Environmental Science and Pollution Research (2021) 28:12889–12897
https://doi.org/10.1007/s11356-020-11311-z

RESEARCH ARTICLE

Goatskin preservation with plant oil: significant chloride reduction in tannery wastewater

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Received: 2 March 2020 / Accepted: 18 October 2020 / Published online: 23 October 2020

Abstract

Preservation of raw hide/skin is the prime technique to stop bacterial deterioration. Universally, salt curing is the most prevalent technique for hide/skin preservation. In this study, extracted oil from *Aphanamixis polystachya* seed was evaluated for the preservation of goatskin to reduce the chloride in tannery wastewater. The oil-induced goatskin preservation method was assessed observing diverse factors, e.g., hair slip, odor, moisture content, bacterial colony counting, total Kjeldahl nitrogen, and hydrothermal stability in evaluation to the conventional salt curing method. Results indicate that 15% oil-induced preservation technique could preserve the goatskin for 30 days. A small-scale experiment was performed for the preservation of goatskins. The preserved goatskins both monitoring and study samples were processed for shoe upper leather. The leather quality was evaluated by examining physical properties and fiber structure by a scanning electron microscope (SEM). Pollution load generated during leather making was determined in terms of total dissolved solids (TDS), chloride (Cl^-), chemical oxygen demand (COD), and biochemical oxygen demand (BOD). The proposed oil-induced preservation method reduced Cl^-, TDS, and BOD by 98.3%, 82.3%, and 86.8%, respectively, in the soaking liquor. The leather readied from the empirical goatskin shows the equipotential properties of leather from conventional goatskin. The oil-induced preservation method is substantiated to be favorable nonconventional preservation instead of conventional wet-salting for the contraction of pollution from soaking operation.

Keywords *Aphanamixis polystachya* · Total dissolved solids · Salinity · Chemical oxygen demand · Leather

Introduction

Hide/skin, the outer covering of animals is the basic raw materials for the leather industry which is converted into leathers (Wu et al. 2017). Hide/skin contains water (60–70%) and protein (25–30%), which makes it susceptible to microbial attack (Balada et al. 2008). It is vulnerable to the microbial attack that starts in 5–6 h after being removed from the carcass (Kanagaraj et al. 2005). Saprophytic bacteria exist in the hide/skin or microbe from the environment decay proteins and yield in lower grade leather (Vijayalakshmi et al. 2009). Bacteria may penetrate through the corium from the flesh side of raw hide/skin in 8–12 h; it causes serious grain peeling in 15–18 h as well as creates voids in hide/skin (Aslan and Birbir 2011). The intact hide/skin protein is directly related to the quality of leather. Hence, preventing hide/skin protein degradation from the microbial attack in applying a suitable preservation method is essential before leather processing.

The protein breakdown of hide/skin can be stopped either instantly starting the tanning process or by preserving properly. Instantly, starting the tanning process is difficult for not having available tanneries in the outlying areas where maximum animals are slaughtered. Besides, it is impossible to tan a large number of collected hide/skin at the time of a special occasion, e.g., Eid-Ul-Azha (Muslim festival) immediately. Therefore, suitable curing is the best choice to keep the hide/skin safe until it reaches the tanneries. For a short time hide/skin preservation, sodium chloride (common salt) is used as a popular curing agent. In hot and humid countries (Bangladesh and India), 40–50% common salt is sprinkled on the flesh side

Responsible Editor: Philippe Garrigues

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to preserve the hide/skin which is termed as 'wet-salting' method (Vankar and Dwivedi 2009). The common salt takes moisture out from the hide/skin which prevents further growth of bacteria (Babu et al. 2009). Worldwide, common salt is used to hide/skin due to low cost, accessible, and easy to apply. However, from the environmental point of view, the application of common salt is a questionnaire. Covington (2011) reported that the wet-salting preservation technique adds more than 55% of chlorides and 40% total dissolved solids (TDS) in the tannery wastewater. Hence, controlling chloride (Cl^-) content is the main criteria to reduce pollution in the environment (Vankar and Dwivedi 2009) because till to date, there is no offered available technology for treating the wastewater comprising elevated concentration of common salt (Kanagaraj et al. 2005) which is considered as a burden to the environment. The high salt content in the effluent contributes to both surface salinity and reduction of soil's fertility with poor crop yielding (Preethi et al. 2006). Researchers have to take challenges to introduce environmental-friendly preservation of hide/skin.

