GENIPIN -ALUMINUM OR -VEGETABLE TANNIN COMBINATIONS ON HIDE POWDER

by

K. DING,[1,2] M.M. TAYLOR,[1] AND E.M. BROWN[1]*

[1]*U.S. Department of Agriculture, Agricultural Research Service*
Eastern Regional Research Center
600 EAST MERMAID LANE
WYNDMOOR, PA 19038

[2]*College of Chemistry & Environmental Protection Engineering*
Southwest University for Nationalities
CHENGDU 610041, P. R. CHINA

ABSTRACT

Genipin, a naturally occurring protein crosslinking agent, isolated from the fruit of *Gardenia jasmindides Ellis*, is beginning to replace glutaraldehyde as a fixative for biological tissues. Earlier research in this laboratory demonstrated that the apparent shrinkage temperature of hide powder could be increased from 60°C to 79°C by treating the powdered hide with 5% genipin at pH ~ 7 and 35°C for 24 hr, a significant improvement, but not enough for most leather uses. Typical tannages proposed to reduce the use of chromium are combinations of vegetable tannins or aldehydes with each other or with minerals. In this study, the tanning effect on bovine hide powder of genipin in combination with aluminum or vegetable tannins has been investigated. In terms of thermal stability, vegetable tannins in combinations with genipin appear to offer little advantage over either component alone, suggesting little if any synergy. Likewise, aluminum tanning over a genipin pretannage appears to provide little advantage. However, when hide powder was first tanned with 8% aluminum and then retanned with genipin the thermal stability increased linearly with increasing concentrations of genipin (2% to 10%). The apparent shrinkage temperature was > 100°C for aluminum post-tanned with 8%-10% genipin, suggesting the possibility that a practical combination tannage based on genipin could be designed.

ABSTRACTO

Genipin, un agente reticulante de origen natural para proteínas, aislable del fruto de la *Gardenia jasmindides Ellis*, empieza ya a reemplazar al glutaraldehído como fijador para tejidos biológicos. Investigaciones anteriores en este laboratorio demostraron que la aparente temperatura de encogimiento de polvo de piel se podría incrementar de 60°C a 79°C tratando piel en polvo con 5% de genipin a un pH ~7 y a 35°C durante 24 horas, una mejoría significante, pero insuficiente para la mayoría de los usos del cuero. Los curtidos típicamente propuestos para reducir el uso del cromo constan de combinaciones de taninos vegetales o aldehídos juntos, o con minerales. En este estudio, el efecto curtiente de genipin sobre piel bovina en combinaciones con el aluminio o curtientes vegetales ha sido investigado. En términos de estabilidad térmica, taninos vegetales en combinación con genipin aparentemente ofrecen solo poca ventaja sobre un curtido único con uno solo de los componentes, sugiriendo poco efecto sinergético. Similarmente el curtido por aluminio sobre un precurtido de genipin aparentemente tampoco ofrece mayor ventaja. Sin embargo cuando polvo de piel fue inicialmente curtido por 8% de aluminio y seguido por un recurtido con genipin, la estabilidad térmica resultante aumentó linealmente con la aumentada concentración del genipin (2% hasta 10%). La aparente temperatura de encogimiento fue >100°C en el caso del curtido con aluminio y recurtido con 8%-10% de genipin, sugiriendo que la posibilidad de un curtido práctico combinado basado en genipin fuera diseñable.

INTRODUCTION

Quality, cost and yield have long been the main concerns of leather manufacturers, and were the driving force behind the development of chrome tanning. In recent years, environmental impact has become a worldwide concern for manufacturing processes. This environmental concern, combined with consumer preference, particularly in the European market, for chromium-free leather is now driving research into the development of reduced chrome and chrome-free tanning methods.

Chrome-tanned leathers are stable in the presence of heat and moisture. The characteristic shrinkage temperature (Ts) of at least 100°C for chrome-tanned leather is a standard to which leathers tanned by other processes are compared. Considerable research has shown that the tanning effects of minerals other than chromium (Al, Zr, Ti, or Fe) are enhanced when they are used in combination with vegetable tannins, aldehydes, or other organic molecules.[1-6] Tannages comprised of vegetable tannin-aluminum,[3] vegetable tannin-aldehyde,[7-9] and aluminum-aldehyde[4] have been shown to be effective. Leather tanned by these combinations had Ts of near 100°C and physical-mechanical properties adequate for a variety of applications. Nevertheless, these combination tannages have not been widely adopted.

