Development of an Alternative Low Salt Bovine Hide Preservation using PEG and Crude Glycerol, Part I: Evaluation of PEG Molecular Weight Fractions

by

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Abstract

Brine curing using sodium chloride is widely used for the short-term preservation of cattle hides. Due to economics and efficiency, it has become the traditional method of hide preservation used by meat packers, hide processors and tanners worldwide. But brine curing is known to cause serious effluent pollution problems in the environment. The main objective of the current study was to develop an alternative brining process that requires less salt to effectively preserve bovine hides by incorporating dehydrating agents. In low salt preservation, the anticipated hardening effect due to over dehydration caused by polyethylene glycol (PEG) polymers was compensated by adding crude glycerol and sodium carbonate. Low molecular weight PEGs were more effective than high molecular weight polymers. Preservation was found to be effective because no sign of putrefaction was observed in alternatively cured hides, and the properties of these hides were comparable to those of traditionally preserved hides. When PEG was incorporated, the salt concentration required for hide preservation could be reduced.

Introduction

Currently, almost 95% of the total U.S. hides (40 million annually) are exported abroad where they are processed to higher value leather, which is used for car seats, garments, shoes, and other products. Proper preservation of U.S. hides is essential during storage and transit because the hide will rapidly decay without preservation.[1,2] The most common preservation method utilized by the hides and leather industry is brining or salt curing. Salt works by abstracting water from hide so that the growth of bacteria is inhibited. There are a number of advantages of salt curing namely low cost, reliability, wide availability, ease of handling, and low toxicity, when safety measures are observed.[2] But the disadvantages are also significant as large quantities of salt are used (40-50% based on the raw hide weight), and processing time and high water consumption are needed to wash out salt and rehydrate the raw stock. The plant effluent contains high salinity pollution load if the spent salt is not isolated. The spent salt is rich in bacteria and therefore reusing it is not recommended. The disposal cost for the spent salt is quite expensive.[3-5] Because of the negative environmental impact of the high salt preservation, alternative low salt and salt-less preservation processes are urgently needed. The main objective of the current study is to develop an alternative brining process that requires less salt to effectively preserve bovine hides. The protocol is to investigate potential effects of incorporating polyethylene glycol (PEG) of different molecular weights[5] with lower salt concentrations in the presence of crude glycerol and sodium carbonate[6,7] for curing bovine hides for short-term preservation.

Previous researchers have tried several alternative salt-less or low salt preservation techniques, with chemicals or with radiation, as summarized shortly. The two principal radiation types used are gamma rays (photon emissions from radioactive materials) and electron beams.[8] High-speed electrons are used to sterilize the hides. If the hides are sterile and the enzymes in them are inactivated, non re-infected hides will retain the properties of fresh raw hides.[8] Prior efforts at improving hide preservation used boric, benzoic and acetic acids,[3,9] sulfites,[4,9] and PEG.[5] In Zimbabwe, the short term preservation of hides is based on sun drying and chilling, as well as treatment with bactericides such as benzalkonium chloride, boric acid, vantocil (IB), Busan 30 and Busan 52 that have been reported

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to yield better results in comparison with conventional salt cured method.[10] Research was conducted by the Agricultural Research Service, U.S. Department of Agriculture and Kalium of Canada, Ltd. to determine the effectiveness of potassium chloride (KCl, one form of potash) as a substitute for sodium chloride (NaCl) for the brine curing of cattle hides.[11] The potassium chemical can provide a necessary plant macro nutrient and the spent brine can be applied directly to the soil as a fertilizer. In fact, a large scale demonstration of raceway curing of over eighteen hundred hides showed that KCl was technically a viable alternative to NaCl.[11] The KCl-cured hides were found to be comparable, with only minor differences, to NaCl-cured hides for leather production. The only drawback then is that KCl costs more than NaCl, and the use of KCl is not economically viable even considering the cost of NaCl disposal.[11]

