2016 IULTCS/IUR – Young Leather Scientist Grant

A novel method to preserve goat skin with indigenous plant extract to reduce chloride load in the effluent

Md. Shahruk Nur-A-Tomal

Department of Leather Engineering, Khulna University of Engineering & Technology (KUET), Khulna-9203, Bangladesh

1. INTRODUCTION

Hides/skins, the outer coverings of animals, are used as the basic raw material for the tanning industry. After flaying hides/skins are susceptible to bacterial attack which starts within 5–6 h after the animal death.[1] Bacteria either in native or derive from the air or soil, putrefy the proteins and makes hides/skins inapt for the production of quality leather.[2] Animal death causes a dramatic metabolic change in hides/skins due to not supplying oxygen and nutritional components. As a result, accumulation of toxic substance leads to cause inactivation of some coenzymes; autolysis starts to decompose the protein to peptide and finally amino acids. Autolysis products are further broken down through the secondary process by the action of putrefactive bacteria.[3]

To stop the decomposition of hides/skins protein after flaying two possible options are available i) instantly start tanning process and ii) properly preservation. The first option is not possible and even in some cases impossible because a lot of hide/skin are slaughtered in the remote where there is no tanning facility as well as a large number of hide/skin is collected in the especial occasion for example during Eid-Ul-Azha (Muslim festival) period, which cannot be processed at the collection time. Therefore, proper preservation is the best option to save the hide/skin before transport to the tanning industry.

In tropical countries like Bangladesh and India, conventionally fresh hides/skins are preserved following wet-salting method where 40–50% common salt (sodium chloride) is applied immediately after flaying.[4] The dual actions of sodium chloride: i) dehydrating and ii) bacteriostatic properties are being exploited in this preservation method.[5]

Although sodium chloride is cost effective, available, and easy to practice hide/skin, the method suffers heavily from the environmental perspective. It is reported that only after soaking 70% total dissolved solids (TDS) of the entire leather processing is released in the effluent as chloride ion.[6] Processing of one ton conventional wet-salted hides/skins contributes

about 350-450 kg of salt as TDS in the wastewater. Chlorides remain a burden to the environment as it is not affected by the effluent treatment because of its high solubility. The high amount of salt contained in the effluent will increase surface salinity, thus reducing the fertility of soil resulting in the poor yield of crops.[7] An eco-friendly preservation of raw hides/skins has become a great challenge for the researchers and scientists.

In the last few decades, numerous works have been carried out with various curing agents efficiently to preserve the hides/skins to reduce salinity load from the final effluent. A great deal of research has done to preserve hides/skins chemically, which generate secondary contaminants that need to be treated. Some physical preservation methods e.g. electron beam irradiation, gamma radiation were developed. But, the developed techniques is facing challenges because of high establishing cost, high operating cost, as well as low preserving efficiency. To overcome these problems some plant based preservation have been investigated to preserve hides/skins.[2,6,7,8,9,10] Unfortunately, they are not commercially accepted because of their limitations.

An investigation was carried out to use indigenous plant extract which has the preserving potentiality and available even in the remote area of Bangladesh. One of the most common negative images for Bangladesh is not to preserve the hides/skins properly after flaying in the remote area. In the long run, many hides/skins are putrefied causing lots of materials are down graded or even rejected due to their defects.

In the present study, goat skins were preserved with the extracted oil from the seed of *Aphanamixis polystachya*. The outcome of this research would be very effective for the environment as well as the economy.

2. MATERIALS AND METHODS

2.1 Goat skin collection

Freshly flayed goat skins of average weight 1.4 kg per skin were collected from a local slaughterhouse located at Khulna, Bangladesh and immediately transported to the laboratory for experimentation. The collected skins were firstly washed with water to remove adhering blood, dung, and dirt etc. The washed skins were hanged for 30 min to drain extra water.

