EVALUATION OF DEGREASERS AS BRINE CURING ADDITIVES

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ABSTRACT
The length of time needed for brine curing of raw hides and skins, a minimum of 18 h, is a time-consuming process. In this paper we initially report the results of an investigation of the stratigraphic distribution of sodium chloride and water in fleshed hides cured for varying intervals of time. We demonstrated that salt entered the hide mainly from the flesh side. Water, on the other hand, was withdrawn from both sides of the hide; the epidermis acted as a semipermeable membrane. Three commercial degreasers as well as a glycolipid surfactant (sophorolipid) were tested as brine curing additives and their efficiency evaluated according to the moisture, salt, salt saturation and fat content levels in the brine-cured hide. One of the commercial degreasers, when used at 0.5% w/w, significantly removed fat from the hide as well as enhanced the uptake of salt. The sophorolipid also was an effective degreasing agent, decreasing the fat content of the brine-cured hide and, if used in excess, significantly increasing the uptake of salt. The data presented here confirmed that the usage of an appropriate degreasing agent in the brine is a suitable option for reducing the turn-around times in raceways and thus creating additional curing capacity.

RESUMEN
El tiempo requerido en la preservación de pieles crudas por la acción de salmuera, un mínimo de 18 horas, es un proceso que consume tiempo. En este trabajo reportamos inicialmente los resultados de una investigación estratigráfica de la distribución del cloruro de sodio y del agua en pieles previamente descarnadas y tratadas durante diferentes intervalos. Hemos demostrado que la sal penetró la piel principalmente por el lado de la carne. El agua, diferentemente, fue extraída a través de ambas direcciones en la piel; la epidermis se comportó como una membrana semipermeable. Tres desengrasantes comerciales así como también un tensoactivo glucolipido (soforolipido) fueron ensayados como aditivos auxiliares para la preservación por salmuera y su eficacia evaluada de acuerdo a los valores determinados por la humedad, sal, saturación salina, y contenido de grasa en la piel preservada por salmuera. Uno de los desengrasantes, cuando se utilizó al 0.5% en peso, significativamente desengrasó la piel así como aumentó la absorción de la sal. El soforolipido fue también un efectivo desengrasante, disminuyendo el contenido graso de la piel tratada por salmuera y, si su empleo fuese en exceso, significativamente aumentaría también la absorción de sal. Los datos aquí presentados confirmaron que el uso de desengrasantes en la salmuera es una opción apropiada para reducir el tiempo de rotación en los tanques de salazón y así aumentar la capacidad adicional del proceso de preservación.
INTRODUCTION

Curing of raw hides and skins with common salt (sodium chloride) is the most popular preservation method used currently. Sprinkling of solid salt on the flesh surface of the hide, known as green-salting, is extensively used in warm countries like India. Conversely, most American and European hide processing facilities typically treat their hides in raceways filled with a highly concentrated solution of sodium chloride (brine). The economics of brine curing of hides and skins have been affected recently by increasing commodity prices for sodium chloride. Furthermore, the large amount of salt needed creates environmental issues when it is soaked out, contributing to 40% of the total dissolved solids generated in the entire process of leather manufacturing.

In our previous paper we developed a mathematical model that described the diffusion of sodium chloride into the hide during the curing process. We concluded that the usage of saturated brine as well as a minimum float of 500% yielded an optimal diffusion rate. We also proved by means of epifluorescence microscopy that salt diffused into the hide mainly from the flesh side, which presents a less compact structure and greater porosity than the grain and upper corium. However, the fatty or adipose tissue present on the flesh side was reported to retard the diffusion of salt into the hide. Thus, a poor fleshing of the hide prior to the cure may further aggravate this phenomenon.

The use of degreasing agents in the leather manufacturing process is quite widespread and they are mainly utilized during soaking, liming and deliming to decrease the fat content of the wet blue below the norm value. However, there is no literature regarding the usage of these degreasers in the cure. If the use of a degreasing agent along with the brine could accelerate the penetration of salt into the hide, then turn-around times in the raceways would be reduced and thus additional curing capacity would be created.

