

The mechanisms of effective soaking: Fresh and brine-cured hides

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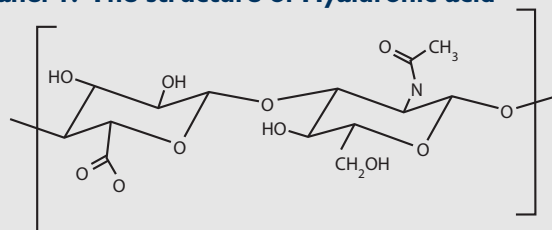
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The soaking of fresh cattle hides differs from the soaking of salted or brine-cured cattle hides. This paper reveals the differences between these two types of raw material, including the relationship between hyaluronic acid extraction from the hide into the float and effective soaking, and other related issues.

Since the early 1900s, soaking was regarded as an equilibrium process. Soaking for two to three days in fresh, cold water was the norm. Eventually, scientists^{1,2} began to unravel the biochemistry of the non-collagen impurities that inhibit the isolation of collagen for tanning.

Identification of the glycosaminoglycans that retard the opening up process has led to a more direct approach to the removal of these impurities. Among these glycosaminoglycans, hyaluronic acid is a long, non-branching polysaccharide chain with a high molecular weight (10^4 to 10^7 Da)³. It is highly hydrophobic and the structure is shown in *Panel 1*:

Panel 1: The structure of Hyaluronic acid



When fully hydrated, the hyaluronic acid molecule occupies nearly twice the volume of the non-hydrated counterpart. The net effect is to impede the migration of water and chemicals through the interfibrillar spaces within the hide. Accordingly, until this gel-like substance is removed, it inhibits the removal of other non-collagen proteins, thus slowing the opening up process, one of the main objectives of the beamhouse.

For adequate opening up of hide fibre, two major cementing substances (mucopolysaccharides) need to be removed. Strongly acidic dermatan sulfate is the smaller of the two. The more significant is hyaluronic acid, which appears to combine with proteins to yield proteoglycans. Alexander showed that hyaluronic acid is completely removed after soaking for 48-hours.

As green-salted or brine-cured hides are washed or soaked, and as the salt concentration in the interfibrillar spaces decreases, hyaluronic acid causes a dramatic increase in viscosity and the hide swells. Further soaking removes significant amounts of hyaluronic acid, with the subsequent release of water, bringing the hide closer to the desired flaccid condition.

Nearly 65-years ago, McLaughlin⁴ et al. observed exactly such a response, where, during the third day of a three-day soak, they observed a reversal in the direction of the swelling curve. They concluded that "coagulable proteins" are removed during soaking, aided by the presence of sodium chloride. It now seems

likely that the reduced swelling on day three was due, at least in part, to the removal of hyaluronic acid.

Many believe that cattlehides should be well soaked within 4 to 6 hours⁵. A pH of 9.5 to 10.5, and enzymatic soaking preparations, in combination with a suitable surfactant, can help reduce the required soaking time. Moreover, the use of proteolytic enzymes for breaking down interfibrillar proteins during soaking has been recognised for nearly 40 years. A float density of 4.0-6.0° Beamé indicates that equilibrium has been achieved and soaking is complete.

However, there is a common misconception that because fresh hides are not dehydrated on arrival at the tannery they do not require a significant soaking. Leafé has suggested that more intensive soaking is required for fresh hides because the non-structured protein in fresh hides have not been subjected to the degradation that occurs during the storage of salted or brine-cured hides.

Investigations into soaking fresh and cured hides

A series of investigations were made under both laboratory control and full-scale tannery practice to observe the effects of soaking fresh and brine-cured hides under different conditions.

LABORATORY TRIALS

The soaking of fresh hides: In the first series of trials, five adjacent pieces of fresh hide were treated with combinations of soda ash, an ethoxylated linear alcohol surfactant (Busperse® 2196), and proteolytic enzyme (Buzyme® 148). The floats were sampled at intervals of 5-minutes, 1-hour, 2-hours and 4-hours. Each hide piece was weighed and the float was tested for temperature, pH and density. The fifth hide piece in the series was processed with a combination of all three chemicals, but was soaked for 24 hours overall and sampled at 8-hour and 24-hour run times.

