PRESERVATION OF BOVINE HIDE USING LESS SALT WITH LOW CONCENTRATION OF ANTISEPTIC

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Abstract. A Conventional technique of bovine hide preservation requires approximately 40-50% sodium chloride or table salt on raw hide weight or 95% saturated brine in case of wet salting. This salt resides in wastewater after the soaking process and generates a huge environmental pollution in the form of total dissolved solids (TDS) and chloride (Cl^-) during leather processing. The current research has developed an antiseptic based hide curing formulation using 45% saturated brine solution which reduces 50% salt usage in compare to the traditional method. For hide preservation, it is essential to arrest microbial attack on hide as the main constituent of raw hide is protein which is very susceptible for bacterial degradation. The newly developed formulations have been found more effective in limiting microbial growth on cured hide than the conventional method preserving the bovine hide for more than 30 days. In-process analysis of cured hides during storage period reveals the compatibility of the alternative curing process. Post-leather analysis e.g. grain pattern, scanning electron microscopic images, mechanical properties and organoleptic evaluation reveal that the crust leather produced from alternatively cured hides are comparable to the control obtained from traditionally preserved hide. The efficacy of the alternative system is also assessed by monitoring the environmental impacts caused by the leather processing effluents on the basis of TDS and chloride content, total solids (TS), total aerobic bacterial counts in soaking liquor, Bio-Chemical oxygen demand (BOD) and Chemical oxygen demand (COD). The environmental advantages of the alternative hide curing method are determined particularly by 50% reduction of TDS and chloride content. Therefore, this new development will not only preserve hide through better protection from microorganisms but also offer improved conservation of the environment.

1 Introduction

Animal hides and skins are valuable byproducts of meat industry because they are used to produce leather. Preservation of raw hide has always been a challenge for leather manufacturing companies as they are putrefied rapidly. The integrity of raw hide is the key to produce good quality leather. Animal hide contains a great variety of microorganisms, which are derived from air, water, soil, manure and extraneous filth.[1] In a living animal, bacteria and microorganism on its skins are held in control by the metabolic defences of the animal, but the flayed skins become vulnerable for bacterial attack within 5-6 h of removal.[2,3] Skin microorganisms produce proteolytic and collagenolytic enzymes resulting in putrefaction of hide. The leather quality depends on the presence of necessary protein levels in raw hide. Therefore, it is extremely important to conserve protein from degradation in skin during the process of preservation.

Prevention of putrefaction is the main objective of hide curing process which can be accomplished by limiting or controlling microbial attacks on hides, either by killing the microorganism which is called bactericidal method or creating unfavourable conditions for the microorganisms to thrive, known as bacteriostatic method. The bactericidal method employs chemicals that are usually harmful for humans or living species and costly. On the other hand the bacteriostatic method utilizes dehydrating agents in bulk such as sodium chloride which generates pollution problems in terms of total dissolve solid (TDS) and chloride content in the resulting effluent from the soaking operation of leather production.

In traditional hide preservation process, 95% saturated brine solution or 40%-50% w/w sodium chloride on the raw hide's weight is used.[4] Almost 75% of the salt ends up in the effluent stream

during soaking, which contributes to 40% of total solid content in the tannery effluent[5] creating major salt pollution in the environment. Concentrated tannery effluent severely affects the germination of seeds and hampers the growth of seedlings and other floras when used for irrigation purpose or simply discharged to the field[6]. When the soils are irrigated with saline effluent, salts accumulate, unless they are leached out. Furthermore, saline irrigation water along with low-soil permeability, inadequate drainage, low rainfall and poor irrigation management, all cause salts to accumulate in soil, which has deleterious effects on crop production. Degradation of soils by salinity and sodicity profoundly affects environmental quality.[7] The salts also affect release and solubility of heavy metals in solution, with potential adverse effects on water quality and plant growth.[8] High concentration of sodium (Na^+) and chloride (Cl^-) affect plants directly by causing excessive uptake of these ions or indirectly by increasing soil pH. Therefore, it is very important to pursue new and more environmentally friendly methods of hide preservation.

