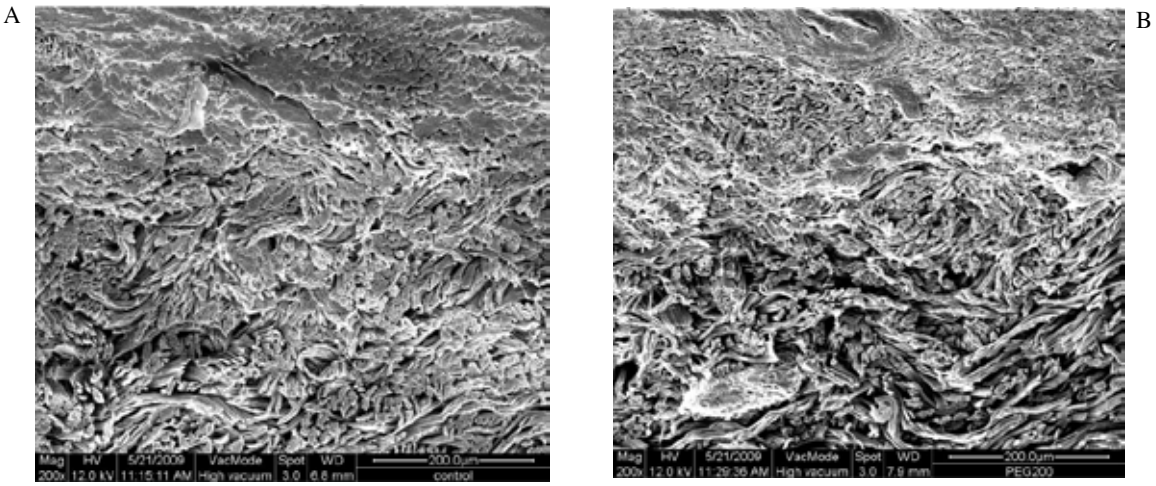

Figure 8. Scanning electron micrographs of crust leather showing the cross section at a magnification of 200× from a) Sodium chloride b) PEG200 preservation

leather preserved using PEG200 shows a uniform and clear organization of fiber bundles similar to that of wet salted preserved crust. The uniform orientation with well split fiber bundles observed in both samples clearly shows the crust of PEG200 has the same physico-mechanical changes during beam house operation as that of wet salted preserved crust.

Physical and Organoleptic Properties

The strength properties such as tensile tear and grain crack strength values were obtained by standard physical testing methods and are presented in Table III. It is seen that both control and experimental leathers exhibit comparable tensile, tear, grain crack and bursting strength values to that of Bureau of Indian Standards(BIS) norms (IS 576, 1989).

Control and experimental crust leathers were evaluated for various organoleptic properties by hand evaluation. The average of the rating for the five leathers corresponding to each experiment was calculated for each functional property and is given in Fig. 9. Higher numbers indicate better property. The experimental leathers exhibit better fullness compared to control leathers. Other properties such as softness, grain tightness and smoothness are comparable to that of conventionally processed leathers. In general, the appearance of experimental leathers is also similar to that of control leathers.

Cost Analysis

Preliminary cost-benefit analysis for the developed preservation method has been carried out as compared to conventional salt based preservation. This analysis is only an indicative of the developed methodology for economic feasibility. The utilization of salt and PEG per ton of raw goat skin is 24 and 42 $ respectively. But treatment cost applies for wet salted goat skin which is about 12 $ and labor cost is equal for both preservation method which is 4 $. The analysis data indicates marginal increase in the cost for PEG based preservation only by 6 $ compared to wet salted preservation. However, this methodology provides a cleaner option for preservation.

CONCLUSION

The development of a salt free curing system would go a long way in addressing the pollution problems faced by the leather industry. The PEG-water interaction is remarkable because a minimum of 2–3 water molecules per PEG monomer is required to complete basic hydration. This characteristic of PEG is utilized in the present work to completely replace salt in curing. The PEG with molecular weight of 200 at an offer of 3% has been standardized based on the dehydration and rehydration behavior of the preserved skins. Scanning electron microscopy study reveals that the skins preserved by PEG200 shows well separated fiber bundles with uniform fiber structure and also clean, smooth grain without any damage. The efficiency of PEG based preservation was found to be better than the wet salted method based on the results obtained from the estimation of hydroxyproline in soak liquor and also the determination of bacterial count in the soak liquor at various time intervals. Input-output analysis reveals that the PEG200 based preservation process reduces the chemical input by 92.5%. This method also reduces the BOD, COD, Cl^- and TDS loads in the soak liquor by 71, 34, 99 and 93% respectively. Thus the developed preservation process is a technically and economically viable alternative for salt based preservation.

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