2.2 Extraction of oil

The seeds of *Aphanamixis polystachya* were collected from Sirajganj, Bangladesh which is abundantly available. The collected seeds were washed with water and dried well under shade at room temperature. The dried seeds were grinded to fine powders. The powders were subjected to heating with water at water bath for several hours. After clearly separated oil from the seed, the mixture was then transferred to the separating funnel and finally, oil was separated from the aqueous phase. The residual water in oil was then distilled off.

2.3 Chemicals

Sodium chloride (commercial grade) used for the preservation experiments was purchased from local market. Surfactant, bactericide, sodium sulfate, lime, sodium sulfide, ammonium chloride, ammonium sulfate, formic acid, sulphuric acid, sodium bicarbonate, sodium formate, basic chromium sulfate, vegetable tannin, syntan and fungicide which were used for pre-tanning, tanning, and post-tanning operations to manufacture crust upper leathers were procured from a tannery. Analytical grade chemicals were used for determination of total extractable nitrogen, bacterial count, chloride (Cl^-), biological oxygen demand (BOD), and chemical oxygen demand (COD).

2.4 Preservation experiments

Preliminary experiments were conducted with 5%, 10%, 15%, and 20% oil only to know the minimum quantity of oil required for skin preservation. Four (04) samples of size 6 inch × 6 inch were cut from the butt portion of a freshly flayed goat skin. The different percentages (w/w) of oil were applied on the flesh side of the skins. Skins were assessed periodically for two months by observing physical changes like odour, and hair slip for confirmation of good preservation. The optimum concentration of oil for preservation was found to be 15% (w/w). After assuming the required amount of oil in the proposed preservation method; it was compared with the conventional wet salting preservation method. A freshly flayed goat skin was cut into halves along the backbone to avoid skin to skin variation. The left half was taken for the control sample (50% sodium chloride) and the right half was used as the experimental sample (15% oil). Each experiment was repeated for three times. The efficacy of the preservation method was periodically (after 1^{st}, 4^{th}, 7^{th}, 14^{th}, 21^{st}, and 30^{th} days of preservation) assessed by analysis of the preserved skin pieces for total extractable nitrogen, bacterial count, hydrothermal stability, and moisture content.

2.5 Effectiveness observation of the proposed method

2.5.1 Preparation of skin extract

The preserved skin pieces of known mass were kept in sterile water (ten times by volume of skin mass), shaken well in an orbital shaker at 200 rpm for 30 min. The extract liquor was filtered through a filter paper (Whatman No. 1); the filtrate was further used for nitrogenous and bacterial analysis.

2.5.2 Determination of total extractable nitrogen content

The extract liquor was digested using sulphuric acid, potassium sulfate, and copper sulfate in a Kjeldahl flask providing the temperature 375-385°C for the effective digestion. The nitrogen

content was determined by the Micro-Kjeldahl method according to the standard method of APHA.[11] Each experiment was conducted in triplicate.

2.5.3 Determination of bacterial count

A volume of 1 mL extract liquor was taken in 9 mL of sterile water into a vial and shaken well to make uniform suspension of bacteria. After that, 0.1 mL of the corresponding diluted solution was poured in a sterile Petri plate and molten nutrient agar at 40°C was poured. The Petri plate was shaken gently to get uniform distribution of the bacteria. The Petri plate was incubated at 37°C for 48 h. The number of colonies on the agar media was counted using colony counter. Each experiment was conducted in triplicate.

2.5.4 Determination of hydrothermal stability of the skin

The hydrothermal stability of the skin is typically evaluated by shrinkage temperature. The shrinkage temperature (°C) of the preserved skin was determined using a shrinkage tester (SATRA STD 114, UK) according to the ISO 3380:2015[12] standard. Each experiment was conducted in triplicate.

2.5.5 Determination of moisture content

The moisture content of the skins was determined by following Bureau of Indian Standards.[13] About 5 g persevered skin samples were cut and weighed. The samples were dried in a drying oven at 50-60°C temperature for 5-6 h. The dried samples were cooled in a desiccator. The weight loss was calculated. Each experiment was conducted in triplicate.

