Soaking formulations that can Soften and Remove Hardened Bovine Manure: Part II, Effects on Quality of Leather

by

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Abstract

Previously developed new soaking formulations have been shown to soften and eventually remove adobe type manure and its damaging effects on bovine hides. The mechanical stress due to the weight and pressure of hard-to-remove adobe-type manure on bovine hides can cause unwanted holes in the finished leather. The incorporation of crude glycerol and sodium carbonate with or without sophorolipid (a biodegradable surfactant) in the soaking solution was found to be effective in the softening and subsequent removal of the adobe-type manure. The leather industry is interested in the potential effects of these newly developed soaking formulations on leather quality. The standard soaking solution that the hide industry is generally utilizing is composed of a high concentration (0.10-0.15%) of biocide and surfactant. In this study, we report that by utilizing crude glycerol and sodium carbonate, the inclusion of only ~10-25 % of the standard soaking solution has the potential to soften and facilitate the removal of adobe-type manure. The mechanical properties of the leather products made from the hides soaked in these newly developed formulations are superior thus improving the overall quality of the finished leather.

Resumen

Nuevas formulaciones de remojo desarrolladas previamente han demostrado ablandar y eliminar finalmente el estiércol del tipo adobe y sus efectos dañinos en cueros bovinos. La tensión mecánica debido al peso y a la presión del estiércol del tipo adobe difíciles de eliminar en pieles bovinas pueden causar orificios indeseados en el cuero acabado. La incorporación de glicerina cruda y carbonato de sodio, con o sin soforolípido (un agente tensioactivo biodegradable) en la solución de remojo, se encontró eficaz en el reblandecimiento y posterior eliminación del estiércol del tipo adobe. La industria del cuero está interesada en los efectos potenciales de estas formulaciones de reciente desarrollo de remojo sobre la calidad del cuero. La solución estándar de remojo que la industria de la piel utiliza generalmente se compone de una alta concentración (0,10-0,15%) de biocida y tensioactivo. En este estudio, nosotros reportamos que mediante la utilización de glicerina cruda y carbonato de sodio, la inclusión de sólo ~10-25% de la solución de remojo estándar tiene el potencial para suavizar y facilitar la eliminación de estiércol del tipo adobe. Las propiedades mecánicas de los productos de cuero elaborados a partir de las pieles remojadas con estas formulaciones recientemente desarrolladas son superiores mejorando así la calidad general del cuero acabado.

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Introduction

New soaking methods are urgently needed to effectively clean raw hides for storage and shipment of bovine hides being exported abroad. Recently, it was reported that about 92% of the total annual hide production (~35M hide pieces) in the US is exported with an average annual revenue of about $2.2B.[1] These revenues can be increased by improving and maintaining the quality of the hides exported to other countries where they are processed to leather. The number one hurdle that the hides and leather industry is facing constantly is the problem of putrefaction and the damaging effects of adobe type manure on finished leather products. When the animals are overcrowded in the feedlots during the winter months, the formation of the "adobe" type manure becomes apparent. For the tanner, avoiding hide damage in the form of large holes can be achieved by removing the manure balls prior to fleshing. To assure the best return to the hide dealer, the removal as early removal is imperative prior to curing for a more effective preservation and for longer storage during shipment.[2] As a result of our recent publication on soaking formulations,[4] questions have arisen concerning the possible effects of these formulations on the final leather products.

Various techniques were mentioned in our previous studies [4] that were tested by industry to remove hardened manure. Some of the processes were very labor intensive and others generated huge amounts of wastewater. Some attempts were to add chemicals to the process; however, the efficiency of manure removal was poor and the costs tended to be worse than the gain. Furthermore, when dealing with fresh hides, one must be aware of the consequences that these products may have in the rendering and wastewater stream. [3, 4]

In our current research project, glycerol has shown promise when included in hide cleaning formulations. The versatility of glycerol is generally acknowledged and accepted because of its presence in most cleaning and moisturizing products.[6] Glycerol has an amphiphilic characteristic and can be inserted into surfactant micelles and modify the interfacial tension of water. It has been used as a freezing agent in membrane protein preservation in its native state. [7] Therefore, it is understood that glycerol has the potential to enhance the solubility of hydrophilic as well as the hydrophobic materials. This could potentially provide a new outlet for the abundant amount of crude glycerol that is produced as a byproduct of the growing biodiesel industry.

