

A Method for Removing Adobe-type Manure from Hides Using an Oxidizing Agent

by

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Abstract

Adobe-type (hardened) manure attached to bovine hair is a major source of meat contamination, hide quality deterioration, and devalued leather products. Therefore, it is important to develop cleaning solutions that can rapidly remove adobe-type manure to improve the quality of hides delivered to tanners for leather processing. Oxidative chemicals such as sodium percarbonate (SPC) have been used as an environmentally friendly alternative to dehair bovine hides in 1-4 hours. In this study, we do not aim to dehair the hide with SPC, but to weaken and shorten the hair enough to remove the adobe-type manure from the hide in under 30 minutes. Our formula substantially removed adobe-type manure in less than 30 minutes using a combination of SPC and sodium hydroxide (NaOH) as a soaking solution in a tumbling drum. Fourier Transform Infrared (FTIR) spectroscopy showed the oxidation of cystine disulfide bridges in keratin, the structural protein of hair, indicating that our formula weakened the hair after treatment. A decrease in hair diameter, or hair shortening, was also observed with Scanning Electron Microscopy (SEM) analysis after treatment of the hides with our formula. Our environmentally friendly formula for oxidatively removing adobe-type manure is a viable option to enhance both meat and hide quality.

Introduction

Animal hides are a large agricultural export of the United States, estimated at \$2.2 billion annually.¹⁻³ Unfortunately, the quality of hides delivered to tanneries suffers from putrefaction and damage from hide processing.²⁻⁴ A major source of hide deterioration and leather quality stems from hardened (adobe-type) manure clad to the hide hair. Accumulation of heavy hardened adobe-type manure causes microbial contamination

and creates mechanical stress on the hide.² On average, 3.7 kg of adobe manure can accumulate on a hide in the close quarters of cattle where manure balls typically form during the winter months.^{5,6} The mechanism of adobe manure formation has been compared to a freeze-thaw process where the manure accumulates on the hide hair and hardens as the temperature approaches freezing.⁶ As the cycle repeats it creates hardened manure that becomes exceedingly attached and entangled with the hide hair. A hide clad with manure also has an effect on the quality of milk and meat.⁶⁻⁹ Therefore, improving the removal of manure from the hide will alleviate some of the problems traditionally associated with the cattle industry.

Adobe manure balls often remain attached to the hair when the hides are delivered to tanneries for processing. Manure removal is sometimes done simultaneously with fleshing the hide. In a fleshing machine, dull blades scrape the side containing the hair and manure while sharper blades flesh the underside.¹⁰ This process causes mechanical damage to the hide by forcefully removing the manure balls from the hair creating holes in the grain. Manure balls can also burst through the hide after they are wedged between the blades and the hide. This results in unusable hides or poor quality leather products. Washing the hides has mitigated some of the problem, but manure balls tend to remain strongly attached to hide hair.

Other methods have been employed to alleviate the adobe-type manure problem. These include the development of soaking formulations that contain enzymes. Enzyme formulation studies included protease for dehairing and mixtures of cellulose, xylanase, and laccase that proved to be effective at removing adobe-type manure, but the process of isolating the enzymes for their use was too lengthy (10-20 days) for industrial applications.^{5-7, 11, 12} Other unpublished techniques using a freshwater soak in the raceway were unsuccessful because they did not satisfactorily soften or remove the manure in addition to

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creating undesirable effluent. Some studies have developed promising soaking formulations that add crude glycerol, sophorolipids, and industrial detergents to soften and remove adobe-type manure.^{2, 3} Sophorolipids are biodegradable surfactants and are attractive for being environmentally friendly.^{2, 13, 14} These methods were effective at softening the manure, removing microbial contaminants, and improving some leather qualities. However, the length of time for removing the manure was still unsatisfactory for industrial practices.

The objective of the current study is to remove strongly attached adobe-type (hardened) manure balls from hides in 30 minutes or less through the use of oxidative chemicals such as sodium percarbonate (SPC). Oxidative chemicals have been used in the past as an environmentally friendly alternative to sulfide dehairing of bovine hides.¹⁵ Oxidative chemicals used for dehairing hides in the beamhouse include alkaline peroxide, calcium or magnesium peroxide, sodium perborate, and SPC.¹⁵⁻²¹ SPC has been demonstrated as a useful reagent for dehairing hide in under an hour.^{15, 20} However, this study does not aim to dehair hides, but to weaken or shorten the hair so that the adobe manure is loosened and removed in 30 minutes or less. Because our aim is to only weaken or shorten the hair, the amount of SPC and sodium hydroxide (NaOH) traditionally used in dehairing is substantially decreased making their use more environmentally friendly.

