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

by

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Abstract

The past three decades have heralded dramatic changes in raw material for tanning in the U.S. Modern blueing facilities lie close to the source of hides and are either captive to the packinghouses or have established long-term contracts for purchase and receipt of the fresh hides on a continuing basis. Typically, tanners are required to receive the hides daily, in concert with the slaughter schedule, with little to no variance possible. Tanners often process both brine cured hides as well as fresh, thus optimizing time management and production demands.

It is commonly accepted that variations in processing time, float, type of equipment (paddle or drum), bactericide, pH, wetting agents and protease-based enzymes are necessary in response to both end-product requirements as well as raw material. Little has been reported in the technical literature, on modern soaking practices and their relationship to the shift in our major raw material.

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

Resumen

Las últimas tres décadas han anunciado dramáticos cambios en la materia prima básica para curtición en los EE.UU. Las facilidades para producir el wet-blue se encuentran cerca de la fuente de pieles y son estrechamente vinculadas las procesadoras de carne o bien tienen contratos de compra y recepción de pieles frescas sobre una base continuada. Típicamente, los curtidores están obligados de recibir las pieles diariamente, en coordinación con la programación del matadero, con muy poca o ninguna variación posible. Los curtidores procesan a menudo pieles saladas y frescas, optimizando el manejo de tiempos y las demandas de producción.

Es comúnmente aceptado que las variaciones en tiempo de proceso, baño, tipo de equipo (molineta o fulón), bactericida, pH, agentes humectantes y enzimas basadas en proteasas son necesarias en respuesta a los requerimientos del producto terminado como también por la materia prima. Muy poco ha sido reportado en a literatura técnica, sobre las prácticas modernas de remojo y su relación a los cambios ocurridos en la gran mayoría de la materia prima.

Esta publicación compara prácticas de remojos modernos con los métodos históricos, con énfasis en los aspectos que generalmente son aceptados como críticos en la optimización de la química en la ribera. Examinamos las prácticas hoy en uso y retamos las medidas comúnmente aceptadas para determinar el remojo óptimo de pieles verdes y las tratadas con salmuera. Demostramos las diferencias con respecto a la remoción de componentes no colagénicos en la piel, ácido hialurónico en la varias químicas del procesamiento de pieles de ganado tanto verdes como tratadas porsalmuera.

Introduction

Since the early 1900s, soaking has been regarded as an equilibrium process. Practitioners and scientists[1,2,3,4] of the time recognized the importance of a thorough soak for the proper rehydration of hides and skins. Soaking for two to three days in fresh, cold water was the norm. Textbooks from that era gave only passing reference to the practice, relegating the identification of the best soaking procedure to the tanner for empirical determination.

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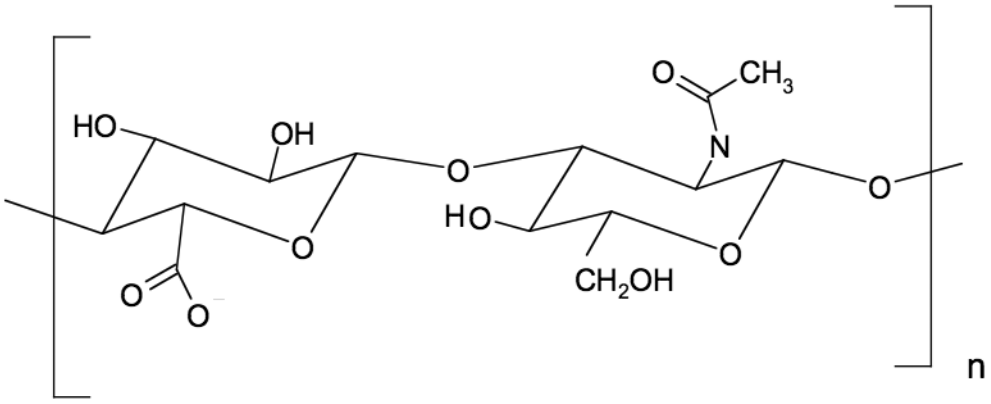

Figure 1: hyaluronic acid

During the decade of the eighties though, significant new insight provided much more specific objectives for optimal beamhouse processing and thus offered building blocks for improvements that promised faster and more effective soaking. In particular, Bienkiewicz[5] and Alexander[6], *et al*. 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.

According to Bienkiewicz, glycosaminoglycans are acidic and neutral polysaccharides that complex with proteins to yield mucoids. They consist of hyaluronic acid, chondroitin sulfate, chondroitin, dermatan, keratin sulfates and heparin. He reports that there are a variety of glycosaminoglycans (formerly called mucopolysaccharides) present in hide. They are polyelectrolytes, whose purpose is to control the viscosity of organic fluids in the extra cellular spaces of the hide and they help manage the flexibility of animal tissue by controlling plumpness. Glycosaminoglycans also affect metabolic processes by regulating the passage of all substances from cell to cell through the extra cellular spaces.

Bienkiewicz is quite graphic in his description of the impact of hyaluronic acid, in particular, on the effective soaking of hides and skins. 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. This gel-like substance, until removed, inhibits the removal of other non-collagen proteins and slows the opening up process, one of the main objectives of the beamhouse.

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 water, bringing the hide closer to the desired flaccid condition.

McLaughlin[7] *et al*. observed exactly such a response during the third day of a three-day soak. They concluded that "coagulable proteins" are removed during soaking, aided by the presence of sodium chloride. It now seems likely that the reversal of direction of the swelling curve that McLaughlin observed was due, at least in part, to the removal of hyaluronic acid.

Alexander[8] *et al*. reported that for adequate opening-up of the 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. He showed that hyaluronic acid is completely removed after soaking for 48-hours.