Many researchers have investigated and proposed alternative hide curing methods which can be divided in to two categories: physical and chemical methods of hide preservation. As physical methods can be mentioned cooling[9], cooling wit addition of ice[10], cooling in vacuum[11], drying chamber[12] and irradiation based curing either by using gamma rays (photon emission from radioactive materials) or electron beams[13]. Although these physical methods are very convenient, as there is no chemical used, but have been found expensive and difficult to adopt in a hide processing facility. Therefore, chemical methods of hide preservation are more welcome due to simplicity of use and needless of expensive special equipment. The possibilities of using potassium chloride[14], soda ash[15], preservatives such as benzalkonium chloride[16], antibiotics such as auromycin and terramycin[17], neem oil[18] and boric acid[19] for preserving the hide have been explored. Higher cost, sub-optimal preservation efficiency, toxicity, poor quality of leather and adverse environmental impact are the main factors that explain why these methods have not been adopted commercially. Therefore, an alternative approach needs to be developed that eliminates/mitigates the problems associated with the existing hide preservation techniques.

In first part of this current research, a lower salt curing method was developed where, 45% saturated brine solution with low concentration of environmentally benign antiseptic was used to preserve bovine hide for more than 30 days.[20] This method reduced salt usage by more than 50% compared to the conventional method where 95% saturated brine solution is used. The efficacy of hide preserving formulations was assessed and reported[20] by monitoring a variety of parameters (i.e. microbial growth, water activity, moisture content, texture analysis, hair slip, odor, microscopic analysis and rehydration) of alternatively cured hides throughout the preservation period. The alternative method provided better resistance to microbial growth on skin than the conventional method during a 30 days storage period. This paper reports the 2nd part of low salt curing research which deals with the characteristics of leather produced from the alternatively cured hides and the environmental impacts caused by the method in compare to that generated by the traditional method of hide preservation.

2 Materials and Methods

Freshly flayed and de-fleshed bovine hides were acquired from a local meat packing facility, courtesy of JBS Packerland (Souderton, PA). Each hide was split down the back into left and right segments. The sides were then cut into pieces that weighed approximately 800 – 1000 g, with dimensions of 12 in x 12 in. All chemicals used for hide preservation listed in **Table 1** were of commercial grade. alkyltrimethylammonium bromide (ATMAB), chlorhexidine di-gluconate (CDG), lactic acid solution ≥ 85%, peracetic acid solution, hydrogen peroxide were purchased from Sigma Aldrich Chemical company (Milwaukee, WI). All other reagents used for the formulations were of the highest purity available from commercial suppliers. Brine solutions were prepared by dissolving

specific amount of common salt (sodium chloride) in water and a salometer was used to measure their saturation level. The preparation of all curing formulations was carried out as detailed in Table 1, where mixed or dissolved in tap water at room temperature (~21 0C). All formulations were prepared ~12 h prior to the experiments.

Table 1. Composition of the developed curing formulations for hide preservation

Formulations	Composition
F-A (control)	95% saturated brine soln. + 0.043% NaOCl (v/v)
F-B	45% saturated brine soln. + 0.6% ATMAB (wt./v) + 0.06% CDG (v/v)
F-C	45% saturated brine soln. + 0.6% ATMAB (wt./v) + 0.06% CDG (v/v) + 0.043% NaOCl (v/v)
F-D	45% saturated brine soln. + 0.6% ATMAB (wt./v) + 0.06% CDG (v/v) + H_2O_2 (135 ppm) + Peracetic Acid (80 ppm)
F-E	45% saturated brine soln. + 0.6% ATMAB (wt./v) + 0.06% CDG (v/v) + 0.043% NaOCl (v/v) + 2% Lactic Acid (v/v)
F-F	45% saturated brine soln. + 0.6% ATMAB (wt./v) + 0.06% CDG (v/v) + H_2O_2 (135 ppm) + Peracetic Acid (80 ppm) + 2% Lactic Acid (v/v)

2.1 Laboratory Scale Protocol for the Alternative Hide Preservation

A 150 % float (volume of sol/w of hide or v/w) was used for preservation treatment. Hide pieces were soaked individually in the 6-in-1 Dose drums (Dose Maschinenbau GmbH, Lichtenau, Germany) with in respective solutions for 18 h. During the treatment, the 6-in-1 Dose drums controls were set to 6 rpm for tumbling. A 95% saturated brine solution with 0.043 % (v/v) bleach (NaOCl) was utilized for the control (F-A, **Table 1**). This formulation is being used commercially for conventional hide preservation. For alternative methods (F-B to F-F, **Table 1**), a 45 % saturated brine solution was used along with other additives which cut the salt usages by more than 50 %. After 18 h of treatment, the hide samples were hung to dry, folded and stored in a humidity chamber at the temperature of 38-40 ^{0}C, and were monitored periodically for physical changes such as smell and hair slip which are the indications of putrefaction.[21] The effectiveness of the developed curing formulations was assessed by determining different parameters of cured hides during storage period and the results were recorded and published.[20]

