Limiting Microbial Activity as an Alternative Approach of Bovine Hide Preservation Part II: Impact of Developed Formulations on Leather Quality and the Environment

by
Majher I. Sarker,* Hailemichael O. Yosief, Cheng-Kung Liu and Nicholas P. Latona
*U.S. Department of Agriculture,** Agricultural Research Service,*
Eastern Regional Research Center, Biobased and Other Animal Co-Products Research Unit
600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

Abstract

Wet salting of bovine hide commonly utilizes 95% saturated salt solution or 40-50% salt (w/w) on raw hide weight for preservation. The salt used for the hide preservation ends up being in wastewater and generates enormous environmental pollution. To minimize the environmental pollution problem associated with the traditional method of hide preservation, alternative formulations containing antimicrobial agents and less amount of common salt (35% saturated brine) have been developed. The alternative formulations were found to be more effective in deterring microbial growth than the traditional formulation as demonstrated by the total aerobic bacterial count of the preserved hide soaking liquor. The effect of the newly developed formulations on leather quality was assessed by analyzing the mechanical properties, scanning electron microscopic images, grain pattern and organoleptic properties of the finished leather. The quality analysis of the crust leather revealed that, the leather panels produced from the traditionally and alternatively preserved hides were comparable. The environmental impact of the newly developed formulations was also evaluated by monitoring the leather processing effluents for the pollution indicators such as total solids (TS), total dissolved solids (TDS), chloride content, Chemical oxygen demand (COD) and Bio-Chemical oxygen demand (BOD). Overall, the environmental impact of the newly developed hide preserving formulation was less severe than the traditionally used formulation. Since the newly developed formulations did not affect the quality of the leather produced and their impact on the environments is minimum, they could be considered as viable options for combatting pollution problems associated with the traditional salt curing method.

Introduction

Bovine hides and skins are non-edible byproducts of the meat industry and they are the main raw materials for leather production. Preservation of raw hides is the first step in the leather production process and it must start right after the raw hide is collected from the slaughterhouse, otherwise the hide will start losing its integrity because of putrefaction. The major component of fresh hide is moisture (60-70%) and the proteins (collagen, elastin, keratin) constitute about 30% of the hide. The integrity of raw hide is an important factor in producing a good quality leather. A variety of microorganisms are found on animal hide and these microorganisms come from different sources such as air, water, soil, manure and others.[1,2] The natural defense system protects skin of living animal from being attacked by bacteria and other microorganisms but the flayed skins become a source of bacterial growth within 5 to 6 h of removal because of the favorable moisture conditions.[3] The normal microflora growing in a hide produces proteolytic and collagenolytic enzymes that damage structural proteins resulting in deterioration of hide. Since the quality of the produced leather relies on the amount of important proteins in raw hide, it is vital to protect proteins from decomposition during hide preservation process.[4]

The main objective of hide preservation is to prevent putrefaction and this is achieved by either killing the microorganism (using bactericidal agent) or by inhibiting the growth of the microorganisms (using bacteriostatic agents) on hides. The bactericidal agents used to kill the microorganisms are usually expensive and not safe to humans or living species. Bacteriostatic agents are dehydrating agents commonly used in bulk like sodium chloride which is responsible

*Corresponding author e-mail address: Majher.Sarker@usda.gov
Manuscript received September 20, 2019, accepted for publication November 7, 2019.
Based on Presentation delivered at 114th ALCA Annual Convention, 2018, Itasca, IL. Title: Limiting Microbial Activity as an Alternative Approach of Bovine Hide Preservation With Less-Salt

**Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture (USDA). USDA is an equal opportunity provider and employer.

for generating salt pollution problems. Pollution problem is assessed by measuring total dissolved solid (TDS) and chloride content in the salt bearing effluents that come from leather processing facility because of the soaking operation.[5]

Traditionally, hide curing is accomplished by soaking the raw hide in 95% saturated salt solution. Close to three fourth of the salt used goes to the discharged stream during soaking, which contributes to more than 40% of total dissolved solids in the tannery wastewater creating huge amount of salt pollution in the environment.[6,7] When the tannery wastewater is dumped to the open field, the high salinity prevents seed germination and the growth of seedlings and other floras.[8] Furthermore, when soils are watered with saline waste, salt is likely to accumulate in the field due to low-soil permeability, inadequate drainage and rainfall. The accumulated salt has a harmful effect on crop production and seriously impacts the environmental.[9,10,11] The discharged salt also causes adverse effect on quality of water and growth of plants by influencing the solubility and release of heavy metals.[12,13,14] Therefore, it is very important to develop eco-friendly hide preservation method where no salt or less salt is utilized.

Many researchers have been actively involved in the development of alternative hide preservation methods with less environmental impact. The alternative methods that have been published could be categorized into the physical and chemical methods of curing hides.[15] The physical methods include cooling,[16] cooling in vacuum,[17] drying chamber[18] and gamma rays or electron beam based irradiation.[19,20] Even though these physical methods are environmentally friendly, most of them are expensive and they have not been adopted in a hide processing facility. For the chemical methods, the chemicals that have been explored for hide preservation include potassium chloride,[21] soda ash,[22] benzalkonium chloride,[23] antibiotics,[24] neem oil[25] and boric acid.[26] However, because of various reasons such as high cost, toxicity, inadequate curing efficiency, severe environmental impact and poor leather quality, most of the chemicals have not found commercial application.

As part of our effort to develop an alternative and environmentally friendly curing method, we previously reported[27] the effectiveness of 35% saturated brine solution in combination with three different biocides in preserving hides for more than a month. This method decreased salt usage by around 60% when compared to the commonly used 95% saturated salt solution. The effectiveness of the developed formulations on hide preservation were evaluated and reported by monitoring different parameters including water activity, microbial growth, texture, moisture content and microscopic analysis of the cured hide during the preservation period. The developed formulations were found to be effective in limiting bacterial growth, controlling yeast and mold growth during more than 30 days of storage.[27] This study is a continuation of the previously reported findings and here we report the effect of the developed formulations on produced leather quality and the impact of the formulations on the environment compared to the conventional hide preservation method.