Thorstensen[9] suggested in 1976 that the ideal soak temperatures should be determined empirically, balancing the beneficial dispersion of globular proteins with indicators of excessive soaking like looseness, veininess and coarse fiber.

Recognition in the literature of the utility of proteolytic enzymes for soaking corresponds with the greater understanding of the chemistry of hide proteins. Sharphouse[10] was among the first authors to recognize, in 1971, the utility of proteolytic enzymes for breaking down interfibrillar proteins.

Leafe[11] presented a helpful representation of modern soaking practice. He recognized that good soaking is a vital precursor to unhairing and liming and said that it optimizes fiber opening up and the resulting improvements in finished product quality. He reported that soaking should be at a pH of 9.5-10.5 to speed up rehydration and that enzymatic soaking preparations, in combination with a suitable surfactant, are effective in reducing the required soaking time. He went on to say that it was beneficial to soak the hides until sodium chloride reaches equilibrium between the hide and the float. This equilibrium is best gauged by monitoring float density in degrees Baume'. Typical 20-36 kg cattlehides should be well soaked within 4 to 6 hours, when the float density should measure 4.0-6.0° Baume'.

Finally, Leafe[12] recognized the significant place that fresh hides have as a raw material in modern tanning practice. He stated

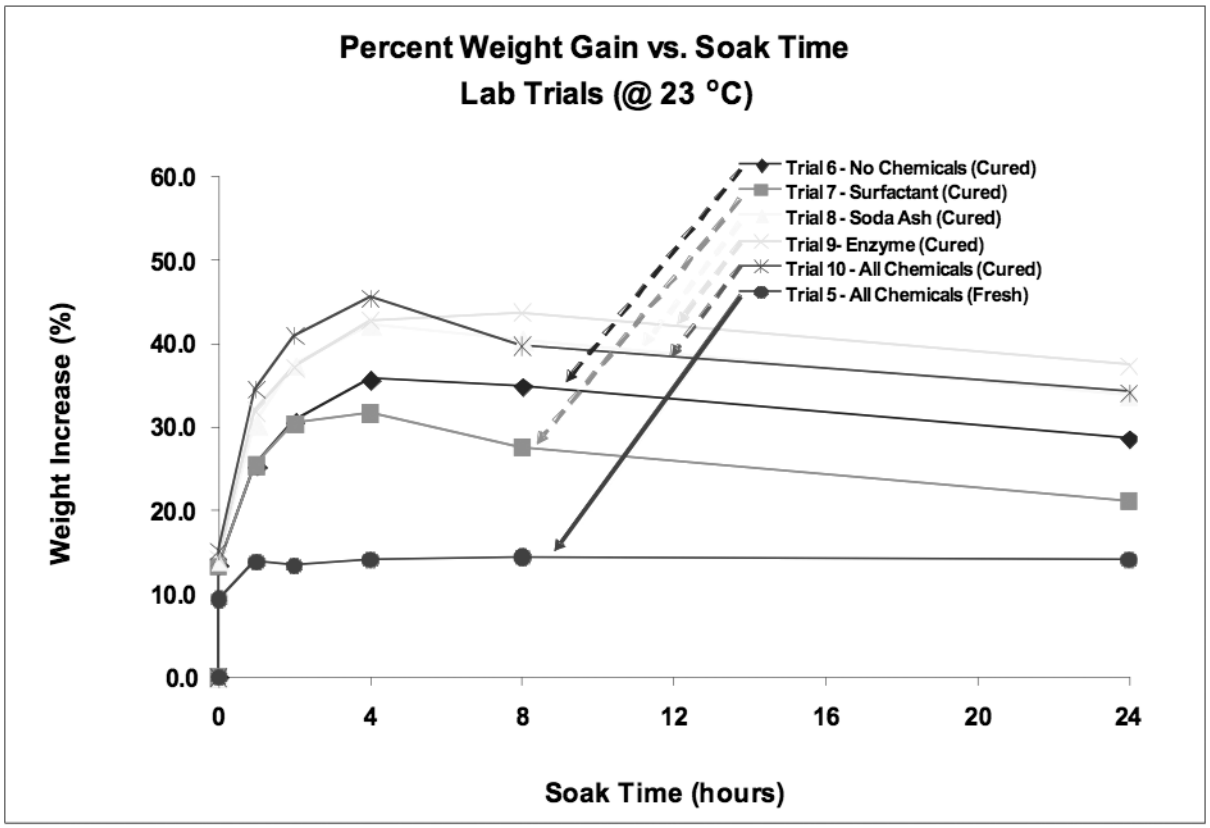

Figure 2

that there used to be a widely held opinion that fresh hides did not require significant soaking, since they were not dehydrated on arrival at the tannery. He further explained that this is inaccurate 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. He proposed, in fact, that more intensive soaking is required for fresh hides.

To date, nobody has presented a comprehensive review of the state-of-the-art for soaking bovine hides as it relates to the theory of hydration of the hide and preparation of the hide matrix for subsequent leathermaking 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 effect of soaking on the progress of hyaluronic acid extraction from the hide into the float and its affect on hydration. This paper addresses these issues and others relating to soaking that are of immediate interest for modern leather processing.

Experimental

Laboratory trials

Lab scale trials were conducted in the Buckman Product Development Laboratories in Memphis Tennessee during the month of March 2007. Ten trials were run in Dose PFI Type 30034 drums (300mm X 160mm) - five with fresh cattlehide pieces and five with cured cattlehide pieces. For each hide type, five adjacent 200g rectangles were cut from the center of a single side. The float was fixed at 400ml of water at 23°C.