Sophorolipids are naturally-produced, renewable glycolipid surfactants [8, 9] that possess environmentally benign qualities. However, since sophorolipids are not yet commercially available on a large scale, working on the soaking solution that is commonly used in the industry was considered. To generate a more environmentally friendly soaking solution, we developed formulations that require only a fraction of what is currently in use.

Experimental

Materials and Methods

The Crude Glycerol (~ 80% Glycerol) was obtained from Griffin Industries (Butler, KY) and was used as received. The composition of the crude glycerol was ~77% glycerol, 0.5% methanol, 0.4% other organic materials including fatty acids and ~22% water. The sophorolipids were biosynthesized at ARS-ERRC as summarized below but detailed elsewhere. [8, 9] All other chemicals were analytical grade and used as received.

Fresh bovine hide samples were collected from the local meat packing company (JBS, Souderton, PA). To have a more uniform textured sample, the core portions from both sides of the hide which were equidistant to the backbone, were chosen for the current study. The irregular portions close to the butt, belly and shoulder were cut off and discarded. Hide samples of comparable quality and quantity were soaked in each of the soaking formulations for 2 h and then processed to shoe upper leather products following the standard USDA tanning protocol.[5]

Sophorolipid Synthesis, Isolation, Purification and Analysis

The sophorolipids utilized in this study were synthesized at the 10-L scale through a fed-batch fermentation protocol in a New Brunswick Scientific Bioflo 3000 bench-top fermenter (Edison, NJ) using the yeast *Candida bombicola* ATCC 22214. The inocula for each of the sophorolipid fermentations were prepared using Candida Growth Medium (CGM), which consisted of 100 g/L glucose, 10 g/L yeast extract, and 1 g/L urea, as previously published.[8] Oleic acid or palmitic acid was used as the lipid co-substrate and was added at a final concentration of 2% (*w/v*). After 2 days, an additional 7.5% (*w/v*) dry glucose and 2% (*w/v*) fatty acid (oleic acid or palmitic acid) were added to the fermentation. At 5 days post inoculation 1% (*w/v*) fatty acid was added and at 6 days an additional 0.5% (*w/v*) fatty acid was added and the fermentations allowed to proceed to completion for 1 additional day (total duration of the fermentation was 7 days). [8, 9]

Sophorolipids were isolated by lyophilizing the entire culture to dryness and then extracting the dried culture 3 separate times with excess ethyl acetate. The combined ethyl acetate fractions were concentrated by evaporation and added to 2-L of hexane to precipitate the pure sophorolipids. The pure sophorolipids were recovered from the hexane through filtration through Whatman #2 filter paper and vacuum dried in a desiccator to obtain a fine white powder. The powdered sophorolipids were analyzed by a previously described liquid chromatography/mass spectrometry (LC/MS) procedure [10] to determine their chemical makeup.

Preparation of Soaking Formulations

The initial soaking formulations were prepared based on the critical micelle concentration (CMC) of commercially available surfactant representing the anionic, cationic and zwitterionic type.[11, 12] The washing efficiency of a surfactant is improved when all the monomeric molecules are in a micelle form. An optimum solubilizing capacity of a surfactant is attained by using concentrations above the CMC, thus using 0.10%(w/v) is suggested for surfactants like the Sophorolipids with CMC of 0.05%(w/v). We experimented using only 10-25% of the standard soaking solution by including crude glycerol in the formulation. Since the softening of the hardened manure is the key to its removal during the demanuring process, the experiments presented in the previous paper [4] and in the current study, were designed such that the changes in the "hardness" of the manure balls were monitored. Deformation on different manure samples, before and after soaking in the respective formulations, was measured by a texture analyzer so that the efficiency of softening and removal could be determined. [4]

A series of 8 different soaking formulations, listed in Table I, were prepared to include the application of sophorolipid (SL), which was found to be quite efficient among the different surfactants investigated previously and published,[4] by combining with other ingredients to study the effects on the quality of each leather product.

