Powdered Hide Model for Vegetable Tanning

by

Eleanor M. Brown,* Renee J. Latona, Maryann M. Taylor and Andrew G. Gehring
U.S. Department of Agriculture, Agricultural Research Service
Eastern Regional Research Center
600 East Mermaid Lane
Wyndmoor, PA 19038

Abstract

To demonstrate the utility of the powdered hide model for vegetable tanning studies, interactions of quebracho with samples of powdered hide prepared from partially processed hides were investigated. Hides were dehaired by a typical sulfide or oxidative method and carried through the delime/bate step of a tanning process. Prior to tanning, the powdered hide from oxidative dehairing was on average slightly more susceptible to attack by collagenase than was the powdered hide from sulfide dehairing. After tanning with as little as 20% quebracho, powdered hide from both processes was well protected against collagenase degradation. Apparent shrinkage temperatures ranged between 79°C and 87°C , increasing with increased quebracho offer. Shrinkage temperatures for quebracho-treated oxidatively powdered hides were generally 2°C lower than for sulfide dehaired samples. This contrasts with no difference in shrinkage temperature for chrome tanned powdered hides. Comparison of micrographs of powdered hide treated with crude and purified quebracho suggest that the tanning effect of quebracho is both a function of quebracho/collagen interactions, and the filling effect of other components of the crude quebracho.

Introduction

Tanning, the conversion of animal hides into leather, is a multistep process that continues to evolve. Driving forces in this evolution include customer specifications, environmental concerns, leather quality, yield and cost. Tanners also adjust their processes in response to the availability of new formulations and chemicals from suppliers, or in anticipation of new regulations on currently used chemicals. Tanning processes have developed in a mostly empirical fashion, and the effects of individual steps in the process on the hide substance are poorly understood. A seemingly minor change in one part of a beamhouse process may lead to the need for additional changes in later stages.

One of the early steps in the production of leather is the removal of hair from the hide or skin. Dehairing (or unhairing) is traditionally accomplished with the use of significant amounts of sodium sulfide. Although sulfide dehairing is relatively inexpensive, rapid and efficient, the associated odor and the potential for the conversion of sodium sulfide under acid conditions to the toxic hydrogen sulfide have led to a search for other dehairing technologies. Germann's 1997 review of the status of hair removal processes in the leather industry culminated in a prediction of future sulfide-free processes based on either selective enzymes or alkaline peroxide.[1] Since that time, research in these two areas has resulted in more than 100 publications. During the decade following Germann's publication, scientists at the Eastern Regional Research Center, ARS, USDA explored several alkaline peroxides as the bases for rapid oxidative dehairing technologies.[2-5] In each case, the hair-free hide was chrome tanned and compared favorably with sulfide-treated controls at the wet blue and crust stages. More recently, we have used our powdered hide model system to show that there was little difference in the behavior of sulfide and oxidatively dehaired hides through the chrome tanning process.[6-7]

*Corresponding author e-mail address: eleanor.brown@ars.usda.gov
Presented in part at the 109th annual American Leather Chemists Association Meeting, June 20 - 23, 2013 Pinehurst, NC.

Manuscript received September 20, 2013, accepted for publication November 5, 2013.

Aldehyde-crosslinked wet white, and vegetable tanned hides are significant contributors, in addition to the dominant chrome-tanned wet blue hides, to current leather production. Vegetable tanning, the oldest of the currently used tanning technologies is, the least well understood from a mechanistic perspective. Some combination of hydrophobic interactions and hydrogen bonds between polyphenolic vegetable tannins and collagen are likely[8] as is a filling action in the gap portion of the collagen fiber.[9] In this study, we used the powdered hide model to begin an exploration of possible vegetable tanning mechanisms, and to compare effects of sulfide and oxidative dehairing in vegetable tanning. Quebracho, a condensed gallotannin from the bark of several South American hardwood trees was chosen as the model tannin.

EXPERIMENTAL

Materials

Fresh hide obtained from a local abattoir was sided, fleshed, and soaked prior to the start of the experiments. Bacterial collagenase (361 units/mg) isolated from *Clostridium histolyticum*, pepsin from gastric mucosa, and Sephadex LH-20 were obtained from Sigma-Aldrich, St. Louis. Quebracho was obtained from Hermann Oak Leather Company (St. Louis, MO) and used as obtained, except where purified quebracho was used for comparisons. All other chemicals were reagent grade from various suppliers.

