Sodium Chloride Substitute for Lower Salt Goat Skin Preservation: A Novel Approach

by
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Abstract

Animal skin is the basic raw materials for the tanning industry, which is the by-product of meat industry. The degradation of skin starts within several hours after the death of animal if it is left untreated. Application of common salt (sodium chloride, NaCl) is the most popular preservation method of skin; it preserves the skin by its dehydrating ability and bacteriostatic effect. The negative effect of sodium chloride is that it generates a huge amount of pollution in the form of total dissolved solids (TDS) during leather processing. In this present study, an investigation was made to preserve goat skin using plant leaf paste. The preservation process was evaluated by monitoring different parameters e.g., shrinkage temperature, hair slip, putrefaction, odor, moisture content, extractable nitrogen, and bacterial count in comparison to the conventional wet salting method. Results indicate that the leaf paste could be used as curing agents to preserve goat skin. Combination of 10% leaf paste + 10% NaCl could preserve the goat skin for a period of 30 days. In this less-salt preservation method, pollution load was reduced e.g., chlorides and TDS in soaking operation by 45.2% and 49.8%, respectively.

Introduction

Animal skin is the basic raw materials for the leather industry. About 60–70% (w/w) moisture and nearly 25–30% (w/w) protein are the main constituents of skin, which makes the materials susceptible to bacterial attack. The degradation of skin starts within 5–6 h after the death of the animal if it is left untreated.[1] The bacteria on the raw skin may penetrate the most important part of the skins (corium) from the flesh surface in 8–12 h; bacteria may also form serious grain peeling and voids in the skin in 15–24 h.[2] The quality of leather depends on the presence of intact protein materials. Therefore, proper preservation process is important to prevent the proteins degradation of the skin due to bacterial attack before being processed into leather.

Common salt (sodium chloride) is a widely popular agent used for the short term preservation of skins. Application of 40–50% salt preserves the skin by its dehydration ability and bacteriostatic effect. It reduces the moisture content of skin from 70% to 30%, which makes the skin unfavorable for bacterial growth while bacteriostatic effect checks the bacterial growth on animal skin.[1,3] The most negative effect of sodium chloride (NaCl) is that it generates a huge amount of pollution in the form of total dissolved solids (TDS) and chloride in leather processing. The wet salting preservation method pays more than 40% of TDS and 55% chlorides in the tannery effluent.[4] It is reported that there is no available technology for treating the effluent containing a high concentration of neutral salt especially sodium chloride.[1] The significant level of chlorides renders the ground water salinity and reduces the fertility of the soil.

To reduce pollution during tanning or cleaner tanning, numerous researchers are continuously trying to develop the alternative preservation methods with salt-free or less-salt. Several alternative preservation methods are being developed e.g., physical and chemical methods. Physical preservation methods are energy intensive and economically impracticable, alternative chemicals methods are either not practicable or have other environmental impacts. The attempts reveal several chemical preservation methods e.g. potassium chloride,[5] boric acid,[6] soda ash,[7] benzalkonium chloride,[8] antibiotics,[9] bacteriocin,[10] formaldehyde,[11] silica gel,[3] Vantocil IB,[12] chlorites and hypochlorites,[13] sulphates,[14] and bisulphite-acetic acid.[15] These approaches are good, but in some cases they are potentially hazardous or are not virtually possible or are not cost-effective. A solution of this problem lies in developing green technologies to keep a check on pollution levels. The present study was attempted with the use of *Clerodendrum viscosum* leaf paste combination with less-salt. The plant has antimicrobial activity and widely distributed throughout tropical and subtropical regions of the world.[16] In Bangladesh, the plant is available near road side and forest.

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In this present study, an attempt was made to introduce ecofriendly preservation for goat skin with *Clerodendrum viscosum* leaf paste with or without sodium chloride. So far authors' knowledge, nobody uses this plant for the preservation of goat skin/hide and this is the first attempt to use the plant as a curing agent. The curing process was monitored for a month and evaluated by examining different parameters e.g., moisture content, hair slip, bacterial count, extractable nitrogen, and shrinkage temperature in comparison to the conventional wet salt preservation method.