Experimental

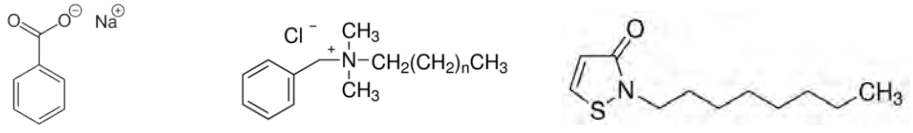
Materials:

Fresh bovine hides were obtained from the local meat packing company, JBS Packerland (Souderton, PA). Each hide was cut into smaller pieces of equal size (12 in x 12 in) that weighed approximately 420–700 g. All the compounds used to prepare the formulations are described in Table 1. They were purchased from vendors as mentioned in part 1 published article[27] and they were of laboratory grade at the time of use. Brine solutions were prepared by dissolving the required amount of salt in tap water at room temperature and the saturation level was confirmed with a salometer. The formulations were prepared as described in Table I by dissolving the chemicals in tap water at room temperature (~22°C) and ~12 h prior to conducting the experiments.

Table I

Composition of the developed hide preserving formulations

Formulations	Composition
F-A (control)	95% saturated brine soln. + 0.043% NaOCl (v/v)
F-B	35% saturated brine soln. + 0.4 % ŧBAC (v/v) + 0.25% ŧNa-B
F-C	20% saturated brine soln. + 1% LA + 0.25% Na-B
F-D	35% saturated brine soln. + 1% LA + 0.25% Na-B + 0.15% NaOH + 0.1% Tween 20
F-E	35% saturated brine soln. + 1% LA + 0.25% ŧOIT + 0.1% Tween 20
F-F	35% saturated brine soln. + 1% LA + 0.4 % BAC (v/v)



ŧNa-B: *Sodium benzoate* ŧBAC: *Benzalkonium chloride* ŧOIT: *2-n-octyl-4-isothiazolin-3-one*

Laboratory Scale Hide Preservation Protocol

The hide pieces were cured by soaking them in 150% float (volume of solution/weight of hide) separately in a 6-in-1 Dose drums which was set to tumble the hide at 6 rpm (Dose Maschinenbau GmbH, Lichtenau, Germany) for 18 h. The control was treated with saturated salt solution (95%) and 0.043% (v/v) bleach (NaOCl) (F-A, Table 1) which is the traditional hide preserving formulation. For the alternative formulations (F-B to F-F, Table 1), a 35% saturated salt solution with different antimicrobials was used in common except F-C, where 20% saturated brine was used. After allowing the treatment for 18 h, extra water was removed from the hide surface and then, they were folded and stored in a humidity chamber at 38-40°C. The hide pieces were periodically observed for possible physical changes such as smell and hair slip which would indicate hide deterioration. The effectiveness of the new hide preservation formulations was evaluated by determining different parameters as described in our previously published studies.[27]

Analysis of Soaking Liquor Generated in Leather Processing

After 35 days of preservation, the hide samples were soaked with 200% (v/w) float of water for 4 h. Then, the soaking liquor of the differently preserved hides was quantitatively measured and analyzed for various pollution load parameters including total solid (TS), total dissolved solid (TDS) and chloride (Cl^-) content according to accepted analytical procedures.[28,29,30,31] Aerobic bacterial colony count, Total carbon (TC) and organic carbon (TOC), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of soaking liquid were also measured to evaluate the overall pollution load resulting from the soaking operation, the first step of leather processing.[28]

Aerobic Bacterial Count of the Soaking Liquor

The soaking liquors from the preserved hide pieces were collected separately and placed in sterile vessels to determine residual aerobic bacterial concentrations. Sterile water was used to prepare serial of dilutions of the collected 1 ml soaking solution. The diluted soaking liquor sample was spread on Tryptic Soy Agar plate and incubated at 37°C for ~24 h. The bacterial colonies were enumerated on the agar and the bacterial population is reported in log CFU/ml. All soaking liquor samples were repeatedly checked three times.

Yeast and Molds Colony Count of Soaking Liquor

Previously published procedure[27] was followed to count yeast and molds colonies in the soaking liquors. Dichloran Rose Bengal chlortetracycline (DRBC) agar was used for this analysis, where DRBC is selective for yeast and mold growth and inhibits bacterial growth. Yeast and molds colonies were enumerated after 4 days of incubation at 22-25°C and expressed as log CFU/mL.

Measurement of TOC and COD in Soaking Liquor

TOC and COD of the spent liquor from the soaking operation were quantitatively determined according to the procedure described in our previous article.[28] The results are reported in terms of g/kg of cured hide.

BOD Analysis of Soaking Liquor

The biochemical oxygen demand (BOD) of soaking liquor represents the amount of oxygen utilized by the microorganisms (biochemical processes) to degrade the organic matters present in the sample. This is a parameter of measuring biodegradable materials in soaking solutions. The BOD measurement was conducted for 5 days according to the previously published procedure.[28]

Tanning Operation of Cured Hides

Following the soaking operation, the hide pieces cured with developed formulations were put in one hair removing drum and the control was put in a different drum. The dehairing process was carried out according to the USDA tanning protocol.[32,33,34] Then all the hide samples were put into a single drum for pickling, tanning, re-tanning, coloring, and fat liquoring steps. The cured hides were processed into crust upper shoe leather and stored in a controlled environment, where the temperature and humidity are maintained at 21°C and 50% (relative humidity) respectively, until the quality tests were performed as described below.