Sophorolipids (SL) are glycolipid surfactants produced in large quantities by the yeast Candida bombicola. Among their desirable properties, they are biodegradable, non-ecotoxic, and non-foaming. Sophorolipids are currently used in the cosmetic industry and as an active ingredient of dishwashing detergents. The potential for their use in the leather industry as a degreasing agent is therefore worthy of consideration.

In the present paper we first carried out a stratigraphic study to monitor the distribution of sodium chloride and water in a hide throughout the curing process. The effect of the cure on the collagen denaturation temperature was also examined. Furthermore, we used scanning electron microscopy in backscattering electron mode to establish changes in the distribution of sodium chloride within the hide. Next, we evaluated the effect that commercial degreasers exerted on the penetration of salt into the hide, as well as their fat removal efficiency. Finally, the possibility of using SL as brine curing enhancers was investigated.

EXPERIMENTAL

Materials

Two fresh cow hides were purchased from a local abattoir. They were soaked in 200% water with 0.15% Boron TS (Rohm & Haas Co., Spring House, PA) and 0.10% Proxel (Chemtan Co. Inc., Exeter, NH) for 2 h and then fleshed. Approximately 6 x 10 in (15 x 25 cm) pieces were cut and stored at -20 °C. They were thawed at 4 °C just before use. Food grade sodium chloride of minimum purity 99.82% (US Salt Corporation, Watkins Glen, NY) was used in the brine preparation. Three commercial degreasing agents marketed to the leather industry and identified as degreasers 1, 2 and 3, as well as the experimental sophorolipid (SL) developed in our research facility, were used without further purification. All other chemicals were reagent grade and used as received.

Stratigraphic Study

Thawed hide was cut into square pieces of approximately 4 x 4 in (10 x 10 cm) with an average weight of approximately 100 g. They were transferred to a Dose drum (PFI 300-34; Dose 131 Maschinenbau GmbH, Lichtenau, Germany) and tumbled at 6 rpm in a 500% float of 95 °SAL brine solution (25.1 g NaCl/100 g brine) for varying time intervals, after which they were removed from the drum, hand-squeezed to wipe off excess water, split into three layers (flesh, middle and grain), sealed in plastic bags and stored at 4 °C until analysis.

Degreaser Study

Pieces of hides of approximately 100 g were tumbled at 6 rpm in a 500% float of initially saturated brine (100 °SAL, 26.4 g NaCl/100 g brine) for 16 h. To the test samples, the required volume of degreasers 1, 2 or 3 was added to the brine held in the drum and tumbled for five minutes to ensure a uniform distribution of the product within the brine. Due to its low solubility, the SL solution was prepared in the laboratory by adding the required amount of SL powder to the saturated brine solution and stirring for at least 24 h, after which the solution was dumped into the drum. An SL solution that had been filtered through a sintered glass funnel immediately after 24 h of stirring was also tested. Control samples to which no degreaser was added were also run for all experiments. The concentration of degreasers or SL was always expressed with respect to the combined weight of hide and brine. No further salt or brine was added to the drums during the experiments. Brine density was determined by gravimetric analysis. Hide density was assumed to be 1 g/cm³.
Determination of Moisture and Ash Content
An oval arch punch (C.S. Osborne & Co., Harrison, NJ), 7/8 inches (2.2 cm) in diameter, was used to sample the pieces of hide. Samples were clipped to minimize variability due to long hair. Next, they were weighed on an analytical balance into dry tared crucibles and dried in a vacuum oven at 80 °C for 18 h. After cooling in a desiccator they were weighed and percentage moisture was calculated. Dry samples were placed in a muffle furnace and ashed at 600 °C for 2 h. After cooling in a desiccator, they were weighed and percentage ash was calculated. All samples were run at least in triplicate.

Determination of Fat Content
In order to accurately compare results among different samples, fat content is expressed in terms of moisture and ash free basis (MAFB). All samples were run in triplicate.

Determination of Thermal Stability
Differential scanning calorimetry (DSC) analysis was performed on a Multi-Cell Differential Scanning Calorimeter CSC-4100 (Calorimetry Sciences Corporation, Lindon, UT). An aliquot of raw or cured hide of approximately 100 mg was weighed into a stainless steel pan and placed in the calorimeter. Another pan containing approximately the same weight of a 5% NaCl solution was used as a reference. When raw hide samples were analyzed, nanopure water was used as a reference. Both sample and reference capsules were weighed before and after the DSC run in order to determine any possible weight loss by leakage. Samples were heated from 10 °C to 120 °C at 1.5 °C/min. The peak maximum temperature was taken as the hide’s denaturation temperature (T0). All samples were run in duplicate.