In the last few decades, many chemical preserving agents are introduced to reduce salinity load from the effluent (Hashem et al. 2017). These methods are worthy, but in some cases, they are not environmentally-friendly and lack of cost-effective. Other physical methods, e.g., electric current (Birbir and Birbir 2006), chilling (Babu et al. 2012), and vacuum (Gudro et al. 2014), require high setup and maintenance cost but exhibit poor curing adeptness. Most recent phyto-based preservation methods are explored as an eco-friendly common salt alternative preserving agents for curing of hide/skin (Mohammad et al. 2016; Vijayalakshmi et al. 2009; Hashem et al. 2018; Tamilselvi et al. 2015; Vinodhkumar et al. 2016; Sivabalan and Jayanthi 2009). However, these curing agents reduce the moisture content from the hide/skin considerably which results in difficulties at the time of rehydrating before tanning. In this work, an investigation was performed to introduce completely new and inborn *Aphanamixis polystachya* plant seed oil that contains both preservation capability and availability even in the outlying region of Bangladesh. The *Aphanamixis polystachya* is a medicinal plant; extracted oil from the seeds has antibacterial and antifungal activity (Khare 2007). In Bangladesh, mostly, hide/skin is not preserved properly just after flaying in the remote area due to not having available tannery. As a result, valuable hide/skin gets downgraded or even repudiated for having defects.

In this present work, preservation of goatskin was performed with the extracted oil from *Aphanamixis polystachya* seeds. The obtained results of this investigation would be an approach to find out an economical and environmentally-friendly preserving agent in Bangladesh. After oil extraction, oil cake was used as an adsorbent to remove chromium from the wastewater.

Materials and methods

Preparation of goatskins

About 1.4 kg per skin weighted and immediately flayed goatskins were assembled from a local abattoir located at Fulbarigate, Khulna, Bangladesh, which was then quickly transferred to the leather manufacturing workshop for investigation. The skins were cleaned with water to separate adhered blood, grime, etc. After that, the cleaned skins were hung for 30 min to remove extra water.

Oil extraction

A traditional device known as Ghani was used to extract oil from the seeds of *Aphanamixis polystachya.* Generally, Ghani is used in the village to extract oil from the mustard, sesame, etc. With a timber structure, the device contains a container at the top. To produce oil from the seeds, the seeds need to pour into the container. After that, seeds are pressurized to extract oil from the seeds. Both mechanical or animal driven can be applied to run the Ghani. In Bangladesh, one bullock is placed to exert pressure in the Ghani and the eye of the Bullock is trapped with bamboo-made barrier to block their side vision. This is mainly done to keep the concentration of the animal in the moving direction. The produced oil is received through an iron lane which is again collected in a pot under the iron lane. From another lane, the oil cake (khail) is recovered. Recirculation of oil cake was repeated until completing extraction.

Reagents

Commercial grade common salt purchased from a local shop was used for this experiment. Reagents used for beamhouse, tanning, and post tanning processes: surface-active agent, bactericide, sodium sulfate, calcium hydroxide, sodium sulfide, ammonium muriate, diammonium sulfate, sulfuric acid, methanoic acid, sodium formate, sodium hydrogen carbonate, chromium hydroxide sulfate, vegetable tannin, synthetic tannin, and fungicide were applied to produce crust upper leathers which were collected from a tannery. To determine total Kjeldahl nitrogen, bacterial count, chloride (Cl^-), chemical oxygen demand (COD), and biochemical oxygen demand (BOD) in the wastewater, analytical grade reagents were used.