Determination of Leather Quality

The effect of the newly developed hide preserving formulations on leather quality was determined by measuring the mechanical properties of the finished crust leather. The mechanical properties, which included tensile strength, elongation ("stretchability"), Young's Modulus ("stiffness") and fracture energy ("energy required to open unit area of crack surface") were determined as per the procedures mentioned in previous article.[28] Subjective tests (break, handle, fullness and color) on the finished leather products were conducted by in-house (USDA) leather expert.

Microscopic Leather Surface Analysis

Finished leather panels produced from the control and alternatively cured hides (F-A to F-F) were analyzed under a stereo microscope to determine if there is any difference in the grain structure of the finished leather panels. Moreover, scanning electron microscope (SEM) images were examined to evaluate the impact of the new hide preserving formulations on the leather surfaces at microscopic level. The stereo microscope and SEM images were taken according to the published procedures.[28]

Results and Discussion

Five newly developed formulations were evaluated for their effectiveness in preserving bovine hides as well as for their environmental impact. The formulations were made by mixing 35% saturated brine solution (in one case 20% saturated brine solution) with different biocidal agents. These new formulations reduce salt consumption by about 60% in comparison to the traditional hide curing method. The efficacy of these alternative formulations in hide curing has been communicated in recently published article.[27] To address the pollution problem associated with the leather processing, an alternative method to conventional salt curing needs

to be developed. For the five alternative formulations, benzalkonium chloride (BAC), sodium benzoate (Na-B), 2-n-octyl-4-isothiazolin-3-one (OIT) and lactic acid (LA) have been used as biocides dissolved in low concentrated brine as described in Table I. To briefly describe some of the chemicals used in the formulations: sodium benzoate (Na-B) is an FDA approved food additive and is capable of stopping bacteria from reproducing. It also inhibits fungus growth.[35] BAC is a quaternary ammonium compound which is active against gram-positive and gram-negative bacteria and it is also used as a hide preservative.[36] 2-n-octyl-4-isothiazolin-3-one (OIT) is a biocide which has found application in paints, coatings, inks, household-cleaning products, building materials, plastics, textiles and wood treatment solutions.[37] OIT is water miscible and gets rapidly biodegraded to materials that are in turn readily biodegradable, therefore it is unlikely to persist in the environment. In addition, biological wastewater treatment can remove OIT from wastewater, so it has a low risk to be accumulated in the food chain and exhibits low toxicity to soil microorganisms, earthworms and birds.[38] Lactic acid is an FDA approved antimicrobial agent which is commonly used to control microbes in animal carcass and it is also known to be used as a humectant to attract moisture and enhance hydration of the skin, making it useful in reducing the roughness and scaling of the skin.[39,40,41] This research is conducted to overcome the pollution problem associated with the use of saturated brine as a hide curing solution. The new alternative hide curing formulations reduce the salt usage by 60% compared to conventionally used formulation. In this article, we have reported the impacts of using the newly developed hide preserving formulations on the quality of leather and on the environment. In terms of impact, also a comparison study between the alternative and traditional method of leather processing has been evaluated.

Effect of the Developed Formulations on Limiting Bacterial Growth

The effectiveness of the newly developed hide curing formulations on limiting bacterial growth was determined by counting the aerobic bacterial colony of the soaking liquor of the preserved hide samples after 35 days of storage. The bacterial count was quantified and expressed in log CFU/mL as shown in Figure 1. Hide sample treated with F-C was putrefied after 21 days of storage and removed from further investigation. Deterioration of the hide piece was characterized by discoloration, full hair slip and pungent odor. This may have resulted due to the insufficient amount of dehydrating agent (salt) used with F-C. In general, the bacterial counts for all other alternative formulations are lower than the control except for F-D. Especially, F-E and F-F showed the best efficacy in limiting the aerobic bacterial growth on hide surface throughout the storage time of 35 days. Soaking liquors for F-E and F-F count 2.92 and 6.38 log CFU/mL less than the control suggesting that the newly developed formulations are more effective to preserve hide from bacterial attack compared to the control during the storage period.

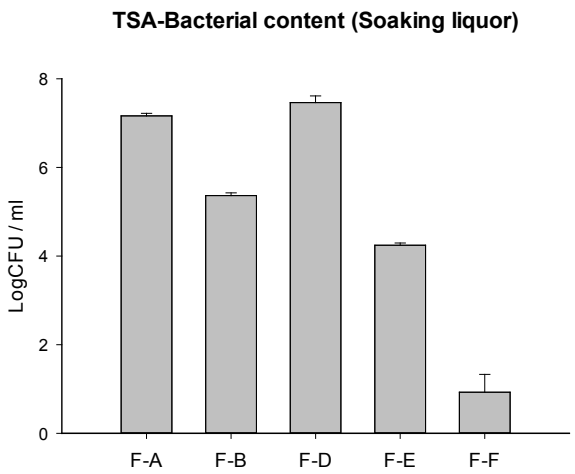


Figure 1. Total Aerobic Bacterial colony count of the soaking liquors from differently preserved hide samples

Effect of the Developed Formulations on Limiting Yeasts and Molds

All the newly developed formulations are found more effective compared to the control (F-A) in limiting yeast and mold growth according to the colony counts in soaking liquors (Figure 2). Yeast and mold recoveries from the soaking liquors are lessen by 4.21, 1.14, 3.88 and 2.64 log CFU/mL for F-B, F-D, F-E and F-F respectively in comparison to the control (F-A).

Determination of Chloride Content

The chloride content in soaking liquor directly reflects the salinity in tannery wastewater.