Back-scattered/Low Vacuum Scanning Electron Microscopy (SEM-BSE)
Backscattered electrons consist of high-energy electrons originating in the electron beam that are reflected or backscattered out of the specimen interaction volume. The brightness of the SEM-BSE image tends to increase with the atomic number. Two major advantages over conventional SEM in the secondary electron image mode are the high-brightness of the SEM-BSE image tends to increase with the atomic number contrast images produced and the simplicity of the technique, which does not require a conductive coating.

A piece of raw hide of approximately 1 x 1 in (2.5 x 2.5 cm) was immersed in a beaker containing 500% v/v float of a saturated brine solution and gently agitated for varying intervals of incubation time. A 2-3 mm wide slice of the sample was excised manually with a stainless steel razor blade (cutting from flesh surface toward the grain), mounted on aluminum stubs, and then irradiated at an accelerating voltage of 25 kV in low vacuum mode (spot size 3.0, pressure 0.98 Torr) using a Quanta 200 FEG environmental scanning electron microscope (FEI Company, Hillsboro, OR). Samples were imaged as montages at a magnification of 50x. Individual montages of images at each curing time were processed and analyzed using Fovea 3.0 plug-ins (Reindeer Graphics, Asheville, NC) for PhotoShop, v. 7 (Adobe Systems, Inc., San Jose, CA). The montages were spatially filtered with a Gaussian blur (3 pixels) to reduce local noise and a line profile was drawn in each image extending from a point at the grain surface to a point at the flesh surface. The line profiles were then plotted as gray level versus distance (data not shown), and the ranges of gray levels for different curing times were plotted in Figure 3.

Statistical Analysis
Data were analyzed by analysis of variance followed by a Dunnett’s test to compare each treatment (degreasing agent) with the control. Further comparisons were made using orthogonal polynomial contrasts to test the linear and quadratic trends due to degreaser concentration. An orthogonal contrast was also used in the sophorolipid study to determine the significance of the filtering effect.

RESULTS AND DISCUSSION
Stratigraphic Study
The layer-wise distribution of water and salt (ash) in a hide cured for various intervals of time was monitored (Figures 1a and 1b). Typically, the flesh layer included the flesh itself and the attached adipose tissue; the middle layer represented the majority of the corium or true skin; and the grain layer included the junction of grain and corium and the epidermis. The initial moisture content was highest in the region of the epidermis and decreased toward the center of the corium. Whereas the diminishing moisture content of the flesh layer leveled off at 54% after 2 h of cure, the moisture content of the middle and grain layers continued to diminish over a 24-h period to less than 45% (Figure 1a). The salt content of a raw hide was found to be less than 0.5%. As curing progressed, salt content increased in all three strata: the flesh layer contained the highest amount of salt at all curing times (Figure 1b). Noteworthy, the salt content of the flesh layer after 30 min and 1 h of cure was 6.7 and 7.1%, respectively, but only 1.6 and 1.9%, respectively, in the grain layer.

These figures demonstrated that salt entered the hide mainly from the flesh side, which is in agreement with the findings of McLaughlin and Theis. It was also confirmed that the epidermis acted as a semipermeable membrane, which allowed the withdrawal of water by osmosis but did not allow salt to penetrate through it. Thus, whereas water was extracted from the hide by both sides, only the flesh side was susceptible to extensive absorption of salt, which then diffused into the hide and towards the grain. The porous nature of the hide may have also contributed to the diffusion of salt through the hide by capillarity.