Preservation experiment

At first, 5%, 10%, 15%, and 20% extracted oil were applied during preliminary experiments for the quantification of oil required for the goatskin preservation. The four pieces were cut into 6″ × 6″ size from the butt portion of goatskin. Then, oil was employed on the flesh side of the skins following different

percentage (*w*/*w*). Physical characterization, e.g., odor and hair slip, was observed for 2 months periodically for the confirmation of good preservation as well as for the assessment of skins. According to the preliminary tested results, 15% (*w*/*w*) oil was optimum to preserve the goatskin. In calculation, the amount of required oil was determined which was correlated with the conventional preservation method. Then, one goatskin was divided in the direction of two towards the spinal column line to prevent confusion of variation on skin characterization. For monitoring sample, 50% sodium chloride was applied on the left half portion and 15% oil was applied on the right half portion as a study sample. The adaptability of the preservation method was examined on fresh (raw), 1st, 4th, 7th, 14th, 21st, and 30th days by analyzing total Kjeldahl nitrogen, bacterial count, shrinkage temperature, and moisture content for both monitoring and study samples.

Effectiveness monitoring of proposed method

Preparation of skin extract

Known mass of preserved skin pieces was kept into the sterile water with its ten times by volume, shaken well for 30 min at 200 rpm in an orbital shaker. Then, the extracted suspension was filtrated through the filter paper (Whatman No. 1). Finally, the permeate was used for analyzing total Kjeldahl nitrogen and bacterial count.

Determination of total Kjeldahl nitrogen

The obtained extract was dissolved using copper sulfate, sulfuric acid, and potassium sulfate in a Kjeldahl apparatus to check the effective digestion maintaining temperature 375–385 °C. Total Kjeldahl nitrogen was assessed by following the micro-Kjeldahl method as stated in the standard method of APHA (2012).

Determination of bacterial count

A total of 1 mL of extracted solution was added in 9 mL of desolated water and shaken well to produce a consistent solution containing bacteria. One Petri dish was readied with pouring melted agar-agar at 40 °C, and then, 0.1 mL was taken from the diluted uniform solution that was poured in the Petri dish. The Petri dish was shaken carefully for uniform dispersion of the solution. The Petri dish was kept for 48 h at 37 °C. By using a colony counter, the number of colonies was enumerated.

Determination of shrinkage temperature

Shrinkage temperature is the evaluation of hydrothermal stability. The shrinkage temperature (°C) was assessed by applying the samples in a shrinkage tester (SATRA STD 114, UK) as stated in ISO 3380:2015 standard (ISO 2015a, b).

Determination of moisture content

At different time interval, both monitoring and study sample moisture content of the preserved goatskin was determined according to the Bureau of Indian Standards (1971) method.

Leather preparation

Both monitoring and study samples had gone under the manufacturing process after completing 30 days of observation to prepare upper crust leathers following commonly practiced leather manufacturing process.

Pollution load generated in leather making

The soaking liquor of monitoring and study samples was characterized by determining Cl^-, TDS, BOD, and COD according to the standardized methods of APHA (2012).

Characterization of leather

Physical strength of leather

Leather prepared both from monitoring and study samples was compared by the physical strength characteristics of leather. Physical strength characteristics of leather determined through conditioning the prepared crust upper leathers at about 22 °C and 65 ± 2% of relative humidity for 48 h following ISO 2419:2012 standard (ISO 2012). Then, the test specimens were taken following sampling location according to ISO 2418:2002 standards for physical testing (ISO 2002). After that, different strength properties, for example, elongation at break, tensile strength, and bursting strength, were determined as per ISO 3376:2011 standard (ISO 2011) and ISO 3379:2015 standard (ISO 2015a, b). Triplicate measurement was conducted for all the experiments.

SEM analysis

Leather prepared both from monitoring and study samples was assessed by scanning electron microscope (SEM) analysis to compare the effect on grain as well as on the fiber structure of the final leather. After placing leather samples on conducting carbon tape, both monitoring and study leather samples were assessed by scanning electron microscope of the model (JEOL JSM-6490, USA). Images of the grain surface were collected by employing a scanning electron microscope at voltage 20 kV and 5000× magnification.

Small-scale experiment

After collecting the immediately flayed six pieces of goatskins from the slaughterhouse of Khulna, Bangladesh, a small-scale experiment was performed. Then, extracted oil was spread over the flesh side following the optimized amount 15% (*w*/*w*) and kept these samples for 30 days of periodical observation. Finally, water content (%), hydrothermal stability, organoleptic properties, hair slip, and odor were evaluated on fresh (raw) 1st, 4th, 7th, 14th, 21st, and 30th days.

