Soaking: Balancing Operational and Quality Issues Using both Fresh and Brine Cured Hides*

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Abstract: This paper compares contemporary soaking practices to historical methods, emphasizing those aspects of beamhouse chemistry that are widely acknowledged as critical for optimization. We examine current practice and challenge the commonly accepted measurements used to gauge optimal soaking of fresh and brine cured hides. We identify differences with respect to removal of the non-collagenous hide component, hyaluronic acid, for various process chemistries on both fresh and cured cattlehides.

Key words: beamhouse chemistry; soaking; hyaluronic acid

1 Introduction

This paper reviews the state-of-the-art for soaking bovine hides as it relates to the theory of hydration and preparation of the hide matrix for subsequent leather making operations. Several questions beg attention. Most significant among them is, "How does the soaking of fresh cattlehides differ from that of brine-cured cattlehides?" Similarly, there has been no significant reporting of the relationship between hyaluronic acid extraction from the hide into the float and effective soaking.

Since the early 1900s, soaking was regarded as an equilibrium process. Practitioners and scientists[1-4] of the time recognized the importance of a thorough soak for proper rehydration of hides and skins. Textbooks from that era gave only passing reference to the practice. Soaking for two to three days in fresh, cold water was the norm. Eventually, scientists[5, 6] began to unravel the biochemistry of the non-collagen impurities that inhibit isolation of collagen for tanning. Identification of the glycosaminoglycans that retard the opening up process has led to a more direct approach to removal of these impurities.

Glycosaminoglycans are acidic and neutral polysaccharides that complex with proteins to yield mucoids. They consist of hyaluronic acid, chondroitin sulfate, chondroitin, dermatan and keratin sulfates and heparin. These polyelectrolytes control the viscosity of fluids in the extracellular spaces of the hide and they help manage the flexibility of animal tissue by controlling plumpness. Glycosaminoglycans also affect metabolic processes, regulating passage of all substances from cell to cell through the extra cellular spaces.

Hyaluronic acid is a long, non-branching polysaccharide chain (see Figure 1) that is highly hydrophilic. 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. Until this gel-like substance is removed, it inhibits the removal of

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other non-collagen proteins and slows the opening up process, one of the main objectives of the beamhouse.

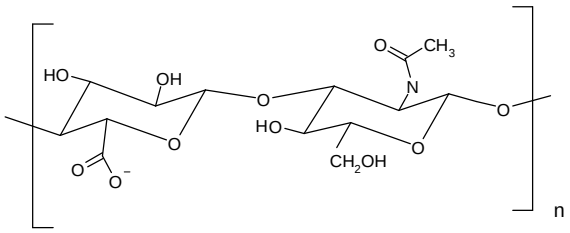

Fig. 1 Hyaluronic acid

For adequate opening up of hide fiber, two major cementing substances (mucopolysaccharides) need to be removed. Strongly acidic dermatan sulfate is the smaller of the two. The other is the very high molecular weight, hyaluronic acid, which does not appear to be bound to the collagen. Alexander showed that hyaluronic acid is completely removed after soaking for 48-hours.

Glycosaminoglycan solutions, including those of hyaluronic acid, exhibit a dramatic increase in viscosity with decreased salt concentration. As green-salted or brine cured hides are washed or soaked and as the salt concentration in the interfibrillar spaces decreases, 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, McLaughlin[7] ***et al.*** observed exactly such a response. 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. Since then, others have suggested that excessive removal of glycosaminoglycans[8] during soaking comes at the expense of looseness, veininess and coarse fiber. The utility of proteolytic enzymes for breaking down interfibrillar proteins during soaking has been recognized[9] for nearly 40-years.

While there is a wide range of opinion with respect to what defines adequate soaking, many believe that 20-36 kg cattlehides should be well soaked within 4 to 6 hours.[10] 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. A float density of 4.0-6.0° Baume′ indicates that equilibrium has been achieved and soaking is complete.

There is a common misconception that fresh hides do not require significant soaking since they are not dehydrated on arrival at the tannery. Leafe has suggested that in fact, more intensive soaking is required for fresh hides, since 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.