In the fresh hide soaking trials, one sample was processed without any chemical addition, one with 0.25% of a nonionic 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. All of the hide pieces were run continuously at slow speed and the drums were stopped at predetermined intervals for sampling. The drums were interrupted at 5-minutes, 1-hour, 2-hours and 4-hours after commencement of the soak, the floats sampled and the hide pieces weighed. The float was tested for temperature, pH and density. After weighing and float analyses, the hide pieces and float samples were returned to the drum and the process continued. This procedure was modified for the fifth trial in which 20ml 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 interrupted and sampled at 8-hours and 24-hours run times.

For the second 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 from 0.25% to 0.40% and the soda ash dosage was increased from 0.67% 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

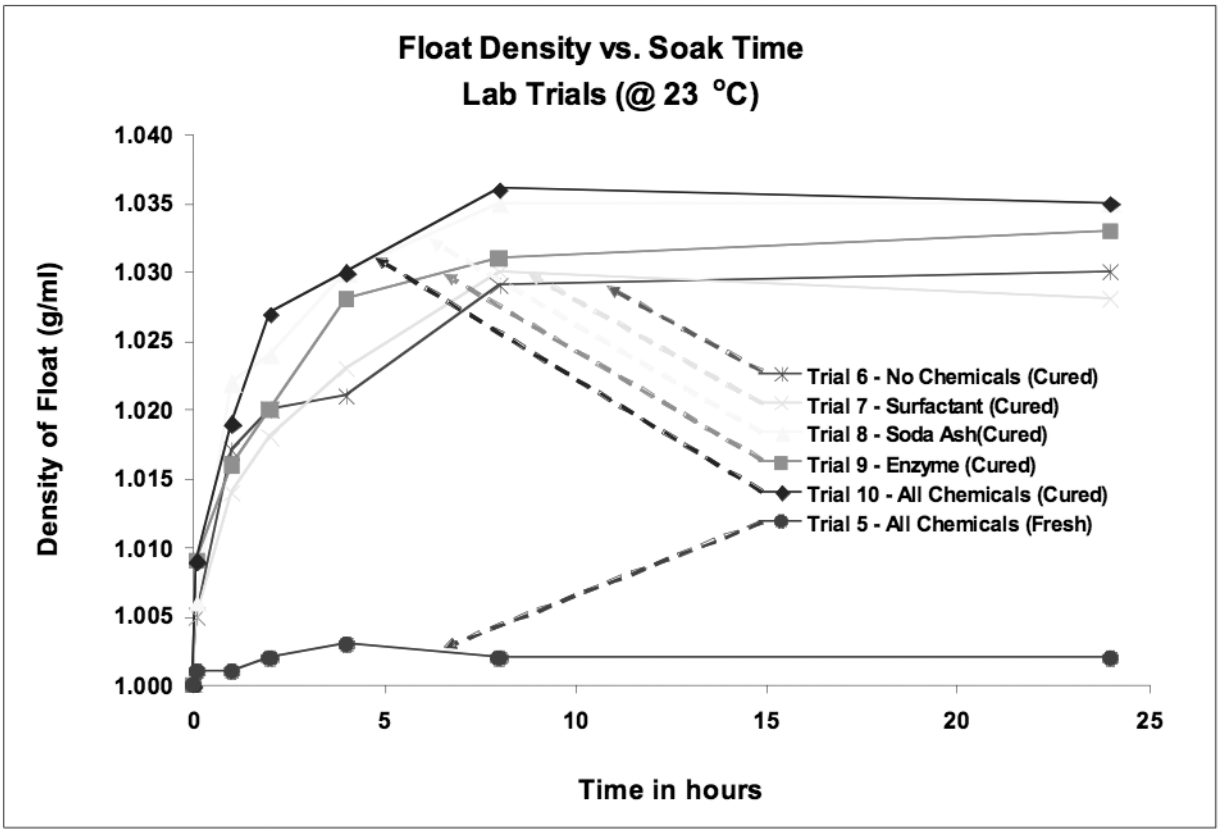

Figure 3

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.

Tannery trials

Full-scale tannery trials were conducted at Prime Tanning in St. Joseph, MO, also during March 2007. These trials were conducted according to a generic soaking protocol, except the chemical additions were varied. 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 a temperature of 29°C. Chemicals were offered in some of the trials at normal dosages, while some trials were run with elevated dosages of soda ash, enzyme. For example, soda ash was increased to 1.25%, and the enzyme was increased to 0.54% in several of the trials. In addition, some drums were run with specific chemicals omitted.

We used an identical soaking protocol for both fresh and cured hides, except that for cured hides we increased the addition of surfactant to 0.40% and soda ash to 1.60%. In addition to these levels, several trials were run with chemical combinations that included increased soda ash at 2.40% and increased enzyme to the 0.54% level. Again, select trials were run with specific chemicals omitted from the soak. For most of the fresh hide and cured hide trials the floats were sampled at T = 5-minutes and T = 5-hours, the normal soak time. For one each of the fresh hide and cured hide trials, four float samples were taken, at intervals: 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 Laboratories, Inc. in Memphis, TN, for further testing.

Measuring hyaluronic Acid

Hyaluronic acid is a high molecular weight (1×10^6 - 5×10^6 Daltons) anionic polysaccharide composed of repeating disaccharides of glucuronate acetylglucosamine. We decided to utilize the HA-ELISA method which is a quantitative enzyme-linked immunoassay designed for the in vitro measurement of HA levels in human or animal biological fluids (blood, serum, urine, diffusate, synovial fluid) or cell-culture supernatant (Hyaluronic Acid ELISA Kit produced by Echelon Biosciences of Salt Lake City, Utah). 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 is determined using a standard curve produced from known amounts of HA.