Genipin, an iridoid crosslinking-agent, which can be isolated from the fruits of *Gardenia jasmindides*,[10] has recently come to the attention of the biomaterials industry. The feasibility of using genipin as a fixative for biological tissues was demonstrated by Sung *et al.*[11] The report by that same research group[12] that genipin was 10,000 times less cytotoxic than glutaraldehyde inspired research that showed genipin to be an excellent natural cross-linker for biopolymers, including gelatin, and chitosan.[12-17]

Recent research in this laboratory[18] showed that treatment of hide powder with 5% genipin in phosphate buffered saline at pH 7 and 35°C for 24 hr resulted in an increase in the apparent shrinkage temperature from 60°C to 79°C. In the present work, we evaluate the effect on the thermal stability of hide powder when treated with genipin in combination with aluminum and vegetable tannins.

EXPERIMENTAL

Materials

Bovine hide powder was prepared in this laboratory for an earlier study[19] and stored dry. Genipin (MW= 226.23, approximately 98% by HPLC) was purchased from Challenge Bioproducts Co. LTD, Taiwan, ROC. Mimosa and chestnut tannins were from Pilar River Plate Corporation, Newark, NJ. Glutaraldehyde (25% solution) and other reagents were from Sigma-Aldrich, St. Louis, MO. All reagents were of the highest grade available and used as received. Citrate-masked aluminum (Al/Cit) tanning solution was prepared from aluminum sulfate ($[Al^{3+}]$ = 0.2 M) and sodium citrate ([Cit]/[Al3+] = 1/4, pH 3.2) and stored at ambient temperature for more than 24 hr before use, to assure the stability of the masked aluminum complexes.

All experiments were performed at least twice to evaluate repeatability.

Combination tanning of hide powder

Genipin-aluminum

Genipin-tanned hide powder was prepared as described previously,[18] except that the tanning was stopped after 16 hr. Hide powder (0.5 g) was first hydrated with distilled water at a 15/1 (v/wt) ratio in a flask overnight at ambient temperature, filtered and resuspended in phosphate buffered saline (PBS) at pH ~ 7. Genipin at 2%-10% (0.01-0.05 g for 0.5 g of hide powder) was added and the tanning continued for 16 hr. At the completion of genipin tanning, the resulting mixture was filtered, and the tanned hide powder was washed with distilled water until the effluent was clear. The genipin-tanned hide powder was then transferred to a flask, with 7.5 ml Al/Cit tanning solution (pH = 3.2), and placed in a shaking bath at 35°C. The pH was increased to about 3.5 after 6 hr and to 4.8-5.0 after an additional 3 hr. Aluminum tanning was continued for another 6 hr for a total tanning time of 15 hr. The tanning mixture was filtered and the tanned hide powder washed 3 times with 10 ml distilled water and then dried at ambient temperature.

Aluminum-genipin

For aluminum pretanning, the hydrated hide powder was pickled with acetic acid (pH 2.5 ~ 3.0) and filtered to remove excess moisture. The Al/Cit tanning solution was added to the pickled hide powder at a 15/1 (v/wt) ratio (the offer was equivalent to 8% Al), 20% NaCl was added, and the pH was adjusted with sodium bicarbonate to about 3.5 after 6 hr; and to pH 4.5-5.0 after an additional 3 hr. Tanning was continued for another 6 hr. Genipin retanning of aluminum tanned hide powder was then carried out in PBS (pH = 6.8-7.2) with 2%-10% genipin for 16 hr. The tanned hide powder was then washed and dried at ambient temperature.

Genipin-vegetable tannin

Genipin-tanned hide powder was prepared as described above with a 6% genipin offer, 0.03 g genipin for 0.5 g hide powder. Vegetable retanning of genipin-tanned hide powder was accomplished with 25% or 50% wt/wt (0.125 g or 0.25 g for 0.5 g hide powder) mimosa or chestnut at pH 6 for 24 hr. The tanned hide powder was then washed and dried at ambient temperature.