Experimental

Materials

Fresh hides were obtained from a local meat packing company, courtesy of JBS Packerland in Souderton, PA. Three types of samples were cut from fresh hides for experiments: i. pilot scale samples with manure attached to test the efficacy of manure removal, ii. pilot scale samples without manure to test the effects of the oxidative manure removal formula on the final leather product, and iii. bench scale samples without manure for laboratory tests.

Pilot scale pieces with manure attached were selected with similar thicknesses and manure ball size, but were cut asymmetrically to maximize the area with manure balls attached (approximately 200 x 200 mm). Pilot scale samples without manure were cut into 200 x 200 mm pieces (approximately 300-500 g) according to ASTM method D2813 and subsequently tanned. Three samples each were cut from matched sides (one side as a control) from the tail toward the neck. Bench scale samples were cut into smaller pieces (75 x 75 mm or approximately 30-70 g).

Sodium hydroxide (NaOH) was obtained from Mallinckrodt (Phillipsburg, NJ). Sodium percarbonate ($\text{Na}_2\text{CO}_3 \cdot 1.5\text{H}_2\text{O}_2$) or SPC was obtained from Alfa Aesar (Ward Hill, MA).

Oxidative Manure Removal Treatment

A 200% float (volume of water / weight of hide or v/w) was used for both bench and pilot scale experiments. NaOH (1-5% weight of NaOH / volume of float or w/v) was added to the float and allowed to dissolve prior to adding the hide pieces. The float containing NaOH was brought to temperature (40°C) using a hot plate for bench scale experiments or set using automated controls of a 6-in-1 drum set-up (Dose Maschinenbau GmbH, Lichtenau, Germany) for pilot scale experiments. Bench scale float temperatures were monitored using a thermometer throughout experiment. The hides were then added to the float followed by SPC at 5% w/v of the float.

In bench scale experiments, the hide pieces were plunged into the float 5 to 10 times causing the SPC to dissolve. The hide pieces were plunged or agitated in the solution four to five times every min to simulate a tumbling action. During pilot scale experiments, the 6-in-1 Dose drums controls were set to 10-15 RPM for tumbling. Control samples went through the same process without the addition of NaOH or SPC. Manure removal was monitored for 30 min.

Tanning and Mechanical Properties

After the oxidative manure removal treatment, pilot scale hide pieces and their controls were washed 3 times for 10 min with a 200% float of tap water in the 6-in-1 Dose drums. They were then processed into crust upper shoe leather (1.7-2.2 mm) following a revised version of the standard USDA tanning protocol.²² The revised tanning protocol includes the replacement of the retanning formula with 4% Mimosa and 4% Quebracho vegetable tanning agents. The leather samples were kept in a temperature (21°C) and humidity (50% relative humidity) controlled environmental chamber for 5 days until mechanical tests were performed. Five dog bones (1x10 cm) were cut from each hide piece perpendicular to the back bone and mechanical property values were averaged (tensile strength, Young's Modulus, fracture energy, and elongation-to-break) according to ASTM methods D2813 and D2209 to verify the effects of the oxidative manure removal formula on the quality of leather. An MTS Insight 5 kN mechanical property tester and Testworks-4 data acquisition software (MTS Systems Corp, Minneapolis, MN) was used to test the mechanical properties. The strain rate was set to 25.4 cm/min with a grip distance of 10.16 cm.

Hair Integrity (FTIR and SEM Analysis)

Bench scale hide pieces without manure were subjected to the oxidative manure removal treatment to determine hair weakening via the oxidation of cystine disulfide bridges in keratin to cysteic acid. The samples were treated with 5% SPC and NaOH (1, 2, 5%) for 30 min and analyzed by a Fourier Transform Infrared (FTIR) Thermo Scientific Nicolet 6700 spectrometer (each sample scanned 64 times with a 4.00 cm^{-1}

resolution). Prior to FTIR analysis, the liquid extracts from the hide pieces (after oxidative formula treatment) were placed in a 0.875 in. diameter Spectrum™ Spectra/Por™ 4 RC Dialysis Membrane Tubing (Fisher Scientific) with a 12,000 to 14,000 Dalton molecular weight cutoff and continuously stirred in a glass cylinder containing nanopure water at 5 times the sample volume. The nanopure water was exchanged after two and four h followed by an additional 48 h of dialysis.