Results and discussion

Optimization of oil concentration in preservation

Hide/skin is extremely vulnerable to microbial attacks because their main constituent is protein contributing about 30% of the weight of the hide/skin (Kanagaraj et al. 2005). The protein degradation process emits amino acid and further breaks down to ammonia (Vedaraman et al. 2016; Berber and Birbir 2010; Covington 2011). Therefore, the beginning of putrefaction is clear by the obnoxious smell of ammonia gas. On the other hand, hair follicles assist many kinds of bacteria to settle on the hide/skin effortlessly. Thus, bacteria exist in hairs first start attacking the nearby protein molecules. The earliest sign of putrefaction just when bacteria perform the degradation of the protein matters existing in the hair bulb in the early stage of putrefaction (Mohammed et al. 2016). Hence, odor emission and hair slip are considered as tangible parameters to judge the efficacy of the curing method because they indicate the onset of the putrefaction process.

The experimental results of the optimization of oil concentration in the preservation of goatskin are reported in Table 1. In this case, 15% (*w*/*w*) oil was considered as the optimum amount for preserving the goatskin as long as 30 days because there was no putrefaction odor and hair slip, indicating the absence of bacterial attack. The extracted oil from the seed of *Aphanamixis polystachya* has antibacterial activity (Khare 2007); therefore, there was no sign of putrefaction of the preserved goatskin.

Table 1 Optimization of oil concentration

S.N.	Oil applied (%)	Effectiveness of curing method	
		Putrefaction odor	Hair slip
01	5	Light odor	Light hair slip
02	10	Light odor	No hair slip
03	15	No odor	No hair slip
04	20	No odor	No hair slip

Effectiveness of proposed preservation method

Total Kjeldahl nitrogen content

Total Kjeldahl nitrogen content determination is a crucial part to evaluate the potency of the curing method. Bacteria degrade the skin proteins and generate comparatively low molecular weight nitrogenous compounds (Mohammed et al. 2016). Thus, the presence of extractable nitrogenous compounds in the hide/skin indicates the extent of putrefaction caused by microorganisms. The monitoring and study sample of the preserved goatskin is shown in Fig. 1 to compare the visual effectiveness of both methods.

In this study, the nitrogenous compounds released from the preserved goatskins (both study and monitoring) were extracted in water, and further, the total Kjeldahl nitrogen content of the aqueous solutions was determined. The occurrence of putrefaction imparts Kjeldahl nitrogen content in the obtained liquor. Figure 2 presents the total Kjeldahl nitrogen content for both the present study and monitoring (conventional) preservation methods.

The total Kjeldahl nitrogen in the study sample was represented underneath values. In the case of the monitoring sample, the amount of releasing nitrogen was moderately higher than the study sample after 1 week of preservation. The oil-induced preservation method stabilizes the polypeptide linkage; therefore, no putrefaction was observed (Babu et al. 2012). The outcomes disclosed that the lower value of total Kjeldahl nitrogen in the study sample was because of the oil-introduced, which obstructs the action of bacteria and stops putrefaction.

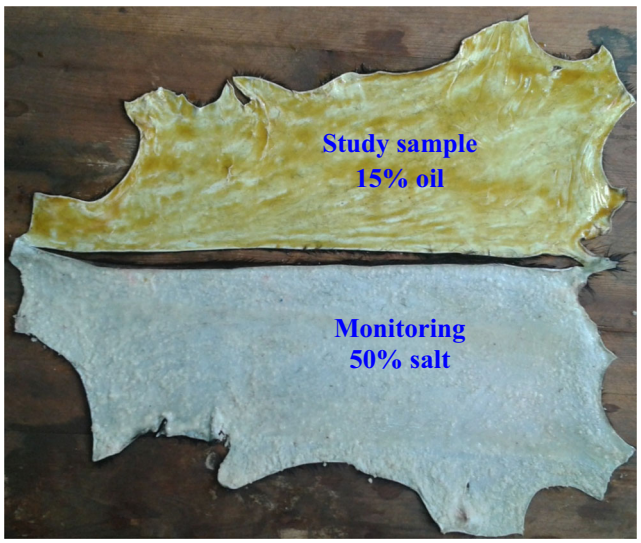

Fig. 1 Preservation with the present study (15% oil) and monitoring (salt 50%) methods