2.6 Leather making

After 30 days of preservation, both the control and experimental goat skins were processed to manufacture upper crust leathers as per conventional leather making procedures.

2.7 Pollution load generated in leather making

Pollution load generated in the soaking operation during leather processing was determined. The wastewater samples were collected from the control and experimental soaking operation during leather manufacturing and analyzed for chlorides (Cl^-), total dissolved solids (TDS), biochemical oxygen demand (BOD), and chemical oxygen demand (COD) following the standard methods of APHA.[11] Each experiment was conducted in triplicate.

2.8 Properties of manufactured leather

2.8.1 Physical properties of leather

The manufactured leather samples were tested for their physical characteristics to compare proposed oil preserved leather with conventional wet-salted preserved leather. After conditioning the crust leathers at 20 ± 2°C and 65 ± 2 % of relative humidity over a period of 48 h according to ISO 2419:2012[14] standard, the samples were taken from specified sampling location as per ISO 2418:2002[15] standard for physical testing. The properties such as tensile strength, elongation at break, and bursting strength were assessed following ISO 3376:2011[16] and ISO 3379:2015[17] standard. Each experiment was conducted in triplicate.

2.8.2 Scanning electron microscope (SEM) Analysis

The manufactured control and experimental crust leathers were subjected to assess the effect of proposed preservation method on fiber structure of leather. Firstly, leather samples from the same area have placed on conducting carbon tape. After preparing, the samples were analyzed by a SEM (JEOL JSM-6490, USA). The photographs of the grain surface were obtained by operating the SEM at an accelerating voltage 20kV with magnification 5000X.

3. RESULTS AND DISCUSSION

3.1 Optimization of oil concentration for preservation

The hides/skin is composed of proteinaceous substances which are susceptible to microbial attract. Proteinaceous substances are hydrolyzed to amino acids by proteolytic enzymes produced from bacteria; bacteria further hydrolyze the amino acids and liberate ammonia gas. Therefore, the odour of ammonia gas is considered as the initiation of putrefaction. Hair follicles on the hide/skin are fairly appropriate structures for many species of bacteria to easily colonize. Therefore, hair slip is the first indication of putrefaction as the protein present in the hair bulb is degraded by the bacteria during the beginning of putrefaction. Hence, putrefaction odour and hair slip were monitored as the physical evaluation to determine the effectiveness of preservation method.

Table 1 Optimization of oil concentration

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

The standardization of optimum concentration of oil for the preservation of goat skin is depicted in Table 1. It seems from results that dose of 15% oil was found to be effective in

preservation the skin for two months. There was no putrefactive odour or hair slip observed indicating no putrefaction of the goat skin.

3.2 Effectiveness of the proposed preservation method

3.2.1 Total extractable nitrogen content

Total extractable nitrogen is a vital factor for assessment the effectiveness of preserved skins. The putrefaction of skin proteins results the release of nitrogenous components which leads the emission of putrefaction odour and hair slip. The putrefaction was measured by the amount of nitrogen extracted in water. Fig. 1 shows the total extractable nitrogen content in the preserved goat skins. It is clearly seen that the putrefaction contributes extractable nitrogen in the extract liquor. The release of total extractable nitrogen in the experimental skin showed lower values. The control experiment showed slightly higher total extractable nitrogen content after 7 days of preservation. The results reveal that the decrease in total extractable nitrogen content in the experimental skin is due to the oil is strongly responsible for impeding the bacteria from putrefaction.