From Figure 3, it is clearly shown that, the amount of chloride content in soaking liquors from alternatively preserved hides is much less than that from conventionally cured hide (F-A). The chloride content reduction in soaking liquors resulted from the less usage of salt during hide preservation. In comparison to the control, F-D, F-E and F-F formulation treated hides generate 60, 52 and 26% less salt-waste respectively, in their soaking liquors. The variation in chloride content recovery was evolved mainly from the differences among the hide samples, although some of the saturated brine solution was used for curing. The more the

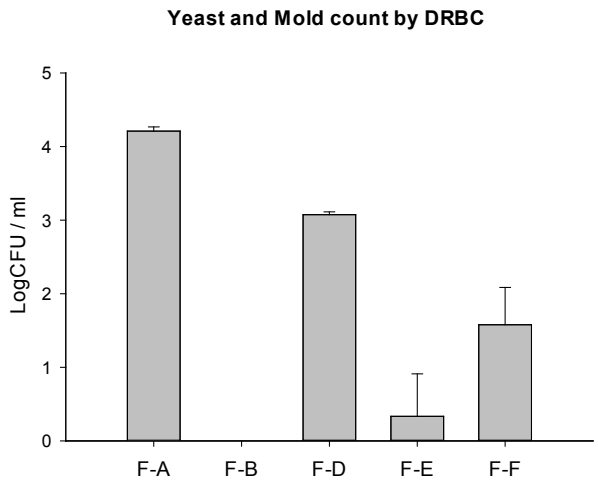


Figure 2. Yeast and molds colony counts of soaking liquors from differently preserved hide samples

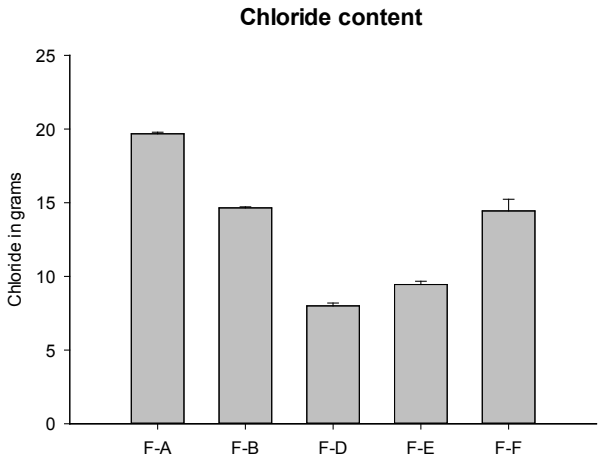


Figure 3. Chloride content (in gram) of used liquid produced from soaking 1 kg of preserved hide.

salt hide samples absorb during curing the more they will release during soaking. The salt absorbing capability of hide samples may vary for the presence of different amount of unexpected materials on internal hide surface, such as fats or associated meat even after using fleshed hide. However, the overall result demonstrates that, upon adoption, the alternative formulations will have much less post leather processing impact to the environment in comparison with the control.

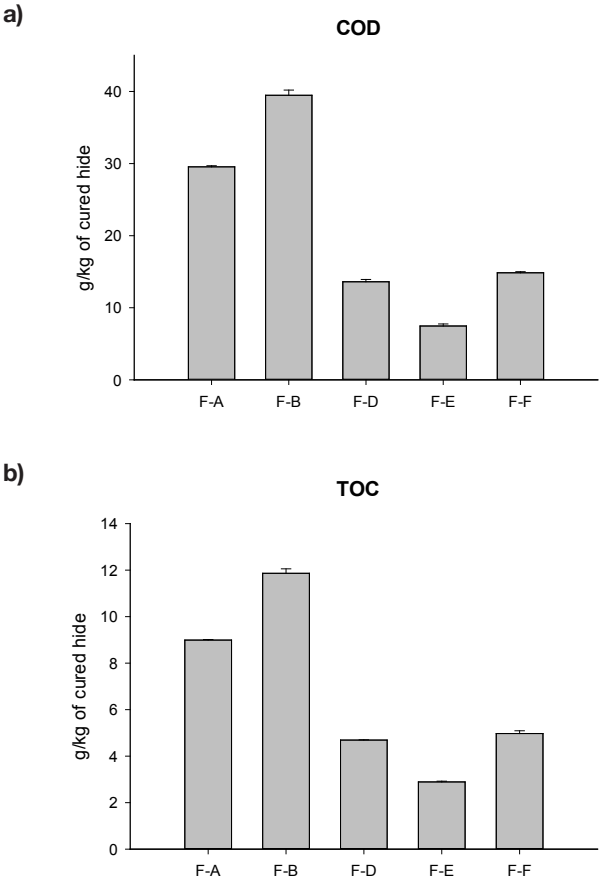


Figure 4. a) Chemical Oxygen Demand (COD) b) Total Organic Carbon (TOC) values of the soaking liquors of the hides preserved by each formulation. Both the values are expressed in g/Kg of cured hide.

Measurement of TOC and COD in Soaking Liquor

The COD and TOC results are found to be similar to each other with the highest value for F-B treated sample (Figure 4). For the other alternatively treated samples (F-D, F-E and F-F), COD and TOC values are significantly less than the control. The possible reason of having the highest TOC and COD values for F-B is that it contains the antimicrobial agents with the high number of carbon atoms compared to other antimicrobial agents used and therefore, a large amount of oxygen is needed to oxidize those carbons coming from the antimicrobial agents. On the other hand, the high COD and TOC values for the control (F-A) is related to the growth of bacteria, yeast and molds which is consistent to the results found in Figure 1 and 2. The presence of high level microorganisms during the hide preservation leads to degradation of proteins resulting in high carbon content in the soaking solution, which in turn requires large amounts of oxygen for the oxidation process.

Determination of BOD in Soaking Liquors

BOD analysis on the soaking solutions of the differently cured hides was carried out over a five-day period. As shown in Figure 5 the BOD load of the soaking liquor of the control is higher than the soaking liquors of alternatively cured hides. According to 5th day data, a 31 to 94% lower BOD load is recorded for the soaking liquors of alternatively preserved hides than the traditionally cured hide. The high BOD value for the control (F-A) could be because of the presence of high concentration of living microorganism (bacteria, yeast and mold) and also the subsequent microbial degradation of the hide. This is consistent with the bacterial, yeast and mold colony count results shown in Figure 1 and 2. The rapid increase of BOD value for F-A in the first 40 h also suggest the presence of high concentration of living microorganism in soaking liquor initially. In addition to the other biochemical substances, the dead cells of microorganisms could serve as a source of nutrients for the living cells present in the soaking liquor.