The sample extracts were then freeze-dried for five days using a Labconco lyophilizer and prepared into a KBr pellet (2 mg sample to 250 mg KBr) for FTIR analysis. The formation of cysteic acid in the hide extracts was monitored at 1040-1045 cm^{-1} and compared against a cysteic acid standard calibration curve (1-10 mg standard to 250 mg KBr). Untreated hair was freezer-milled, made into a KBr pellet (1 mg sample to 250 mg KBr) and also compared against the cysteic acid standards. All FTIR absorbance values were normalized to baseline signals at 3950 cm^{-1} .

Additionally, representative treated and untreated samples (~2.5 x 2.5 cm) were cut from pilot scale pieces that went through the following sequential steps: i. oxidative manure removal formula treatment (when applicable), ii. sulfide dehairing, and iii. reliming and analyzed by Scanning Electron Microscopy (SEM) to observe hair shortening and surface changes. The samples were then cut in half, placed in a test tube, covered with nanopure water, and lyophilized for 5 days. Two pieces (1.5 mm) were cut from each of the dry samples and mounted on to the surfaces of carbon adhesive tabs with the help of Duco cement. After drying for at least 1 h, silver paint was applied to the exposed surface

area around the samples. The samples were sputter-coated with a thin layer of gold using a Scancoat Six Sputter coater. Next, the surfaces and hair content (for visual damage and hair diameter) of the samples were analyzed by a Quanta 200 FEG (FEI Company, Oregon) field-emission environmental scanning electron microscope. The SEM was operated in high vacuum-secondary electron imaging mode at a voltage of 10 kV, pressure of 0.3 torr, and a spot size of 3.0. Digital images were collected at 50, 250, 500, and 1000 magnification.

Results and Discussion

Adobe-type manure was progressively removed from pilot scale hide samples after tumbling in a 6-in-1 drum at 40°C with 5% SPC and 2.5% NaOH (the oxidative manure removal formula) as shown in Figure 1. Figure 1a shows an untreated hide with hardened manure attached to the hide hair. After 10 min (Figure 1b), weakening of the hide hair begins with minor removal of manure and at 20 min (Figure 1c) the manure balls are considerably removed. By 30 min the hide hair is significantly weakened and 100% of the manure balls were removed into the effluent. Untreated control samples subjected to a 40°C float (water only without SPC and NaOH added) did not remove or loosen the manure (images not shown).

Hide samples were not fully dehaired after treating them with the oxidative manure removing formula (Figure 1d) leaving much of the shortened hair intact on the hide. The width of the hide hair before treatment (~30 μm , Figure 2a) decreased to about 30% of its original size after the hide was treated (~10 μm , Figure 2b). Past studies have shown that alkaline solutions containing hydrogen peroxide or SPC (a 3:2 adduct of hydrogen peroxide and sodium carbonate) can be used for dehairing hides in the beamhouse.¹⁸⁻²⁰ The conjugate base (HOO^-) of hydrogen peroxide (H_2O_2) in the SPC is formed at high pH and readily oxidizes hair.^{20, 23-25}

The remaining weakened / shortened hair, left after the oxidative manure removal treatment, was completely removed after sulfide dehairing, as seen in the SEM micrographs (Figures 2c-2d). Past studies that have used oxidative formulas to rapidly dehair hides sometimes observed hair immunization on the hide even after sulfide dehairing or reliming due to the high alkalinity of the oxidative treatment.^{15, 19, 20} This was not observed in our samples and may be attributed to the substantial decrease in NaOH (2.5%) in our treatment opposed to 7% NaOH used in the oxidative dehairing studies.^{15, 19, 20} Additionally, after treating the hide with the oxidative manure removal formula followed by the sulfide dehairing step (Figure 2d) there appears to be flaking, perhaps from hair pulp, left on the hide surface, but is largely removed with the relime step (Figure 2f).