The soaking of brine-cured hides: In the second series of trials, five brine-cured hide pieces were soaked following the same protocol used for the fresh hide pieces, except the surfactant and soda ash dosages were increased. The soaking time for the cured hide pieces was extended to 24 hours for all five trials. The 4-hour float samples were retained for hyaluronic acid determination. For the fifth trial, which was processed with all three process chemicals, 20ml samples of the float were retained after each interruption of the process. Float samples were tested for the same parameters as in the fresh hide trials.

TANNERY TRIALS

Full-scale tannery trials were conducted at Prime Tanning in St



Joseph Missouri in March 2007. These trials were based on normal soaking procedures, but with significant variations in chemical dosages. The generic procedure is to soak fresh hides in drums with 0.25% of an ethoxylated alcohol surfactant, 0.67% soda ash and 0.36% of a proteolytic enzyme for five hours in a 110% float at 29°C. In some of the trials chemicals were offered at normal dosages, and at elevated dosages in others. For example, soda ash was doubled and the enzyme was increased by 50% in several of the trials. Some drums were run with specific chemicals omitted.

An identical soaking protocol was used for brine-cured hides as that used for fresh hides, with appropriate adjustments to the chemical dosages. The normal soaking time was 5 hours, but for most of the fresh and cured hide trials the floats were sampled at 5 minutes and 5 hours. For one of the fresh hide trials and one of the brine-cured hide trials, float samples were taken after 5-minutes, 1 hour, 2 hours and 5 hours. All of the float samples were analysed on site for temperature, pH and Baume, and aliquots were chilled and sent to Buckman in Memphis, for further testing.

MEASURING HYALURONIC ACID

Hyaluronic acid was measured using the HA-ELISA (*Enzyme-Linked Immuno Sorbent Assay*) method. This is a quantitative enzyme-linked immunoassay designed for the in vitro measurement of hyaluronic acid levels in human or animal biological fluids (*Echelon Biosciences, Salt Lake City*). The concentration of hyaluronic acid in the sample was determined spectrophotometrically, using a standard curve produced from known amounts of hyaluronic acid.

Findings from the investigations

Two different parameters were investigated as part of the soaking mechanism:

1) WEIGHT GAIN AND FLOAT DENSITY AS MEASURES OF SOAKING

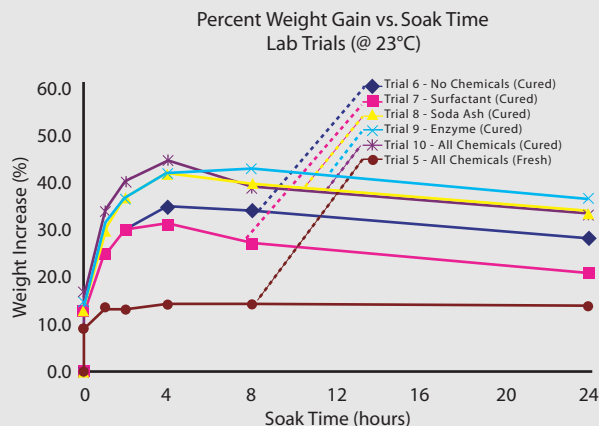
When weight increase is the objective, the laboratory trials confirmed that the most commonly used soak time in the United States of 4 to 6 hours as the optimum. The percent weight gain for brine-cured hides steadily increased until it peaked at four hours, but after this time the weights of four of the five brine-cured hide pieces declined significantly. The greatest weight gain was 45%, recorded when all three of the chemicals used in this study were offered at the same time (*Trial 10*). These findings are shown in *Panel 2*:

Panel 2 also shows the weight increase for the single fresh hide piece that was soaked for a full 24 hours. *Trial 5 (which utilised all three chemicals offered)* had the highest degree of water absorption of any of the five fresh hide pieces. For this trial, the weight was nearly stable after only about one hour, with a weight gain of only 14%, this time being much less than for brine-cured hide pieces.