By means of the equation,
\[
\text{Saturation} = \frac{\text{%Ash}}{\text{%Moisture}} \times \left( \frac{1}{0.359} \right) \times 100
\]
the hide salt saturation levels at various curing times were calculated (Figure 1c). O'Flaherty claimed that a 70% salt saturation of the water remaining in the hide with a maximum of 50% moisture was needed for a proper cure. Later on, a new minimum standard of 85% salt saturation was established by the U.S. Hide, Skin & Leather Association. The salt saturation of the flesh layer was the highest of all three layers at all curing times. The reason was found in the rapid dehydration experienced in the early stages of the cure along with the almost exclusive penetration of salt from the flesh side of the hide. The differences of hide salt saturation levels among the three layers become less pronounced after 16 h of cure. Although we did not sample the hides beyond the 24 h curing time frame, one could assume that the hide and surrounding brine reached equilibrium after about one day of brining. In fact, it was previously reported that a hide continuously brined for 48 h took up moisture as well as salt; brining a hide beyond the equilibrium did not necessarily entail a more proper cure.

The denaturation or melting temperature ($T_D$) of the grain, middle and flesh layers of a hide cured for various time intervals was monitored (Figure 2). The hide's collagen, upon heating, undergoes a transition from the triple helix to a randomly coiled form in which the three chains are separated. The flesh layer was the first to reach a constant $T_D$ of 78 °C after only 2 h of cure. On the other hand, about 8 h of cure were needed to level off the temperature curve of the middle and grain layers ($T_D = 82 °C$). The increase of the thermal stability of the hide upon brining could be ascribed to two factors. The first and most important factor is the dehydration of the hide. Kopp et al. developed a mathematical function that showed an exponential decrease of $T_D$ with increasing moisture content. The second factor is protein salting out and aggregation. The brine, despite the disruption of some hydrogen bonds due to the withdrawal of water, induces this phenomenon, which was reported to further stabilize the collagen molecules, thus increasing the values of $T_D$.
below 200 (16 h), and the higher values were located within the flesh side of the cross-section, with a boundary slowly moving toward the grain side of the cross-section as the hours of curing increased (Figure 3).

Degreasing Study
The effect of three commercial degreasers on the content of water, salt and extractable fat of the hide after 16 h of brine curing was evaluated (Table I). A degreaser concentration of 0.5% (w/w) with respect to the combined weight of hide and brine was used. Samples that had been brine-cured in the presence of degreaser 1 showed a significantly higher salt content than the control, to which no degreaser had been added. Furthermore, degreaser 1 was shown to be the most efficient as far as its fat-removal capacity, significantly decreasing the extractable fat content with respect to the control. Samples that had been treated with degreasers 2 and 3, despite tending to increase salt content and decrease extractable fat, were not statistically more efficient than the control. The composition of degreasers 2 and 3 was likely the cause of a lower efficiency than degreaser 1. Although the specific compositions of these commercial products are not disclosed, they are typically composed of a blend of nonionic surfactants, modifiers and adjuvants. The hydrophilic-lipophilic balance (HLB) is a widely used scale in the technology of surfactants; HLB values run from 0 to 20 and indicate whether a surfactant has greater wetting (low end of the scale) or emulsifying properties (high end of the scale). It is likely that degreaser 1 had a slightly higher HLB than the other two products and hence a higher tendency toward forming oil-in-water emulsions. As a matter of fact, the emulsification of fat in water was reported to be a crucial step for the efficiency of the degreasing process.\textsuperscript{19}

Next, degreaser 1 was selected to study the effect of its concentration on the content of water, salt and extractable fat of a hide after 16 h of curing with brine (Table II). All three tested concentrations of degreaser (0.25, 0.5 and 1% w/w) significantly defatted the hide. Furthermore, hides treated with a concentration of 0.5% also had significantly higher salt and salt saturation values. The statistical analysis showed evidence of a significant quadratic trend with increasing concentration of degreaser 1, with a maximum occurring at a critical concentration between 0.5% and 1%. The usage of a concentration larger than this critical point neither further increased the uptake of salt nor additionally decreased the fat content. That could be ascribed to the fact that the concentration of surfactants in degreaser 1 was well above the critical micelle concentration.