Experimental

Materials

To examine the possibility of *Clerodendrum viscosum* for the preservation of skin, freshly flayed goat skins of average weight 1 kg per skin were purchased from a nearby local slaughter house at Khulna, Bangladesh. The *Clerodendrum viscosum* plant leaf was used for this study, which was collected from the university campus, Khulna University of Engineering & Technology, Khulna, Bangladesh. The plant leaf was pasted using laboratory mortar and used in the preservation method.

Chemicals

Analytical grade chemicals were used for the determination of biochemical and pollution parameters. Commercial sodium chloride was used for the preservation experiments and commercial auxiliaries were used for pre-tanning and post-tanning processes to make shoe upper leathers.

Experimental Systems for Preservation

Preliminary experiments were conducted to define the minimum quantity of salt required for the preservation. Four (04) samples of size 30 cm × 20 cm was cut from the freshly flayed goat skin. The different percentages (w/w) of preserving materials were applied with different combinations based on the raw goat skin weight as shown in Table I and assessed periodically (fresh, 1st, 2nd, 4th, 7th, and 14th days of preservation) for the physical changes e.g., odor, hair slip, and moisture content.

Table I
Leaf paste optimized in preservation method.

No.	% of curing agents
01	10% leaf paste
02	5% NaCl + 10% leaf paste
03	10% NaCl + 10% leaf paste
04	15% NaCl + 10% leaf paste

Based on preliminary experimental results, the optimum concentration of salt for preservation was found to be 10% (w/w) with the leaf paste. After assuming the amount of salt in the proposed preservation method; the method was compared with the conventional wet salting preservation method. Freshly flayed two (02) pieces goat skin was collected from the local slaughter house; one piece was taken for the control (50% NaCl) and another piece was used as experimental (10% NaCl + 10% leaf paste). After applying preserving materials, the skins were folded at the backbone level and kept for preservation at surroundings temperature (28±2°C) for 30 days. The preservation methods were critically assessed determining moisture content (%), shrinkage temperature, hair slip, odor, bacterial count and extractable nitrogen at different intervals. To carry out these analyses, a small piece of goat skin samples was collected on fresh (raw), 1st, 2nd, 4th, 8th, 16th, 24st, and 30th day of preservation time.

Monitoring the Preservation Method

Determination of Moisture Content

Moisture content (%) of the goat skins were determined by taking about 5 g of the preserved skin pieces from the experimental and control samples at different periods of curing storage according to the standard procedures.[17] The samples were weighed in a suitable silica crucible and placed it for 3 h in an oven at 105±1°C. The samples were cooled in a desiccator and weighed. The procedures were repeated for extra 1 h or more until the mass of the sample remains constant (within 0.1 mg difference). Each experiment was conducted in triplicate.

Determination of Extractable Nitrogen

The preserved samples of known weight (5 g) were taken in distilled water ten times of its weight, shaken well in a conical flask at 200 rpm for 30 min to extract the soluble nitrogenous compounds. The extracted liquid was then filtered through a filter paper (Whatman No. 1). The filtrate was digested with sulphuric acid, potassium sulphate and copper sulphate in a Kjeldahl flask providing temperature 375–385°C for the effective digestion. The nitrogen content was determined according to the standard method of APHA.[18] Each experiment was conducted in triplicate.

Determination of Bacterial Count

A volume of 1-mL filtrate (same as done determination of extractable nitrogen) was taken in 9 mL of sterile water in 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. Finally, the number of colonies on the agar media was counted using colony counter.

Hydrothermal Stability Determination

Hydrothermal stability of the preserved goat skins was typically evaluated by the shrinkage temperature. The shrinkage temperature (°C) of the preserved goat skins was determined using a shrinkage tester (SATRA TD 114, UK) according to the ISO 3380 standard.[19] Each experiment was performed in triplicate.

Leather Processing

Conventional leather processing method was performed for the preserved goat skins. After 30 days of preservation, both the control and experimental goat skins were processed to manufacture shoe upper crust leathers following the conventional leather making procedures.