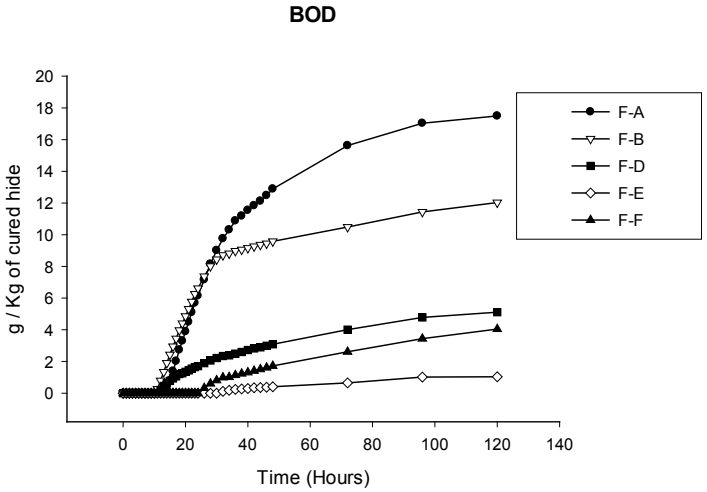


Figure 5. Biochemical Oxygen Demand (BOD) values of the soaking liquor measured over 5 days period.

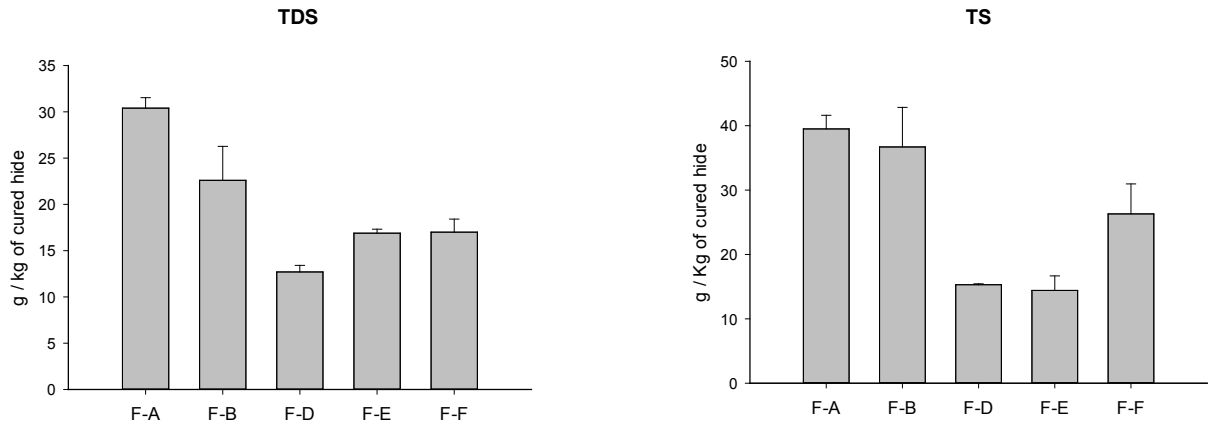


Figure 6. Solid pollutants generated from soaking process of 1 kg of differently cured hides: Total Solid (TS) and Total Dissolved Solid (TDS).

Determination of Solid Pollutants in Soaking Liquors

According to Figure 6, most of the soaking liquors from alternatively cured hides result in a significant decrease in both total solid (TS) and total dissolved solid (TDS) loads. Specially, F-D and F-E brought down the solid pollutants by more than 50% when compared to the control (F-A). This significant reduction in the TS and TDS values is because of the reduction in the amount of salt used for preservation with the newly developed formulations.

Quality Analysis of Crust Leather

Following the USDA standard tanning protocol, the hide pieces, preserved by the traditional and the newly developed formulations, were processed into crust upper shoe leather.

Grain Surface Analysis of Leather

The grain structure of each finished leathers was analyzed under a stereo microscope. The analysis was conducted to see the effect of each formulation on the surface fineness or coarseness of the crust leather. As shown in Figure 7, no noticeable difference was observed between the grain structure of leather produced from the traditionally cured hides and leathers produced from the alternatively cured hides. To further evaluate the surface feature of the crust leather a stereo microscopic image was captured at the crease upon folding the leather panels (Figure 8). Again, there was no noticeable difference between the leathers produced from the differently cured hides, indicating that none of the developed formulations caused harm to the hide grain.

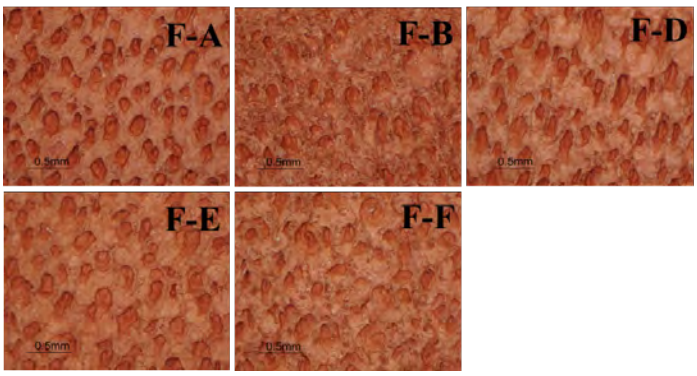


Figure 7. Stereo microscopic images of the crust shoe leather made from hides cured with different formulations.