2.2 Analysis of Soaking Liquid Generated in Leather Processing

After preservation for 35 days, the hide samples were subjected to soaking with 200% float of water for 4 hours. Then, the spent liquors from the soaking operation were collected and analyzed for different pollution parameters such as, total dissolved solid (TDS), chlorides (Cl^-) content, total solid (TS) using standard analytical procedures.[22,23,24] Aerobic bacterial colony count, total carbon (TC), total organic carbon (TOC), chemical oxygen demand (COD), biochemical oxygen demand (BOD) of soaking liquid were also determined to assess the overall pollution load from soaking operation in preparation of leather from the alternatively cured hides.

2.2.1 Total aerobic bacterial colony count of soaking liquor

The samples were collected independently in sterile containers to analyze residual aerobic bacterial concentrations in the soaking liquors, 1 ml of sample solution was serially diluted in sterile water. Diluted sample was then plated on Tryptic Soy Agar and incubated at 37 ^{0}C for ~24 hours and bacterial counts were reported in log CFU/ml. with the lowest detection level of 1 log/CFU ml. All samples were conducted in triplicate.

2.2.2 Analysis of TOC and COD in soaking liquor

To determine TOC and COD of the soaking liquids, a Quick COD/TOC ultra single-stream analyzer purchased from Liquid Analytical Resources LLC (LAR), West Bend, WI was used. LAR's high temperature TOC analyzers provide a summary measurement of organic contaminants by oxidizing a sample's carbon compounds (Total Carbon, TC) in a high temperature furnace (1200 ^{0}C). A Near Infrared Detector then measures the resulting CO_2. Total Inorganic carbon (TIC) is measured separately, then subtracted from the total carbon, thus providing Total Organic Carbon (TC-TIC=TOC). This method is derived from EPA Standard Method 415.1.

LAR's COD analyzer's operating method is formalized as ASTM D6238-98. This method also combusts the sample at 1200 °C, but uses a nitrogen carrier gas and measures oxygen consumption using a zirconium dioxide O_2 gas detector. LAR's high temperature method provides accurate, repeatable results in three minutes, uses no reagents and achieves far greater accuracy and repeatability of 3% coefficient variation (CV), compared to ±20% which follows US EPA Method 410.4.

2.2.3 BOD analysis of soaking liquor

The biochemical oxygen demand (BOD) in soaking liquor is the amount of oxygen that is consumed during the degradation of organic substances through biochemical processes. For BOD measurement, each sample was analyzed for 5 days using a Lovibond BOD-System BD 600 (Tintometer Inc. Sarasota, FL). BOD measurement was carried out by means of pressure differential in a closed system (respirometric BOD measurement). The BOD measuring unit comprising test bottle and BOD sensor, is a closed system. There is a gas compartment with a defined quantity of air in the test bottle. The bacteria in the soaking water filled in the bottle consume the oxygen dissolved in the sample over the course of BOD measurement. It is replaced by air oxygen from the gas compartment of the test bottle. The simultaneously developing carbon dioxide is chemically bound by the potassium hydroxide in the seal cap of the test bottle. As a result a pressure drop occurs in the system, which is measured by the BOD sensor and shown directly in the display as a BOD value in mg/l O_2. The system records a measurement every hour on the first day, every other hour on the second day, and once every 24 hours starting on the third day up to 5th day.

2.3 Tanning of Cured Hides

After soaking operation, the alternatively cured hide samples were placed in one dehairing drum and the control sample panel was placed in another dehairing drum and de-haired per the USDA tanning protocol.[25,26,27] All the hide panels were combined into one drum for the pickle, tanning, re-tanning, coloring, and fat liquoring steps. The samples were tanned into crust upper shoe leather and kept in a temperature (21 ^{0}C) and humidity (50% relative humidity) controlled environmental chamber (Caron Environmental Chamber, Marietta, OH) until subjective, mechanical, and microscopic analyses were performed.