Pollution Load Generated During Leather Making

Pollution load in soaking operation of leather processing was determined. The wastewater in soaking operation both from the control and experimental was collected and analyzed for chlorides (Cl^-), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), and total suspended solids (TSS) following the standard methods of APHA.[18] All the experiments were analyzed in triplicate.

Physical Strength of Leather

Determining physical strength of leathers, produced crust leathers were conditioned at temperature 20 ± 2°C and relative humidity 65 ± 2% over a period of 48 h; samples were taken from the specified sampling location. The properties such as tensile strength, elongation at break, and bursting strength were assessed following ISO 3376[20] and ISO 3379.[21]

Scanning Electron Microscope (SEM)

Crust leathers from the preserved control and experimental goat skins were subjected to assess the effect of proposed preservation method on fiber structure of leather. Firstly, leather samples from the same area were placed on conducting carbon tape. After preparing, the samples were analyzed to a SEM (JEOL JSM-6490, USA). The photographs of the grain surface were obtained by operating the SEM at an accelerating voltage 20 kV with magnification 10,000X.

Results and Discussion

Optimization of Leaf Paste

The percentage of leaf paste combination with NaCl for the preservation of goat skin is shown in Table II. It seems that with the all conditions preserved goat skin was intact. But the physical feel (hand feel) was different. Preservation without salt the skin was very hard and it will be difficult to process in a drum or in the paddle. Preservation of 10% leaf paste and 5% NaCl, the skin was medium hard that will be a hindrance to process in drum or paddle. Therefore, in this approach preservation with 10% leaf paste and 10%, NaCl was considered to carry out the experiment.

In Table III and Table IV show the shrinkage temperature and moisture contents of the new approach preservation method. The shrinkage temperatures of the preserved goat skins with the various conditions were unchanged. It seems that based on shrinkage temperatures, the preserved goat skins were in good condition. It is clear from Table IV that moisture contents of the preserved goat skins were gradually decreased. In case of goat skin preservation with 10% leaf paste and 5% NaCl + 10% leaf paste, on 14th day moisture contents was 41.0% and 49.3% respectively. On the other hand, on the 14th day, moisture contents in the preserved goat skins with 10% NaCl +10% leaf paste and 15% NaCl + 10% leaf paste were 41.4% and 50.3%, respectively. It is obvious that preservation using only leaf paste goat skin was flint like feel and it was difficult to rehydrate. It is also noticeable that with increasing (5%–10%) NaCl in preservation method, moisture content was increased in the preserved goat skin due to NaCl having the hygroscopic property.

Table II
Leaf paste optimized in preservation method (21 days).

No.	% of curing agents	Hair slip	Odor	Physical feel
01	10% leaf paste	No	No	Hard
02	5% NaCl + 10% leaf paste	No	No	Medium hard
03	10% NaCl + 10% leaf paste	No	No	Flexible
04	15% NaCl + 10% leaf paste	No	No	Soft and flexible

Table III
Shrinkage temperature of the preliminary experiment (14 days).

Duration	10% leaf paste	5% NaCl + 10% leaf paste	10% NaCl + 10% leaf paste	15% NaCl + 10% leaf paste
Fresh	64.5	64.3	65.1	64.5
1st day	64.8	65.1	64.3	65.8
4th day	65.4	65.6	64.2	66.2
7th day	66.2	65.4	64.3	65.8
14th day	65.9	64.7	64.8	65.1

Effectiveness of the Preservation Method

Total Extractable Nitrogen

The extractable nitrogen content in experimental and control is depicted in Fig. 1. The extractable nitrogen was calculated by the amount of nitrogen extracted in the aqueous phase. Total extractable nitrogen is the best indicator whether bacteria degrades the animal skins or not. The putrefaction of skin proteins results the release of nitrogenous components, which lead the emission of putrefaction odor and hair slip.

In this study, physical assessments: hair slip and odor of the preserved goat skin by control and experimental were observed; there were no hair slip and odor during preservation period up to 30 days. In the fresh goat skin, extractable nitrogen contents were 1.5 g/kg and 2.6 g/kg for control and experimental respectively. On the 1st day and 4th day extractable nitrogen contents in goat skins were different but there were no hair ship or odor of the preserved skins by control and experimental. On the 8th day, extractable nitrogen contents were almost same both for the experimental and control goat skins. On the 16th day, 24th day, and 30th day nitrogen contents were different but there was no hair slip or odor. It is noticeable that combination of 10% leaf paste + 10% NaCl preserves the goat skin for 30 days.