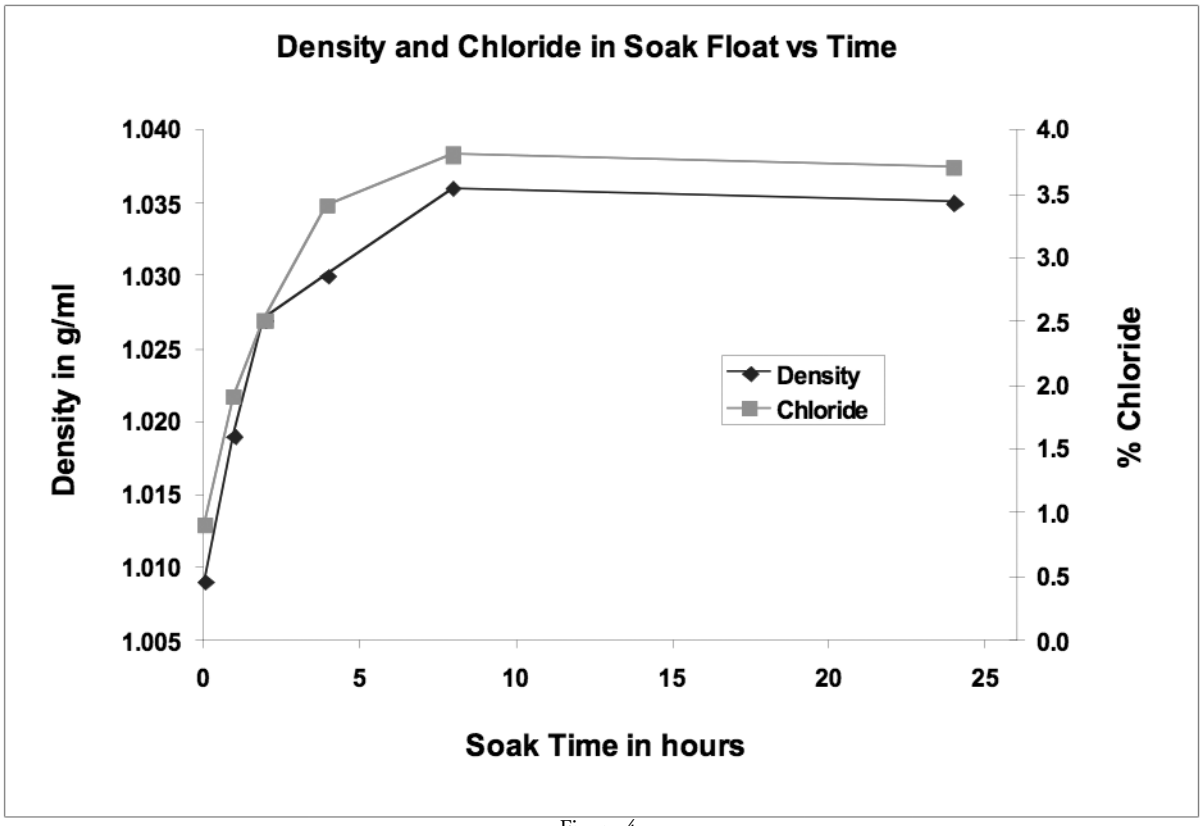

Figure 4

RESULTS

Various measures are used to track the extent of soaking. Among these, percent weight gain is one of the most frequently used indicators. 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. Only the piece treated with enzyme alone failed to decline in weight. The results indicate that adding soda ash or enzyme by itself increased the rate of water absorption. 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 a nonionic surfactant 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 (This trial 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 14%. Note that the percent weight gain for Trial 5 was significantly less than that for any of the cured hide pieces.

Another commonly used measure for gauging the progress of soaking is float density. For cured hides, this is largely a surrogate for the measurement of sodium chloride diffusion from the hide into the float. Common measures of density are degrees Baume', Specific Gravity or % Salometer. In our laboratory trials, we chose to measure float density directly, by weighing an aliquot of the float. In Figure 3, it is immediately obvious that there is a vast difference in the float density for cured vs. fresh hides. **For cured hides, the optimal soak time, as measured by maximum float density, is eight hours or more, depending on the chemistry used.** The significantly higher overall float densities for Trial 8 and Trial 10 are probably explained by the contribution of sodium carbonate. The float densities for these two trials peaked at 1.035 and 1.036, respectively. **In contrast, the float density increase for fresh hides comes at only four hours and thereafter significantly declines.** For Trial 5, in which all three chemicals were added, the final level was less than 1.002 g/ml, after 24 hours. Again, it is likely that a substantial part of the increase is due to the addition of soda ash.

The fact that float density is an excellent surrogate for salt removal from cured hides is demonstrated in Figure 4. Chloride was measured by potentiometric titration with silver nitrate solution. Float density clearly mimics total chloride. The chloride results indicate that in these trials, maximum salt removal did not occur until 8 hours of soaking.