2.4 Evaluation of Leather Quality

To assess the effects of the hide preserving formulations on leather quality produced from alternatively cured hides, the mechanical properties of the produced crust leather were measured. The mechanical properties included tensile strength, Young's Modulus ("stiffness"), elongation ("stretchability"), and fracture energy ("energy required to open unit area of crack surface") were conditioned and tested as per the ASTM methods D1610 and D2209 to verify the effect of the newly developed hide preservation formulas on the quality of leather. Five dumbbell shaped leather samples were cut from each leather piece following the protocol in ASTM D2209 parallel to the backbone. The average thickness range of the leather samples were observed from 2.13 to 2.82 mm. An Insight-5 test frame and Testworks-4 data acquisition software (MTS Systems Corp., Minneapolis, MN) were used to evaluate the mechanical properties of the leather samples. The strain rate and the grip distance for this study were set to 24.5 cm/min and 10.16 cm respectively. Samples were tested in a room set at 23±3 °C and 50±5 % relative humidity. Tannery subjective tests (break, handle, fullness, and color) were conducted by an expert in-house USDA tanner.

2.5 Microscopic Imagining

Representative crust leather samples produced from the hides which were cured by individual hide preserving formulation (F-A to F-F, Table 1) were inspected under a stereo microscope (Nikon Digital Microscope SMZ-2T, Melville, NY) to determine any detectible changes in the hide grain structure from curing. Additionally, scanning electron microscope (SEM) images were taken to identify potential finer structural changes in the surface of the leather. For SEM images, samples were mounted on stubs and sputter gold coated for 1 minute (EMS 150R ES, EM Sciences, Hatfield, PA). Samples were viewed with a FEI Quanta 200 F Scanning Electron Microscope (SEM), (Hillsboro, OR, USA) with an accelerating voltage of 10KV in high vacuum mode.

3 Results and Discussion

Five novel formulations have been developed and tested to preserve bovine hide where, a 45% saturated brine solution in combination with bactericidal antiseptics is used. This new development reduces salt consumption by 50% from the conventional curing process, where 95% saturated brine solution is being used. The effectiveness of the newly developed formulas in hide preservation has been reported in previously published article.20 This technology is adopted principally to address the pollution problem created from conventional curing methods either by the soaking liquor discharged to the environment during leather making process or/and tannery effluents. To develop the reported five formulations, a surfactant (ATMB) and an antimicrobial agents (CDG) have been used in common. ATMB is a quaternary ammonium compound which in addition to possess antibacterial properties.28,29 ATMB is able to damage cell membranes and destroy the cellular structure of various microorganisms including fungi, bacteria and other single cell organisms. ATMB is non-toxic when applied directly to the skin. Chlorohexidine salt (CDG) dissociates in water and releases positively charged chlorhexidine cation which results bactericidal effect through the binding of this cationic molecule to negatively charged bacterial cell walls.30 CDG is active against Gram-positive and Gram-negative organisms, facultative anaerobes, aerobes, and yeasts.30 Among the additives, lactic acid (a alphahydroxy acid) is a well-known antimicrobial31 and also acts as humectant32 which attracts water and improve hydration of the stratum corneum of the skin. Alphahydroxy acid such as lactic acid also increases cohesion of the stratum corneum cells and thus reduces roughness and scaling. Also two combinations of spore killing agents, hydrogen peroxide with peracetic acid33 (F-D and F-F, Table 1) and sodium hypochlorite34 (F-C and F-E, Table 1) have

been added to enhance the antimicrobial properties of the particular formulations. In this paper, the environmental impacts of leather making from the alternatively versus traditionally cured hides have been evaluated and also the impact of the developed curing formulations on leather are reported.

3.1 Soaking Liquor Analysis

3.1.1 Aerobic bacterial colony count determination

Aerobic bacterial colony counts were conducted to determine the bacterial concentration per mL of soaking liquor up to 5 log CFU/mL. Figure 1 shows all the formulations control bacterial growth significantly better than the control even in spent liquor. A 10 fold dilution of soaking liquor from traditionally preserved sample (F-A) were TNTC (Too Numerous to Count) thus they had counts in excess of 5 log CFU/mL. However, colony counts for F-B and F-E were 2.96 and 2.30 log CFU/mL respectively. No microbial growth was observed from the other soaking liquors in a 24 hours incubation period at 37 ^{0}C. This results demonstrate the effectiveness of these formulations in limiting microbial growth compare to the control which consisted of the industry standard of 95% brine solution.