Powdered Hide Preparation

One side of the hide was sulfide dehaired and relimed with lime/sulfide as described by Cabeza et al.[10] The other side was oxidatively dehaired and relimed without sulfide as described by Marmer and Dudley.[4] Both sides were then taken through the delime/bate step and powdered as described previously.[6]

Quebracho Purification

Purified quebracho was extracted from commercially available crude quebracho powder as described by Hagerman.[11] One gram of crude quebracho in 10 ml 80% ethanol was filtered through Whatman #1 filter paper in a Buchner funnel with gentle vacuum and washed with 80% ethanol. A slurry of Sephadex LH-20 in 80% ethanol was added to the filtrate with stirring for 3 m, then filtered through sintered glass by gravity and washed with 95% ethanol until the eluates were colorless. The Sephadex LH-20 was then washed with acetone:water (1:1) until the Sephadex was white and the eluate nearly colorless. Acetone washes were saved and the acetone removed under reduced pressure. The resulting aqueous sample was extracted three times with an equal volume of ethyl acetate, each time discarding the acetate layer. The aqueous layer was lyophilized and yielded 0.16 g purified quebracho.

Tanning

Initially, 1.0 gram each of sulfide and oxidatively dehaired powdered hide was hydrated overnight at 20˚C, 30˚C or 40˚C in 15 ml of phosphate buffered saline (PBS) at pH 6.0. The hydrated powdered hide was filtered to remove the buffer and rehydrated in fresh buffer. Quebracho powder 10% or 20% (based on the weight of the powdered hide) was added to each sample. The samples were tanned overnight in a shaking water bath at 20˚C, 30˚C or 40˚C. The following morning, they were filtered, and 0.5 g samples reserved damp for differential scanning calorimetry (DSC); the rest was allowed to air dry. In a second series of experiments, 0.25 g each of sulfide and oxidatively dehaired powdered hide was hydrated overnight at room temperature in PBS containing various concentrations of quebracho. Hydrated samples were filtered with gentle vacuum on Whatman #1 paper. The damp filter cake was reserved for analysis.

Analyses

Calorimetry

Hydrothermal stability of powdered hide was determined on a Multi-Cell Differential Scanning Calorimeter (DSC) (model CSC-4100) from Calorimetry Sciences Corporation, Lindon, UT, as previously described.[12] Moist, blotted samples (100 - 250 mg) were weighed into ampoules that were sealed and placed in the calorimeter. The calorimeter was programmed to record heat flow as mcal/°C while the temperature was increased from 30°C to 130°C at 1.0°C /min with an equilibration period of 600 s at the start. The temperature at the peak of the calorimetry trace, $T_{p,}$ was considered to be an apparent shrinkage temperature.

Microscopy

Hydrated samples of sulfide and oxidatively dehaired powdered hide from the delime/bate step of processing and after treatment with quebracho or purified quebracho were lyophilized. Lyophilized samples were mounted on the surface of carbon adhesive, the entire specimen was sputter-coated with gold using a Scancoat Six Sputter Coater (Edwards, Wilmington, MA). Samples for SEM were imaged using a Quanta 200 FEG environmental scanning electron microscope, (FEI Company, Hillsboro, OR). SEM images were captured as described previously.[13]

Collagen Extractibility

Soluble collagen was extracted from tanned or untanned powdered hide with 0.5 M acetic acid as described earlier.[6] Collagenase susceptibility of powdered hide that was untanned, or tanned with 20% quebracho was analyzed in triplicate as described previously.[7]

Characterization of Extracted Collagen

The amount of collagen extracted from the powdered hide at the delime/bate stage before and after quebracho tanning was estimated from the absorbance in 0.05 M acetic acid at 218 nm using the absorption coefficient 9.43 cm^{-1}-ml-mg^{-1} determined by Na.[14] The degree of native structure was estimated from the circular dichroism spectrum (AVIV 420 Spectropolarimeter, AVIV Biomedical Inc., Lakewood, NJ), and the thermal

stability of native collagen structure was determined from the temperature dependence of the circular dichroism signal at 223 nm as described previously.[7]