Table IV
Moisture content of preliminary experiment (14 days).

Duration	10% leaf paste	5% NaCl + 10% leaf paste	10% NaCl + 10% leaf paste	15% NaCl + 10% leaf paste
Fresh	69.9	70.0	69.1	65.3
1st day	60.7	60.0	50.3	62.7
4th day	54.1	52.4	52.7	59.0
7th day	45.0	52.3	45.9	54.2
14th day	41.0	49.3	41.4	50.3

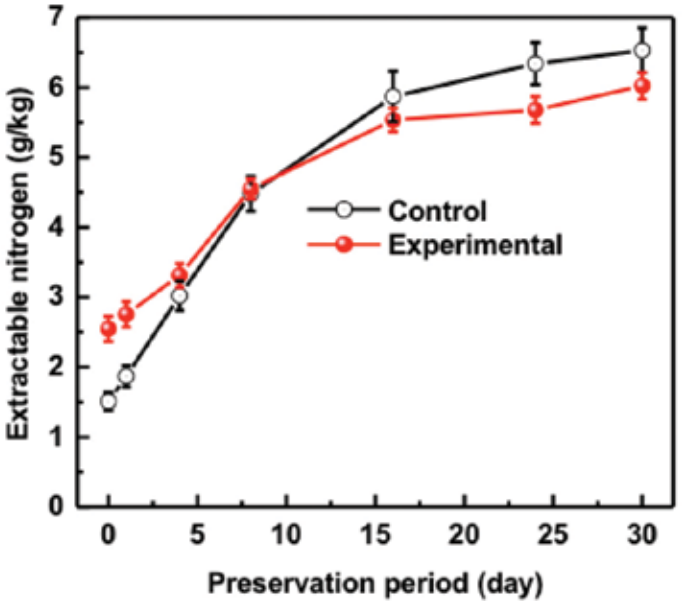

Figure 1. Extractable nitrogen content in preserved goat skin by control and experimental methods.

Bacterial Count

Bacterial count of the control and experiment preservation of the goat skins is shown in Table V. In raw goat skins bacterial count for control and experimental was 3.2×10^3/g and 1.2×10^4/g respectively. On the 4th day, the bacterial count for control and experimental was 3.9×10^9/g and 1.9×10^6/g respectively. On day 8th, and 16th bacterial count of the experimental preserved goat skin was less in comparison to the control preserved goat skin; preservation in the present approach (10% NaCl and 10% leaf paste) has biocidal effects which inhibit the bacterial population. Although on the 24th day and 30th day, bacterial count was higher in the experimental preserved goat skin than in control preserved goat skin. However, there were no hair slip, odor in the present approach preservation method by using 10% NaCl and 10% leaf paste.

Moisture Content

Raw skin contains about 60–70% moisture, which is favorable condition for bacterial growth. The moisture content (%) of preserved skin is considered an important indicator that can be used to evaluate preservation method. The moisture content in the experimental (preservation with 10% leaf paste + 10% NaCl)

Table V
Bacterial count (CFU/g) in the preserved goat skins.

Duration	10% NaCl + 10% leaf paste	50% NaCl
Fresh	1.23×10^4	3.2×10^3
1st Day	2.52×10^4	8.4×10^9
4th Day	1.88×10^6	3.9×10^9
8th Day	2.31×10^6	2.1×10^7
16th Day	2.34×10^6	4.6×10^6
24th day	2.26×10^6	1.7×10^6
30th day	3.13×10^6	5.1×10^5

goat skin in comparison with the conventional method is shown in Fig. 2 during the period of 30 days. There is no significant variation in moisture content between the experimental and control.