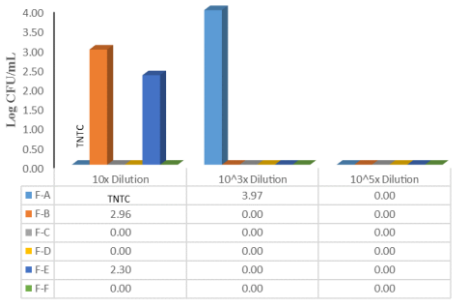

Fig. 1. Bacterial colony count per milliliter of soaking liquors of hide samples preserved by the different formulations

3.1.2 Chloride content determination

The determination of chloride content in spent soak liquor directly correlates to the salinity in tannery waste water.

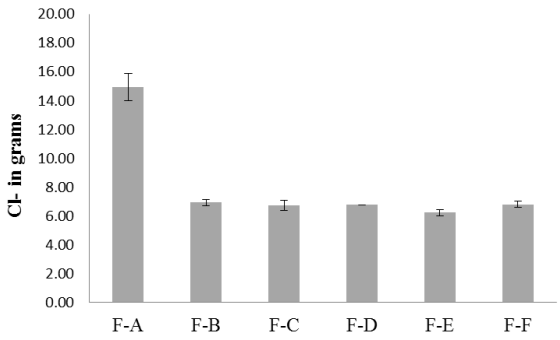

Fig. 2. Chloride content of spent liquors generated by soaking of 1 kg of preserved hide

The results in Figure 2 clearly shows that, comparing to conventional treatment (F-A), chloride content in spent soaking liquor can be reduced by 50% or more if any of the developed formulation is used for hide preservation. This significant reduction in salinity and chloride loads will help in achieving cleaner and greener leather processing.

3.1.3 Determination of TOC and COD in soaking liquor

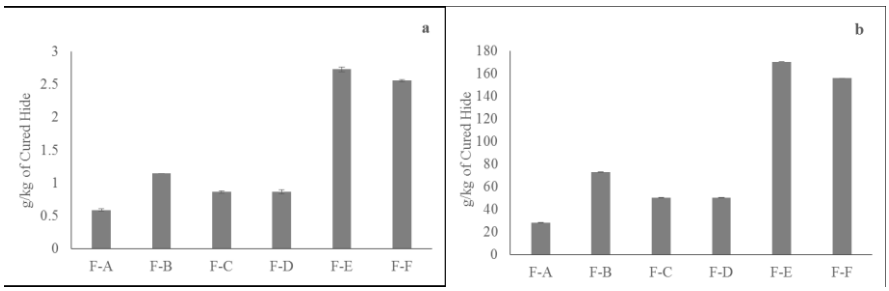

Fig. 3. a) Total Organic Carbon (TOC) and b) Chemical Oxygen Demand (COD) values (in gram) of soaking liquors processing 1 Kg of cured hide.

The results of COD and TOC analysis are consistent to each other. Higher carbon content present in the soaking liquor will presumably result higher COD. Compared to the control (F-A), both TOC and COD loads are increased for the alternative treatments (F-B through F-F). This is due to the addition of antimicrobials in newly developed hide preserving formulas. However, the significant increase in TOC and COD loads for F-E and F-F could be accounted for the use of 2% lactic acid in the formulations.

3.1.4 Determination of BOD

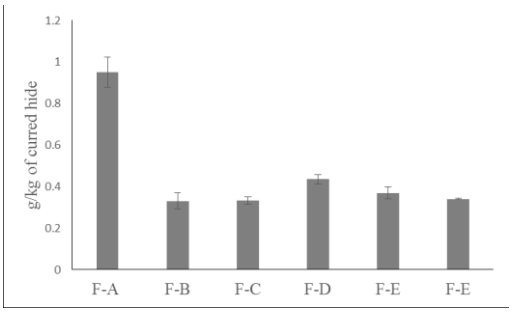

Fig. 4. Biochemical Oxygen Demand (BOD) values (in gram) of soaking liquors processing 1 Kg of cured hide.

