Microbiological and Near IR Studies of Leather From Hides Presoaked in Formulations that can Remove Hardened Bovine Manure

by

Mila L. Aldema Ramos*[1], Zerlina E. Muir[1], Daniel K.Y. Solaiman and Suzanne Schreyer[2]
[1]*U.S.D.A., Agricultural Research Service, Eastern Regional Research Center*
600 E. Mermaid Lane, Wyndmoor, PA 19038, USA;
[2]*Chemometrics Applications, Thermo Fisher Scientific*
Tewksbury, MA 01876

Abstract

New efficient eco-friendly soaking methods are urgently needed to clean manure from raw hides for storage and shipment abroad of the~ 35M bovine hides annually produced in USA. High concentrations of surfactant (~0.15% (w/w)) and biocide (~0.10%(w/w)) in commonly used presoaking solution are functional for eliminating microbial contamination, but these are quite unfriendly to the environment and are also inefficient in removing the damaging adobe-type manure. We found that a much lower concentration of surfactant (~0.0375% (w/w) and biocide (~0.025% (w/w) are sufficiently effective when crude glycerol and sodium carbonate are incorporated in the presoaking formulation. Using lower concentrations, the efficiency in removing hardened manure improved and ~70% microbial growth inhibition was still observed. Furthermore, significant manure smell reduction and additional microbial inhibition are observed when chlorine dioxide is also added. No chemical and structural alterations on finished leather samples made from the presoaked hides were observed based on non-denaturing Near Infrared (NIR) spectroscopy analysis. The leather products made from hides that were presoaked in newly developed formulations showed comparable or even better mechanical properties than the control leather products obtained from traditionally treated hides. Considering the low cost in the procurement and the presence of other ingredients in it that have enhanced the efficiency in softening and removing of adobe type manure, crude glycerol is quite desirable as an ingredient in the new eco-friendly presoaking formulation.

Resumen

Nuevos métodos ecológicos eficientes de remojo se necesitan con urgencia para limpiar el estiércol de las pieles crudas para almacenamiento y envío al extranjero de los ~35 millones de cueros crudos bovinos producidos anualmente en EE.UU. Las altas concentraciones de tensoactivo (~0,15% (p/p)) y biocida (~0,10% (p/p)) soluciones comúnmente utilizadas en un pre-remojo son funcionales para la eliminación de la contaminación microbiana, pero éstos son bastante desagradables para el medio ambiente y también son ineficaces en eliminar el daño por el estiércol de tipo adobado. Hemos encontrado que una concentración mucho más baja de tensoactivo (~0,0375% (p/p) y biocida (~0,025% (p/p) fueron suficientemente eficaces cuando glicerol crudo y carbonato de sodio se incorporan en la formulación de pre remojo. Usando concentraciones más bajas, la eficiencia en la eliminación de estiércol ya endurecido mejora y el ~70% de inhibición del crecimiento microbiano se ha observado. Además, una reducción significativa del olor a estiércol y una inhibición microbiana adicional se observa cuando también se añade dióxido de cloro. Ni alteraciones químicas ni estructurales en muestras de cuero acabados obtenidos a partir de las pieles pre-remojadas se observaron basada en espectroscopia infrarroja cercana no desnaturalizada (NIR). Los productos de cuero hechos a partir de pieles que se pre-remojaron en las formulaciones desarrolladas recientemente mostraron propiedades mecánicas comparables e incluso mejores que los productos control obtenidas a partir de pieles tratadas tradicionalmente. Teniendo en cuenta el bajo costo en la adquisición y la presencia de otros ingredientes en el mismo, que han mejorado la eficiencia en el ablandamiento y la eliminación del estiércol tipo adobado, glicerol en bruto es muy deseable como un ingrediente en la nueva formulación de pre-remojo ecológicamente amigable.

*Corresponding author e-mail address: mila.ramos@ars.usda.gov.
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Introduction

Putrefaction and the damaging effects of adobe-type manure on finished leather products are among the major problems that the hides and leather industry are constantly facing. New soaking methods are urgently needed to effectively clean raw hides prior to storage of ca. 35M bovine hides produced in the USA annually and shipped abroad.[1, 2] Revenue can be increased by improving and maintaining the quality of US hides that are exported to countries where they are processed to leather.[1, 3]