TABLE I
Preparations of a new series of 8 soaking formulations

Trial #	Soaking solution ingredients
SF-1	10% Pure Glycerol
SF-2	10% Crude Glycerol (c-glyc)
SF-3	10% c-glyc+ 1 % sodium carbonate, Na_2CO_3 (SC)
SF-4	10% c-glyc+ 0.1% SL-A or SL-oleic acid +1.0% SC
SF-5	10% c-glyc+ 0.1% SL-A or SL-palmitic acid + 1.0% SC
SF-6	25 % std SF +10% c-glyc+ 1.0% SC
SF-7	50% std SF
SF-8	Control, 100% std soaking formulation, SF

Protocol developed for soaking with the different formulations.

The hide samples were weighed individually. About 200% (w/v) float using the prepared formulation was added to each respective sample (for 500 g hide, 1000 ml of the desired soaking formulation was added). The hide sample was introduced into the mini drum and set to room temperature (~27 °C) and spinning at 16 RPM for 2 h. At the end of 2 h, the soaking formulations were carefully drained and washed with 500% (w/v) float tap water. The presoaked and washed hide samples were then processed to shoe upper leather following the standard USDA tanning protocol.[5] To convert the standard soaking solution into a more eco-friendly formulation, another series of soaking formulations were prepared by using only a fraction of the typical concentration that the hide industry uses. The mechanical properties were determined after tanning the presoaked hides with and without the incorporation of crude glycerol, sodium carbonate and other cleansing agents such as chlorine dioxide to the formulation with lower concentration of biocide and surfactant.

Microscopic Images

Representative hide samples were inspected under the stereo- and scanning electron microscope and images were taken (Microscopic Images- ERRC core facility) to determine if there were any visible changes in the hide grain structure after soaking in the different formulations. The field-emission environmental scanning electron microscope (ESEM) model Quanta 200 FEG, FEI Company, Oregon). It was operated at low vacuum (0.3Torr) with voltage set at 15kV, spot size 5.0 and working distance of about 10mm. The samples were uncoated, thus preserving its original characteristics.

Determination of Mechanical Properties:

To verify the effects on leather quality, the mechanical properties (Young's modulus, tensile strength, fracture energy, elongation -to-break) were measured. Dog bone shaped leather samples (1- × 10-cm) were cut near the standard test area as described in ASTM D2813-03[13] with the long dimension parallel to the backbone of the bovine hide. The average thickness of the leather samples varied from 2 mm to 2.7 mm. An MTS Insight 5 mechanical property tester and Testworks-4 data acquisition software (MTS Systems Corp., Minneapolis, MN) were used throughout this work. The strain rate was set to 25.4 cm/min with a grip distance of 10.16 cm. The samples were tested in a room set at 73 ± 3 °C and 50 ± 5% relative humidity. Each test was conducted on five samples to obtain an average value.

RESULTS AND DISCUSSIONS

Among the 5 different surfactants, 4 of which represented different types of commercially available detergents, the sophorolipid containing formulation gave the best overall efficiency in terms of manure softening when each surfactant was incorporated with crude glycerol and sodium carbonate.[4] The efficiency in softening the "adobe-type" manure by different soaking formulations can be determined quantitatively following the protocol stipulated and discussed in detail in Part I of this study.[4] The percentage softening at specific times after soaking is the difference between the hardness at t = 0 minus the hardness at that certain time (e.g. t = 60 min) divided by the hardness at t = 0 then multiplied by 100. The softening can be related to manure removal. The manure removal can also be monitored by measuring the relative work (in mJ) needed to cause a preset deformation at peak (def@peak) of 5 mm into each of the manure samples before and after soaking using a CT3- texture analyzer.[14]

The equations were utilized to measure the relative manure removal based on the softening of the hardened manure as in Eqtn 1 and 4.