Vegetable tannin-genipin

For vegetable pretannages the hide powder was hydrated overnight in PBS, 5%, 10% or 50% (0.01, 0.05, or 0.25g) chestnut or mimosa was added and the mixture was maintained at pH 6 for 24 hr. Genipin retanning of 5% or 10% vegetable-tanned hide powder was carried out in PBS

* Corresponding Author-E-mail: ellie.brown@ars.usda.gov
K. Ding was a visiting scientist at ERRC 2004-2006; his permanent address is at Southwest University for Nationalities.
Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.
Manuscript received December 5, 2006, accepted for publication December 5, 2006

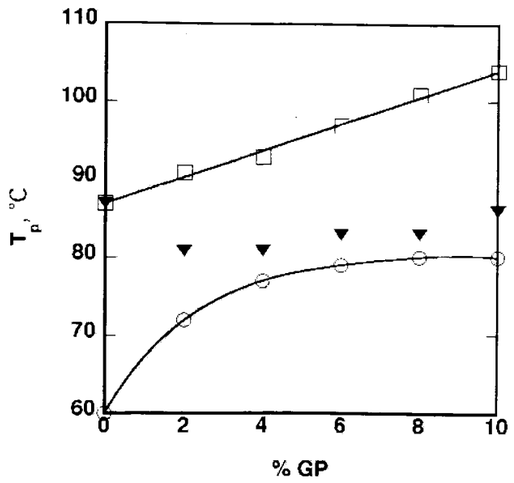

Figure 1. - T_p of hide powder as a function of tanning process: circles represent hide powder tanned with increasing concentrations of genipin alone; triangles represent genipin-tanned hide powder after retanning with 8% aluminum; squares represent hide powder tanned with 8% aluminum and retanned with increasing concentrations of genipin.

(pH = 6.8-7.2) with 1%, 3% or 5% genipin for 16 hr. For 50% vegetable-tanned hide powder, the genipin offers were 5% or 10%. The tanned hide powder was then washed and dried at ambient temperature.

Aluminum-glutaraldehyde and aluminum-mimosa combinations

Hide powder samples tanned with combinations of aluminum and glutaraldehyde or mimosa were prepared to serve as controls. For Al-glutaraldehyde tanning, 0.5g Al pretanned hide powder was put in a flask with 7.5 ml PBS and 4 ml glutaraldehyde (25% solution) and placed in a shaking water bath at 35°C for 24 hr, the combination-tanned hide powder was rinsed and dried at ambient temperature. For Al-mimosa tanning, Al-pretanned hide powder was retanned with 25% or 50% wt/wt mimosa at pH 6 for 24 hr. For glutaraldehyde-Al and mimosa-Al tanned hide powder, glutaraldehyde- and mimosa-pretanned hide powders were prepared, and retanned with Al/Cit tanning solution as described above.

Determination of thermal stability

Thermal stability of tanned hide powder was determined on a Multi-Cell Differential Scanning Calorimeter (model CSC-4100) from Calorimetry Sciences Corporation, Lindon, UT, as previously described.[18] Hide powder samples were prepared for DSC experiments by soaking in distilled water overnight and then blotting on filter paper. Moist, blotted samples (100-250 mg) were weighed into ampoules that were sealed and placed in the calorimeter. The temperature was programmed to record from 30°C to 130°C at 1.5°C/min with an equilibration period of 600 sec at the start. The temperature at the peak of the calorimetry trace, T_p, was considered to be an apparent shrinkage temperature. Samples were dried, and the initial moisture content (usually 150%-250%) of each sample was calculated.

RESULTS AND DISCUSSION

Combination tanning of hide powder

In a preliminary study of the reaction of genipin with hide powder,[18] the apparent shrinkage temperature of hide powder that had been treated with 5% genipin at pH ~ 7 and 35°C for 24 hr was increased from 60°C to 79°C. This increase in thermal stability was similar to the increase in the shrinkage temperature of sheepskin from 65°C to 79°C - 80°C achieved by Covington and Shi[8] with oxazolidine. In the present study, combinations of genipin with aluminum or vegetable tannins were evaluated in the effort to further increase the thermal stability of the hide powder.

Aluminum is often the metal of choice to replace or augment chromium in tanning processes. The solution properties[20,21] of aluminum in the presence of masking agents as well as the mode of binding to collagen[22,23] have been well studied. The shrinkage temperature of aluminum-tanned leather depends on the masking ions and ranges between 80°C and 90°C.[3,24,25] The thermal stability imparted by aluminum makes it an excellent component of combination tannages with organic or vegetable tannins.