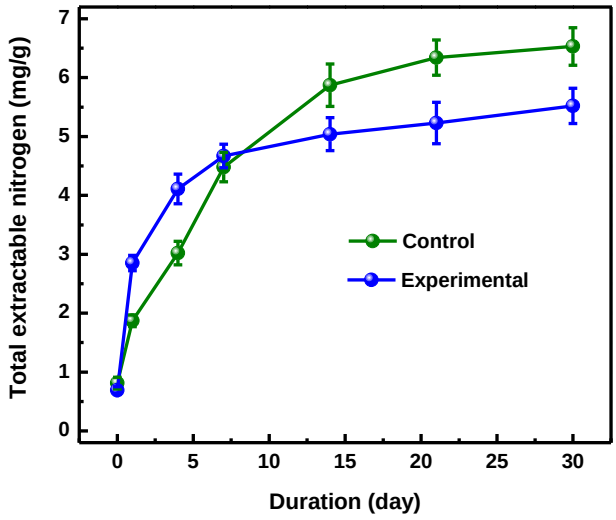

Figure 1 Total extractable nitrogen of the preserved control and experimental goat skins

3.2.2 Bacterial count

The bacterial count in preserved skins was performed to determine the number of bacteria present in the skins. The effectiveness of preservation principally depends on the development of inhibitory properties for bacteria on the skin. The bacterial count reveals the degradation of skin i.e. the presence of bacteria on the skin during preservation. The bacterial count of the control skin and experimental skin at different intervals are inserted in Table 2. The experimental skin exhibited relatively lower bacterial count in comparison to the control skin.

The results disclose that the oil starts preservation skin not only at the initial stage but also at later stages. This clearly validates the antibacterial property of oil.

Table 2 Bacterial count of the preserved control and experimental goat skins

Duration (day)	Bacterial count (CFU/g)	
	Control	Experimental
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}

3.2.3 Hydrothermal stability

The hydrothermal stability of collagen is considered as a significant property for the assessment of the hides/skins quality because it shows indirectly any structural deterioration of the hides/skins protein. The purpose of this parameter of the study was to know whether the proposed preservation method had any effect on the deterioration of collagen matrix. The shrinkage temperature of preserved skins in different periods is represented in Fig. 2.

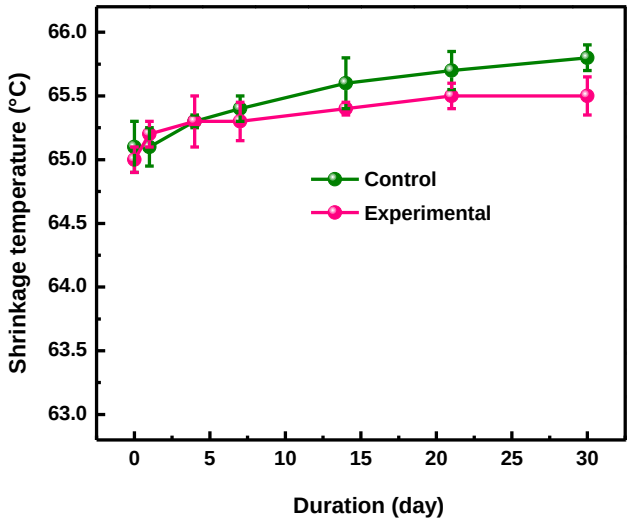

Figure 2 Shrinkage temperature of the preserved control and experimental goat skins

No remarkable change in the shrinkage temperature of control and experimental skins was observed. It could be concluded that the preserved skins were not deteriorated during preservation.

3.2.4 Moisture content

The moisture content of preserved skin is important to assess the effectiveness of preservation method. The moisture content of the preserved control and experimental skins at different time intervals are depicted in Fig. 3. The moisture content of control and experimental skins were reduced 19.3% and 50.0% respectively after 4 days. The moisture content was reduced to 34.5% and 13.2% respectively for control and experimental skins at the end of 30 days. The greater reduction in moisture content in the experimental skin could be due to the hygroscopic properties of the oil.