One of the goals of our work is to monitor the softening of adobe type manure before and after soaking in the differently prepared formulations. We previously found that the incorporation of crude glycerol and sodium carbonate with or without a biodegradable surfactant in the newly developed soaking formulations (SF) was effective in the softening and subsequently in the removal of adobe type manure.[4] This led to the quantitative measurement of the relative efficiency of manure removal using a Texture Analyzer instrument.[4, 5] We had further determined the mechanical properties of the resultant finished leather made from presoaked hides compared to the control leather made from traditionally treated hides.[6]

To generate an eco-friendly formulation, the ingredients chosen should be non-toxic to the tanner and the environment. Thus, the inclusion of chlorine dioxide (ClO_2) was considered. Chlorine dioxide is a broad spectrum biocide [7-9] with more than two times the oxidizing capacity of chlorine. It is one of four EPA approved disinfectants for drinking water. Chlorine dioxide has unique biocidal properties and has been used effectively as a disinfectant, algaecide and oxidizer in influent process waters and waste waters.[7-9]

In the present study, our goal is to determine if there are any structural changes that take place during the soaking of the hides and if there is any microbial growth inhibition by the soaking formulations. The chemical structure at the surface of the leather product can be analyzed by utilizing a non-destructive technique such as Near InfraRed (NIR) Spectroscopy. The NIR signals arise from the excitation of vibrational modes within molecules of which, the strongest signals correspond to functional groups containing NH, OH or CH bonds.[10-12] The vibrational modes that absorb most strongly are those with dipole moments. Bonds with dipole moments are mostly heteronuclear, like C-H, and are off the molecular backbone. [11] A robust handheld NIR instrument could give such information and does not require rigorous sample preparation.[13] Principal component analysis[14] (PCA) is performed on the NIR spectra of each sample so that the similarity and/or variation of the different samples can be determined. PCA is a statistical calculation with a multidimensionality reduction method used for the extraction of systematic variations in a data set such as NIR spectra. PCA models the variance/covariance of the data structure such that the significant information can be extracted in the first most important PCs and the remaining PCs will successively model the lower variation or noise portion of the data.[14]

Experimental Methods

Materials

All the chemicals used for the conventional processes are of commercial grade. The chemicals used for the preparation of the different soaking formulations are of analytical grade. The crude glycerol was obtained from Griffin Industries (Butler, KY) and was used as received. The different components present in the crude glycerol were found to be ~77% glycerol, 0.5% methanol, 0.4% other organic materials including fatty acids and ~22% water. The sophorolipids were biosynthesized at the ARS-ERRC and detailed elsewhere.[15, 16] The chlorine dioxide used is from a commercially sold AquaDry3000 from Beckart Environmental, Inc (Kenosha, WI).[7]

Soaking/Manure Removal Processes

The fresh hide sample with high amount of adobe type manure was divided into two groups numbered as shown in Fig 1. The hide pieces close to the backbone (considered prime hide samples) were used for quality of leather measurements after soaking in newly developed soaking formulations (SF). The results were compared to the control, which was the hide soaked in traditional (standard) soaking formulation composed of (0.15% Boron TS, 0.1% Proxel GXL) biocide and surfactant.

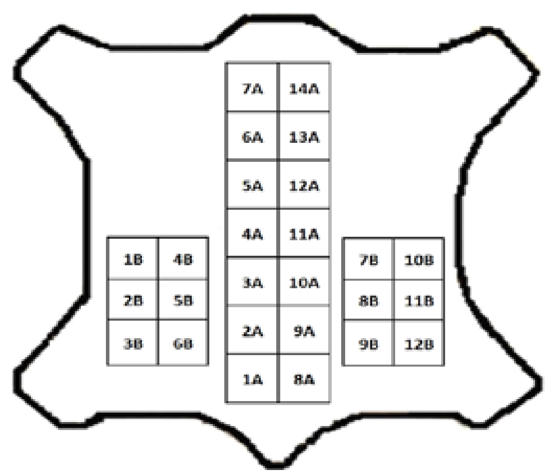

Figure 1. Sampling of fresh cattle hide for soaking then tanning trials.

Group 1: samples for soaking then tanning trials were taken from areas labeled 1A to 14A. Group 2: samples for soaking and manure removal trials were taken from areas 1B to12B, hide areas where hardened manure samples are located.