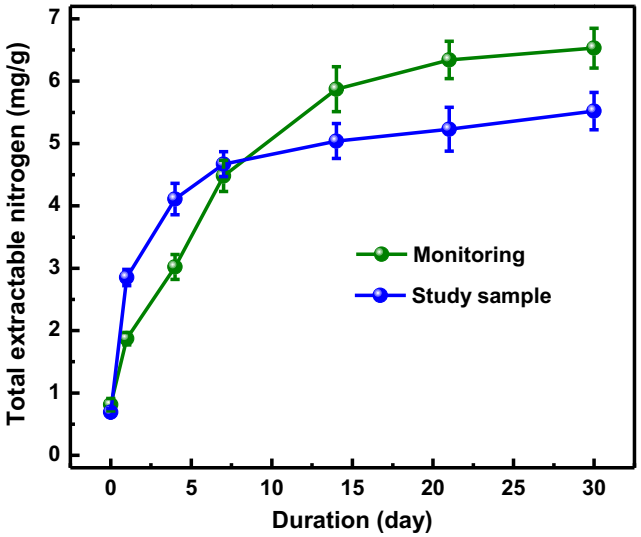

Fig. 2 Total extractable nitrogen content of preserved monitoring and study goat skin samples

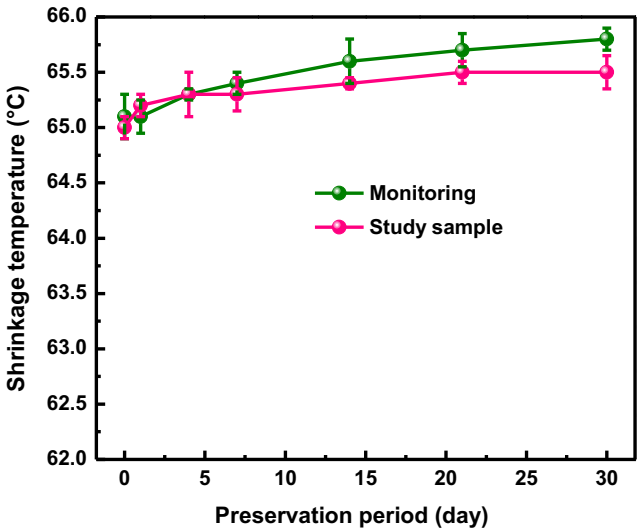

Fig. 3 Shrinkage temperature of preserved monitoring and study goatskin samples

Hydrothermal stability

Degradation of skin collagen leads to putrefaction. Hydrothermal stability is one of the most important parameters for assessing the quality of raw hide/skin, which indicates the structural stability of the hide/skin (Iyappan et al. 2013; Ramesh et al. 2009). The main objective of estimating shrinkage temperature was to know whether the proposed preservation method had any sign of deteriorating on the collagen matrix of the preserved skin. It also indicates whether the curing agent acts as a tanning agent.

The value of shrinkage temperature of hide/skin decreases when structural deterioration occurs and increases when tanning occurs. The obtained shrinkage temperature of both the samples at a different time interval is shown in Fig. 3. The outcomes represent that there was no outstanding transformation in the shrinkage temperature of monitoring and study samples. Hence, no deterioration and tanning have taken place in the study sample while preserving with the oil.

Bacterial colony counting

The potency of the preservation methods is indicated by the obstructive characteristics of the curing agents on bacteria. The higher the growth of bacteria intensifies the putrefaction of the goatskin during preservation. The deterioration of the skin is implied by the number of bacterial colony presence on the skin at the time of preservation. Table 2 illustrates the number of bacteria colony count present in the monitoring and study samples on fresh (raw) 1st, 4th, 7th, 14th, 21st, and 30th days.

The outcomes disclosed that the number of bacteria existing in the study sample is relatively lower than the monitoring sample both at earlier and later stages. Consequently, the antibacterial property of oil is affirmed by the outcome of this investigation.

Moisture content

In preservation, the moisture content is one of the most significant aspects to assess the efficiency of the methods. Figure 4 depicts the estimated moisture contents of both monitoring (50% salt) and study sample (15% oil) at different time intervals.