During long term storage, the drying of the salted hides provides some extra stability.[1] Adding a dehydrating agent such as PEG that is known to absorb moisture, is one way to reduce the amount of salt needed in hide preservation.[5] In a different study, high molecular weight, solid PEGs were successfully used as dehydrating agents in concentrating purified dilute membrane protein samples through dialysis bags for crystal growing trials, and the protein still maintained its native state.[12] In this study crude glycerol and sodium carbonate are incorporated into the brining process. The crude glycerol is a bountiful and inexpensive co-product of biodiesel production. In our recently concluded research project, the inclusion of crude glycerol and sodium carbonate in the presoaking solution proved to be beneficial in enhancing the efficiency of the adobe type manure removal from bovine hides.[6, 7] The added benefit observed was the improvement in the overall quality of finished leather derived from them.[6] Sodium carbonate and bicarbonate have shown limited effectiveness against bacteria, particularly strains of halophilic bacteria,[3] and therefore 1% sodium carbonate has been utilized in our current study to help inhibit some bacterial growth.

PEG refers to a polymer or an oligomer of ethylene oxide and is also known as polyethylene oxide (PEO) or polyoxyethylene (POE), depending on its molecular weight. The three names are chemically synonymous, but historically PEG referred to oligomers and polymers with a molecular mass below 20,000 g/mol, PEO to polymers with a molecular mass above 20,000 g/mol, and POE to a polymer of any molecular mass.[13] PEG and PEO are liquids or low-melting solids, depending on their molecular weights. PEGs are prepared by polymerization of ethylene oxide and are commercially available over a wide range of molecular weights from 200 g/mol to 10,000,000 g/mol. While PEG and PEO with different molecular weights find use in different applications, and have different physical properties (e.g. viscosity) due to chain length effects, their chemical properties are nearly identical.

Experimental

Materials

All the chemicals used for the conventional processes are of commercial grade. The chemicals used for the preparation of the different alternative brining formulations are of analytical grade. The crude glycerol was obtained from Griffin Industries (Butler, KY) and was used as received. Fresh hides were obtained from a local beef cattle processing plant (JBS, Souderton, PA). The different PEGs and sodium carbonate were obtained from Sigma-Aldrich, (St. Louis, MO). The bulk NaCl, TX-10, was obtained from Superior Salt LLC, (Watkins Glen, NY).

Laboratory Scale Protocol for the Alternative Hide Preservation

Freshly washed and defleshed hides were collected and split down the back into left and right segments. The sides were then cut into groups of 2 that weighed approximately 600g each, with dimensions of ~6 in x 8 in, with the longer side parallel to the backbone. The procedure developed by Kannan *et al*.[5] for preservation of goatskins provided a basis for this study. Modifications to that procedure were made because of differences in substrate, thick cattle hides rather than the thinner goatskins, and to make the process more compatible with current US brining operations. These initial trials (Table I) were run with 20% NaCl alone, and 10% NaCl plus 5% crude glycerol (CG) and 1% sodium carbonate (SC) as controls. PEGs with molecular weights of 200, 400, 600, 1000, 1400, 3000 and 8000 were added to the low salt brining solution at a 2.5% concentration. PEG 200, 400 and 600 were in liquid state. While PEG 1000, 1400 (waxy solid), 3000 and 8000 were in solid state as flakes. PEG1400, 3000, and 8000 were each used twice. In drums D#3 - D#5, the solid polymers were applied to the hide pieces after they were soaked in 10% NaCl, 5% CG, and 1% SC. In drums D#10 – D#12, the high molecular weight PEGs were dissolved in the soaking solution before the hide pieces were added. The hides were allowed to soak in their respective solutions in individual drums overnight at 6 rpm. The following day, the differently cured hides were hung to dry and the moisture content or rate of dehydration was monitored.