The Group 1 sample pieces for soaking and tanning trials were taken close to the backbone and labeled 1A-7A on the left side and 8A-14A on the right side as shown in Fig 1. Each piece had a dimension of 20.3 x 30 cm, the 20.3 cm side parallel (//) to the backbone. This was done in order to obtain five dog bone-shaped pieces parallel to the 20.3 cm side when the mechanical properties determination were performed on the crust leather. The pelts of Group 1 were dehaired, relimed, bated, pickled, and chrome tanned according to the conventional procedures for obtaining shoe upper leather.[17,18] The crust leather was split to obtain grain layer with a thickness of ~2 mm, and the mechanical properties in the grain layer of each leather samples were analyzed. Five dog bone-shaped samples based on the ASTM standard protocol were obtained to give five trials each. [19]

Group 2 samples were taken for soaking and manure removal experimental trials. The sample pieces were taken from the belly part on the left and the right side of the hide and close to the butt region that had large amount of adobe type manure. The samples were labeled 1B to 6B on the left and 7B to 12B on the right as shown in Fig 1. Smaller pieces with dimensions of ~ 10.2 x 15.2 cm were taken. Aligning the longer side parallel to the backbone was unnecessary because there is no need to tan the samples afterwards. More importantly, the initial amount of manure in each hide sample had to be almost uniform so that comparing the efficiency of the different soaking formulations in manure softening and eventually the removal, could be feasible.

The volume of the soaking formulation used was equivalent to 200% float (i.e., for every gram of sample, 2 mL of the solution were added). The tanning mini drum set-up (GmbH, Germany) was utilized, and soaking was set at room temp (~27°C) for 1 h (then for 2 h) with tumbling at ~16K RPM. Qualitatively, the manure softening and removal was inspected after 1 h soaking. The weight of the hide with remaining manure was measured. It was compared to the initial weight of the hide with manure, immediately after it was immersed into the respective formulation (at t = 0). The excess solution was allowed to drain for a uniform length of time before the weight of the soaked hides were measured individually. The weight lost was treated as the amount of manure removed from the hide. The softening effect was assessed to the touch by checking the changes in hardness of the representative removed manure samples. The relative softness of the still adhering manure was rated and tabulated in order to obtain qualitative comparison. [18]

Microbial Growth Studies

Microbial growth inhibition studies were done using the SFs before and after soaking of hides with manure as well as from soaking hardened manure balls. Manure ball samples were chosen based on almost uniform size, shape, and weight. Each manure sample was weighed and immersed in the designated formulation and placed in a sealed 4 x 10.2 cm Ziploc polyethylene (PE) bag. The volume of the respective soaking formulations was measured at 250% float (for every 1 g of manure, 2.5mL of the formulation were added), enough to completely submerge the manure sample. Then the samples in solution were agitated on a Gyrotatory ® Shaker - Model G2 at approximately 120 RPM for 1h. Texture analysis was performed using the CT3-texture analyzer with the smallest stainless steel cylindrical probe, CT-14.[4,5] The amount of work needed to attain the same deformation at peak load, equivalent to the percentage of softening of the hardened manure were determined at 30 min and 60 min after soaking in the respective solution. [4-6] The same pressure, probe, and load weights were applied, and corresponding readings were taken and tabulated.

Aliquots of 1 ml were withdrawn from each formulation at the end of the soaking experimentation and were allowed to settle down with a quick spin (8,000 x g for 3 min) in a microcentrifuge (Eppendorf). From each trial, 50_L of the supernatant was diluted to 1 ml of doubly deionized water (ddw) from which a 10_L aliquot was inoculated on agar plates in order to determine and detect microbial growth.[20] The widely used medium called Luria-Bertani broth (1% (w/v) tryptone, 1% (w/v) yeast extracts, 0.5% w/v NaCl) with 1% agar was used to prepare the agar plates because the medium was capable of sustaining growth of various types of microbes. The microbial growth of each formulation was investigated before and after soaking the respective ball-like adobe-type manure samples.

Near-IR Analysis of Manure and Leather Product Samples

To determine if there were any structural changes that took place after soaking in the newly developed formulations, the Near InfraRed (NIR) spectra of the finished leather samples were taken. The NIR spectra of the manure samples before and after soaking were also taken to monitor the softening and structural changes. The NIR spectra were taken by using a Phazir handheld near IR instrument capable of scanning a wavelength range from 1600nm to 2400nm with an optical resolution of 11nm.[11, 13] The Phazir™ Handheld NIR Material Analyzer[13] (Thermo Scientific, Tewksbury, MA), is a point-and-shoot NIR measurement instrument capable of real-time, instantaneous, qualitative and quantitative materials analysis. All data are stored in memory, which can then be exported to a PC for logging or additional processing using formats such as a Microsoft Excel spreadsheet.