The results represent that the moisture content of the monitoring and study sample was lessened to about 19.3% and 50.0%, respectively, on the 4th days. On 30th days, moisture content was in the monitoring and study samples by 34.5% and 13.2%, respectively. The decrease in moisture content was an indication of absorbing moisture from the study sample by the oil-induced preservation. Lower moisture content in the preserved goatskin has two benefits: less transport cost and higher the antibacterial activity.

Table 2 Bacterial count of preserved monitoring and study goat skin samples

Duration (day)	Bacterial count (CFU/g)	
	Monitoring	Study
Fresh	3.2×10^3	2.5×10^3
1	8.4×10^9	6.1×10^7
4	3.9×10^9	1.7×10^7
7	2.1×10^7	5.3×10^6
14	4.6×10^6	2.9×10^5
21	1.7×10^6	4.0×10^4
30	5.1×10^5	1.9×10^4

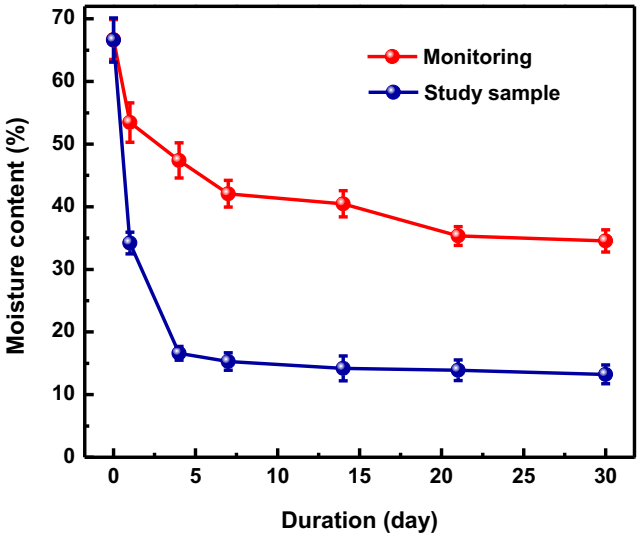

Fig. 4 Moisture content of preserved monitoring and study goatskin samples

Pollution load generated in leather making

The wastewater obtained from the soaking of the preserved monitoring and study samples was characterized to determine the pollution load contributed by both preservation methods that is shown in Table 3.

The Cl^-, TDS, BOD, and COD in the soaking liquor were significantly lessened in the case of oil-induced preservation in the place of common salt (sodium chloride). However, the amount of BOD was slightly increased in the soaking liquor of the study sample in comparison with monitoring. The extracted oil might contain organic substances which lead to increase BOD value in the soaking liquor. After all, this oil-induced preservation method was reduced in Cl^-, TDS, and COD severally by 98.4%, 82.3%, and 86.8% in the effluent of soaking liquor comparing with the monitoring (salting) method.

Properties of manufactured leather

Physical strength

The physical strength characteristic of leather is usually determined to predict its performance during service (Covington 2011). The results of different physical strength characteristics obtained from the testing for both monitoring and study leather samples are tabulated in Table 4. No remarkable difference in the physical characteristics in the leathers manufactured from the monitoring and study samples was observed.

Moreover, the outcomes meet the standard requirement of physical characteristics, e.g., tensile strength, elongation at break, distension at grain crack, and load at grain crack. After considering the outcomes of physical characteristics, it can be concluded that the oil-induced preservation is effective and also keeps the skin structure intact.

Scanning electron microscope analysis

The cross-sectional SEM photographs of the upper leathers manufactured from the monitoring and study samples are shown in Fig. 5. From SEM analysis, it is clear that the leather processed from the goatskin preserved by using oil (15%) did not represent any destruction and enough finer in comparison with the leather processed from the goatskin preserved by using salt (50%). The outcomes of SEM analysis represent that the proposed curing agent did not influence the texture of the final leather.

Small-scale experiment

Moisture content of small-scale experiment

During the preservation period of 30 days, the percentage of moisture content of the small-scale experimental sample (15% oil) is represented in Fig. 6. The outcomes of this experiment did not represent any notable differences of the moisture content from the skin to skin of the small-scale experiment. The moisture content was nearly stable for every skin sample from the 14th to the 30th day. There was no skin deterioration like hair slip, odor, etc.

Hydrothermal stability of small-scale experiment

During 30 days of preservation, the hydrothermal stability of the small-scale experimental sample (15% oil) is represented in Fig. 7. The outcomes of this test did not represent any significant differences in the shrinkage temperatures of the small-scale experiment.