Tannery results for soak liquor density resemble those seen in the lab. As shown in Figure 5, 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

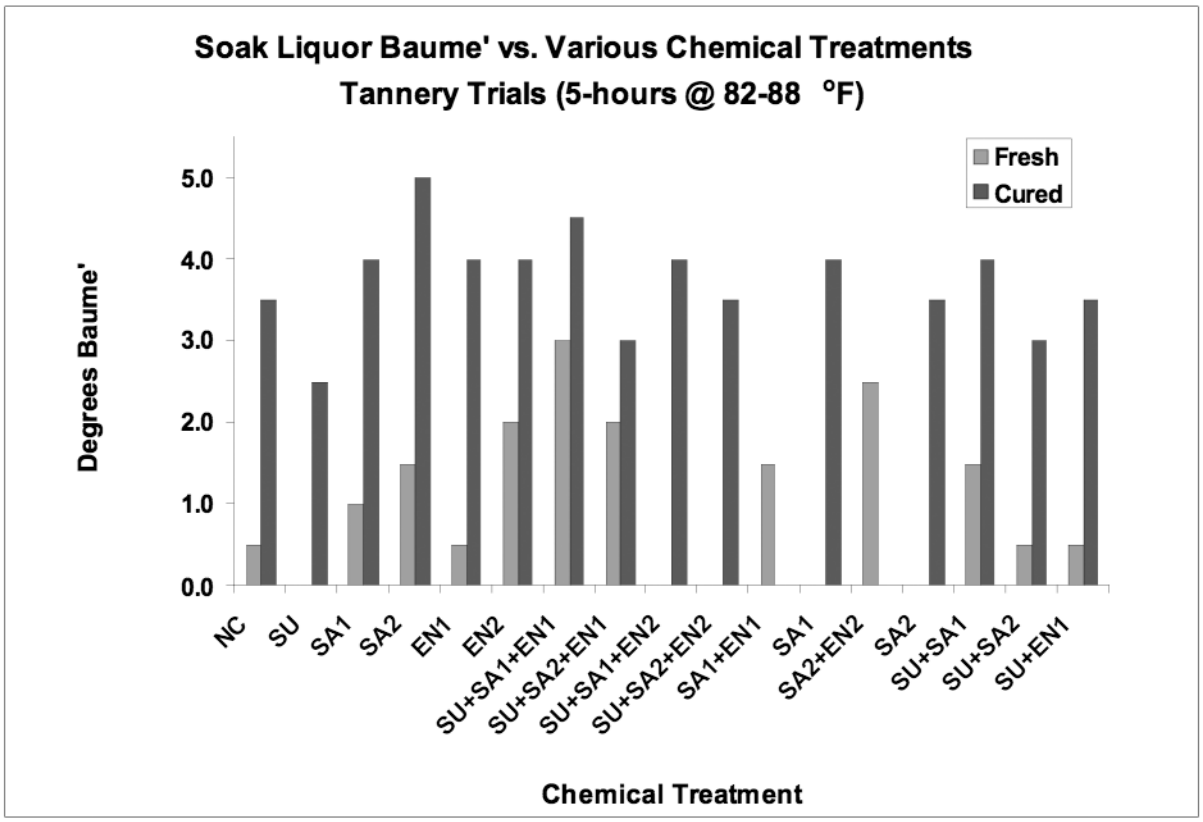

Figure 5

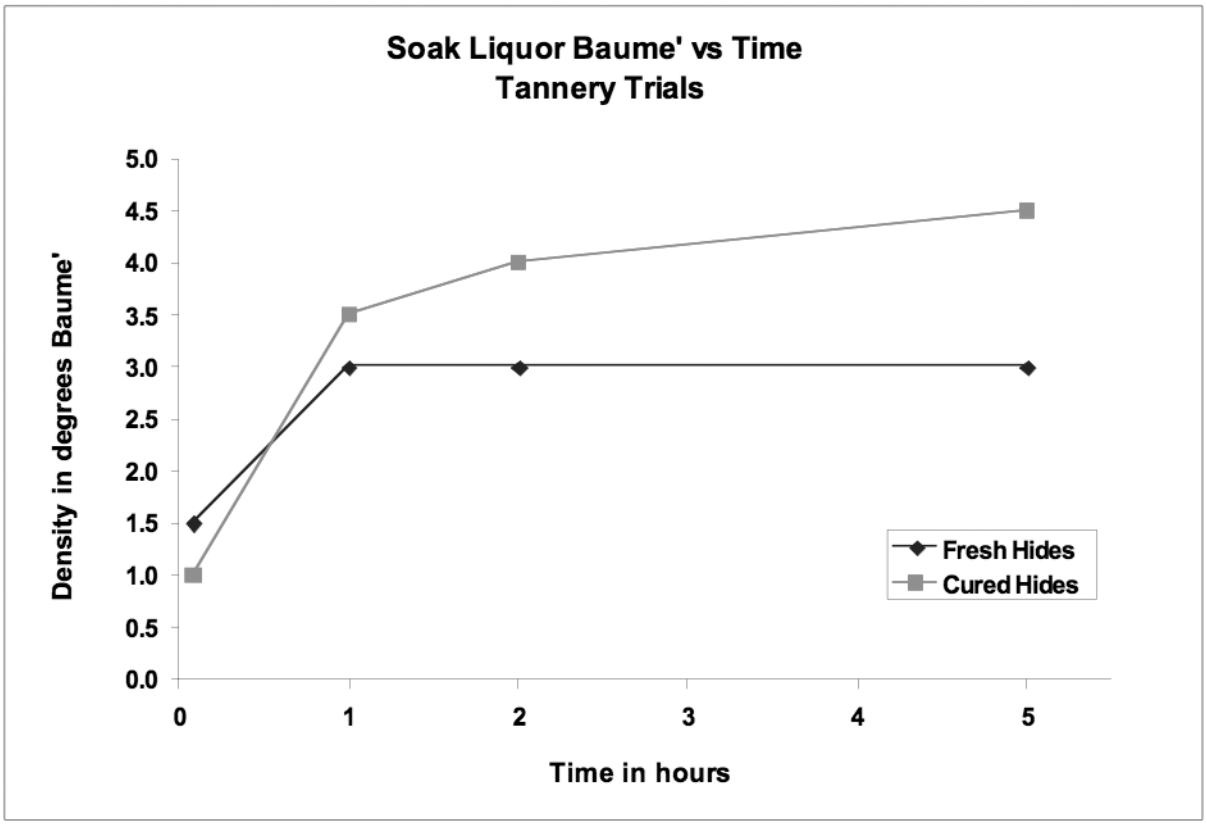

Figure 6

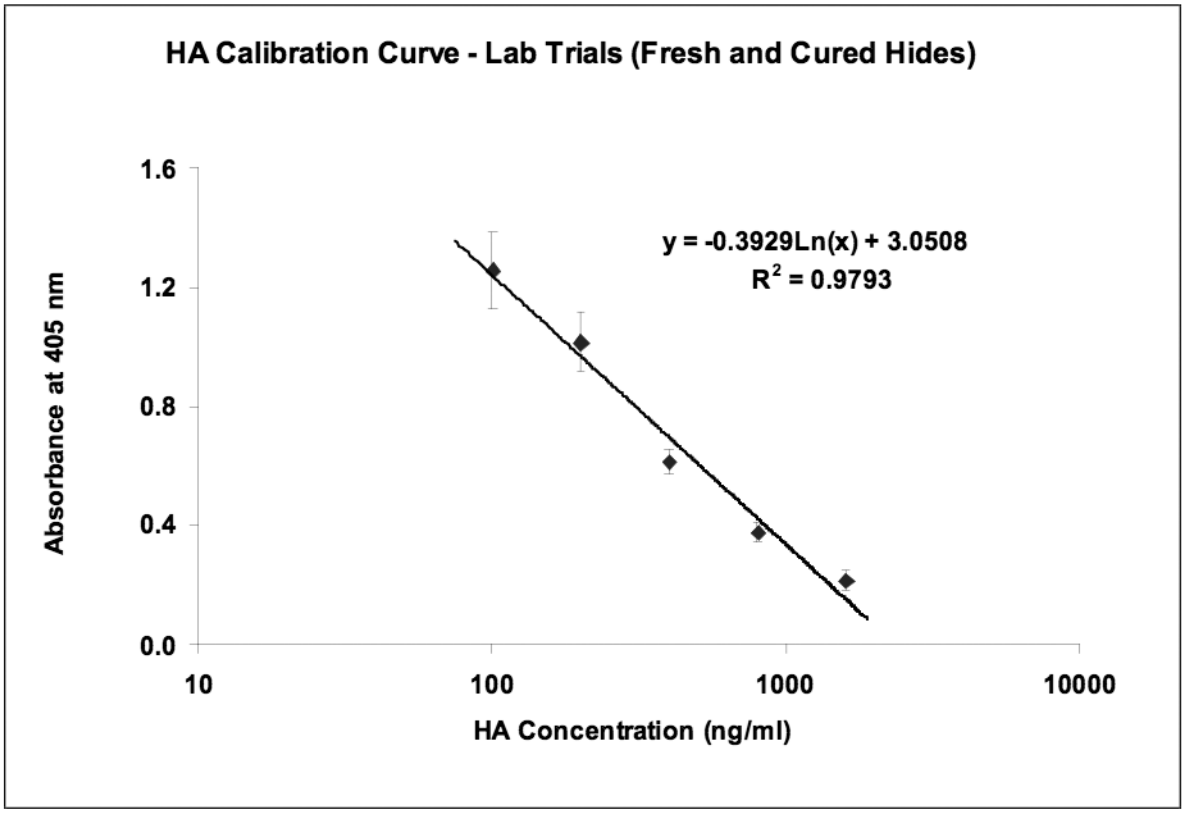

Figure 7

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.

The influence of soak time on float density in the tannery trials is depicted in Figure 6. 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, even after five-hours soaking, the soak density for the cured hide trial with the same chemical additions was still increasing.

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 is shown in Figure 7 as a logarithmic function. Individual standards were run in triplicate and the standard error (+/- one standard deviation) is indicated by the error bars. The relative standard deviation of all points averaged +/-10%. The least squares regression analysis yielded an R2 value of nearly 0.98, indicating a good correlation.

The mass of hyaluronic acid extracted from 200g fresh or cured hide pieces during the laboratory soaking trials are shown in Figure 8. For the cured hide trials, the average relative standard deviation for measurements made in either triplicate or quadruplicate was +/- 13%. For the fresh hide trials, the average relative standard deviation for measurements made in either triplicate or quadruplicate was +/- 8%. The maximum theoretical extraction of hyaluronic acid, based on the report of Alexander, *et al.*, is calculated at 192mg. Plotting HA-Extraction versus Soak Time reveals a logarithmic relationship that is characteristic of extractions in general. For a cured hide piece soaked with all three of the subject chemicals added, the total hyaluronic acid extracted measured 93mg and 144mg after 8-hours and 24-hours of soaking, respectively. This represents just less than 50% and approximately 75%, respectively, of the total theoretical HA present in untreated hide substrate. 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 only 20.5mg, or less than 11% of the theoretical amount.

The effect of chemical treatment for the laboratory soaking is shown in Figure 9. It is immediately obvious that all trials on cured hides yielded significantly more hyaluronic acid in the float after 4-hours soaking, than when fresh hide pieces were soaked. For fresh hide pieces, it appears that soda ash and proteolytic enzyme positively influence 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.