Scanning Electron Microscopy

From the preserved hide samples, ~1.5mm pieces were cut and mounted on stubs and sputter-coated with thin layer of gold using a Scancoat Six Sputter Coater (Edwards Scancoat 6, West Sussex, UK) for 90 seconds in two orientations. The samples were then observed with FEI Quanta 200 FEG Environmental Scanning Electron Microscope (SEM) (Hillsboro, OR, USA) in high vacuum-secondary electron imaging mode with an accelerating voltage of 10KV. Digital images were collected at 100, 500, 1000, 5000 and 10,000 x magnification.

TABLE I
List of alternative curing formulations.

Sample code	Curing solution ingredients
D#1	20% NaCl
D#2	10% NaCl + 5% Crude Glycerol (CG) +1% Sodium Carbonate (SC)
D#3	10% NaCl + 5% CG + 1%SC + 2.5% PEG 1400 (added solid)
D#4	10% NaCl + 5% CG + 1% SC + 2.5% PEG 3K (added solid)
D#5	10% NaCl + 5% CG + 1% SC + 2.5% PEG 8K (added solid)
D#6	10% NaCl + 5% CG + 1% SC + 2.5% PEG 200
D#7	10% NaCl + 5% CG + 1% SC + 2.5% PEG 400
D#8	10% NaCl + 5% CG + 1% SC + 2.5% PEG 600
D#9	10% NaCl + 5% CG + 1% SC + 2.5% PEG 1000
D#10	10% NaCl + 5% CG + 1% SC + 2.5% PEG 1400
D#11	10% NaCl + 5% CG + 1% SC + 2.5% PEG 3000
D#12	10% NaCl + 5% CG + 1% SC + 2.5% PEG 8000

Moisture and Ash Content Determination

The percentage of moisture and ash were determined by applying the American Standard Testing Method International D7476-08. [14] Dehydration was monitored by analyzing the moisture content at the end of the designated time periods from 0 to 192 h. For the dehydration study, 0 h was at the completion of a 24 h soak. Approximately 2g samples were taken from each treated hide to be used for percent moisture and ash testing in triplicate trials. Samples were weighed in a crucible, and then placed in a vacuum oven for 3 h at 60°C and 100°C overnight. The percent moisture was calculated from the weight of the sample obtained the following day after equilibration at ambient temperature and pressure. The dried samples were placed in a furnace for 1 h at 100°C and then at 600°C overnight.[14, 16] The percent ash was calculated from the weights of the ash of the samples compared to their original weights. To evaluate the amount of salt retained by the cured hides, the percent brine saturation value (% BSV) was obtained by the following equation

$$\% \text{ BSV} = (\text{ash/moisture} \times 100) / 35.9$$

where 35.9 is the percent salt to moisture ratio in a saturated NaCl (brine) solution.[14, 1]

Texture Analysis of the Cured Hides

To compare the relative hardness of the resulting cured hides, texture analysis was performed on dried hide samples using a Brookfield CT3 texture analyzer, Middleboro, MA.[15] Using the TA10 probe at a 20g load for 5-7 mm of penetration, the hardness, hardness work cycle, adhesiveness, total work, load at target, and deformation at target were determined. The amount of work needed to attain the same deformation at certain predetermined peak load on the cured dry hide, the ratio of total work done to total hardness was calculated for each sample.[6,15] These data points were taken three times at different locations on the sample to improve homogeneity of the results.

Rehydration of Cured Hide Samples

The control and alternatively cured bovine hide samples were stored for 2 weeks and then rehydrated. The preserved hide pieces were soaked to remove excess salt and to determine the rehydration rate. Soaking was in 150% float of water and biocide (0.15% Boron TS and 0.10% Proxel, based on recorded dry weight) in Dosemat mini drums for 18 hours at 6 rpm. During the first 6 hours of the rehydration study, aliquots from the float solution were extracted and a hide sample was cut at each hour to determine the concentration of sodium chloride suspended in the float and brined hide sample.