Five day analyses was carried out for the BOD determination of the soaking solutions of differently cured hides. Results show a significant decrease of 54 to 65% in BOD load for the soaking liquors of alternatively cured hides in compare to traditionally preserved hide. The higher BOD value for the control is due to the presence of high concentrations of bacteria in the soaking liquor which is consistent with the bacterial colony count analyses as shown in Figure 1. Beside the availability of other biochemical substances, the dead cells of microorganism also provide with the source of nutrients for the living cells present in the soaking effluents.

3.1.5 Determination of Solid Pollutants

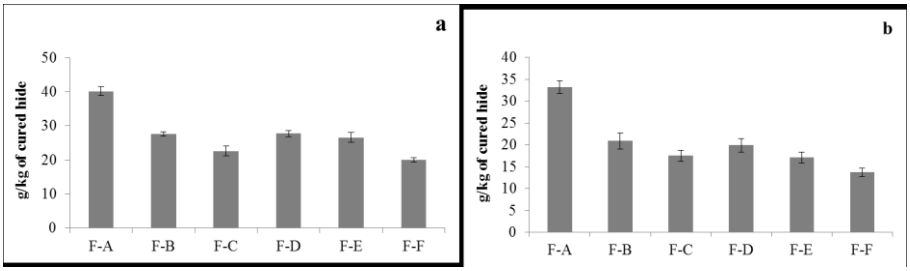

Fig. 5. Pollution load generated in the soaking process of hides cured by the different formulations: a) Total Solid (TS) and b) Total Dissolved Solid (TDS)

The data in Figure 5 represent the amount of solid pollutants in the soaking liquors contributed by the different curing chemicals. The results clearly show that there is substantial reduction in the values of the pollution parameters, Total solid (TS) and Total dissolved solid (TDS) for the alternative methods compared to the conventional treatment. These changes are caused mainly due to the reduction of salt in the preservation step.

3.2 Quality Determination of Crust Leather

After the preservation period of 35 days, the cured hides were processed into crust upper shoe leather following the standard tanning protocol.

3.2.1 Grain surface pattern study

In-order to study the surface finesse or coarseness of the crust leather, the grain pattern was studied. The grain structure of the leathers made from the traditionally (F-A) and alternatively cure hides (F-B, F-C, F-D, F-E and F-F) were analyzed under a stereo microscope. There was no discernable difference between the grain structure of leather made from conventionally treated hide and leathers made from the experimentally treated hides (Figure 6). Additionally, the leather panels were folded, and a stereo microscopic image was taken at the crease to assist with analysis of the surface features (Figure 7). Again, there was no discernable difference between the leathers made from the hides treated with the control and developed formulas. No sueding (fraying) was observed from any of the samples. This indicates that none of the developed formulation for hide preservation damage the hide grain.

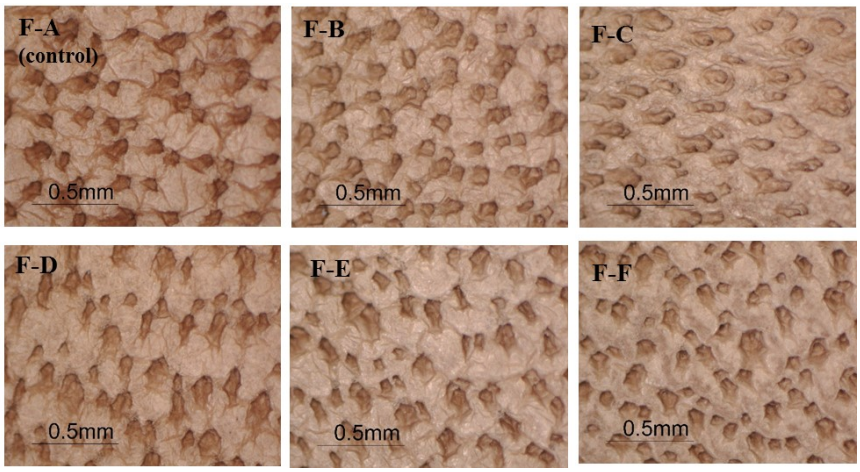

Fig. 6. Stereo microscopic images of the leather made from the differently preserved hides. Bars represent 0.5mm.