RESULTS AND DISCUSSION

Soaking/manure Removal Processes

The efficiencies of the different formulations in removing the adobe type manure adhering to the hide samples were qualitatively determined and the summary of the tabulated results are shown in Table I. The efficiency was rated 1X - to - 5X, with 5X being the most efficient and 1X, the least efficient.

TABLE I
Qualitative comparison of adobe type manure removal from hide sample with manure clinging tightly through the hair. Removal efficiency is directly proportional to the # of X after 2h soaking.

Soaking	Formulation	Removal efficiency	observation after 2 h soak
1	10% pure glycerol	XXX	minimum manure left; relatively soft manure
2	10% crude glycerol	XXX	minimum manure left; relatively soft manure
3	10% crude glycerol + 0.1% SL-A	XXXX	mostly removed manure- manure soft, easy to pull off
4	10%crglyc+0.1% SL-A+1.0% SC	XXXX	manure mostly gone- manure soft
5	10%crglyc+1.0% SC+100ppmClO_2	XXXXX	almost all manure gone-- the winner
6	10% crude glycerol + 1.0% SC	XXX	some manure left- but quite easy to pull
7	10%CG+0.1%SL-A +1% SC+ 100ppmClO_2	XXX	few manure left- easy to pull but quite hard manure- crushed
8	1.0% Sodium carbonate (SC)	XXXX	few manure left- easy to pull but quite hard manure- crushed
9	std washing-0.15 %TS+0.10%Prxl	XX	the most manure left among trials; hard to pull
10	10%Stdsoak+10%CrG+1% SC	XXXX	manure mostly gone- manure soft

Formulation 5 (F5) composed of 10% (v/w) crude glycerol + 1% (w/V) sodium carbonate (SC) + 100 ppm ClO_2 gave the best results where almost all of the manures was removed. It was followed by F3 (10% crude glycerol+1% sodium carbonate), F4 (10% crude glycerol+1% sodium carbonate + 0.1% SL-Oleic) and F10 (10% of the standard presoaking solution + 10% c g + 1% SC) with the same relative efficiency where the manure was almost entirely gone, leaving the soft to the touch and easy to pull manure off the hide. Formulation 8 (1% SC) was relatively efficient with little manure left that crushed to the touch. The 100% standard presoaking solution (F9) was observed to be the least efficient in removing the manure and what was left was quite hard to pull off the hide.

Another set of presoaking formulations were prepared utilizing only a fraction (10 to 25%) of the standard SF, and

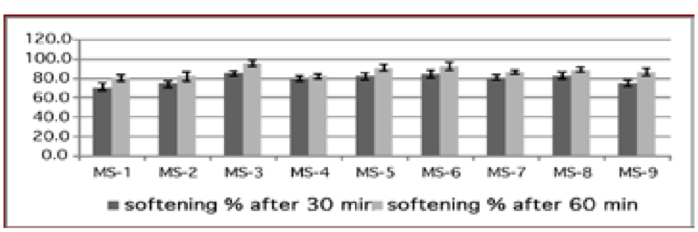

Figure 2. % Softening of hardened manure after soaking for 30 minutes and 1 h with formulations listed in Table II.

results are tabulated in Table II. The corresponding % softening of hardened manure is shown in Fig 2.

Results showed that the softening efficiency of presoaking formulations with crude glycerol and sodium carbonate (MS-3, MS-5 to MS-9) are better than the treatments without it in MS-2 and MS-4 or compared to the standard SF, MS-1, in the absence of crude glycerol. The 80-90% softening is sufficient in loosening and then removing the hardened manure from tight adherence to the hair of the hides. The softened manure can detach from the hide and go with the washes. In the hide processing facility, if the manure is still attached, it can be removed more readily from the hide by passing through a demanuring machine. The new presoaking formulations with crude glycerol also showed promising effects on the quality of leather products after the corresponding mechanical properties of the crust leather products are obtained as shown in Fig 3.

Based on the tensile strength and softness data in Fig 3A, the crust leather products made from hides soaked in SF with crude glycerol and sodium carbonate were either comparable or more superior (MS-3 (t-test= 4.1), MS-5 (t-test= 2.5), MS-6 (t-test=2.6) and MS-8 (t-test= 3.1) than the control, leather made from the hide treated with standard SF (MS-1). The crust leather appeared to be softer (with lower Young's modulus values) and yet stronger (with higher tensile strength

TABLE II
Presoaking formulations where only a fraction of the standard soaking formulation was used with varied amounts of other cleansing agents.