OR the data can be normalized by using specific (Sp) Energy values which can be obtained by using either Eqtn 2 or 3. The values obtained are found to be very similar in terms of specific energy or work needed in (mJ/g) to cause uniform preset deformation on a given manure sample, before and after soaking.

After just 1 h soaking, most of the formulations containing 10% crude glycerol and 1% sodium carbonate, attained almost 90% manure removal. As shown in Figure 1.

The manure softening of the control, SF-8, a 100% standard soaking formulation, and SF-7, composed of just 50% of the standard solution, were comparable in efficiency. In SF-6, with only 25% of the standard soaking formulation, the manure removal was more efficient even without the high concentration of biocide and surfactant. This could be due to the presence of crude glycerol and sodium carbonate which by itself in SF-2, had shown improved efficiency compared to the control. Soaking formulations SF-4 through SF-6 showed a much improved efficiency in manure removal due to the presence of crude glycerol, sodium carbonate and in combination with surfactants either from the sophorolipid-oleic or SL-A (SF-4) or sophorolipid-palmitic or SL-B (SF-5) and even from the surfactant of the 25% standard solution (SF-6).

When hide samples were inspected under the stereo- and scanning electron microscope and images were taken (Microscopic Images- ERRC core facility), it was found that there was no visible nor microscopic changes in the hide grain structure after soaking in different formulations. The three microscopic images (A-C) that represented the rest of the investigated formulations listed in Table I, as shown in Figure 2 are comparable to the control (D). The microscopic images of the hide grain regions appear to have a tight looking structure and are very similar to each other.

Furthermore, the effects of the different soaking formulations on the quality of leather products were verified by determining the mechanical properties. Mechanical property measurements

$$\% \text{ softening} = 100 - \left[\frac{\text{hardness at } t=0 - \text{hardness at } t=1h}{\text{Hardness at } t=0}\right] \times 100 \qquad \text{Eqtn, 1}$$

$$\text{Specific Energy1} = \text{hardness work cycle(mJ)/ hardness cycle (g)} \qquad \text{Eqtn. 2}$$

$$\text{Specific Energy2} = \text{Total work cycle (mJ)/ load at target (g)} \qquad \text{Eqtn. 3}$$

$$\% \text{ softening} = 100 - \left[\frac{\text{Sp Energy at } t=0 - \text{Sp Energy at } t=1h}{\text{Sp Energy at } t=0}\right] \times 100 \qquad \text{Eqtn. 4}$$

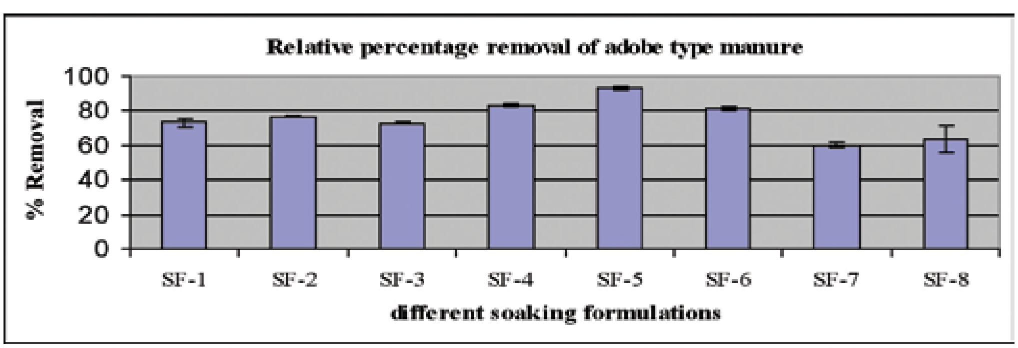

Figure 1. Percentage removal based on % softening of adobe type manure after 60 min soaking in 8 different soaking formulations listed in Table I.