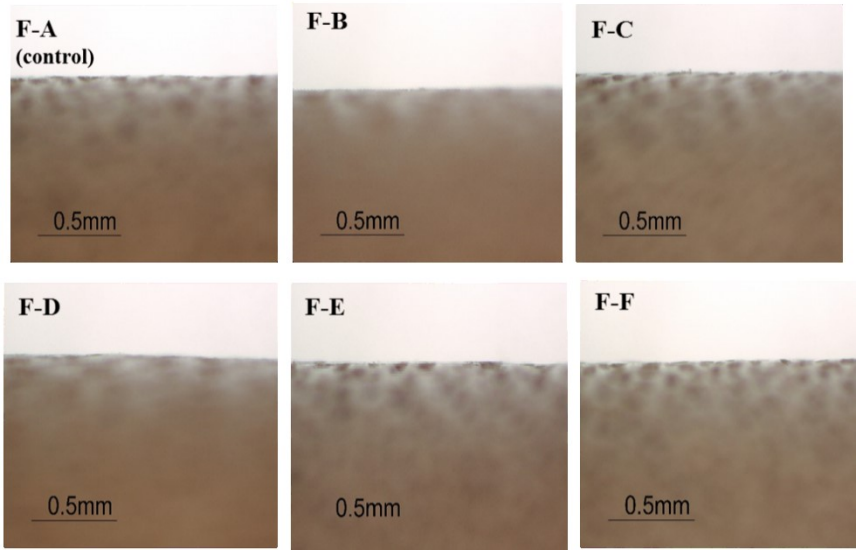

Fig. 7. Stereo microscopic images of the leather at the crease made from the differently preserved hides. Bars represent 0.5mm.

3.2.2 Surface image of crust leather using scanning electron microscope

Surface images of crust leathers from individually cured hides were observed using scanning Electron microscope at 100 x magnifications. The images (Figure 8) of the crust reveal uneven or rough surface of the leather made from traditionally preserved hide (F-A), whereas the leather from alternatively cured hides appears to have smoother and homogeneous surfaces.

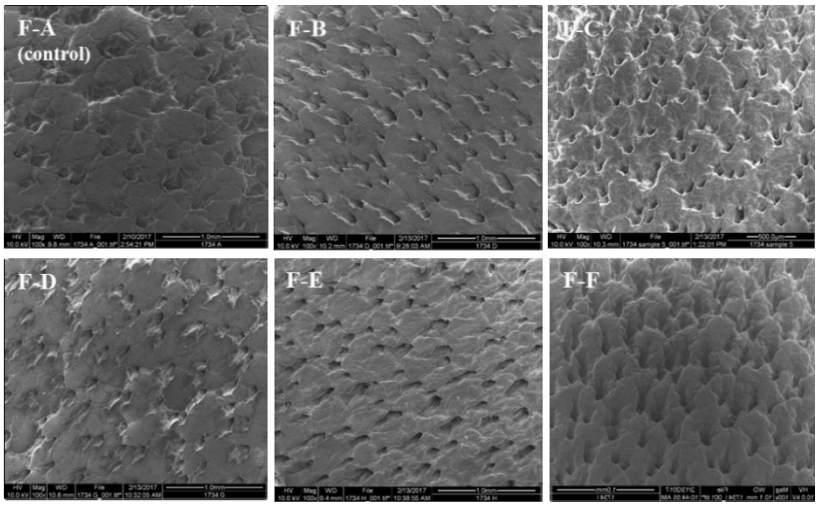

Fig. 8. SEM surface images at 100 x (as shown in bar length) of crust leathers from individually preserved hides.