sple code	Presoaking solution ingredients
MS-1	100% standard solution (0.15% Boron TS, 0.1% Proxel)
MS-2	25% std soln (0.0375% Boron TS; 0.025% Proxel)
MS-3	25% std soln (0.0375% Boron TS; 0.025% Proxel) + 10% cr G + 1% SC
MS-4	25% std soaking soln (0.0375% Boron TS; 0.025% Proxel) + 200 ppm ClO_2
MS-5	10% cr G + 1% SC + 0.1% sophorolipid-Oleic
MS-6	10% cr G + 1% SC + 0.1% sophorolipid-Palmitic
MS-7	10% cr G + 1% SC + 200 ppm ClO_2
MS-8	10% cr G + 1% SC + 0.1% sophorolipid-Palmitic + 200 ppm ClO_2
MS-9	10% crude glycerol (cr G), 1% Na2CO3 (SC)

values). The % elongation-to-break data in Fig 3B is also showing the same trend, that the hides previously soaked in formulations with crude glycerol and sodium carbonate are more stretchable (MS-3, MS-5 to MS-7 and MS-9) than those soaked in formulations without the added humectant (MS-1, MS-2 and MS-4).

Near IR Spectra of the Leather Samples From Presoaked Hide Samples

During the analysis, the instrument collected digitized data of the corresponding overtones and combinations of vibrations that resulted in a spectra characteristic of the material. This was graphically represented by plotting the spectral reflectance versus the wavelength. The near-IR spectra (Fig 4 and 5) and the subsequent principal component analysis (PCA, Fig 6) plot[14] showed differences between the outside or grain side and inside or corium part of the hide samples.

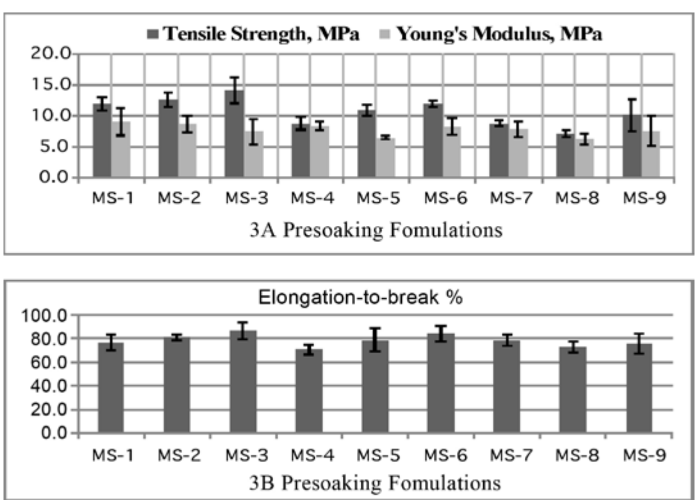

Figure 3. Mechanical Properties of the crust leather made from hides presoaked in nine(9) formulations tabulated in Table II.

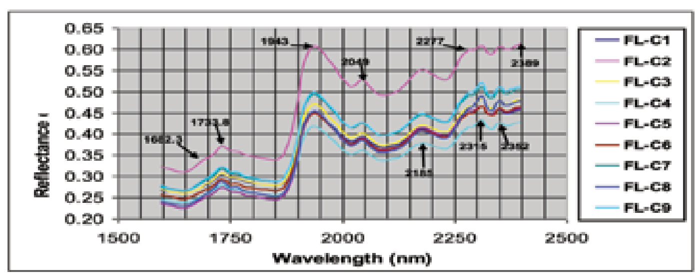

Figure 4. The near infrared (NIR) spectra: Corium(C) sides of the crust leather (FL) after soaking the hide samples in respective soaking formulations.

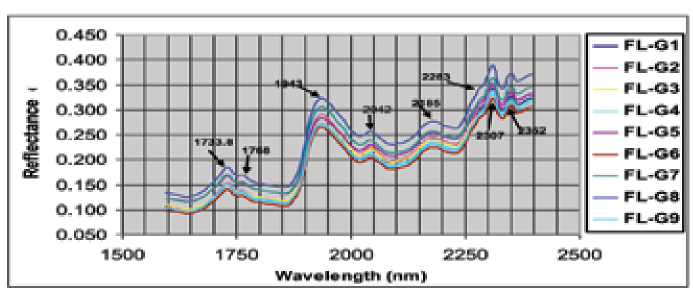

Figure 5. The near infrared (NIR) spectra: Grain(G) sides of the crust leather(FL) samples after soaking the hides in respective soaking formulations.