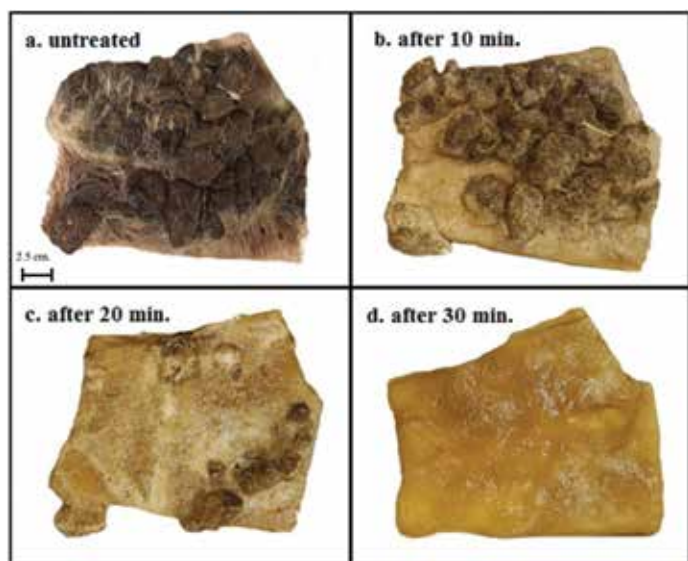


Figure 1. The hair on (a) fresh hide containing adobe-type hardened manure started to weaken after (b) 10 min removing some manure. The manure was considerably removed after (c) 20 min and fully removed after (d) 30 min with significant hair weakening using 5% SPC and 2.5% NaOH, in a 40°C 200% float.

FTIR analysis of the hide extracts show that as you increase NaOH 1 to 5% (with 5% SPC) the formation of sulfonic acid groups also increases (Figure 3). The formation of the sulfonic acid groups is most likely from the SPC oxidation of cystine's disulfide bridges in keratin, the structural protein of hair. Cystine oxidation forms cysteic acid side chains on the protein (i.e. sulfonic acid groups) and appear in the IR region of 1040-1045 cm^{-1} .^{23,26} As the pH or NaOH concentration is increased in our formula (from 1, 2.5, and 5%) the resulting amount of cysteic acid is 30, 44, and 68 $\mu\text{mol g}^{-1}$ of extract respectively, in the treated hair extracts when compared to the untreated hair sample (Figure 3). In fact, the IR spectrum of the untreated hair does not show a peak for cysteic acid in the 1040-1045 cm^{-1} region indicating that the disulfide bridges of the untreated hair are still intact or they did not have the oxidized moieties of sulfonic acid groups, as in our treated samples.

Our oxidative formula does not negatively affect the mechanical properties of the final leather products (Figure 4). Adobe-type

manure is a major source of hide quality deterioration, but it is also important that the oxidative manure removal formula does not decrease the performance of the final leather products. The tensile strength (4a), Young's Modulus (4b), elongation-to-break (4c), and fracture energy (4d) were all statistically the same ($p > 0.05$, $N = 15$) for treated and untreated hides made into crust upper shoe leather. Although the two groups are statistically the same ($p > 0.05$, Figure 4), there appears to be more variability (standard deviation) in all of the mechanical properties for the

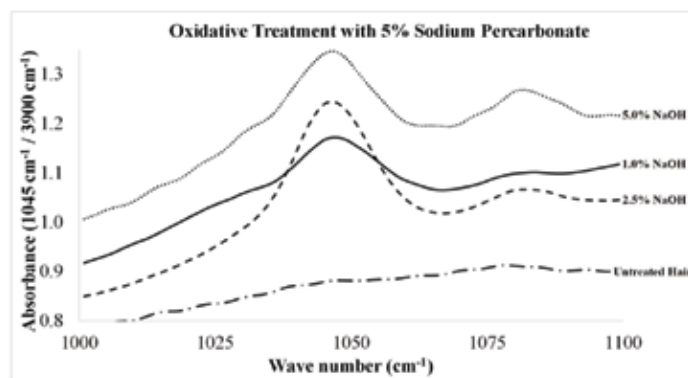


Figure 3. FTIR of hair and hair extracts in the cysteic acid region of 1040-1045 cm^{-1} . Cysteic acid formed in the hair extracts when the disulfide bridges in cystine (of keratin) were oxidized to sulfonic acid side chains. Hide samples were treated with 1% (—), 2.5% (---), and 5% (····) NaOH in the presence of 5% SPC and compared to an untreated hair sample (— ·).