2 Experimental

2.1 Laboratory trials

Five adjacent pieces of fresh hide, each weighing 200g, were individually soaked in bench top drums. The float was fixed at 400 ml of 23°C water. One of the pieces was processed without any chemical addition, one with 0.25% of an ethoxylated linear alcohol surfactant (Busperse® 2196), one with 0.67% of dry powdered soda ash, and one with 0.045% of a commercial proteolytic enzyme (Buzyme® 148). These four hide pieces were soaked a total of four hours each. The fifth hide piece in

the series was processed with a combination of all three chemicals, but was soaked for 24-hours overall. 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 hide pieces and float samples were returned to the drums and the process continued. For the fifth trial, an aliquot of float was removed from the process and retained after each interruption for subsequent hyaluronic acid determination. Float samples from the first four trials were similarly retained after 4-hours running time. The fifth trial, containing all three chemicals, was also sampled at 8-hours and 24-hours run times.

For the second lab series of evaluations, cured hide pieces were soaked following the same protocol as for the fresh hide pieces with a few modifications. The surfactant dosage was increased to 0.40% and the soda ash dosage was increased to 1.00%. The enzyme dosage remained the same as for the fresh hide pieces, at 0.045%. The soaking time for the cured hide pieces was extended to 24-hours for all five trials. All five trials were interrupted at the intervals: 5-minutes, 1-hour, 2-hour, 4-hours, 8-hours and 24-hours. Again, the floats were sampled at each interval and the hide pieces were weighed. Only the 4-hour float samples were retained for four of the five trials. 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.

2.2 Tannery trials

Full-scale tannery trials were conducted at a large side leather tannery in the US, according to a generic soaking protocol, 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. Chemicals were offered in some of the trials 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.

We used an identical soaking protocol for cured hides as that used for fresh hides, except that for cured hides the surfactant was increased to 0.40% and soda ash to 1.60%. Additionally, trials were run with varying chemical combinations that included a 50% increase in soda ash and a 50% increase in enzyme. Again, select trials were run with specific chemicals omitted from the soak. For most of the fresh and cured hide trials the floats were sampled at 5-minutes and 5-hours; the normal soak time was five hours. For one of the fresh hide trials and one of the cured hide trials, float samples were taken after: 5-minutes, 1-hour, 2-hours and 5-hours. All of the float samples were analyzed on site for temperature, pH and Baume′ and aliquots were chilled and sent to Buckman in Memphis, TN, for further testing.

2.3 Measuring hyaluronic Acid

Hyaluronic acid is a high molecular weight ($1x10^6$ to $5x10^6$ Daltons) anionic polysaccharide composed of repeating disaccharides of glucuronate acetylglucosamine. We used the HA-ELISA (Echelon Biosciences, Salt Lake City) method, a quantitative enzyme-linked immunoassay designed for the *in vitro* measurement of HA levels in human or animal biological fluids. The HA-ELISA is a competitive ELISA assay in which the colorimetric signal is inversely proportional to the amount of HA present in the sample. Samples to be assayed are first mixed with the Detector then added to the HA-ELISA Plate for competitive binding. An enzyme-linked antibody and spectrophotometric detection at a wavelength of 405nm is used to detect the HA detector bound to the plate. We measured absorbance on a Molecular Devices Spectra Max® 340 Microplate Analyzer, using SOFTmax PRO software. The concentration of HA in the sample was determined using a standard curve produced from known amounts of HA.

3 Results and discussion

Our laboratory trials corroborate the most commonly utilized soak time in the United States of 4 to 6 hours as the optimum when weight increase is the objective. As can be seen in Figure 2, the percent weight gain for cured hides steadily increased until it peaked at four hours. After four hours, weights of four of the five 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). Equally interesting, the addition of an ethoxylated linear alcohol by itself, inhibited water uptake, compared to the control with no chemicals added. In addition to the results for the five cured hide trials, Figure 2 also shows the weight increase for the single fresh hide piece that was soaked for a full 24-hours. Trial 5 (which utilized 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%, much less than for cured hide pieces.

Float density is another commonly used measure for gauging the progress of soaking. This is largely a surrogate for the measurement of sodium chloride diffusion from the hide into the float. Chloride was measured by potentiometric titration with silver nitrate solution. Float densities were measured directly by weighing aliquots of float.