It can be seen from Fig. 2, moisture content was same both in experimental and control methods within the first 24 h. On the 4th day, moisture content was little higher (7.0%) in experimental but there was no sign putrefaction which may be due to the potential antibacterial aids of the *Clerodendrum viscosum* leaf paste against degrading microorganisms. On the 8th day, moisture content was 8.9% higher than the conventional preservation method. Subsequently, in the both preservation methods, moisture content was gradually decreased and it was lower than the critical moisture content (50%). Moisture content was almost constant from the 16th day in the preserved goat skins for control and experimental. It is obvious that there was no skin degradation e.g., hair slip, odor, etc.

Hydrothermal Stability

The hydrothermal stability (shrinkage temperature) of collagen is considered as a significant property for the assessment of the animal skin quality because it indicates indirectly any structural deterioration of the skin protein. The shrinkage temperature is the measurement of the breakdown of stabilizing linkages and the bases for the type of interactions existing in the collagen matrix[22]. The purpose of this parameter was to know whether the proposed preservation method had any effect on the deterioration of collagen matrix. The hydrothermal stability of the experimental (preserving with 10% leaf paste + 10% NaCl) goat skin in comparison with the conventional method is shown in Fig. 3 during the period of 30 days.

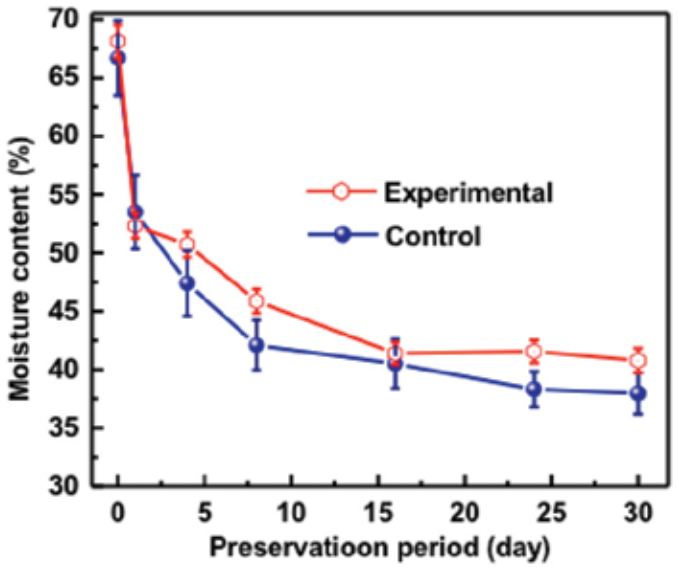

Figure 2. Moisture content of preserved goat skin in control (50% NaCl) and experimental method (10% leaf paste + 10% NaCl).

The shrinkage temperature of experimental skin shows marginal differences in comparison with control preserved skin. In Fig. 3 shows that up to 4th day (fresh, 1st day and 4th day) there was no significant difference in shrinkage temperature for experimental and control methods. On 8th, 16th and 24th day, shrinkage temperatures were little increased for both in experimental and conventionally preserved skins. However, on the 30th day, the shrinkage temperature for experimental and control was (64.8°C) and (65.8°C), respectively. Therefore, it can be said that *Clerodendrum viscosum* leaf paste based preserving does not modify the stability of the collagen protein matrix in goat skin.

Pollution Load in Soaking Operation

Pollution load generated in soaking operation of the preserved goat skins both for control and experimental samples were depicted in Table VI. It seems that the chloride and TDS load were greatly reduced when the 10% leaf paste + 10% NaCl was used in preservation in place of the common salt (NaCl). Even though there was little decrease in the BOD_5 and COD levels in the experimental soaking wastewater compared to the control method. It may be the reason that plant materials contain organic substance which leads to increase BOD and COD. The main