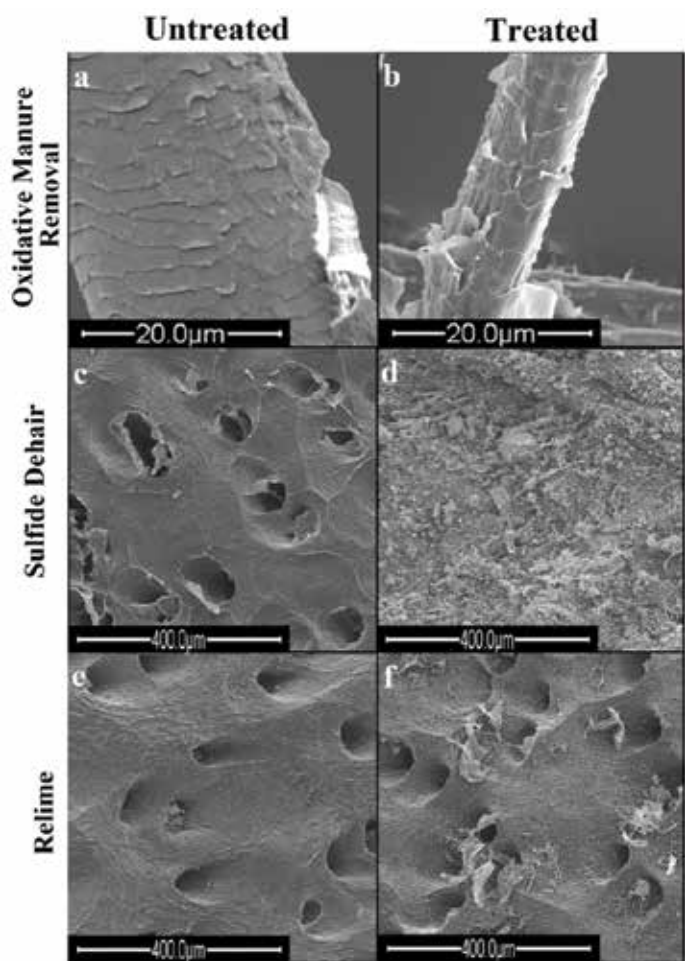


Figure 2. 2500x magnified SEM micrographs of (a) untreated and (b) treated hide with the oxidative manure removal formula. 250x magnification of the surfaces of (c) untreated hide at the sulfide dehairing step, (d) treated hide at the sulfide dehairing step, (e) untreated hide at the relime step, and (f) treated hide at the relime step.

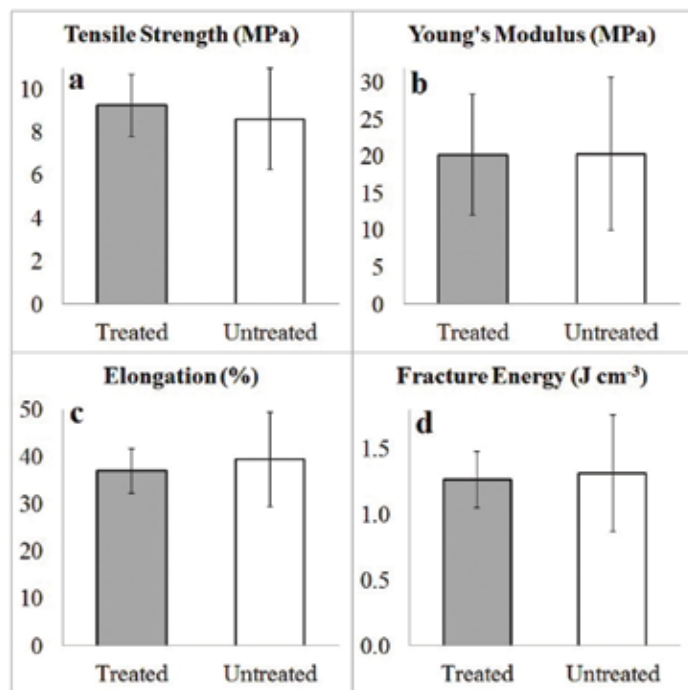


Figure 4. Mechanical testing results are statistically the same ($p > 0.05$, $N=15$) between crust upper shoe leather made from treated hides (grey bars) and untreated hides (white bars) with our oxidative manure removal formula containing 5% SPC and 2.5% NaOH.

untreated samples when compared to those that were treated. Past studies that have used SPC and NaOH to dehair hides noted similar variability, but without any discernable trends in the mechanical properties of the leather made from treated and untreated hides.²⁰

Conclusion

We demonstrated that our oxidative formula containing 5% sodium percarbonate (SPC) and 2.5% NaOH can remove hardened adobe-type manure from hides in 30 minutes or less. The mechanism of the manure removal proceeds through weakening / shortening of the hair via the oxidation of the cystine disulfide bridges in hair keratin. The mechanical properties of the treated and untreated hide made into leather were statistically the same indicating that the oxidative manure removal formula can help enhance the end product by preventing damage of the hide. We believe that our oxidative formula is a viable option for the environmentally friendly removal of manure. Further tests include removing manure in a shorter time frame and may be achieved through a spraying application.

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