The near-Infrared (NIR) spectra of all the leather samples at the grain surface look very similar to each other and comparable to the control. It implies that the composition at the hide grain surface where the site of the NIR analysis was taken were the same or unaffected by the different treatments or when soaked in different formulations. The low-power light source in handheld NIR instruments has insufficient energy to cause damaging effects or decomposition in materials examined. When the sample was exposed to the electromagnetic radiation emitted from the light source, that radiation was reflected, absorbed and/or transmitted. The interaction between this harmless radiation and the compound(s) contained within the sample caused molecular excitation as well as vibrational energy changes. These vibrational changes caused functional groups; especially O-H, C-H, S-H and N-H if they are present within the sample compound(s), to stretch and bend.[11, 21]

The quality of the leather samples made from bovine hide that were presoaked in soaking formulations with sophorolipid-oleic but with 10% crude glycerol and 1% sodium carbonate was comparable to the ones without the biodegradable surfactant. Even the samples co-treated with formulations that included chlorine dioxide (ClO_2) showed similar chemical characteristics as long as the same amount of crude glycerol and sodium carbonate were incorporated. The only difference that was observed among the crust leather samples was the different amounts of water or moisture content. The water peak is observed from 1900 to 2000nm, mostly centered at ~1943 nm. The outside region or grains of the finished leathers are not differentiable from each other. Likewise, the inside region or corium of the hide were also not differentiable from each other. However, a significant difference in the NIR-spectra can be seen when the outside of the hide, better known as the grain, were compared to the inside of the hide, known as the corium. From Fig 4, it can be seen that the corium has an additional reflectance at 1682.3nm. There is a slight shift from 2042 nm on grain side to 2049 nm on the corium side. There is also another slight shift from 2307nm on the grain side compared to 2315nm on the corium side.

The similarity or subtle differences can be illustrated more clearly by applying statistical calculations on the NIR spectra called Principal Component Analysis (PCA).[14] Because of the high signal to noise ratio in NIR instruments, derivatives are frequently used such as the one utilized in PCA, for identification and quantification using these bands. Specifically, PCA for this data set is a method for reducing the 100 variables (wavelength data) in each scan down to just a few important variables. These variables are often referred to as latent variables or principal components (PCs). After calculating the PC values of all scans, the standard deviation for each PC can be calculated that encompasses the limits for each cluster. The limits are drawn on the plot at plus and minus the number of standard deviations specified the Stdev value, centered around the average PC-n value for each PC.[14] The data obtained from the differently treated samples are compared to the control, which was soaked in standard soaking solution normally used by the hides industry.

The NIR spectra of the nine (9) different leather products were not distinguishable from each other, according to sample grouping in the PCA analysis.[14] The major chemical composition of each leather product is observed to be the same overall. The broad bands in NIR spectra arise from overlapping absorption bands of polar functional groups in different intramolecular and extramolecular environments. It implies here that the intermolecular environment of the functional groups affects the anharmonic and dissociation energy of hydrogen bonds and thereby shifting the absorption band.

Microbiological Growth Inhibition Studies

Various presoaking formulations, utilizing only fractions of the standard presoaking formulation (SF) were prepared and used for microbiological inhibition studies on petri plates shown in Table III.

The microbiological study results are shown in Fig 7, where the "as is" standard presoaking formulation (or 100% SF) (ZS-1) before soaking, did not exhibit any growth. After 1 h of soaking, no bacterial colonies were observed either. This implied that the solution was clean and inhibited any bacterial growth coming from the manure after soaking. The "as is" pure glycerol (ZS-2) was also clean but after soaking, numerous bacterial colonies were observed, implying that it was not efficient in inhibiting bacterial growth from manure. The crude glycerol alone (ZS-3) exhibited some microbial growth even before soaking. This implied that originally, crude glycerol had inherent bacterial contamination of its own. The formulation without any standard SF (ZS-4) but with sophorolipid (SL-Oleic) showed some growth before and after soaking; again this could be due to the presence of crude glycerol. In ZS-5, a number of colonies were observed after soaking with manure sample. This implied that 10% standard SF (0.015% Boron TS, 0.01% Proxel GXL) alone is not enough to inhibit microbial growth. By increasing the standard SF to 25% or by including 0.0375% Boron TS and 0.025% Proxel GXL in ZS-6 to ZS-8, a good amount of inhibition were observed before and even after soaking. Additional inhibition was observed with the presence of chlorine dioxide (ZS-7 and in ZS-10) and further microbial growth inhibition was shown with the presence of sophorolipid (ZS-4, ZS-8 and also in ZS-11).