Results and Discussion

Calorimetry

Shrinkage temperature is the leather scientist's common term for the hydrothermal stability of a piece of hide or leather. One objective of tanning is to increase the shrinkage temperature from the 65 - 70°C range characteristic of raw cattle hide to a temperature approaching the 100°C of chrome tanned leather, or >80°C for most other tannages. The phenomenon measured by the shrinkage temperature is the contraction of the collagen fibers as they denature. The denaturation of collagen is monitored by a variety of techniques, depending on the characteristics of the sample and the equipment available, resulting in values that are apparent shrinkage temperatures and must be qualified by reference to the method. Table I details the apparent shrinkage temperatures obtained by DSC with powdered hide samples treated with varying amounts of quebracho. The shrinkage temperature increased linearly with increased offer of quebracho, regardless of whether quebracho was added to hydrated powdered hide or was included in the hydrating solution. At offers below 50% quebracho, shrinkage temperatures for oxidatively dehaired powdered hide were approximately 2°C lower than for sulfide dehaired powdered hide. As the offer was increased, the difference between shrinkage temperatures decreased, becoming negligible at or above 80% quebracho. At 10% and 20%, the temperature of the reaction, up to 40°C had little effect.

In an attempt to better understand the vegetable tanning process, an experiment was done using 7% purified quebracho which would be equivalent to 44% crude quebracho. Interestingly, the shrinkage temperatures of 75.6°C and 73.6°C are considerably lower than might have been anticipated, suggesting that the other components of crude quebracho play a significant role in the tanning process. Also noted in this experiment was a DSC peak at 45°C in the oxidatively dehaired material representing collagen that was not stabilized and was more readily denatured. Although no low temperature peak could be resolved in the sulfide dehaired sample treated with purified quebracho, the raw calorimeter data showed three inflections between 40°C and 60°C . No low temperature peaks or inflections were seen in data from powdered hide treated with crude quebracho. It is notable that the difference in shrinkage temperature between sulfide and oxidatively dehaired powdered hide with vegetable tannin is both smaller and in a reversed direction than when these materials were chromed tanned. (Table I).

TABLE I
Apparent Shrinkage Temperature, °C

Sample[a]	Sulfide dehaired	Oxidatively dehaired
0% Q	68.5 ± 0.7	67.1 ± 0.7
10% Q	80.3 ± 1.3	78.8 ± 1.5
20% Q	82.2 ± 0.1	80.2 ± 2
28% Q	83.5 ± 0.3	80.7 ± 2.8
55% Q	84.8 ± 0.1	83.6 ± 1.2
82% Q	85.9 ± 0.1	85.7 ± 0.1
95% Q	86.7 ± 0.2	86.7 ± 0.1
7% PQ[b]	75.6	73.6
Cr[c]	91.4 ± 0.6	95.9 ± 1.0
Cr[d]	100.8 ± 3.2	103.2 ± 1.5

[a]Values for crude quebracho (Q) represent results obtained with at least two separate samples.
[b]7% purified quebracho (PQ) is equivalent to 44% Q
[c]Chrome tanned powdered hide[7]
[d]Chrome tanned wet blue[2-5]

Microscopy

The characteristic banding pattern of native collagen is discernible in SEM images captured at 50,000x (Figure 1) of lyophilized sulfide and oxidatively dehaired powdered hide from the delime/bate step of processing both before and after treatment with quebracho or purified quebracho. Micrographs of powdered hide from either sulfide or oxidatively dehaired hide at the delime/bate step after lyophilization showed structures that were compacted and flattened. When these powdered hide samples were treated with crude quebracho, individual collagen fibers, coated with a material that fills the spaces between fibers were seen.

Although suspension of crude quebracho in PBS appeared to be a nearly clear solution, only 16% pure quebracho could be extracted from this suspension. Thus 84% of the crude quebracho is soluble material, that is not quebracho, and that appears to provide much of the filling effect seen in the center panel of Figure 1 and is characteristic of vegetable tannins in general. In contrast, when the treatment was with purified quebracho, the fibers were well separated, and not coated as evidenced by the more easily seen collagen-banding pattern. Also visible in the 50,000x micrographs and more clearly in 100,000x micrographs, not shown, are thread-like structures, possibly quebracho oligomers that appear to overlap or connect multiple fibers.