RESULTS AND DISCUSSION

In order to optimize the molecular weight fraction and level of PEG to be used, an initial set of trials was performed using small hide pieces (~600g each) and soaking formulations with 10% NaCl, 5% CG, 1% SC, and various PEGs. The drying process was relatively fast at ambient temperature and pressure because of the small size of the hide samples. All the preserved hide samples appeared similarly cured having dried with no apparent odor or slippage of hair from the grain. The absence of excess moisture in the hide inhibited microbial growth that could have led to hide damage through putrefaction. After allowing the cured hide pieces to dry, all samples were rigid to the touch and those cured with PEGs seemed firmer than the control hides.

Scanning Electron Microscopy of Cured Hide Samples

The microscopic (SEM) images (Figure 1) can demonstrate changes in the fibrillar structure of the cured hides. The first two micrographs show very open structure in the absence of PEG. The next two micrographs for curing formulations containing PEG200 and PEG400 show relatively open interfibrillar structure, suggesting that rehydration may not be overly hindered. With higher molecular weight PEGs, the fibers appear to stick together.

Moisture and Ash Content

Data (Table II) were gathered including moisture and ash content and an estimate of the brine saturation value (BSV) of the cured hide pieces containing 40% or more moisture.[14, 16] The moisture content in raw hide was found to be ~70%. After 24 h curing with 20% NaCl, as a control for the low salt brining in Table II, the

moisture content came down to ~64%. The inclusion of crude glycerol and sodium carbonate with 10% NaCl brought the moisture content down to ~58%. When PEGs were included, the average moisture content was ~53-55%. These values were all too high to effectively inhibit microbial growth. The critical moisture content where microorganisms could not thrive was attained at around 96 h when the moisture content was lower than 50%.[2]

After two weeks of storage at ambient conditions, the PEG dehydrating effect was more pronounced; the moisture content was much lower than the critical moisture content necessary to inhibit microbial growth. BSV values which give an indication of salt retention by the cured hides, were surprisingly similar for hides treated with 20% NaCl with or without the crude glycerol, sodium carbonate, and PEGs, suggesting that these additives make little contribution to the ash content.

Texture Analysis

To obtain a semi-quantitative measure of the hardness of each cured hide sample, and to compare the texture of the control to the differently cured hide samples, texture analysis was performed on samples from the initial trial, and the results are shown in Figure 2.

The hardness of each sample was obtained by calculating the total work required to cause the same preset deformation on each hide sample.[6, 15] The harder or tougher the sample is underneath the grain layer, the higher the total work needed in mJ per g average load at target, or hardness. In the current study, we measured the work needed to cause the same deformation of 5-7 mm into the preserved dried hide samples. Overall, the alternatively preserved hides were slightly softer than the control samples, traditionally cured hides.

Rehydration of the Cured Hides

Before tanning the preserved hides, the first step is to remove the excess salt. At the same time, the relatively dried and hardened hide has to be rehydrated and softened prior to processing so that the beaming and tanning chemicals can be used effectively. After

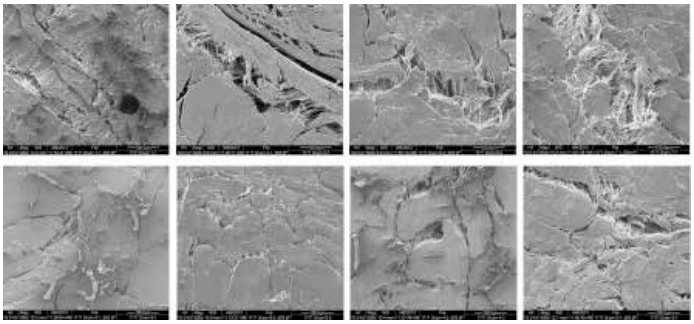

Figure 1. Crosswise SEM views of cured and toggled hide samples at 500x magnification. Curing formulations were top row left to right 20% NaCl; 10% NaCl with 5% CG and 1% SC; 10% NaCl with CG, SC, 2.5% PEG200; and 10% NaCl with CG, SC, and 2.5% PEG400 and lower row left to right 10% NaCl with CG, SC, and 2.5% PEG600; 10% NaCl with CG, SC, and 2.5% PEG1000; 10% NaCl with CG, SC, and 2.5% PEG3000; 10% NaCl with CG, SC, and 2.5%PEG8000.