The organic chemicals, other than vegetable tannins, that are most used for tanning have an aldehydic or acidic group that would be expected to react primarily with the amine groups of collagen. In contrast, chromium, aluminum and other mineral-tanning agents are most likely to react with carboxyl groups on the protein. In an early study of aluminum-acrylate combination tanning, Beebe *et al.*[26] found that pretanning calfskin with acrylates was beneficial because it increased the amount of aluminum fixed and improved the physical properties and the appearance of the leather. On the other hand, pretanning with aluminum followed by an acrylate polymer retannage produced leather with a slightly higher shrinkage temperature, but poorer qualities overall. Thus the tanner could decide which leather properties were most important for the application. More recently, Gangopadhyay *et al.*[27] demonstrated that the shrinkage temperature of resin-tanned goatskin was increased by 20-25°C when it was retanned with aluminum. Although the detailed mechanisms of genipin-collagen interactions are not completely understood,[18] the active structure and binding mode of genipin appear to resemble those of glutaraldehyde.

Genipin and aluminum combinations

Thermal stabilities or apparent shrinkage temperatures, as reported from the peak in the DSC curve, for hide powder tanned with genipin/aluminum, and aluminum/genipin combinations are summarized in Table I. The first four columns show the effect of pretanning with 2% to 10% genipin followed by tanning with 8% aluminum in the form of citrate-masked aluminum sulfate. The solo 5% genipin tannage[18] with T_p = 79°C is included as a reference point, as is the T_p = 87°C for aluminum as a solo tannage.

As was reported earlier,[18] T_p of the genipin-tanned hide powder increased almost linearly up to 5% genipin, and remained nearly constant at 79°C to 80°C at higher genipin levels. After retanning these genipin-tanned hide powder samples with the Al tanning solution, the peak melting temperature was increased slightly to between 81°C and 86°C, approaching but not quite achieving the thermal stability (T_p = 87°C) of hide powder that had been solo tanned with aluminum. In contrast, when the first tannage was with aluminum, followed by genipin, the increase in thermal stability was linear with respect to the amount of genipin used, with a range spanning 17°C and rising to 104°C with 10% genipin. To give perspective, values for glutaraldehyde-aluminum combinations are included in Table I.

The effect of the order of tanning with genipin and aluminum can be clearly seen in Figure 1. Aluminum as a solo tannage on hide powder increased Tp from 60°C to 87°C. Aluminum retanning of genipin-tanned hide powder had little effect on T_p (3-5°C). Only when the first tannage was 10% genipin did T_p = 86°C for the genipin/aluminum combination approach the value (87°C) achieved with aluminum tanning alone. On the other hand, when the order was reversed, so that

TABLE I

Thermal Stability of Hide Powder Tanned with Genipin-aluminum Combinations

Tannage 1	T_p	Tannage 2	T_p	Tannage 1	Tannage 2	T_p
5% GP	79	none	79	Al	none	87
2% GP	72	Al	81	Al	2% GP	91
4% GP	77	Al	81	Al	4% GP	93
6% GP	79	Al	83	Al	6% GP	97
8% GP	80	Al	83	Al	8% GP	101
10% GP	80	Al	86	Al	10% GP	104
20% GLUT	84	Al	86	Al	20% GLUT	93

GP-genipin, Al-8% aluminum on wt of hide powder, GLUT-glutaraldehyde, T_p-peak temperature (°C)
Tanning procedures are detailed in Methods

TABLE II

Thermal Stability of Hide Powder Tanned with Mimosa or Chestnut and Genipin

Tannage 1	Tannage 2	T_p (°C)	Tannage 1	Tannage 2	T_p (°C)
5% Ma	none	69	5% Ct	none	63
5% Ma	1% GP	77	5% Ct	1% GP	73
5% Ma	3% GP	76	5% Ct	3% GP	76
5% Ma	5% GP	77	5% Ct	5% GP	79
10% Ma	none	69	10% Ct	none	65
10% Ma	1% GP	75	10% Ct	1% GP	71
10% Ma	3% GP	78	10% Ct	3% GP	77
10% Ma	5% GP	77	10% Ct	5% GP	79
50% Ma	none	81	50% Ct	none	76
50% Ma	5% GP	93	50% Ct	5% GP	81
50% Ma	10% GP	99	50% Ct	10% GP	83
6% GP	none	79	-	-	-
6% GP	25% Ma	83	6% GP	25% Ct	80
6% GP	50% Ma	88	6% GP	50% Ct	80

GP-genipin, Ma- mimosa, Ct-chestnut, Tp-peak temperature (°C)
Tanning procedures are detailed in Methods

aluminum-tanned hide powder was retanned with genipin, the apparent shrinkage temperature (T_p) increased dramatically to 90°C at the 2% genipin level and higher than 100°C at the 8% genipin level. The aluminum-glutaraldehyde combination tannage, run as a control, showed the same tendency, i.e., pretanning with aluminum resulted in greater thermal stability (T_p =90°C) than did retanning with aluminum (T_p =86°C).