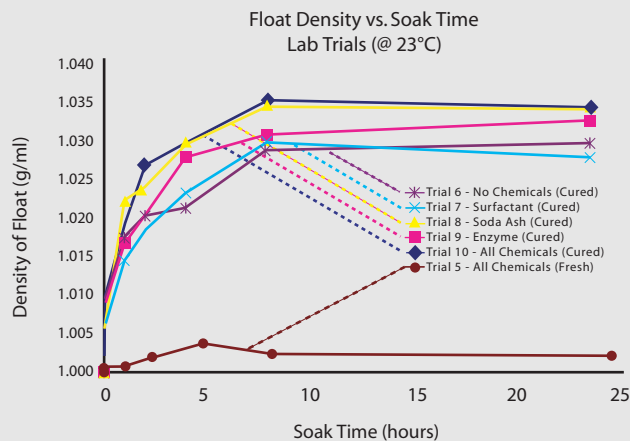
It was also found that both float densities and chloride concentration steadily increased with time in the soak. As expected, the correlation between soak density and chloride concentration was excellent.

For brine-cured hides - in contrast to the weight gain statistics - the optimal soak time (*as measured by either maximum float density or chloride concentration*) is eight hours or more, depending on the chemistry used. However, for fresh hides, the float density peaked at 4-hours, and any increase may not be significant. These findings are given in *Panel 3*:

Panel 2: Weight gain vs. time



Panel 3: Float density vs. time



The tannery results for soak liquor density resemble those seen in the laboratory investigations, and little correlation with chemicals offered was observed. There was, however, a significant difference in the influence of soak time on float density for brine-cured hides compared to fresh hides. The fresh hide trial that incorporated all three of the chemicals at the normal tannery levels showed that the density quickly stabilised after 1 hour at 3.0° Beamé. This density was maintained to the end of the 5 hour soak. On the other hand, in the brine-cured hide trial with the same chemical additions, soak density continued to increase up to five hours.

2) HYALURONIC ACID REMOVAL AS A MEASURE OF SOAKING

Hyaluronic acid removal has been suggested as an alternative measure for gauging the progress of soaking. To that end, the hyaluronic acid extracted during soaking in both the laboratory and the tannery soaking trials was measured. (*A typical calibration curve for the Echelon HA-ELISA method revealed a logarithmic function for which a least squares regression analysis yielded an R² value of 0.98, indicating a good correlation.*)

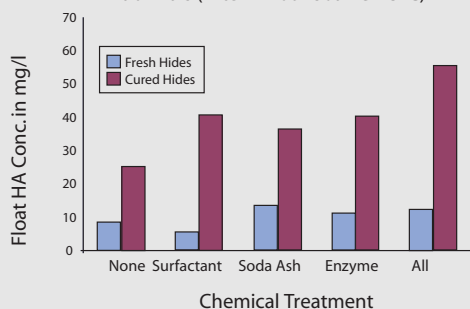
The maximum theoretical extraction of hyaluronic acid, based on the report of Alexander, et al., was calculated at 192 mg. From these trials, for a brine-cured hide piece soaked with all three of the subject chemicals added, the total mass of hyaluronic acid extracted measured just less than 50% of Alexander's figure after 8-hour soaking and 75% after 24 hours.

In contrast, the mass of hyaluronic acid extracted from fresh



Panel 4: Hyaluronic acid removal – laboratory trials

Effect of Chemical Treatment on Hyaluronic Acid Removal
Lab Trials (After 4-hour Soak @ 23°C)



hide pieces differed significantly, with fresh hides yielding very little hyaluronic acid in the float. Even with the full array of chemicals offered in these trials, the hyaluronic acid extraction after the full 24 hours was less than 11% of the theoretical amount. Overall, significantly more hyaluronic acid was removed from brine-cured hides than for fresh hides after 4-hour soaking.