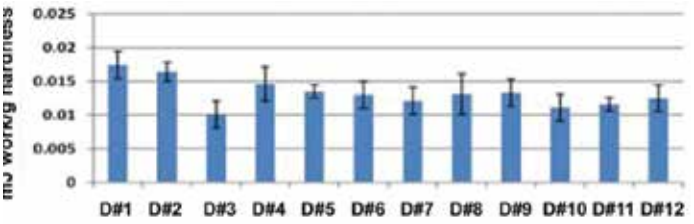

Figure 2. The relative hardness of the differently cured hide samples

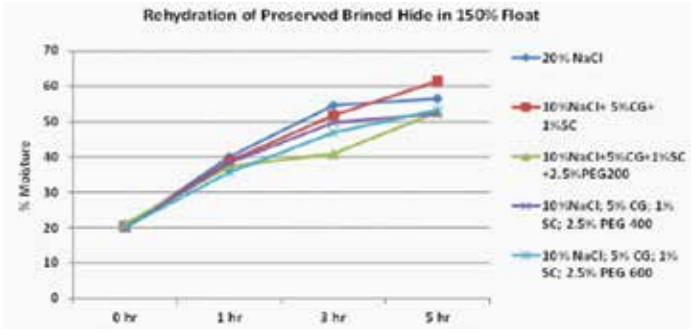

Figure 3. Trend of the rehydration of differently cured hides

TABLE II
Ash to moisture ratio and the percentage brine saturation of the cured hide pieces after 24 h soaking in alternative brining solutions.

Curing solution ingredients	% Ash	% Moisture	A:M ratio	BSV
20% NaCl (W/V)	9.47	64.03	14.8	41.22
10% NaCl; 5% CG; 1% SC	2.72	57.76	4.71	13.12
10% NaCl; 5% CG; 1% SC; 2.5% PEG 200	2.53	54.15	4.68	13.03
10% NaCl; 5% CG; 1% SC; 2.5% PEG 400	2.58	55.15	4.68	13.03
10% NaCl; 5% CG; 1% SC; 2.5% PEG 600	2.73	53.97	5.07	14.12

two weeks storage, the moisture content in each of the preserved and relatively dried hide samples was nearly the same at ~20%.

This was much lower than the critical moisture content where the microorganisms can grow and thrive (>50%), thus the curing was quite effective in all the tested conditions because microbial growth was inhibited. The rate of rehydration using just the base ingredients which were present in all the test solutions, composed of 10% NaCl + 5% crude glycerol and 1% sodium carbonate, was similar to that obtained with 20% NaCl alone. When using the alternative preserving solutions with PEG 400 and PEG 600, relatively stable moisture content of about 50% was reached after 3 h; when using PEG 200, it took nearly 5 h to reach 50%. After 5 h the moisture content increased to nearly 60% for 10% NaCl, 5%CG and 1% SC, ~70% moisture was found originally in raw hides. Potentially, less water might be used in the rehydration and preparation for tanning of these hides to leather because the amount of salt was already quite low. Thus the amount of wastewater discharged into the environment could also be lowered considerably.