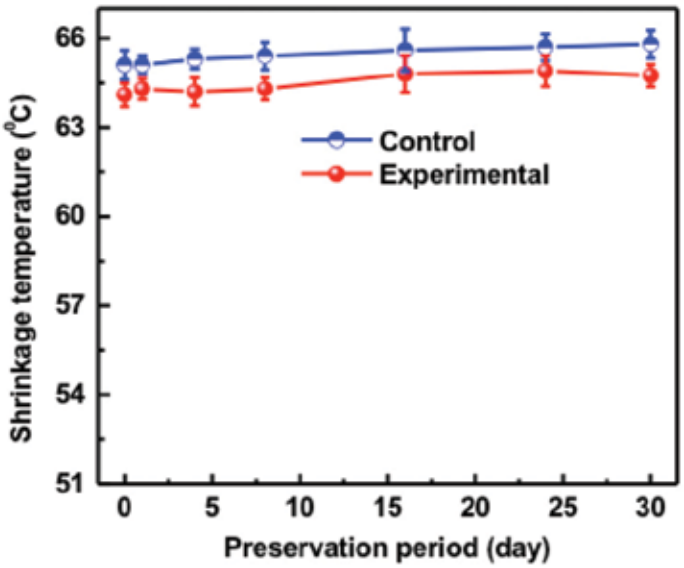

Figure 3. Shrinkage temperature of preserved goat skin in control (50% NaCl) and experimental method (10% leaf paste + 10% NaCl).

Table VI

Pollution load generated in soaking of preserved goat skins.

Sample	Cl^- (mg/L)	TDS (mg/L)	BOD_5 (mg/L)	COD (mg/L)
Control	18223 ± 173	42259 ± 153	1260 ± 36	5250 ± 63
Experimental	9980 ± 14	21215 ± 45	1360 ± 11	5644 ± 23

pollution problem of the leather manufacturing: chloride and TDS were reduced 45.2% and 49.8% respectively in the experimental soaking wastewater. It could be concluded that the present preservation approach for the goat skin in combination with 10% leaf paste + 10% NaCl reduce the salinity in soaking operation.

Physical Properties of Leather

Table VII shows the organoleptic properties and physical strength of the crust upper leather of experimental in comparison with the control. The crust leathers were assessed for softness, grain tightness, fullness, and smoothness. The tabulated physical properties in Table VII indicate that the physical strengths e.g., tensile strength, elongation at break and grain crack of the experimental skin preserved with 10% leaf paste and 10% NaCl were comparable with that of the corresponding control method. The elongation at break (%) and load at grain crack (kg) values were fulfilled the required values.

SEM Analysis

SEM photographs of the leather processed from the control (50% NaCl) and experimental (10% leaf paste +10% NaCl) preserved goat skins are depicted in Fig. 4. SEM analysis exhibits that the leather prepared from the experimental (10% leaf paste + 10% NaCl) goat skin did not show any deterioration compared with the goat skin preserved with 50% NaCl. This shows that proposed preservation did not affect the texture and quality of the goat skin.

Table VII
Physical properties of processed control and experimental leather.

Parameters	Experimental	Control	Requirements[3]
Tensile strength (kg/cm^2)	226.14	244.2	200
Elongation at break (%)	43.5	39.6	40-65
Bursting strength: Distension at grain crack (mm) Load at grain crack (kg)	7.4 40	8.3 43.0	7 20

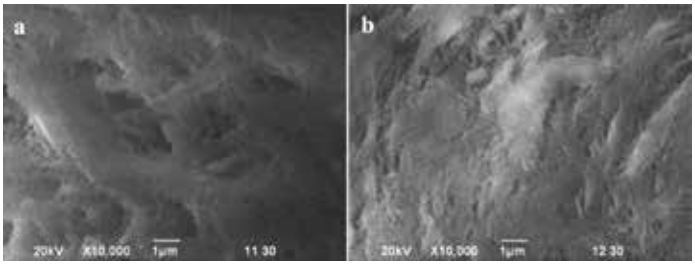

Figure 4. SEM photographs of prepared crust leathers from the preserved goat skin (a) control (50% NaCl) and (b) experimental (10% leaf paste +10% NaCl).

Conclusions

The novel preservation system using *Clerodendrum viscosum* leaf paste in combination with less-salt formulation could be a cleaner preservation of goat skin in place of conventional wet salting preservation method. Application of 10% leaf paste + 10% NaCl could preserve the goat skin for a period of 30 days. The physical properties of the produced leather were fulfilled the requirements of shoe upper. This less-salt preservation method reduced the pollution load, e.g. chlorides and TDS, in soaking operation by 45.2% and 49.8%, respectively. The method could be a viable option to preserve goat skin, which could reduce the pollution load during leather processing.

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