The efficiency in adobe type manure removal was observed (unreported results) to be similar in 20% and 30% (v/v) of the standard soaking formulation if supplemented with 10% crude glycerol and 1% sodium carbonate. An almost uniform 80-90% softening of the hardened manure samples was observed after 1-2 h soaking.[4,6] Using only 10% of the standard

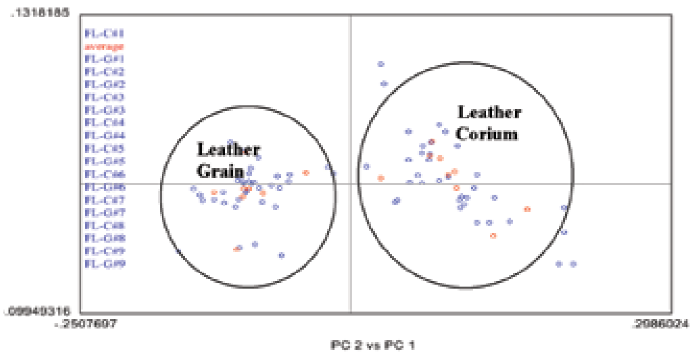

Figure 6. PCA analysis of the NIR spectral data of leather samples in Fig 4 and 5.

TABLE III
Presoaking formulations used for microbiological inhibition studies on petri plates.

Trial #	Soaking Solution Ingredients
ZS-1	100% Standard Soaking formulation (SF) (0.15% Boron TS, 0.1% Proxel GXL)
ZS-2	10% Pure Glycerol
ZS-3	10% crude Glycerol (c-G)
ZS-4	10% c-G +1% Na2CO3 (SC) + 0.1% sophorolipid-oleic (SL-oleic)
ZS-5	10% std SF (0.015% Boron TS, 0.01% Proxel GXL)
ZS-6	25% std SF + 10% c-G + 1% SC
ZS-7	25% std SF +10% c-G + 1%SC + 200 ppm Cl02
ZS-8	25% std SF + 10% c-G + 1% SC + 200 ppm Cl02 + SL-Palmitic
ZS-9	10% std SF + 10% c-G + SC
ZS-10	10% std SF + 10% c-G + SC + 200 ppm Cl02
ZS-11	10% std SF + 10% c-G + SC + SL-Oleic
ZS-12	10% std SF + 1 % SC

soaking solution was not as efficient (from the same unreported data). That is why 25% of the standard presoaking formulation was chosen as an optimum amount (also based on the efficient softening of MS-2 and MS-3 of Table II and the effective microbial growth inhibition of formulations ZS-6, ZS-7 and Z8 of Table III). It is considered eco-friendly because the amount of surfactant and biocide used was only a fraction (1/4) of what the hide industry has been utilizing. Additional microbial growth elimination was observed when about 200 ppm chlorine dioxide, another cleansing and sterilizing agent, was added in the formulation (ZS-7, table III). The addition of sophorolipid-oleic or sophorolipid-palmitic to the 10% crude glycerol and 1% sodium carbonate (ZS-11, table III) improved the manure removal efficiency and the microbial growth inhibition.

Conclusions

The NIR spectra of all the leather samples at the grain surface looked very similar to each other. This implies that the structural and chemical composition at the leather grain surface where the site of the NIR analysis was taken were the same or unaffected by the different treatments or when soaked in different formulations. This demonstrated that the newly developed formulations are as safe and non-invasive as the commonly used soaking solution to the hides and the resulting leather products. In fact, the quality of leather products made from hides soaked in formulations where crude glycerol and sodium carbonate are incorporated, are significantly improved.[6] The quality of the leather samples made from bovine hide that was presoaked in formulations with sophorolipids were comparable to the ones without it as long as 10% crude glycerol and 1% sodium carbonate were present. When crude glycerol and sodium carbonate are included in the presoaking solution, the efficiency in removing adobe type manure is improved even with just a fraction (1/4) of the amount of biocide and surfactant that the hide industry is normally using.

The standard soaking formulation with high concentrations of biocide and surfactant completely hindered microbial growth. Whereas, the soaking formulations with only a fraction of the standard solution but supplemented with crude glycerol and sodium carbonate prevented about 70% of the microbial growth. Microbial growth inhibition of almost 90% was also observed if the said presoaking formulation was supplemented with about 2 times the cmc (~0.1%) of sophorolipid, the biodegradable surfactant. To eliminate almost all microbial growth, about 100 ppm of chlorine dioxide can be additionally incorporated in the presoaking formulations with the added bonus of manure smell removal. When used in water disinfection, it does not form chlorinated or brominated by-products, such as trihalomethanes (THMs) or haloacetic acids (HAAs), both of which cause concern for health and will soon come under strict regulatory limits.[7, 9] In the rendering facilities of the meat packing industry, chlorine dioxide controls odors by destroying hydrogen sulfide through chemical oxidation.[9] Chlorine dioxide's effectiveness is not limited to just odor-related problems it is also readily biodegradable, thus it is environmentally friendly.