Conclusions

The incorporation of low molecular weight PEG with lower salt concentrations shows promise for the development of low-salt preservation of cattle hides prior to tanning. The presence of glycerol counteracted the tendency for over drying caused by PEG, thus ameliorating the negative effects of excessive drying including a tighter or more intact interfibrillar structure that would require a longer rehydration period. In fact, the low salt curing was as effective as the high salt control in hide preservation and yet the hide was softer. Rehydration before tanning would take a shorter time because the amount of chloride is already relatively lower in the newly developed low salt preservation process. The ash to moisture ratio values were also closely related, suggesting that the additives, such as PEG, crude glycerol and sodium carbonate, did not contribute to any additional ash content or its total dissolved solids. The low molecular weight PEGs are relatively inexpensive, and as liquids, easy to work with. For these reasons and because the SEM images and texture analysis suggested that any detrimental effects would be minor, PEG200, PEG400 and PEG600 were selected to pursue further experiments. It is imperative to pursue the promising low molecular weight PEGs in larger scale hide preservation studies, finish the cured hides to crust leather and verify its quality by measuring mechanical properties.

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References

1. Leach, I. and Wilson, R. T.; Higher value addition through hides and skins, FAO Diversification booklet number 8. Rome. 2009.
2. Bailey, D. G.; Preservation of Hides and Skins. *JALCA* **98**, 308-319, 2003.
3. Kanagaraj, J. Chandra Babu, N. Sadulla, K. S., Suseela Rajkumar, G. Visalshi, V., Chandrakumar, N.; A new approach to less-salt preservation of raw hide/skin. *JALCA* **95**, 368-374, 2000.
4. Kanagaraj, J., John Sundar, V., Muralidharan, C., Sadulla, S.; Alternatives to sodium chloride in prevention of skin protein degradation a case study. *J Clean Prod* **13**, 825-831, 2005.
5. Kannan, K. C., Pradeep Kumar, M., Raghava Rao, J., Nair, B.U.; A novel approach towards preservation of skins. *JALCA* **105**, 360-368, 2010.
6. Aldema-Ramos, M. L., Muir, Z. E., Ashby, R. D.; Soaking formulations that can soften hardened bovine manure Part II: Effects on quality of leather. *JALCA* **107**, 1697-1764, 2012.
7. Aldema-Ramos, M., Muir, Z., Wheeler, T., Kalchayanand, N., McAloon, A.; Economic assessment and pathogenic bacteria inhibition of bovine hide presoaking solutions formulated with enzymes that can remove adobe-type manure. *JALCA* **108**, 355-363, 2013.
8. Bailey, D. G., DiMaio, G. L., Gehring, A. G., Ross G. D.; Cattle hide preservation with 10 Mev electron beam irradiation. *JALCA* **91**, 382-392, 2001.
9. Valeika, V., Beleska, K., Sirvaityte, J., Valeikiene, V.; The short term preservation of skins with acids. *J. Soc. Leather Tech. Chem.* **97,** 101-104, 2013.
10. Barret, J.C.; Short term preservation of hides in Zimbabwe. *J. Soc. Leather Tech. Chem.* **70,** 83-89, 1986.
11. Bailey, D. G.; The preservation of cattle hides with potassium chloride. *JALCA* **90**, 13-21, 1995.
12. Aldema, M. L**.**, McMurry, L. M., Walmsley, A. R., Levy, S. B.; Purification of the Tn10-specified tetracycline efflux antiporter TetA in native state as a polyhistidine fusion protein. *J. Mol Microbiol*. **19**, 187-195, 1996.
13. Kahovec, J., Fox, R. B., Hatada, K.; Nomenclature of regular single-strand organic polymers. *Pure and Applied Chemistry* **74**, 1921–1956, 2002.
14. ASTM D7476-08 Standard test method for brine saturation value of cured (salt-preserved) hides and skins, ASTM International, West Conshohocken, PA, 2008.
15. CT3- Texture Analyzer Manual, Brookfield Engineering, Inc, Middleboro, MA. 2009 It can also be found at this website: http://www.brookfieldengineering.com
16. Roddy, W. T.; Moisture and Ash Analysis of Skin. Hide and Leather. The Chemistry and Technology of Leather. Vol IV, p.3 (F. O'Flaherty, W. T. Roddy, R. M. Lollar, editors) Reinhold Publishing Company, NY, NY, 1965.