Surface Analysis of Crust Leather using Scanning Electron Microscope

Surfaces of the crust leather produced from the traditionally and alternatively preserved hides were analyzed using SEM at 100 x magnification. As shown in Figure 9, there was no significant difference

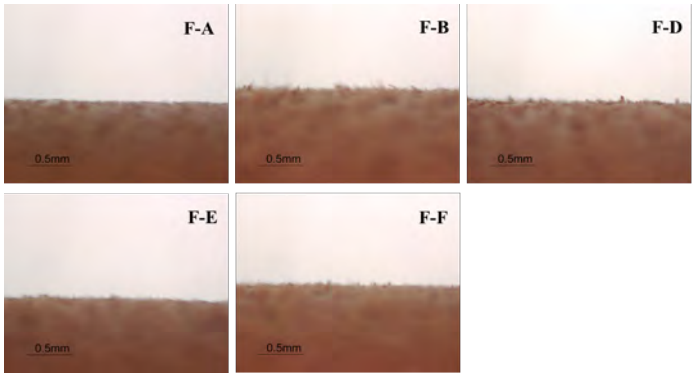


Figure 8. Stereo microscopic images of the leathers at the crease

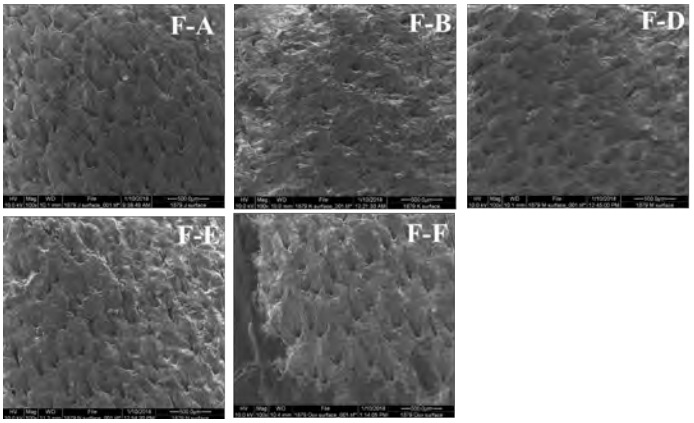


Figure 9. SEM surface images of crust leathers from the differently preserved hides

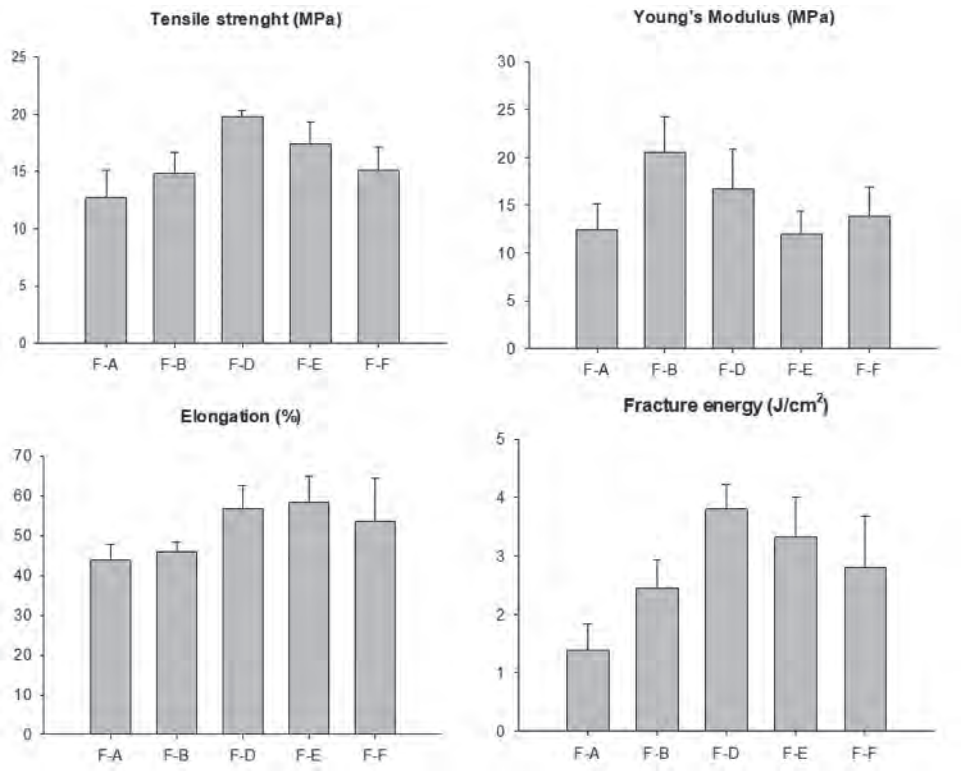


Figure 10. Mechanical properties of the finished leathers made from hides cured with the different formulations

observed when the experimental samples (F-B to F-F) were compared with the control (F-A). Therefore, no detrimental impact from the newly developed hide curing formulations on finished leather.

Determination of Mechanical Properties of Leather

From Figure 10, it is evident that the new hide preserving formulations do not have any adverse effect on the finished leather in terms of property analysis (Tensile Strength, Young's Modulus, Fracture Energy and Elongation). The overall mechanical properties of the leather produced from the experimentally preserved hides were found comparable or even better than that produced from the control. For instance, leather produced from F-E cured hide exhibited improved property in every parameter as shown in Figure 10 in comparison to the quality of leather yielded from traditionally preserved hide sample (F-A).

Subjective Test Analysis of Leather

In-house expert on leather assessed the crust shoe leather panels for their fullness, softness, color, grain tightness (break) and general appearance by hand and visual examination (Figure 11). The subjective test results were expressed on a scale of 0-5 points with 0 being the worst and 5 being the best subjective test outcome.

The leathers produced from the experimentally cured samples displayed similar fullness, grain, handle, color and general appearance. Overall, the alternatively preserved hides produced leathers of similar quality to the traditionally processed leather (F-A), indicating that the biocides utilized with the new formulations do not damage the hides.