The feasibility of using a sophorolipid (SL; a fermentation product, under investigation at this location) as a degreasing agent for raw hides was examined. Due to the low solubility of SL in the brine, the effect of filtering the SL solution prior to its use was also examined. By doing this, we ensured that the brine solution contained the maximum amount of soluble SL. Hides samples cured with the unfiltered brine-SL solution displayed significantly higher values of salt and salt saturation

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**Table I.**

<table>
<thead>
<tr>
<th>Degreaser</th>
<th>Concentration (w/w)</th>
<th>Water (g/100 g hide)</th>
<th>Salt (g/100 g hide)</th>
<th>Extractable Fat (g/100 g hide)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.22 ± 0.02</td>
<td>2.5 ± 0.1</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>Degreaser 1</td>
<td>0.5%</td>
<td>0.18 ± 0.01</td>
<td>4.1 ± 0.3</td>
<td>3.0 ± 0.2</td>
</tr>
</tbody>
</table>

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**Table II.**

<table>
<thead>
<tr>
<th>Degreaser</th>
<th>Concentration (w/w)</th>
<th>Water (g/100 g hide)</th>
<th>Salt (g/100 g hide)</th>
<th>Extractable Fat (g/100 g hide)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.22 ± 0.02</td>
<td>2.5 ± 0.1</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>Degreaser 1</td>
<td>0.5%</td>
<td>0.16 ± 0.01</td>
<td>4.1 ± 0.3</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>Degreaser 2</td>
<td>0.5%</td>
<td>0.18 ± 0.02</td>
<td>4.0 ± 0.4</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>Degreaser 3</td>
<td>0.5%</td>
<td>0.19 ± 0.03</td>
<td>4.2 ± 0.5</td>
<td>3.4 ± 0.3</td>
</tr>
</tbody>
</table>
TABLE I
Effect of Commercial Degreasers on Brine Curing [0.5% w/w]

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Moisture (%)</th>
<th>Salt (%)</th>
<th>Hide salt saturation (%)</th>
<th>Fat (%, MAFB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>46.5 ± 1.5</td>
<td>11.8 ± 0.9</td>
<td>70.9 ± 7.0</td>
<td>11.8 ± 2.6</td>
</tr>
<tr>
<td>Degreaser 1</td>
<td>47.9 ± 3.0</td>
<td>12.9 ± 0.9*</td>
<td>75.2 ± 1.1</td>
<td>7.3 ± 0.6*</td>
</tr>
<tr>
<td>Degreaser 2</td>
<td>46.5 ± 1.0</td>
<td>12.1 ± 0.8</td>
<td>72.1 ± 4.1</td>
<td>8.8 ± 3.6</td>
</tr>
<tr>
<td>Degreaser 3</td>
<td>46.7 ± 1.7</td>
<td>12.2 ± 0.4</td>
<td>73.1 ± 4.1</td>
<td>8.8 ± 2.2</td>
</tr>
</tbody>
</table>

Average of 8 values

Values with * are significantly different from the control (Dunnett’s test, p<0.05)

TABLE II
Effect of Degreaser 1 on Brine Curing

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Moisture (%)</th>
<th>Salt (%)</th>
<th>Hide salt saturation (%)</th>
<th>Fat (%, MAFB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>53.4 ± 2.0</td>
<td>15.2 ± 0.6</td>
<td>79.5 ± 1.3</td>
<td>16.8 ± 2.2</td>
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<td>Degreaser 1 [0.25%]</td>
<td>50.4 ± 2.0</td>
<td>13.8 ± 1.1</td>
<td>76.3 ± 3.5</td>
<td>7.4 ± 2.4*</td>
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<tr>
<td>Degreaser 1 [0.5%]</td>
<td>57.2 ± 4.2</td>
<td>17.1 ± 1.7*</td>
<td>83.5 ± 2.7*</td>
<td>3.8 ± 0.9*</td>
</tr>
<tr>
<td>Degreaser 1 [1%]</td>
<td>56.4 ± 2.9</td>
<td>16.5 ± 0.7</td>
<td>81.7 ± 1.9</td>
<td>9.7 ± 2.9*</td>
</tr>
</tbody>
</table>

Average of 6 values

Values with * are significantly different from the control (Dunnett’s test, p<0.05)

TABLE III
Effect of Sophorolipid on Brine Curing

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Moisture (%)</th>
<th>Salt (%)</th>
<th>Hide salt saturation (%)</th>
<th>Fat (%, MAFB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.0 ± 1.4</td>
<td>11.8 ± 0.6</td>
<td>72.8 ± 1.5</td>
<td>15.3 ± 0.5</td>
</tr>
<tr>
<td>SL Non-filtered</td>
<td>46.6 ± 0.8</td>
<td>12.5 ± 0.3*</td>
<td>74.9 ± 0.8*</td>
<td>9.1 ± 2.3*</td>
</tr>
<tr>
<td>SL filtered</td>
<td>49.7 ± 1.6*</td>
<td>13.0 ± 0.5*</td>
<td>73.0 ± 1.1</td>
<td>11.1 ± 1.9*</td>
</tr>
</tbody>
</table>