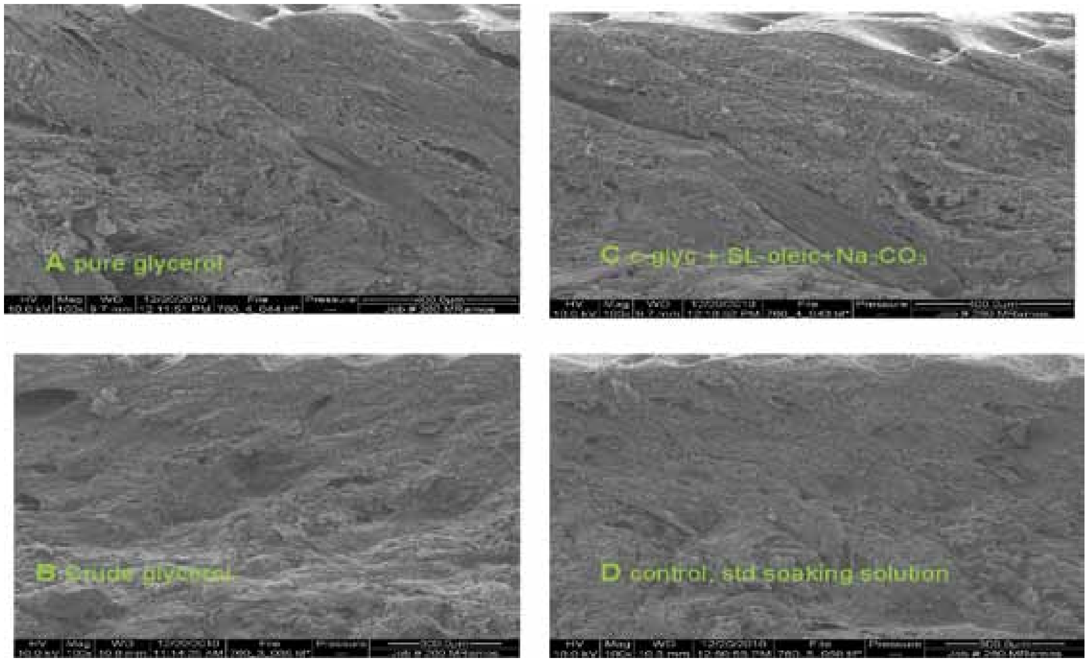

Figure 2. SEM microscopic images (100X magnification) of hide grain samples after soaking in different soaking formulations comparable to control.

TABLE II
Mechanical properties of leather products from the hides previously soaked in 8 different formulations listed in Table I.

Formulation ID	Tensile Strength, Mpa	Elongation-to-break, %	Young's Modulus, Mpa	Fracture E, J/cm^3	Toughness Index
SF-1	5.4 ± 0.7	80.6 ± 5.4	4.3 ± 2.1	1.7 ± 0.5	1.3 ± 0.2
SF-2	6.7 ± 1.2	82.1 ± 2.1	4.1 ± 1.5	1.6 ± 0.3	1.2 ± 0.2
SF-3	7.2 ± 0.6	62.9 ± 6.2	8.4 ± 1.3	1.7 ± 0.3	0.8 ± 0.2
SF-4	6.9 ± 2.0	81.6 ± 6.7	5.8 ± 1.9	1.9 ± 0.7	1.2 ± 0.3
SF-5	4.9 ± 1.0	71 ± 4.1	7.1 ± 0.7	1.3 ± 0.3	0.7 ± 0.1
SF-6	4.4 ± 0	78.1 ± 1.2	4.4 ± 0,5	1.4 ± 0.3	1.2 ± 0.2
SF-7	5.1 ± 0.9	68.5 ± 3.9	8 ± 1.2	1.5 ± 0.2	0.9 ± 0.2
SF-8	4.8 ± 2.2	88.1 ± 5.1	4.3 ± 2.4	1.3 ± 0.7	1.2 ± 0.3

included tensile strength, elongation-to-break ("stretchability"), Young's modulus ("stiffness"), and fracture energy (the energy needed to fracture leather samples, its "toughness"). The data is shown in Table II.