In summary, the stabilization of collagen through complexation of aluminum salts with carboxyl groups favored the crosslinking of amine groups by genipin. This outcome might be predicted on the basis that genipin reaction is favored under neutral to basic conditions,[18] and when acidic groups are complexed with aluminum salts, the local environment of the amine groups may be somewhat more basic. Conversely, when an aluminum tannage was added to hide powder pretanned with as little as 2% genipin, the final apparent shrinkage temperature was less than could be achieved by solo tanning with aluminum, and at higher levels of genipin, the additional stabilization afforded by aluminum remained at about 4°C. Although it has been noted for other aluminum-based combination tannages that the order in which the tannages are applied has more effect on the characteristics of the leather than on the shrinkage temperature,[26,30] an obvious explanation for the lack of effect on thermal stability when genipin-tanned hide powder is retanned with aluminum is not apparent.

Vegetable tannin and genipin combinations

A second category of combination tannages is based on vegetable tannins. Extracts from mimosa, chestnut and quebracho trees have long been part of the tanner's repertory. Prior to the advent of chrome tanning, they were the primary tanning agents. More recently, these polyphenolic compounds are used in combination tannages with minerals and aldehydic crosslinkers. The aldehydic crosslinkers that have been evaluated as components of combination tannages with vegetable tannins include glutaraldehyde[29,30] and oxazolidine[5,9,31] An initial tannage of sheepskin with glutaraldehyde followed by vegetable tanning with mimosa produced better leather, albeit with a slightly lower shrinkage temperature than did the reverse process.[29,30] On the other hand, oxazolidine proved to be a successful retannage (shrinkage temperature >110°C) on mimosa-tanned sheepskin leather or hide powder.[8,31] In this study, the tanning effect on hide powder of genipin in combination with mimosa and chestnut both as a pretannage and a retannage was evaluated. The results are summarized in Table II.

Vegetable pretanning of hide powder with 5% or 10% mimosa raised the Tp of the hide powder from 60°C to 69°C; a genipin second tannage at 1% to 5% further increased T_p to between 76°C and 78°C. In these ranges, the results were not sensitive to the concentration of either tanning agent, nor was the T_p achieved any higher than with 5% genipin alone. A 50% mimosa pretannage followed by a 5% or 10% genipin tannage did produce a T_p higher than 90°C. This result suggests the possibility that at high concentrations of mimosa the genipin may be crosslinking the condensed vegetable tannin as well as the collagen.[32] Hide powder pretanned with chestnut (5% to 50%) and then tanned with genipin (1% to 10%), had at best a T_p of 83°C. Hydrolyzable tannins, such as chestnut, that have been shown to interact strongly with minerals[32] would be less likely to crosslink aldehydes.

When hide powder tanned with 6% genipin (T_p = 79°C) was retanned with 25% and 50% mimosa, T_p increased to 83°C and 88°C respectively. Although as shrinkage temperatures for leather these values would not be impressive, increases in thermal stability of hide powder in the 4°C to 9°C range suggest that further investigations with intact hide might be warranted. Retanning of geninpin-tanned hide powder with 25% or 50% chestnut had no noticeable effect on T_p.

These results are in general agreement with those of Covington and Shi[8] and Lu *et al.*[31] who evaluated combinations of vegetable tannins with oxazolidine. The highest T_p or shrinkage temperature was achieved when hide powder or sheepskin was pretanned with 20% to 50% mimosa and retanned with 4% to 6% oxazolidine. They also noted little advantage to combinations of chestnut with oxazolidine.