Table 3 Pollution load generated in soaking operation of preserved goatskins

Parameters	Monitoring sample	Study sample	Unit	Pollution reduction (%)
Cl^-	18.2 ± 0.2	0.3 ± 0.01	g/L	98.4
TDS	42.3 ± 0.5	7.5 ± 0.1	g/L	82.3
BOD	1.3 ± 0.02	1.4 ± 0.03	g/L	-
COD	5.3 ± 0.6	0.7 ± 0.04	g/L	86.8

Table 4 Physical characteristics of monitoring and study leathers and comparison with the requirement

Parameters	Sample ID		Requirement (Kanagaraj et al. 2001)
	Monitoring	Study	
Tensile strength (kg/cm^2)	245.1 ± 1.2	259.8 ± 1.1	200
Elongation at break (%)	39.8 ± 0.5	39.4 ± 0.5	40–65
Bursting strength:			
Distension at grain crack (mm)	7.9 ± 0.1	7.8 ± 0.2	7
Load at grain crack (kg)	43.3 ± 0.6	40.0 ± 0.6	20

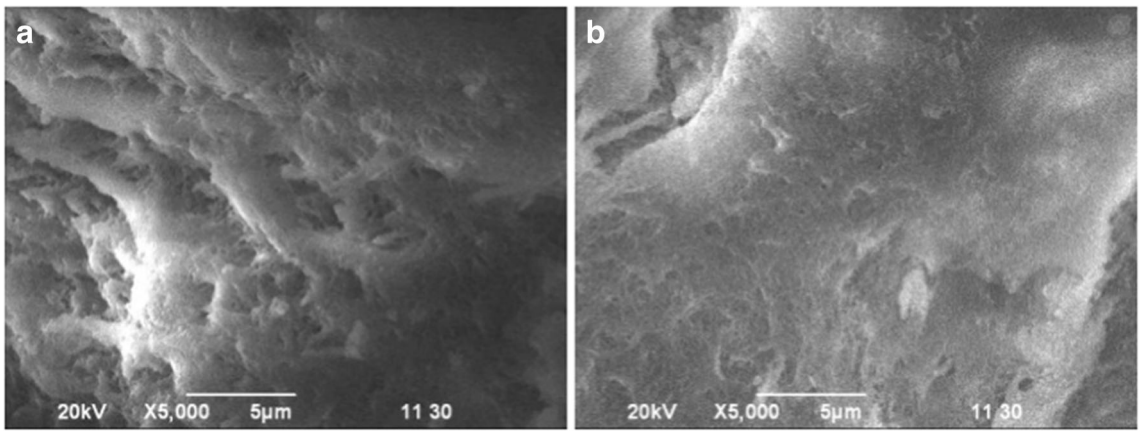

Fig. 5 SEM photographs of manufactured **a** monitoring and **b** study leathers

Physical properties of small-scale experimented leather

Table 5 depicts the results of different physical characteristics obtained from the testing of small-scale experimental processed leathers. The outcomes of the physical characteristics of the upper leathers meet the standard prerequisite. For example, tensile strength was 206–228 kg/cm^2 which was above the standard value (200 kg/cm^2). Likewise, elongation at break, distension at grain crack, and load at grain crack values were within the standard values.

Conclusion

The novel salt-free goatskin preservation system following oil-induced design could be a cleaner alternative conventional