The averaged data was taken from 5 bone-shaped pieces. The data suggests that the hide sample soaked in pure glycerol (SF-1) and crude glycerol (SF-2) as well as the crude glycerol with sophorolipid (SL-oleic acid; SF-4), respectively, showed toughness indices that had no significant difference (1.3± 0.2, 1.2 ± 0.2, 1.2 ± 0.3 respectively) with $p<0.5$. The values obtained were comparable to those soaked in standard soaking solution (SF-8) which is commonly used by the hide industry to remove any dirt and contaminants from the dirty hide samples (1.3 ± 0.5). The standard soaking formulation, SF-8, is usually composed of biocide and surfactant (in this

TABLE III
New set of soaking formulations using only a fraction of the standard soaking solution used normally by the hide and leather industry.

Trial #	Soaking Solution Ingredients
DS-1	Control, 100% Standard solution (0.15% Boron-Ts, 0.1% Proxel-GXL)
DS-2	25% std soln (0.0375% Boron-Ts, 0.025% Proxel-GXL)
DS-3	25% std soln (0.0375% Boron-Ts, 0.025% Proxel GXL)10%crG+1%SC
DS-4	10% crude glycerol (cr G), 1% Na_2CO_3 (SC)
DS-5	10% c-glyc + 1%SC+0.1% SL-Oleic (or SL-A)
DS-6	10% c-glyc + 1%SC+0.1% SL-Palmitic (or SL-B)
DS-7	10% c-glyc + 1%SC + 200 ppm ClO_2
DS-8	10% c-glyc + 1.0%SC + 0.1% SL-Palmitic + 200 ppm Cl02
DS-9	25% std soaking soln(0.0375% Boron-Ts+0.025% Proxel-GXL +200ppmClO_2

experiment, 0.10% Proxel-GXL and 0.15% Boron-TS respectively, were used). Even to the formulation, SF-6, with just 25% of the standard formulation, (0.0375% Proxel and 0.025% Boron-TS) but with the incorporation of 10% crude glycerol and 1% sodium carbonate, the toughness index was almost identical (1.2 ± 0.3). The same trend was also observed in the fracture energy and stiffness or Young's modulus data of the leather sample soaked in the above mentioned formulations.

The stretchability of the finished leather based on % elongation-to-break, showed comparable values to the standard soaking solution. Whereas, the tensile strength values, showed improvement to 6.7 ± 1.2 MPa for SF-2, 7.2 ± 0.6 MPa for SF-3, 6.9 ± 2.0 MPa for SF-4, compared to 4.8 ± 2.2 MPa for SF-8, the control soaking formulation.

Another series of soaking formulations was prepared by using only a fraction of the typical concentration that the hide industry uses, thus lowering the impact to the environment. The sample coding (DS- #) pertains to the tanning mini drums where the hide samples were presoaked in this new set of formulations listed in Table III before tanned to shoe upper leather. The mechanical property results are tabulated in Table IV for easier comparison of data from differently soaked hides in formulations listed in Table III.

In terms of the mechanical properties of the resulting leather, DS-2, which was composed of only 25% of the standard soaking solution, compared to DS-1, the 100 % control that the hide industry typically uses, are quite encouraging because the Tensile strength (t-test = **21**) , elongation (t-test = **4**), fracture energy (t-test = **4.2**) and toughness index (t-test = 3.1) are significantly improved. The lowering of the concentration of biocide and surfactant but incorporated with crude glycerol and sodium carbonate did better on quality of leather products. SL-palmitic in DS-6 worked better compared to SL-oleic in DS-5 in terms of improving quality of leather significantly as shown by the bold t-test values in Table IV; tensile strength (t-test is ~**14**), t-test for young's modulus is ~**6.4**, and the fracture energy t-test is ~**11.2**.