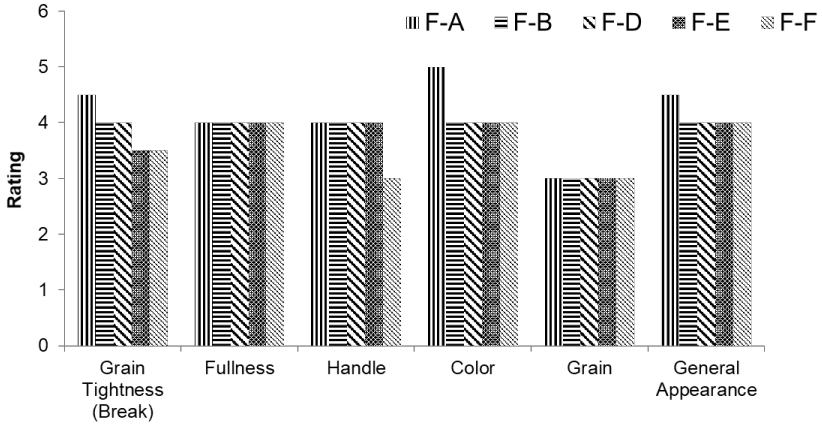


Figure 11. Organoleptic evaluation of crust leathers from differently cured hides

Conclusion

The inventive formulations have demonstrated to be effective in preserving bovine hides by preventing bacterial growth on the hides better than the traditional formulation (95% saturated salt solution) for 35 days or more. The new formulations utilize around 35% saturated brine solution, which is around 60% reduction in salt usage compared to conventional formulation. While the dehydrating brine of the formulation keeps the moisture level of the preserved hide low creating inconvenient condition for the microbes to grow, the low concentration antimicrobial agents inhibits or kills the bacteria simultaneously. The environmental impact studies reveal that pollution loads of the process discharge from the alternatively cured hides are significantly lower than the traditionally cured hide as demonstrated by the measured values of chloride content, TOC, COD, BOD, TDS. From the SEM surface images, mechanical properties, grain pattern analysis and organoleptic evaluation, the newly developed formulations do not appear to have any negative effect on the finished crust leather. The results of all the experiments conducted in this study suggest that, these new formulations could be considered as viable alternatives to the conventional salt curing formulation.

Acknowledgments

The authors would like to thank Vincent Hui and Wilbert Long, III for their help in soaking liquor analysis. We would also like to thank Joe Uknalis for preparing and taking SEM micrographs of our samples.

References

1. Sundar, V. J., Muralidharan, C., Mandal, A. B.; Salinity reduction through synergetic substitutes: Mgo aided low salt skin preservation. *Leather and Footwear Journal.* **12(4)**, 285-294, 2012.
2. Bailey, D.G.; The Preservation of hides and Skins. *JALCA* **98**, 308-319, 2003.
3. Birbir, M., Ilgaz, A.; Isolation and identification of bacteria adversely affecting hide and leather quality. *J. Soc. Leather Technol. Chem*. **80**, 147-153, 1995.
4. Shede. P.N., Kanekar, P.P., Polkade, A.V., Sarnaik, S.S., Dhakephalkar, P.K., Chiplonkar, S.A., Nilegaonkar, S.S.; Effect of microbial activities on stored raw buffalo hide. *J.Environ. Biol.* **30(6)**, 983-8, 2009.
5. Wu, J., Zhao, L., Liu, X., Wuyong Chen, W., Gu, H.; Recent progress in cleaner preservation of hides and skins. *Journal of Cleaner Production*. **148**, 158-173, 2017.
6. Kanagaraj, J., Senthilvelan, T., Panda, R.C., Kavitha, S.; Eco-friendly waste management strategies for greener environment towards sustainable development in leather industry: a comprehensive review. *Journal of Cleaner Production*. **89**, 1-17, 2015.
7. Hashem, A., Arman, N., Sheikh, H. R., and Islam, M.; Sodium Chloride Substitute for Lower Salt Goat Skin Preservation: A Novel Approach. *JALCA* **112**, 270-276, 2017.
8. Hausam, W.; Behavior of hide during salting and the action on the hide protein. *JALCA* 35-44, 1951.
9. Ramasami, T., Rao, J.R., Chandrababu, N.K., Parthasarathi, K., Rao, P.G., Sarvanan, P., Gayathri, R., Sreeram, K.J.; Beamhouse and tanning operation: Process chemistry revisited. *J. Soc. Leather Technol. Chem*. **83**, 39-45, 1999.
10. Eynard, A. Lal, R., Wiebe, K.; Crop Response in Salt-Affected Soils. *Journal of Sustainable Agriculture*. **27(1)**, 5-50, 2005.
11. Erdei, L., Kuiper, P.C.J.; The effect of salinity on growth, cation content, Na+-uptake and translocation in salt-sensitive and salt-tolerant Plantago Species. *Physiol. Plant*. **47(2)**, 95-99, 1979.
12. Valipour, M.; Drainage, waterlogging, and salinity. Archives of Agronomy and Soil Science. **60 (12)**, 1625–1640, 2014.
13. Hanin, M., Ebel, C., Ngom, M., Laplaze, L., Masmoudi, K.; New Insights on Plant Salt Tolerance Mechanisms and Their Potential Use for Breeding. *Front. Plant Sci.* **7 (1787)**, 1-17, 2016.
14. Shrivastava, P., Kumar, R.; Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi Journal of Biological Sciences*. **22**, 123–131, 2015.
15. Sivakumar, V., Mohan, R., Muralidharan, C.; Alternative methods for Salt free / Less salt short-term preservation of hides and skins in leather making for sustainable development – A review. *Text Leath. Rev.* 2 (1) 46-52, 2019.
16. Babu, N.K.C., Karthikeyan, R., Kumari, B.S., Ramesh, R., Shanthi, C., Sadulla, S.; A systematic study on the role of chilling temperatures on the curing efficacy of hides and skins. *JALCA* **107 (11)**, 362-374, 2012a.
17. Gudro, I.; Valeika, V.; Sirvaityte, J.; Short term preservation of hide using vacuum influence on properties of hide and of processed leather. *PLos ONE*. **9(11)**, 1-9, 2014.
18. Waters, J.J., Stephen, L.J., Sunridge, S.; Controlled drying. *J. Soc. Leather Tech. Chem*. **65**, 32, 1997.
19. Bailey, D.G.; Gamma Radiation Preservation of cattle Hides. A New Twist on an Old Story. *JALCA* **70**, 248-260. 1975.
20. Bailey, D.G., DiMaio, G.L., Gehring, A.G. and Ross, G.D.; Electron Beam Irradiation Preservation of Cattle Hides in a Commercial-Scale Demonstration. *JALCA* **96**, 382-392, 2001.
21. Bailey, D.G., Gosselin, J.A.; The preservation of animal hides and skins with potassium chloride. *JALCA* **91**, 317-333, 1996.
22. Rao, B.R., Henrickson, R.L.; Preservation of hides with soda ash. *JALCA* **78**, 48-53, 1983.
23. Cordon, T.C., Jones, H.W., Naghski, J., Jiffee, J.W.; Benzalkonium chloride as a preservative for hide and skin. *J. Soc. Leather Trade Chem*. **59**, 317-26, 1964.
24. Berwick, P.G., Gerbi, S.A., Russel, A.E.; Use of antibiotics for short term preservation. *J. Soc. Leather Trade Chem*. **74**, 142-50, 1996.
25. Venkatachalam, P.S., Sadulla, B., Duraisamy, V.S., Krishnamurthi; Short-term preservation of hide with neem oil. *J. Soc. Leather Tech. Chem*. **61**, 24, 1977.
26. Kanagaraj, J., John Sundar, V., Muralidharan, C., Sadulla, S.; Alternatives to sodium chloride in prevention of skin protein degradation-a case study. *Journal of Cleaner Production*. **13**, 825-831, 2005.
27. Sarker, M.; Long, W.; Liu, C-K.; Limiting Microbial Activity as an Alternative Approach of Bovine Hide Preservation, Part I: Efficacy of Developed Formulations. *JALCA* **114**, 271-278, 2019.