Average of 5 values

Values with * are significantly different from the control (Dunnett’s test, p<0.05)
than the control (Table III). The extractable fat was also significantly lower than the control. When a filtered brine-SL was used, the values for moisture, salt, and extractable fat content were significantly different from those of the control. Hence, SLs were demonstrated to have a degreasing activity on the hide. The percentage of fat removed from the hide was 40.5 and 27.5% for the unfiltered and filtered SL-brine solution, respectively. These figures are similar to those obtained for the commercial degreasers (from 25.4% for degreasers 2 and 3 to an average of 53.4% for degreaser 1). The undissolved SL likely provided a reservoir of active surfactant as the initial concentration of solubilized SL suffered from dilution or inactivation.

From Tables I, II and III it can be seen that test samples with a significantly higher amount of salt than the controls also exhibited a significantly lower fat content. However, this statement was not verified in the other direction. It was also observed that a significant effect of the degreaser on the content of salt and fat did not necessarily entail a significant increase in the salt saturation levels. That could be attributed to the wetting properties of the degreasers, which did not significantly alter the amount of remaining moisture in the hide after the 16 h curing time frame (Tables I and II).

It is important to bear in mind that the results presented in this study are based on the analysis run on different areas of two cow hides. Variance in the results can be attributed to some extent to the variability always found in biological material. In addition, although the hides were washed, fleshed, cut up and stored in the freezer on the same day that the animal was slaughtered, some damage to the hide due to a delayed cure might have taken place. A delayed cure of only a few hours was reported to slow down the diffusion of salt during the early stages of the cure. Furthermore, and for the particular case of fat content, some variability could be attributed to the horizontal distribution of grease, which is more abundant in the looser and more flexible areas (e.g., belly). Despite all these facts, the coefficients of variation (CV) obtained for the determination of moisture, salt and salt saturation were 3.97, 5.76 and 3.54%, respectively, which indicate good precision. In the particular case of the determination of extractable fat, a CV of 21.3% was relatively precise, considering the low analytical values of that parameter.

**Conclusions**

The purpose of research reported in this article was to evaluate the possibility of using degreasers as brine curing additives, with the aim of enhancing the uptake of salt by the hide. In the stratigraphic study carried out in order to get a better understanding of the curing process, we found that the epidermis acted as a semipermeable membrane, enabling the diffusion of water from the hide and into the surrounding float as well as hindering the diffusion of salt into the hide, which occurred from the flesh side overwhelmingly. These findings were corroborated by the pictures taken with a scanning electron microscope run in the back-scattered electron mode. Furthermore, the thermal stability of the hide increased upon curing, mainly due to the simultaneous dehydration and salting out processes of the constituent collagen. If 0.5% (w/w) of a commercial degreaser, made of a blend of nonionic surfactants, had been added to the brine, the fat content of that hide was significantly decreased and the uptake of salt was also significantly enhanced. Since another two commercial degreasers exhibited less stimulation of salt uptake, we concluded that the composition of the degreaser was a critical parameter for this specific purpose. The sophorolipid tested showed remarkable degreasing properties and enhanced the uptake of salt by the hide if it was used above the solubility limit. These facts along with its low-foam properties and low cost (from $1 to 3/kg) make it an attractive choice of surfactant. One may hypothesize that the addition of small amounts of a proteolytic enzyme along with a degreasing agent would enhance the fat removal action, since it would facilitate the breakdown of the proteinaceous membrane of the fat-containing sac. Nevertheless, the detrimental effect that this treatment could have on the grain is an important drawback.

In this paper, we gave an overview of the brine curing process of raw hides and also suggested the use of an additive (e.g., commercial degreasers, sophorolipid) to enhance the uptake of salt and remove a major amount of the fat. It is important to note that actual raceways or vats are operated continuously. Thus, it will be essential to find a way to remove the fat that builds up in the vat.

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**References**