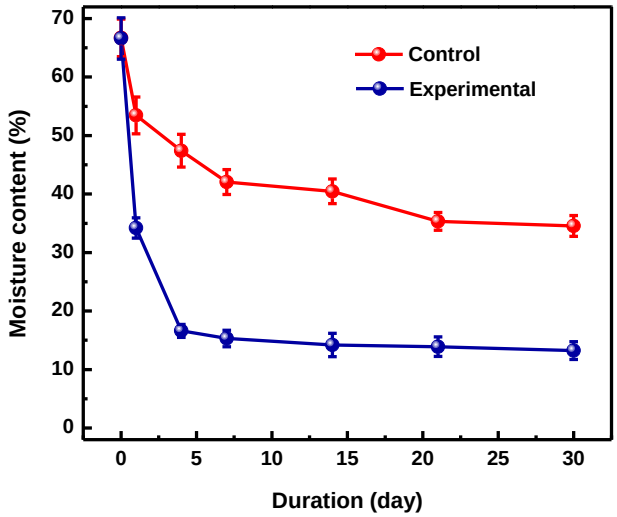

Figure 3 Moisture content of the preserved control and experimental goat skins

3.3 Pollution load generated from leather making

The analysis of pollution load generated in the soaking operation of preserved goat skins for both control and experimental samples were represented in Table 3.

Table 3 Pollution load generated in soaking of the preserved control and experimental goat skins

Sample	Cl^- (g/L)	TDS (g/L)	BOD (g/L)	COD (g/L)
Control	18.2 ± 0.2	42.3 ± 0.5	1.3 ± 0.02	5.3 ± 0.6
Experimental	0.3 ±0.01	7.5 ± 0.1	1.4 ± 0.03	0.7 ± 0.04

The table depicts that the Cl^-, TDS, and COD load were greatly reduced when the oil was used in preservation in place of the control salt. Even though there was little increase in the BOD and levels in the experimental soaking wastewater compared to the control. The main pollution problem of the leather manufacturing: chloride and TDS were reduced 97.8% and 82.3% respectively in the experimental soaking wastewater.

3.4 Properties of manufactured leather

3.4.1 Physical strength

The physical properties e.g., tensile strength, elongation at break, distension at grain crack, and load at grain crack of crust leathers which were obtained by processing of preserved skins are represented in Table 4. The results disclose that there is no significant variation in physical properties between control and experimental leather samples. The values fulfilled minimum physical properties. The result of physical properties clears the effective preservation by oil.

Table 4 Physical properties of the control and experimental crust leathers

Parameters	Control	Experimental	Minimum Requirements[18]
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

3.4.2 SEM Analysis

SEM photographs of the leather processed from salt and oil preserved goat skin are depicted in Fig. 4. SEM analysis exhibited that the leather manufactured from the oil preserved goat skin did not depict any deterioration and was much better to the goat skin preserved with salt. This shows that proposed preservation did not affect the texture and quality of the goat skin.

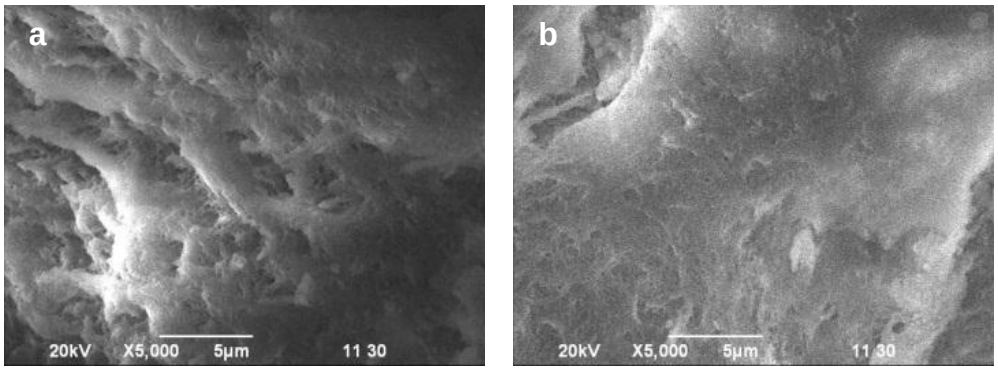

Figure 4 SEM photographs of manufactured (a) control and (b) experimental leathers

4. CONCLUSION

Commercially feasible salt-free preservation development has become a great challenge in recent years to save the environment from pollution. To response, an investigation was made to preserve goatskin using indigenous plant oil. The oil is capable of preserving goat skins for more than a month. The results from experiments indicate that the oil is an effective and eco-

friendly curing agent. The present study averts the limitations: cost, and no availability in remote areas. The novel preservation method based on indigenous plant oil could be a cleaner preservation choice to the conventional wet salting preservation method.