In the laboratory trials (See Figure 3), both float densities and chloride concentration steadily increased with time in the soak. Float densities peaked for Trials 8 and 10 at 1.035 and 1.036 g/ml respectively, in part due to the addition of sodium carbonate. As expected, the correlation between soak density and chloride concentration was excellent. In contrast to the weight gain statistics, for cured hides, 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; the increase may not be significant.

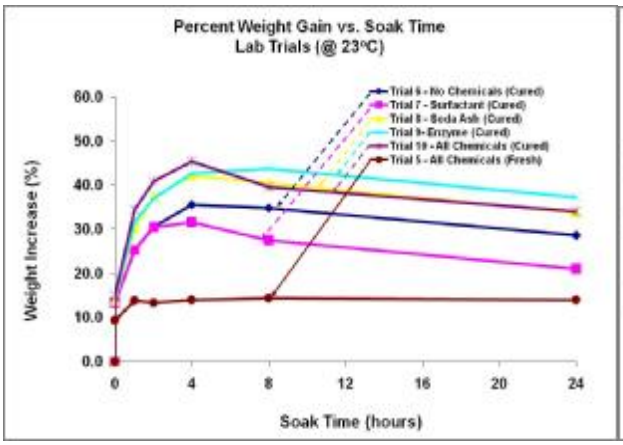

Figure 2 Weight gain *vs.* time

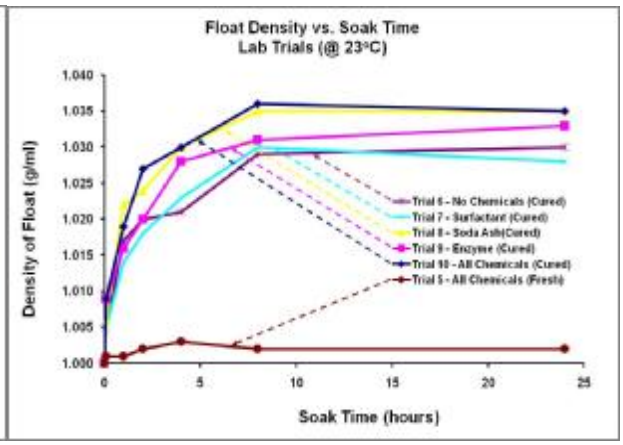

Figure 3 Float density vs. time

Tannery results for soak liquor density resemble those seen in the lab. Soaking of cured hides resulted in significantly higher float densities than for fresh hides. Soaking floats ranged from 2.5 to 5.0 degrees Baume′ (1.018 – 1.035 g/ml), in general agreement with values obtained in the laboratory. We observed little correlation with chemicals offered. For the fresh hides, soaking floats ranged from 0 to 3.0 degrees Baume′ (Sp. Gr. = 0 to 1.02) – generally higher than those observed in the lab. Again, there does not appear to be a correlation with the chemicals added.

In the tannery trials, there was a significant difference in the influence of soak time on float density for cured hides compared to fresh hides. We found that for the fresh hide trial that incorporated all three of the chemicals under consideration at the normal tannery levels, the density quickly stabilized after 1-hour at 3.0° Baume′. This density was maintained to the end of the 5-hour soak. On the other hand, in the cured hide trial with the same chemical additions, soak density continued to increase up to five-hours.

Hyaluronic acid removal has been suggested as an alternative measure for gauging the progress of soaking. To that end, we measured the hyaluronic acid extracted during soaking in both the laboratory as well as the tannery soaking trials. A typical calibration curve for the Echelon HA-ELISA method revealed a logarithmic function for which a least squares regression analysis yielded an R^2 value of 0.98. Individual standards were run in triplicate and the relative standard deviation of all points averaged +/- 10%.

The mass of hyaluronic acid extracted from 200 g cured hide pieces soaked in the lab, differed significantly from fresh hide pieces. The maximum theoretical extraction of hyaluronic acid, based on the report of Alexander, ***et al.,*** was calculated at 192 mg. For a 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-hours soaking and 75% after 24-hours. In contrast, the soaking of fresh hides yields 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 cured hides than for fresh hides after 4-hours soaking.