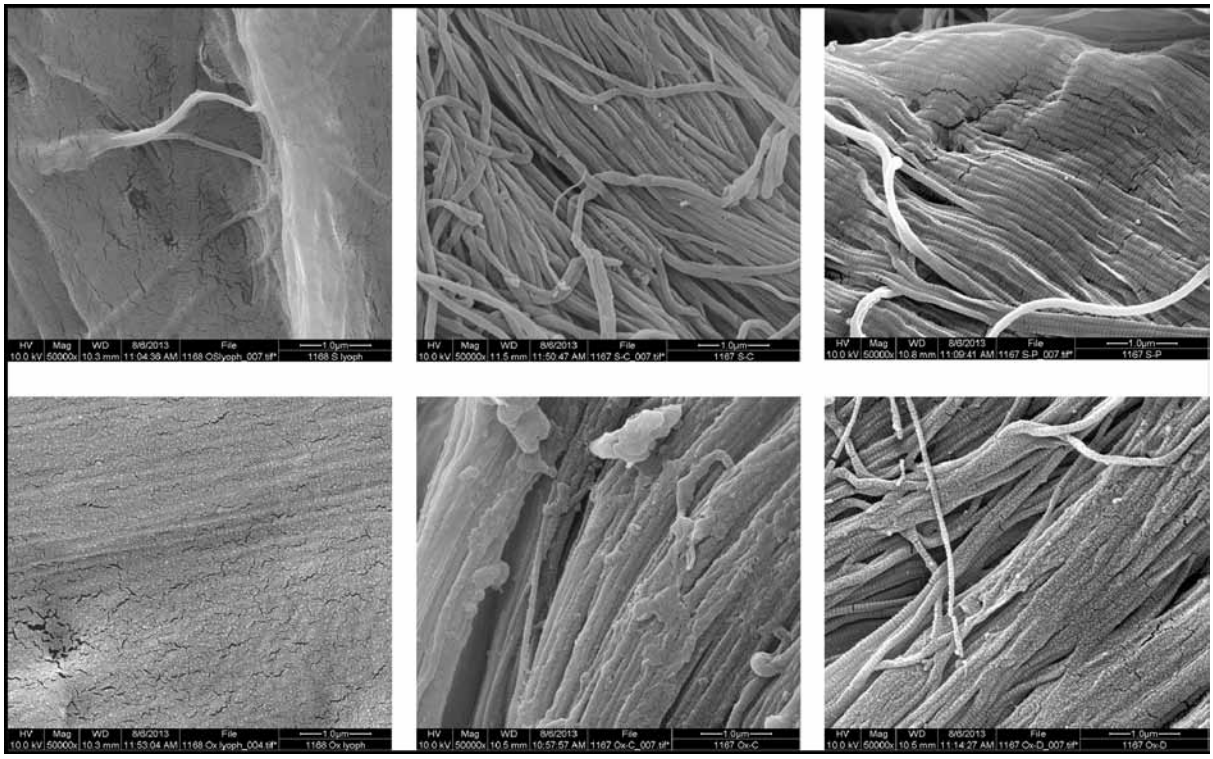

Figure 1. Scanning electron micrographs of powdered hide at 50,000x, the bar at the bottom of each micrograph represents 1 mm. The top row from left to right are lyophilized sulfide dehaired powdered hide from the delime/bate step, after treatment with an 82% offer of quebracho, and after treatment with 7% purified quebracho. The bottom row is for oxidatively dehaired powdered hide under the same conditions.

Stability of Collagen Structure

Extraction of 1.0 g each of untanned sulfide and oxidatively dehaired powdered hide from the delime/bate step, with 0.5 M acetic acid yielded 200 and 300 mg of collagen respectively. Similar extraction of samples that had been tanned with 20% quebracho yielded 18 and 28 mg respectively, showing that even a low level of tanning protected most soluble collagen. Interestingly, the ratio of acid extractable collagen (oxidative/sulfide) is 1.5 both before and after treatment with quebracho. In all cases, the circular dichroism spectrum of the acid extracted collagen showed a positive band in the 220 - 225 nm range and a stronger negative band at 198 nm characteristic of the collagen triple helix. Melting curves were likewise qualitatively similar to those of soluble collagens[7] in that they consisted of a positive flat region representing helical protein, at temperatures below 20°C , and a second negative flat region representing the unfolded protein at temperatures above 40°C. Between these two regions, inflections associated with a pretransition (29°C) and denaturation (35°C) were seen in the derivatives of the melting curves for collagen from sulfide dehaired material. These values are each 5°C lower than typical for soluble collagen.[15] Collagen extracted from oxidatively dehaired samples showed a single transition at 39°C corresponding to the full denaturation temperature.