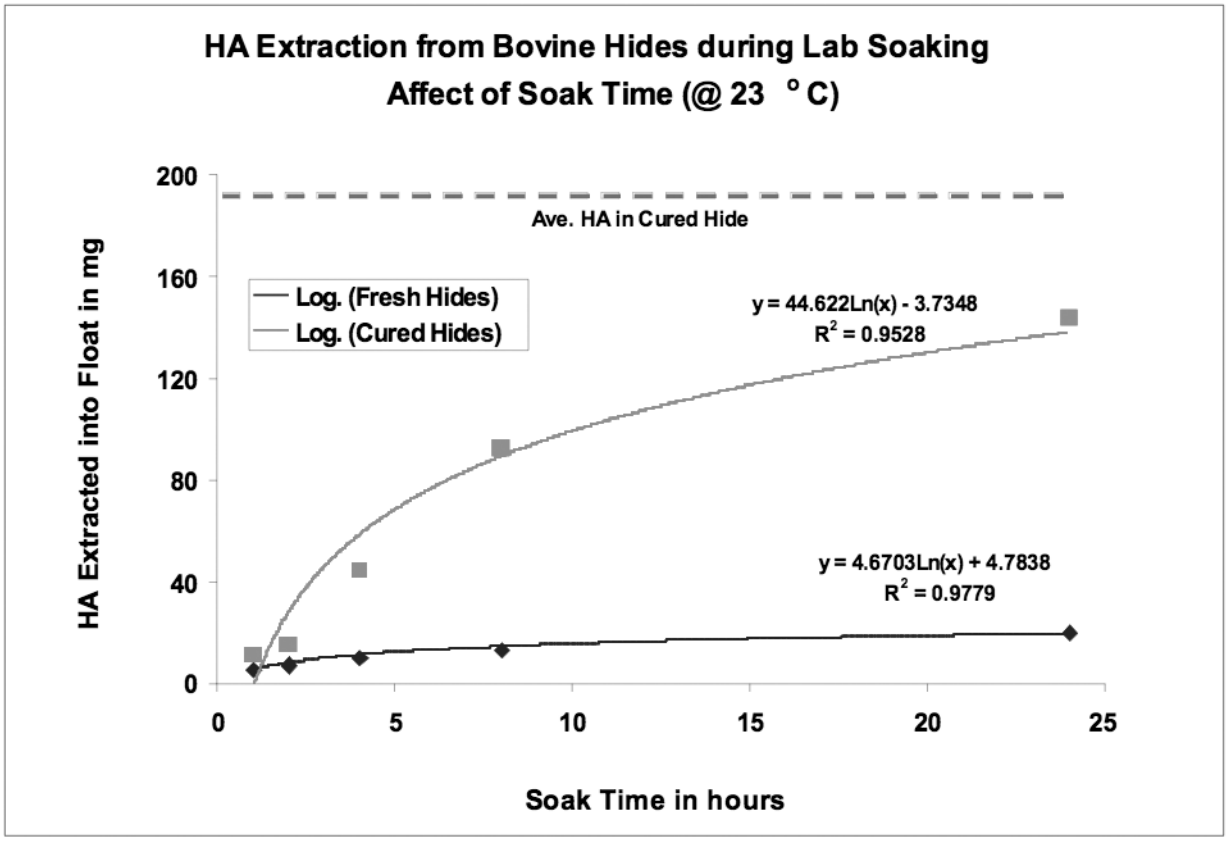

Figure 8

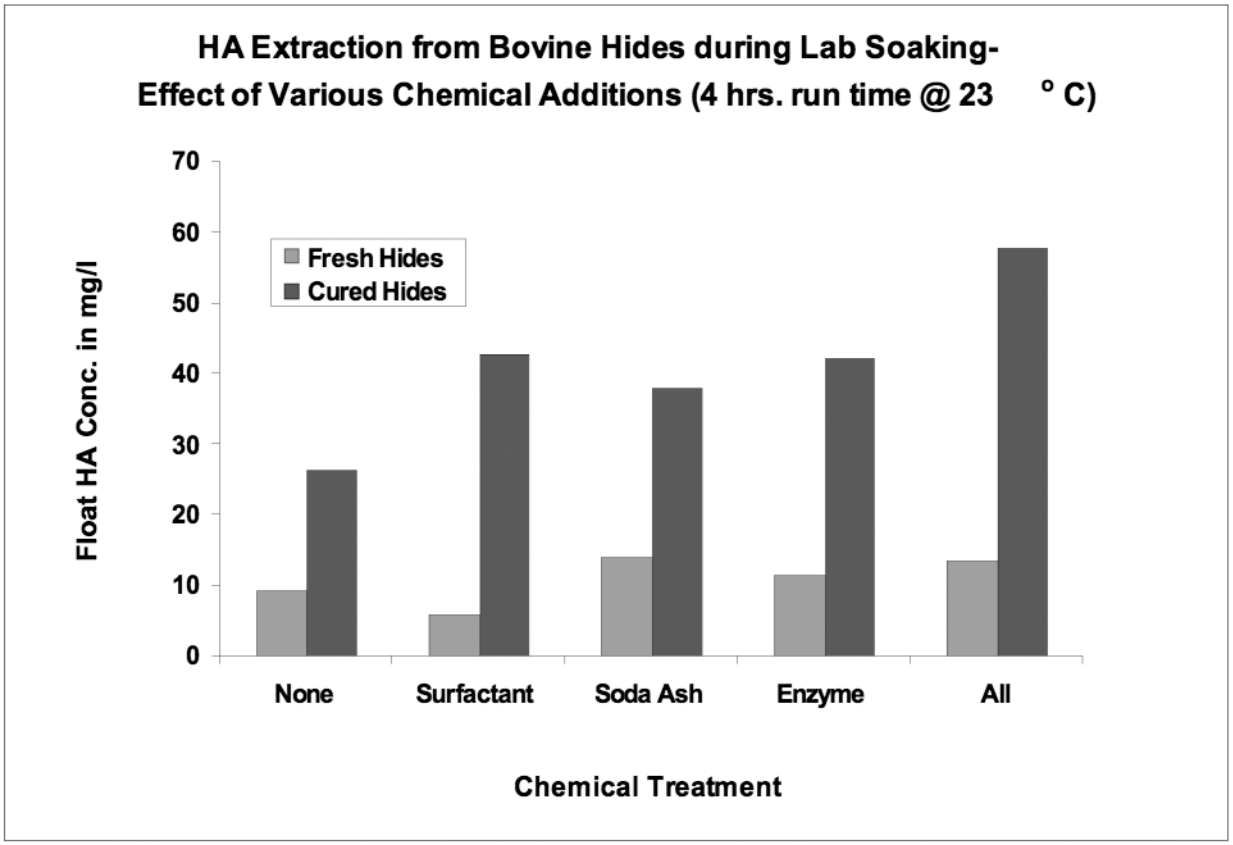

Figure 9

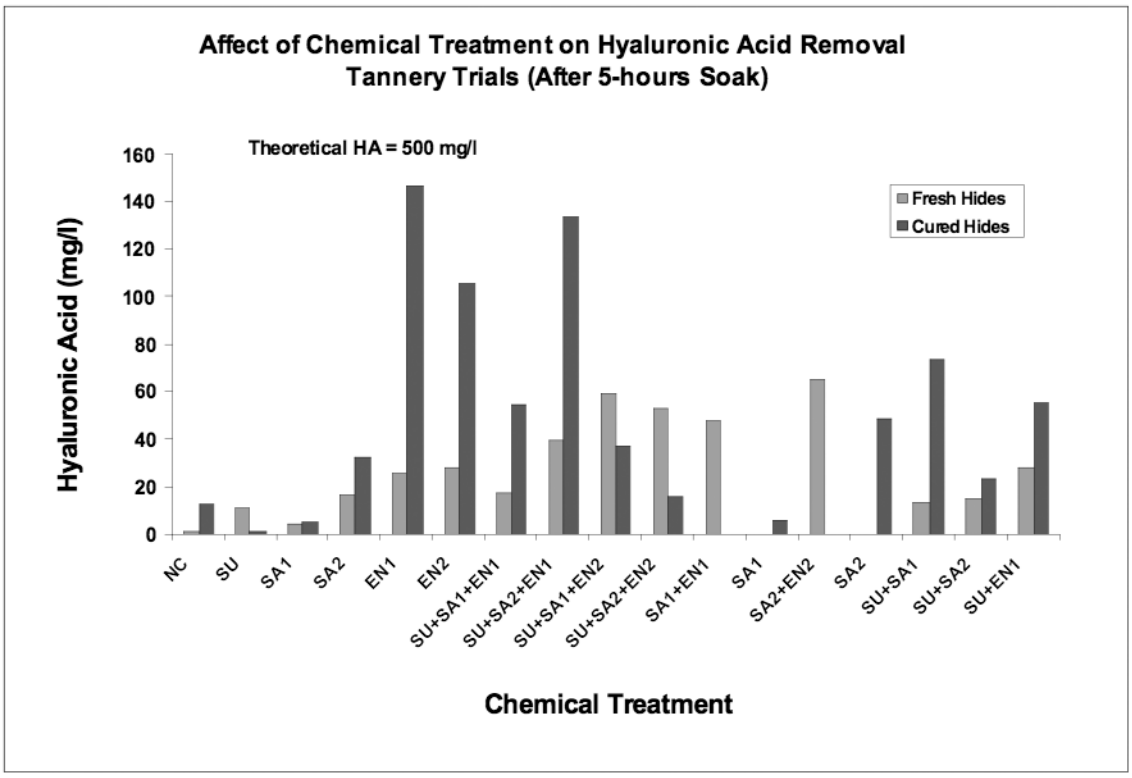

Figure 10

The effect of chemical additions on HA extraction during the tannery trials is depicted in Figure 10. At equilibrium, complete extraction of the hyaluronic acid would yield approximately 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 of 0.36%. This is less than 30% of the theoretical HA value. An increased dosage of enzyme to 0.54%, by itself, also resulted in very substantial HA removal. 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 66mg/l, about 13% of theoretical. In addition, when no chemicals were used or when surfactant or soda ash were used by themselves, the HA levels in the float were markedly lower for both fresh and cured hides.

Discussion

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 practices. 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.

Depending upon the measures one selects for monitoring the progress of the soak, different conclusions must be drawn regarding the relative effectiveness of the soak. 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 is the primary measure, we conclude that optimal soaking occurs around eight hours for cured hides and four hours for fresh hides. If chloride is measured instead of density, the same conclusion results for cured 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 adequate. 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 form 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 increased HA removal. On the other hand, some applications of surfactants may in fact inhibit the removal of this important glycosaminoglycan. 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. While tannery conditions promoted improved HA removal, observed extraction values were 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 penetration of water into 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 agree with the observations made by McLaughlin back in 1923.

Conclusions

We have evaluated the effect of several parameters on the overall efficiency of soaking as measured by the traditional measures of hide weight increase 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 a nonionic surfactant, soda ash and sometimes a proteolytic enzyme), can be expected to maximize water absorption. These soaking conditions appear to maximize the float density for fresh hides. 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.

These results compare favorably with those found during an earlier soaking process evaluation conducted by the tannery. In that earlier evaluation, the glycosaminoglycan contents of the hides were measured before and after soaking. The percent removals of glycosaminoglycan (mainly hyaluronic acid) were quite similar to those calculated from soaking float extraction values reported herein.

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 sub-process. 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.

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