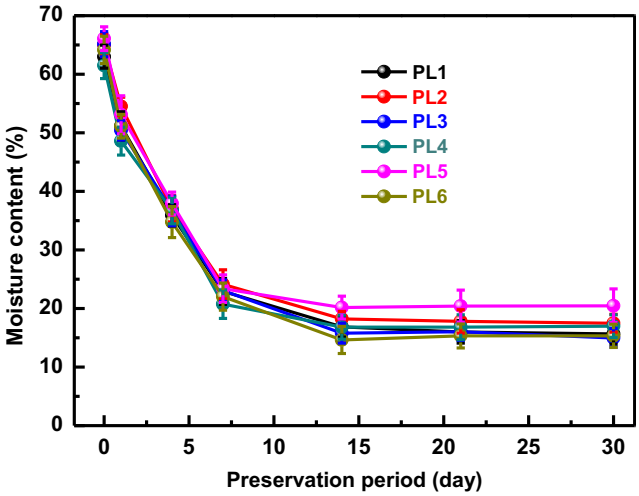

Fig. 6 Moisture content of the small-scale experiment with 15% oil

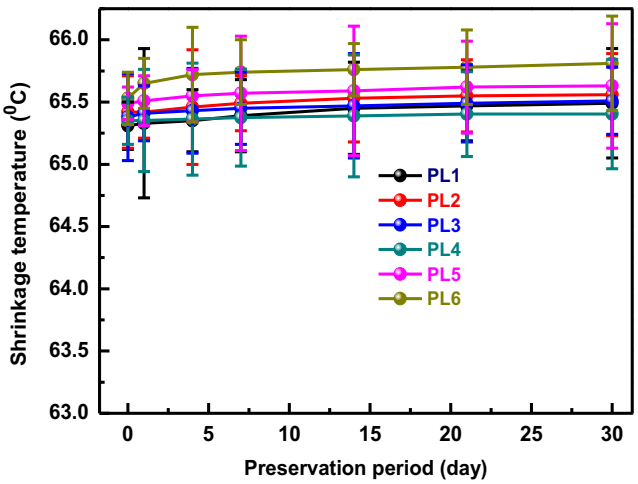

Fig. 7 Shrinkage temperature of small-scale experiment with 15% oil

Table 5 Physical characteristics of the processed small-scale experimented leather

Parameters	PL1	PL2	PL3	PL4	PL5	PL6	Requirement (Kanagaraj et al. 2001)
Tensile strength (kg/cm^2)	220	227	206	215	211	228	200
Elongation at break (%)	45	49	43	46	44	47	40–65
Bursting strength							
Distension at grain crack (mm)	7.9	8.2	8.5	8.6	8.4	8.8	7.0
Load at grain crack (kg)	28.7	30.8	28.6	27.4	30.2	30.3	20

salting method. The preserved goatskin by applying extracted oil from the *Aphanamixis polystachya* seeds was subject to a series of examinations. Formulation of only 15% oil could preserve the goatskin for 30 days. The outcomes of this study demonstrate that extracted oil could expediently be used for the preservation of goatskin. The extractable nitrogen, shrinkage temperature, bacterial count, and moisture content study revealed that the oil is efficient in preserving the goatskin. The method was reduced in Cl^-, TDS, and COD by 98.4%, 82.3%, and 86.8%, respectively, from the effluent of soaking liquor comparing with the monitoring (salting) method. Moreover, no remarkable difference in the physical strength characteristics in the leathers manufactured from the monitoring and study samples was observed. The scanning electron microscopy indicated that the oil does not affect the fiber structure quality of goatskins. Overall, abundantly available, even in remote areas, inexpensive oil might be a green curing choice in the place of the conventional method. In the future, the process could be commercially implemented.

Acknowledgments Authors wish to thank the related personnel for providing all facilities to conduct this research at the Department of Leather Engineering, Khulna University of Engineering & Technology (KUET), Khulna 9203, Bangladesh. Authors also want to extend thanks to Mr. Md. Aminur Rahman, Senior Chemist, Department of Public Health Engineering, Zonal Laboratory, Khulna 9100, Bangladesh.

Authors' contributions Md. Shahruk Nur-A-Tomal: visualization/conceptualization, investigation, methodology, writing-review, and editing. Md. Abul Hashem: investigation, methodology, supervision, data curation, writing-original draft, writing-review, and editing. Md. Enamul Hasan Zahin, Md. Lutful Hossain Pulok, and Moumita Rani Das: sampling and data collection. Sadia Mim: review and editing. All authors read and approved the final manuscript.

Funding This research was financially supported by the International Union of Leather Technologists and Chemists Societies (IULTCS) through the Young Leather Scientist Grant 2016.

Data availability The datasets generated and analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The authors declare that the submitted manuscript is original. They also acknowledge that the current research has been conducted ethically and the final shape of the research has been agreed by all authors. They declared that this manuscript does not involve researching about humans or animals.

Consent to participate The authors consent to participate in this research study.

Consent to publish The authors consent to publish the current research in ESPR journal.

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