The different chemical treatments applied during laboratory soaking influenced HA removal to varying degrees (See Figure 4.) For fresh hide pieces, soda ash and proteolytic enzyme positively influenced HA removal. The addition of surfactant, on the other hand, actually appears to be counterproductive. For cured hide pieces, each of the chemicals offered had a positive effect on HA removal. Addition of all three chemicals together yielded the best results for cured hides, with a total of 58mg/l HA detected in the float after 24-hours, equivalent to 75% of the theoretical HA thought to be present.

The effect of chemical additions on HA 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 the tannery trials, maximum HA extraction of 147 mg/l occurred for cured hides when a proteolytic enzyme was used by itself at the normal dosage. This was less than 30% of the theoretical HA value. When a full array of chemicals was added to the soak, the resulting HA extraction was 134mg/l. 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 HA levels in the float were markedly lower for both fresh and cured hides.

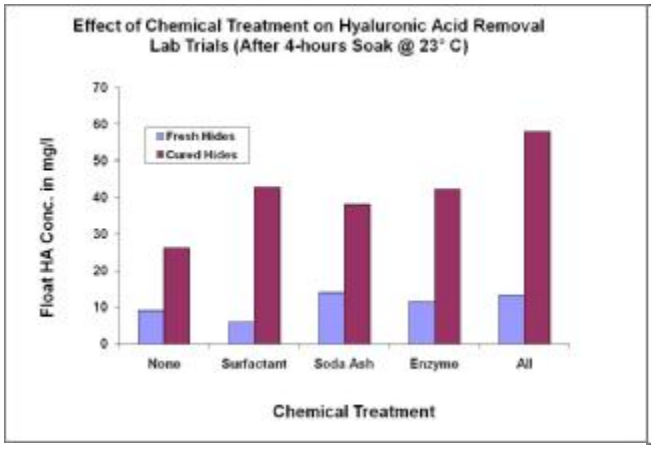

Figure 4 HA removal – lab trials

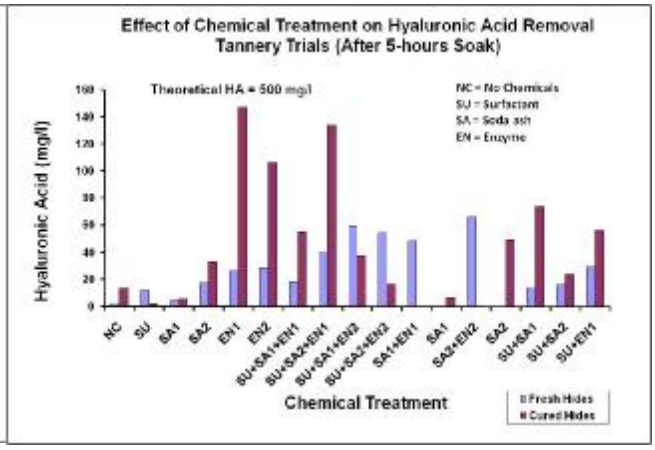

Figure 5 HA removal – tannery trials

Opinions regarding the optimum conditions for soaking cattlehides have changed over the past few decades. Modern soaking practices differ widely from those reported in textbooks still in use in the industry. With better understanding of the chemistry of hide protein and the ancillary compounds surrounding collagen, new theories challenge the very objectives of soaking. In particular, removal of hyaluronic acid as an essential component of the soaking sub-process is now regarded as requisite to the ultimate success of the opening up of 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 though, there has been little reported on the effect of these changes in soaking practice on even the most basic measures of soaking efficiency.

Different measures for monitoring the progress of soaking lead to different conclusions regarding what constitutes optimal soaking. If, for example, we accept that relative weight increase due to water absorption is the most important metric in soaking, then we must conclude that the optimal soak time for cured hides is around four hours. On the other hand, our soaking data indicate that when soaking fresh hides, if percentage weight gain is the principal gauge of soaking progress, then there is little difference between soaking for one hour and soaking for 24-hours.