3.2.3 Determination of mechanical properties of leather

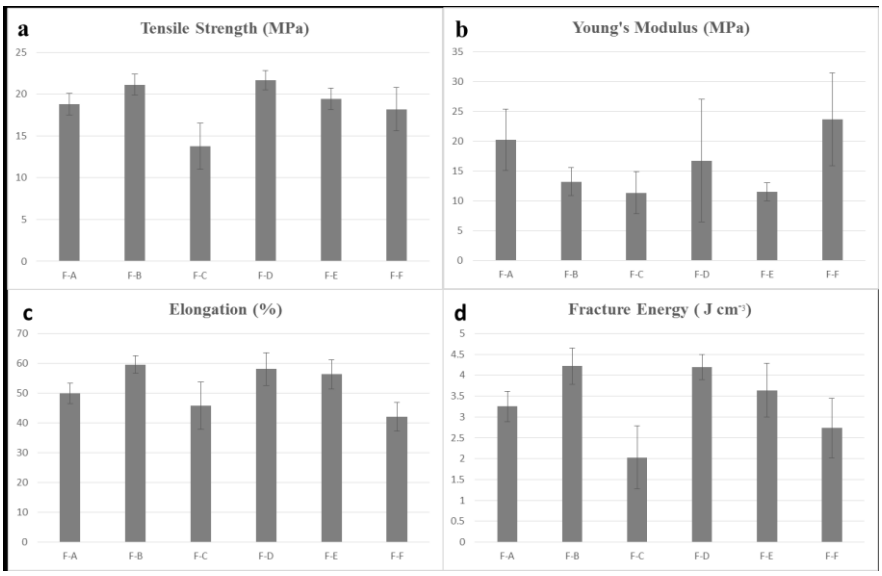

Fig. 9. Mechanical properties of the crust upper shoe leather from differently cured bovine hides

The overall mechanical properties of the resulting leather products from the alternatively cured hides were comparable to that produced from the traditionally preserved hide (F-A). In some cases alternatively cured hides produce better quality of leather. For example, leather yielded from F-B and F-D treated hides showed improved quality in every property shown in Figure 9 in comparison to quality of leather produced from control treatment (F-A). More flexible leathers resulted from alternatively preserved hides in compare to conventionally treated hide (Figure 9b) and this is potentially because of the using of low salt.

3.2.4 Subjective Evaluation of Leather

Crust leathers from preserved hides were assessed for softness, fullness, grain tightness (break), color and general appearance by hand and visual examination (Figure 10). The leathers were rated on a scale of 0-5 points for each functional property where higher points indicate better property.

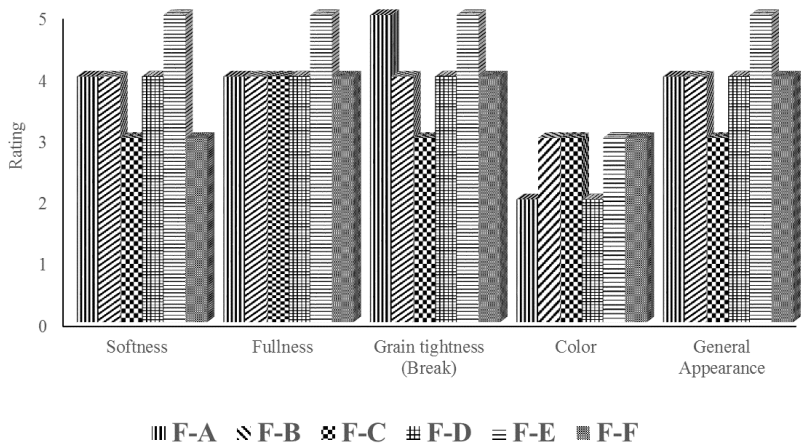

Fig. 10. Organoleptic evaluation of crust leathers from differently cured hides

The leathers from alternatively cured hides by every formulation exhibit better or similar fullness and color in comparison to the leather from a traditionally cured hide. Especially, the leather from the F-E treated hide which has superior rating in almost every property of the subjective test. Other formulated hides produced leathers of comparable quality to the conventionally processed leather (F-A).

4 Conclusions

The developed curing formulas are proven to be effective in preserving bovine hide for more than 35 days limiting microbial growth on hide better than the traditional curing process. The new process utilizes 45% saturated brine solution in general, which offers more than 50% reduction of salt usage in conventional method. The dehydrating brine solution helps to keep the moisture level of cured hides low creating an unhospitable environment for the bacteria to survive and a low concentration of antiseptic kill the bacteria at the same time. The evaluation of environmental impacts from process discharge reveals that the alternative hide curing methods reduce pollution loads significantly in comparison with the traditional method in terms of Chloride content, TDS, TS, BOD and solid pollutants. From grain pattern analysis, surface images, mechanical properties and organoleptic evaluation, no detrimental impact has been identified on crust leather made from the experimentally treated hides. Rather, in many cases, alternatively treated hides appear to produce better quality of leather than the control. The results obtained in this study suggest that this technology can be potentially used as an alternative to traditional salt curing to preserve raw bovine hide.

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