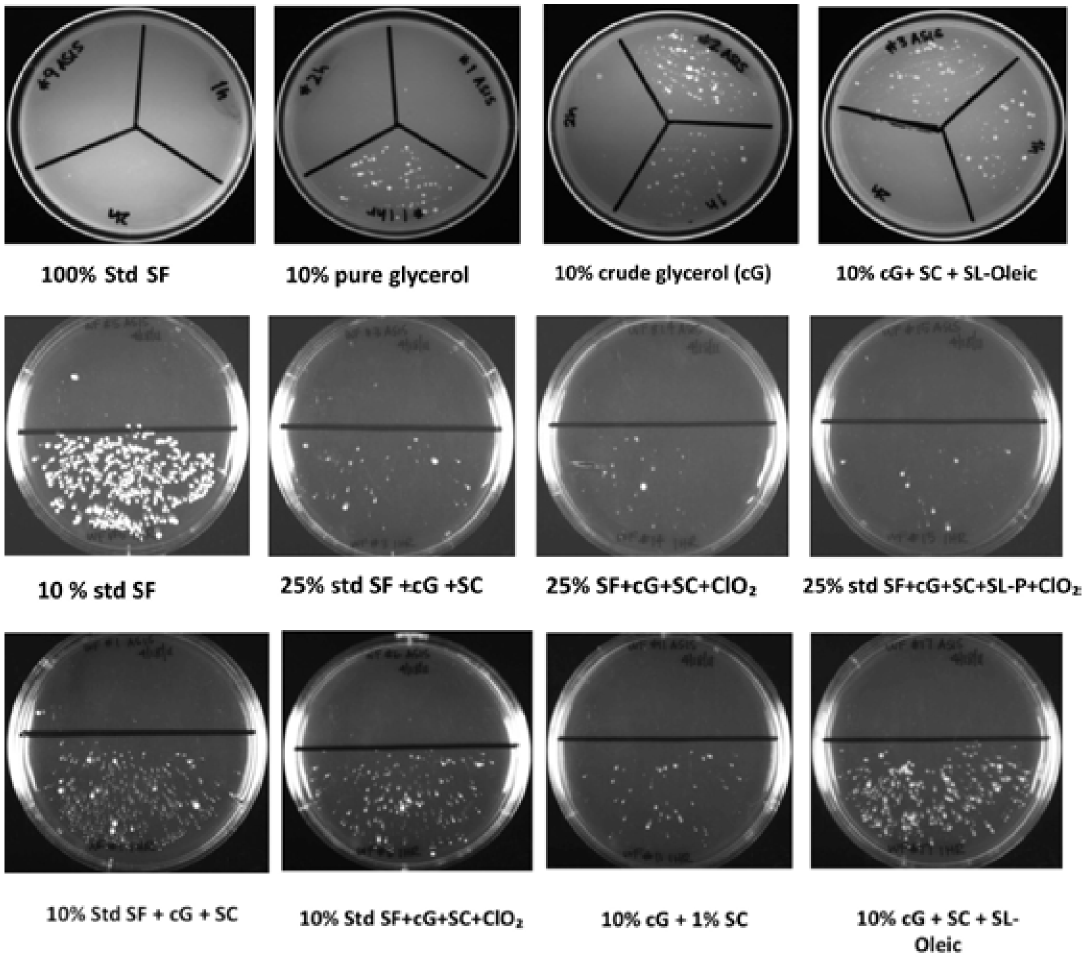

Figure 7. Petri plates showing microbial growth of manure washings from different soaking formulations from table III.

The outcome of this research is valuable to the hides and leather industry to improve the handling and processing of hides and the quality of the leather derived from it. The future direction of this research project will be to make more economical formulations by using lower concentrations of crude glycerol with the incorporation of enzymes such as cellulase (to act on the cellulose component of the manure), laccase (to act on lignin and lipid components of plants in the manrue), and xylanase (to act on plant cell walls known as hemicellulose present in manure balls). These formulations could potentially gain more practical applications in the hide industry as well as food safety in the meat packing industry.

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