Contrast the conclusions drawn when weight increase is the principle measure of soaking, versus using one of the measures of float density. When float density (or chloride concentration for cured hides) is the primary measure, we conclude that optimal soaking occurs around eight hours for cured hides and four hours for fresh hides. If, however, the extraction of hyaluronic acid from the hide matrix is considered a significant measure of soaking efficiency, then we must conclude that current practice for soaking fresh hides are far from optimal. Within the physical constraints of float, time, temperature and mechanical action utilized in these trials, and regardless of the chemistry currently employed in the industry, a small fraction of the hyaluronic acid present in cattlehide is in fact extracted from the hide into the soaking float. In the case of cured hides, the "removal" of hyaluronic acid from the hide matrix varies widely. Clearly, curing itself plays a major role in HA extraction. Similarly, application of specific chemicals in soaking, namely soda ash and proteolytic enzymes, contribute to HA removal. Time is an important variable for HA removal. Under the conditions explored in our laboratory trials, soaking times in excess of 24 hours are needed to approach 100% removal of HA. The tannery conditions encountered in this study resulted in observed extraction values that fell well short of theoretical 100% removal values.

The measurement of hyaluronic acid in the soaking float advances our understanding of the mechanism of effective soaking. The data presented here support the theory that hyaluronic acid is impeding the passage of of water into and through the hide matrix during the soak. In particular, 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.

4 Conclusions

We have evaluated the effect of several parameters on the overall efficiency of soaking as measured by the traditional measures of hide weight and float density. According to those old metrics, some soaking practices currently regarded as state-of-the-art make sense. In particular, soaking either fresh or cured hides for 4-6 hours in 100% float at 20-30° C, with chemical augmentation (a combination of soda ash, a linear alcohol ethoxylate surfactant, and sometimes a proteolytic enzyme), can be expected to maximize water absorption. However, these same conditions appear inadequate for the complete removal of salt (to an equilibrium state) from cured hides; eight hours or more may be necessary.

When one considers a more contemporary model of beaming as requiring the removal of hyaluronic acid to enable subsequent wetting and opening up of the hide matrix, entirely different conclusions are drawn. The soaking of fresh hides in particular, under commonly encountered soaking conditions, is woefully inadequate. With the removal of 13% or less of the available hyaluronic acid, it is unlikely that the fiber network in fresh hides undergoes any meaningful beneficial change. Similarly, it appears that for cured hides, while hyaluronic acid removal is markedly better than for fresh hides, it is far from optimal. Consequently, it is likely that a significant amount of time is spent during liming to remove hyaluronic acid as the requisite first step in opening up of the collagen.

We believe that our observations with respect to modern soaking practices will encourage leather practitioners and scientists alike to take a fresh look at the soaking subprocess. We are hopeful that the reevaluation and restatement of the basic objectives of soaking will lead to improvements in overall soaking effectiveness with concomitant improvements in overall leather processing. In particular, additional work is necessary to further identify those chemistries and practices that will maximize hyaluronic acid removal. It will also be important to relate HA removal to finished leather quality.

References

[1] L. A. Louis; Practical Tanning, Third (ed.), Henry Carey Baird & Co., Philadelphia, 1916

[2] G. D. McLaughlin; D. George; E. R. Theis. The Chemistry of Leather Manufacture, Reinhold, New York, 1945.

[3] J. A. Wilson. Modern Practice in Leather Manufacture, Reinhold Publishing Corporation, New York, 1941.

[4] F. O'Flaherty; et al.. The Chemistry and Technology of Leather, Robert E. Krieger, Huntington, NY, 1978.

[5] K. Bienkiewicz. Physical Chemistry of Leathermaking, pp. 88-95.

[6] K. T. W. Alexander; B. M. Haines; M. P. Walker. JALCA 1986, 81: 85.

[7] G. D. McLaughlin; E. R. Theis. The Chemistry of Leather Manufacture, Reinhold, New York, 1945.

[8] T. C. Thorstensen. Practical Leather Technology Second Revised (ed.), Robert E. Krieger, Huntington, NY, 1976.

[9] J. H. Sharphouse. Leather Technician's Handbook, Leather Producers Association, London, 1979.

[10]M. K. Leafe. Leather Technologists Pocket Book, Society of Leather Technologists and Chemists, 1999.