5. SUGGESTION FOR FUTURE WORK

The research work revealed that indigenous plant extract has great potentiality to preserve raw goat skin. The proposed preservation method could be applied to another hides/skins e.g., cow, buffalo, sheep etc. The process could be commercially implemented.

Moreover, I believe there are more indigenous plants available abundantly having preservation potentiality. Numerous research works could be made in this research area.

6. ACKNOWLEDGEMENTS

I would like to acknowledge the financial support by the IULTCS/IUR through the Young Leather Scientist Grant 2016 to carry out the research project. I wish to give special thanks and appreciation to Dr. Md. Abul Hashem, my advisor, for the input of all his knowledge, experiences, time and patience throughout the research. His constant supervision, constructive criticism, valuable advice, scholarly guidance and encouragement at all stages of this research have made it possible to complete this research work. I wish to thanks the related personnel for providing all facilities to conduct this research at Department of Leather Engineering, Khulna University of Engineering & Technology (KUET), Khulna 9203, Bangladesh. I also want to extend thanks to Mr. Md. Aminur Rahman, Senior Chemist, Department of Public Health Engineering, Zonal Laboratory, Khulna 9100, Bangladesh and Dr. Md. Mominul Islam, Associate Professor, Department of Chemistry, University of Dhaka, Dhaka 1000, Bangladesh.

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Head of the Department
Department of Leather Engineering
Khulna University of Engineering & Technology
Khulna 9203, Bangladesh

বিভাগীয় প্রধানের কার্যালয়
লেদার ইঞ্জিনিয়ারিং বিভাগ
খুলনা প্রকৌশল ও প্রযুক্তি বিশ্ববিদ্যালয়
খুলনা ৯২০৩, বাংলাদেশ

Ref.....

Date.....

Review report on the project "A novel method to preserve goat skin with indigenous plant extract to reduce chloride load in the effluent"

Tanneries recycle animal hide/skin, the meat industries' waste. The degradation of hide/skin starts within several hours after the death of the animal if it is left untreated. Proper preservation is important to save the hides/skins from decomposition before starting the leather processing to produce good quality leather. Hides/skins are putrefied causing lots of materials are down graded or even rejected due to their defects. Conventionally flayed fresh hides/skins are preserved by the wet-salting method using sodium chloride. Although sodium chloride is cost effective, available, and easy to apply, the method suffers heavily from the environmental perspective. Development of salt-free or salt-less preservation has become a great challenge in recent years to save the environment from pollution. In the present study, an investigation was made to preserve goat skin using the extracted oil from the seed of *Aphanamixis polystachya* plant. The oil was applied on the flesh side of the skin in different concentrations. The efficacy of the preserved method was periodically assessed by analysis of the preserved goat skin for hair slip, odor, moisture content, bacterial count, total extractable nitrogen, and shrinkage temperature which was compared with the conventional method.

Finally, I would like to conclude by saying thanks to the selection committee of 2016 IULTCS/IUR-Young Leather Scientist Grant for selecting **Mr. Md. Sharuk Nur-A-Tomal** for the Grant. **Mr. Nur-A-Tomal** carry out his research work successfully and here are major findings:

- Freshly flayed goat skin was preserved with extracted oil more than one month.
- Extracted plant oil preserved goat skin without structural modification.
- Chloride and total dissolved solids reduction were 97.8% and 82.3% reactively.
- Physical properties of the crust leather fulfil requirement of shoe upper

02.02.2017

(Dr. Md. Abul Hashem)
Head of the Department
Department of Leather Engineering
Khulna University of Engineering & Technology
Khulna-9203, Bangladesh
E-mail:mahashem@mail.kuet.ac.bd, mahashem96@yahoo.com