During laboratory soaking, the different chemical treatments influenced hyaluronic acid removal to varying degrees. For fresh hide pieces, soda ash and proteolytic enzyme positively influenced hyaluronic acid removal. On the other hand, the addition of surfactant actually appears to be counterproductive.

For brine-cured hide pieces, each of the chemicals offered had

a positive effect on hyaluronic acid removal. The addition of all three chemicals together yielded the best results, with a total of 58mg/l hyaluronic acid detected in the float after 4 hours. These findings are given in *Panel 4*:

The effect of chemical additions on hyaluronic acid extraction was also measured in the tannery trials. At equilibrium, complete extraction of the hyaluronic acid would yield 500 mg/l in the float. In these tannery trials, the maximum hyaluronic acid extraction of 147 mg/l occurred for brine-cured hides when a proteolytic enzyme was used by itself at the normal dosage. This was less than 30% of the theoretical hyaluronic acid value. In contrast, the highest HA extraction measured for fresh hides was only 66 mg/l, about 13% of theoretical. When no chemicals were used or when surfactant or soda ash were used alone, the hyaluronic acid levels in the float were markedly lower for both fresh and brine-cured hides. These findings are given in *Panel 5*:

The mechanisms of effective soaking

The removal of hyaluronic acid as an essential component of the soaking sub-process is now regarded as requisite to the ultimate success of opening up the hide structure. There has been a significant shift in the art of soaking towards the use of chemical adjuncts such as surfactants and proteolytic enzymes; both are widespread and soda ash remains an essential component. Until now there has been little reported on the effect of these changes in soaking practice on even the most basic measures of soaking efficiency.

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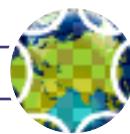
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The measurement of hyaluronic acid in the soaking float advances our understanding of the mechanism of effective soaking. The data presented here supports the theory that hyaluronic acid impedes the passage of water into and through the hide matrix during the soak.

This series of studies shows in particular that where hyaluronic acid removal was highest, water absorption peaked early, followed by a significant reduction in free moisture in the hide. Subsequent to this reversal in the weight gain versus time slope, the salt concentration in the float declines, further indicating the release of free moisture by the hide into the float. These results help explain observations made by McLaughlin back in 1923.

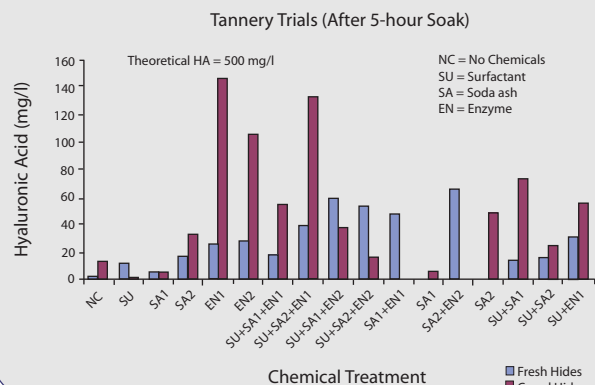
Conclusions

- Different parameters regarding the thoroughness of the soak - weight gain, float density, and hyaluronic acid removal - yield different conclusions.
- Conventional soaking times of 4-6 hours for brine-soaked hides do not give an equilibrium soak.
- Hyaluronic acid removal from brine-cured hides during conventional soaking is incomplete.
- Hyaluronic acid removal from fresh hides during conventional soaking is minimal.
- Chemical augmentation with combinations of soda ash, surfactant and proteolytic enzymes improves hyaluronic acid removal.

Acknowledgements

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Panel 5: Hyaluronic acid removal – tannery trials



annual meeting of the American Leather Chemists Association, June 23, 2007. The original version of this paper was published in JALCA, p76-85, Vol. 103, 2008 under the title "Soaking: Balancing operational and quality issues using both fresh and brine-cured hides".

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