The t-test values in bold font are indicative of significant improvement in terms of leather quality. SL-palmitic-containing formulations (DS-6 and DS-8) showed much better improvement to the overall quality of leather than SL-oleic (DS-5). The formulations that had about 10% crude glycerol (DS-2, DS-3, DS-6, DS-8 and DS-9) generated a tougher leather which was supported by fracture energy values following the same trend with t-tests of relatively high values (when compared to the 100 % standard soaking solution, DS-1). Likewise, the elongation or stretchability also follows the same significant enhancement in leather quality. The presence of crude glycerol and sodium carbonate resulted in softer yet stronger leather products.

Conclusion

There were no visible physical changes or microscopic differences in the hides soaked in our recently developed formulations compared to the hides soaked in standard soaking solution that the leather industry currently uses. This demonstrated that the newly developed formulations were safe and are not detrimental as the standard soaking solution to the hides and the resulting leather products.

TABLE IV
Mechanical Properties of leather products made from hides presoaked in Soaking formulations listed in table III.

new batch Formulation #	Tensile Strength	Young's Modulus, MPa	Elongation-to-break, %	Fracture Energy, J/cm^3	Toughness Index
DS-1	2.7 ± 0.2	7.3 ± 0.2	76.2 ± 6.7	2.9 ± 0.5	1.2 ± 0.3
DS-2	14.5 ± 1.2	8.4 ± 1.4	88.8 ± 2.2	4.1 ± 0.4	1.7 ± 0.2
DS-3	15.1 ± 2.1	8.3 ± 2.0	84.4 ± 6.8	4.1 ± 0.7	1.9 ± 0.4
DS-4	7.4 ± 1.0	8.5 ± 0.7	60.6 ± 4.1	1.6 ± 0.3	0.9 ± 0.1
DS-5	5.1 ± 0.9	4.5 ± 0.3	78.5 ± 10.0	1.4 ± 0.2	1.1 ± 0.2
DS-6	11.9 ± 0.5	8.3 ± 1.3	81.5 ± 6.3	3.2 ± 0.3	1.5 ± 0.3
DS-7	6.9 ± 0.5	5.8 ± 1.2	78.3 ± 4.7	1.9 ± 0.2	1.2 ±0.3
DS-8	7.1 ± 0.6	6.3 ± 1.0	78.3 ± 4.7	1.9 ± 0.4	1.7 ±0.3
DS-9	12.1 ± 2.5	7.3 ± 2.4	80.2 ± 8.2	3.3 ± 0.7	1.8 ±0.3
t-test for					
DS-1 vs DS-2	**21.6**	1.7	**4.0**	**4.2**	**3.1**
DS-5 vs DS-6	**14.7**	**6.4**	0.6	**11.2**	**2.5**
DS-3 vs DS-4	**7.4**	< 0.5	**6.7**	**7.3**	**5.4**
DS-6 vs DS-7	**15.6**	**3.2**	0.9	**8.1**	1.6
DS-7 vs DS-9	**4.5**	1.3	< 0.5	**4.3**	**3.2**

t-test = [DS 1 - DS 2]/sqrt[(SD of DS1)2/5 (SD of DS2)2/5]1/2
if t-test value is >2.3, the change is significant as signified by the bold font.

Furthermore, when the presoaked hides were subjected to uniform standard tanning treatments, significant improvements in leather quality were observed especially when crude glycerol and sodium carbonate were incorporated in the soaking solutions.

If the recycled crude glycerol is included in the formulation, the sophorolipid was observed to be effective in softening and hence aided in the removal of the hardened manure samples, Additionally, using only a fraction (~10 – 25%) of the standard soaking solution together with about 10% crude glycerol and 1% sodium carbonate, showed potential for softening the adobe-type manure and deserves further investigation. The future direction of this research project includes the microbiological study of the washes from newly developed soaking formulation and the chemical studies of the manure, leather products and the hide samples presoaked in newly developed soaking formulations. This will enable us to see if there are any major changes or visible effects brought about by the formulations on the leather products. This formulation could potentially gain more practical applicability in the hide industry.

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