Susceptibility to Collagenase

Collagenases are a class of enzymes that cleave the triple helical structure of collagen, and increase the number of available primary amino groups by exposing buried sidechain groups and creating additional N-terminal amino groups. Before treatment with quebracho, both sulfide and oxidatively dehaired samples were moderately susceptible to collagenase digestion. Treatment of either powdered hide sample with as little as 20% quebracho largely protected the collagen. In contrast to the acid extraction of collagen, there was little difference in collagenase sensitivity between sulfide and oxidatively dehaired powdered hide, either before or after treatment with quebracho.

Molecular Model

In an earlier study, using the collagen microfibril model to explore interactions of a model gallotannin with the collagen microfibril, both hydrogen bonding and hydrophobic interactions were observed.[8] Tannin molecules were docked, near serine residues on the microfibril. Although free to move in any direction, each of the tannin molecules remained near its docking site when the region was subjected to molecular dynamics simulations at 300K - 400K. By the midpoint of the

dynamics simulation, each of tannin molecules had stabilized in a position relative to a collagen side chain atom that could be explained by hydrogen or hydrophobic bonding. In the visual representation (Figure 3) the firmly anchored gallotannin molecules are seen to extend outward where they would be interacting with the next adjacent collagen microfibril, to fill gaps and stabilize the fiber structure.

CONCLUSIONS

The similarities in properties between the powdered hide from sulfide and oxidative dehairing are more notable than the differences. Calorimetry showed that in contrast to chrome tanning shrinkage temperatures for quebracho treated oxidatively powdered hides were generally 2°C lower than for sulfide dehaired samples. The difference diminished as the offer of quebracho was increased. A quebracho offer of 20%, which only increased the shrinkage temperature to 80°C , stabilized the collagen against acid extraction and collagenase digestion. Micrographs at 50,000x clearly show the coating effect of crude quebracho on collagen fibers. The tanning effect is partly a function of quebracho/collagen interactions, and also a function of the filling effect of other components of the crude quebracho.

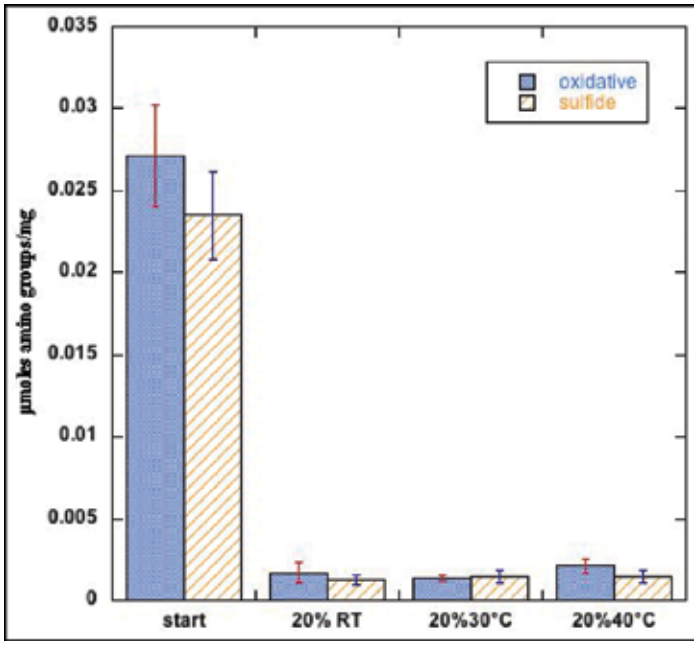

Figure 2. Available primary amino groups in powdered hide from collagenase treated oxidatively (blue) and sulfide (tan) dehaired powdered hide. The samples from left to right are from the delime/bate step, untreated, treated with 20% quebracho at room temperature, 30°C and 40°C , all samples in triplicate.

ACKNOWLEDGEMENTS

The authors wish to thank Lorelie Bumanlag for DSC data, Joseph Uknalis for microscopy, and Joe Lee for tanning advice.