28. Sarker, M.; Long, W.; Piazza, J. G.; Latona, N.P.; Liu, C-K.; Preservation of Bovine Hide using Less Salt with low Concentration of Antiseptic, Part II: Impact of developed Formulations on Leather Quality and the Environment. *JALCA* **113**, 335-341, 2018.
29. Sivaparvathi, M., Nandy, S.C.; Evaluation of preservatives for skin preservation. *JALCA* **69**, 349-62, 1974.
30. Eston, A.D.; Clesceri, L.S.; Greember, A. E.; Standard method of the examination of water and wastewater. *American Public Health Association*, **19**th ed. Washington, DC, 1995.
31. ASTM D4458-94 Standard test method for chloride ions in brackish water, seawater, and brines. *ASTM International*, West Conshohocken, PA, 1994.
32. Taylor, M.M.; Diefendorf, E.J.; Phillips, J.G.; Feairheller, S.H.; Bailey, D.G.; Wet process technology 1. Determination of precision for various analytical procedures. *JALCA* **81**, 4-18, 1986.
33. Cabeza, L.F, Taylor, M. M., DiMaio, G. L., Brown, E. M., Marmer, W. N., Carrio, R., Celma, P. J., Cot, J.; Processing of Leather Waste: Pilot Scale Studies on Chrome Shavings. Part II. Purification of Chrome Cake and Tanning Trials. *JALCA* **93(3)**, 83-98, 1998.
34. Long, W.; Sarker, M.; Marsico, R.; Ulbrich, L.; Latona, N.; Muir, Z.; Liu, C-K.; Efficacy of citrilow and cecure spray wash on prevalence of aerobic and enterobacteriaceae bacteria/gram-negative enteric bacilli and cattle hide quality. *J. Food Safety*. **e12441**, 1-7, 2018.
35. Krebs, H.A.; Wiggins, D.; Stubbs, M.; Sols. A.; Bedoya, F.; Studies on the mechanism of the antifungal action of benzoate. *Biochem. J.* **214(3)**, 657-663, 1983.
36. Garcia, M.R.; Cabo, M.L.; Optimization of E. coli inactivation by benzalkonium chloride reveals the importance of quantifying the inoculum effect on chemical disinfection. *Frontiers in Microbiol.* **9**, 1259, 2018
37. Document II-A: Study Summaries–Dossier for Active Substance 2-Octyl-2H-isothiazol-3-one (OIT), Product type 6, Incan preservatives, RMS: United Kingdom. *Rohm and Haas Company*. Pages 3, 20, 49, 60, 62, 64, 68, 71, 74, 78, 79, and 80, 2008.
38. KATHON™ 893T BIOCIDE Material Safety Data Sheet, Ecological Information, *The Dow Chemical Company*. 2011.
39. Long, W.; Sarker, M., Liu, C-K.; Evaluation of novel decontamination wash formulations on bovine hides for meat safety and leather quality assurance. *Adv. J. Food Sci.Technol.***14(2)**, 33-41, 2018.
40. Mies, P. D., Covington, B. R., Harris, K. B., Lucia, L. M., Acuff, G. R., Savell, J. W.; Decontamination of cattle hides prior to slaughter using washes with and without antimicrobial agents. *J. Food Prot.* 579-582, 2004.
41. Lynde, CW.; Moisturizers: what they are and how they work. *Skin Therapy Lett*. **6(13)**, 3-5, 2001.