REFERENCES

1. Germann, H.P.; John Arthur Wilson memorial lecture: the evolution of the unhairing process as influenced by technological, economic and ecological consideration. *JALCA* **92**, 84-92, 1997.
2. Marmer, W. N., Dudley, R. L., and Gehring, A. G.; Rapid oxidative unhairing with alkaline hydrogen peroxide. *JALCA* **98**, 351-358, 2003.
3. Gehring, A. G., Bailey, D. G., DiMaio, G. L., Dudley, R. L., Marmer, W. N., and Mazenko, C. E.; Rapid oxidative unhairing with alkaline calcium peroxide. *JALCA* **98**, 216-223, 2003.
4. Marmer, W. N., and Dudley, R. L.; Oxidative dehairing by sodium percarbonate**.** *JALCA* **100**, 427-431, 2005.
5. Gehring, A. G., Dudley, R. L., Mazenko, C. E., and Marmer, W. N.; Rapid oxidative dehairing with magnesiumperoxide and potassium peroxymonosulfate. *JALCA* **101**, 324-329, 2006
6. Brown, E. M., Latona, R. J., and Taylor, M. M.; Powdered hide for research on tanning mechanisms. *JALCA* **105**, 116-120, 2010.

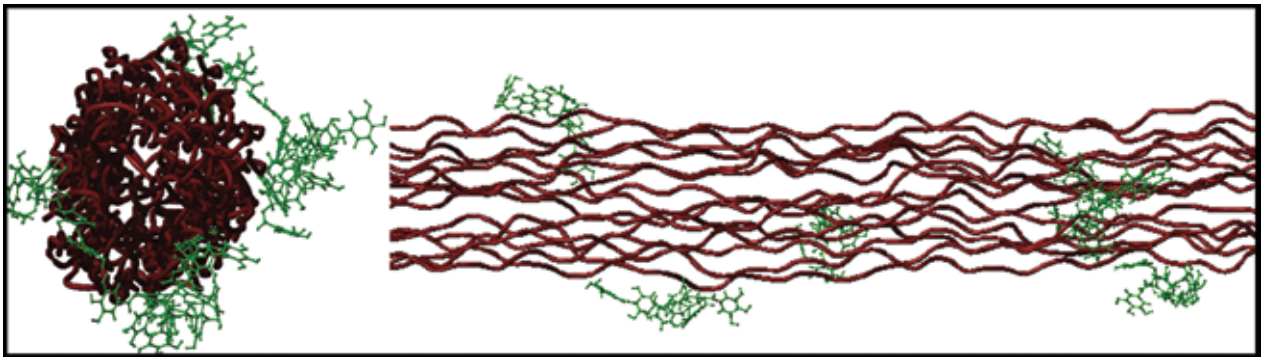

Figure 3. End-on and longitudinal views of a fragment of the collagen microfibril model, displayed as a brown ribbon, with 6 associated gallotannin molecules in green, after molecular dynamics simulation at 400K.

7. Brown E. M., Latona, R. J., Taylor, M. M. and Garcia, R. A.; Effects of pretanning processes on bovine hide collagen structure. *JALCA* **107,** 1-7, 2012.
8. Brown, E. M. and Shelly, D. C.; Molecular modeling approach to vegetable tanning: preliminary results for gallotannin interactions with the collagen microfibril. *JALCA* **106**, 145-152, 2011.
9. Haslam, E.; Vegetable tannage: Where do the tannins go? *J. Soc. Leather Tech. Chem.* **81**, 45-51, 1997.
10. Cabeza, L. F., Taylor, M. M., DiMaio, G. L., Brown, E. M., Marmer, W. N., Carrio, R., Celma, P. J., and Cot, J.; Processing of leather waste: Pilot scale studies on chrome shavings. Part II. Purification of chrome cake and tanning trials. *JALCA* **93**, 83-98, 1998.
11. Hagerman, A. E.; Purification of quebracho tannin. In Tannin Chemistry http://www.users.muohio.edu/hagermae/ accessed September 2013.
12. Ding, K., Taylor, M. M., and Brown, E. M.; Effect of genipin on the thermal stability of hide powder. *JALCA* **101**, 362-367, 2006.
13. Brown, E. M., Stauffer, D. M., Cooke, P., and Maffia, G. J.; The effect of ultrasound on bovine hide collagen structure. *JALCA* **101**, 274-283, 2006.
14. Na, G. C.; UV spectroscopic characterization of type I collagen. *Collagen Rel. Res.* **8**, 315-330, 1988.
15. Brown, E. M., Farrell Jr., H. M., and Wildermuth, R. J.; Influence of neutral salts on the hydrothermal stability of acid-soluble collagen. *J. Protein Chemistry* **19**, 85-92, 2000.