CONCUSIONS

Tannages proposed to replace or reduce the use of chromium are nearly always combination tannages that combine two or more mechanisms for interaction with collagen. Mineral agents generally in the form of oligomeric, sulfate-containing complexes react with the carboxyl groups on collagen. Vegetable tannins, also in the form of oligomers or polymers, stabilize collagen through hydrogen bonding networks, hydrophobic interactions and possibly by filling in gap regions of the fibril.[33] Aldehydes as well as cyclic compounds including genipin and oxazolidine that acquire aldehydic functionality through ring opening mechanisms react with primary amines on collagen. With such a variety of modes of interaction, synergies between different types of tanning agents might be expected. In terms of thermal stability, vegetable tannins in combinations with genipin appear to offer little advantage over either component alone, suggesting little if any synergy. In aluminum-genipin combinations, aluminum tanning over a genipin pretannage appears to provide little advantage. The most exciting finding in this study was the linear increase, with increasing concentrations of genipin (2% to 10%), in apparent shrinkage temperature of hide powder tanned first with 8% aluminum and retanned with genipin. The T_p > 100°C for aluminum post tanned with 8%-10% genipin suggest the possibility of designing a practical combination tannage.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Robert Dudley, Lorelie Bumanlag, Renée Latona, and Joe Lee for technical contributions and helpful discussions. As a visiting scientist at ERRC, ARS, USDA, K. Ding wishes to thank the China Scholarship Council (CSC) for financial support.

REFERENCES

1. Sundarrajan A., Madhan, B., Rao, J. R., and Nair, B. U.; Studies on tanning with zirconium oxychloride: Part I Standardization of tanning process. *JALCA* **98**, 101-106, 2003.
2. Madhan, B., Subramanian, V., Rao, J. R., Nair, B. U., and Ramasami, T.; A semi-empirical quantum mechanical modeling study on the interaction of collagen-like peptides with polyphenolic molecules: An attempt to gain insights into vegetable tanning. *JALCA* **98**, 273 278, 2003.
3. Hernandez J. F., and Kallenberger, W. E.; Combination tannages with vegetable tannins and aluminum. *JALCA* **78**, 182-206, 1984.
4. Zhang, T. Y., Chen, H. L., and Zhang, T. S.; Research on the process of aluminum pretanning wet-white. *Zhongguo Pige* **26**, 29-31, 1997 (Chinese).
5. Peng, B. Y.; Study on the titanium tanning process and its tanning mechanism. Ph.D. thesis (Sichuan University, Chengdu, P. R. China, 1999) (Chinese).
6. Fathima, N. N., Chandrabose, M., Araviadhan, R., Rao, J. R. and Nair, B. U.; Iron phosphonium combination tanning: Towards a win-win approach. *JALCA* **100**, 273 281, 2005.
7. Träubel, H.; A new approach to tanning-An unconventional attempt. *JALCA* **100**, 304-316, 2005.
8. Covington, A. D., and Shi, B.; High stability organic tanning using plant polyphenols: Part 1: The interaction between vegetable tannins and aldehydic crosslinkers. *J. Soc. Leather Tech. Chem.* **82**, 64-71, 1998.
9. Shi, B., He, Y., Fan, H., Zeng, S., Covington, A. D., and Attenburrow, G. E.; High stability organic tanning using plant polyphenols: Part 2: The mechanism of the vegetable tannin-oxazolidine tannage. *J. Soc. Leather Tech. Chem.* **83**, 8-13, 1999.
10. Park, J. E., Lee, J. Y., Kim, H. G., Hahn, T. R., and Park, Y. S.; Isolation and characterization of water soluble intermediates of blue pigments transformed from geniposide of *Gardenia jasminoides*. *J. Agric. Food Chem.* **50**, 6511-6514, 2002.
11. Sung, H. W., Huang, R. N., Huang, L. L. H., Tsai, C. C., and Chiu, C. T.; Feasibility study of a natural crosslinking reagent for biological tissue fixation. *J. Biomed. Mater. Res.* **42**, 560 567, 1998.
12. Sung, H. W., Huang, R. N., Huang, L. L. H., and Tsai, C. C.; *In vitro* evaluation of cytotoxicity of a naturally occurring crosslinking reagent for biological tissue fixation. *J. Biomater. Sci. Polym. Ed.* **13**, 63-78, 1998.
13. Liang, H. C., Chang, W. H., Liang, H. F., Lee, M. H., and Sung, H. W.; Crosslinking structure of gelatin hydrogels crosslinked with genipin or a water-soluble carbodiimide. *J. Appl. Polym. Sci.* **91**, 4017-4026, 2004.
14. Sung, H. W., Chang, Y., Chiu, C. T., Chen, C. N., and Liang, H. C.; Crosslinking characteristics and mechanical properties of a bovine pericardium fixed with a naturally occurring crosslinking agent. *J. Biomed. Mater. Res.* **46**, 757-767, 1999.
15. Bigi, A., Cojazzi, G., Panzavolta, S., Roveri, N., and Rubini, K.; Stabilization of gelatin films by crosslinking with genipin. *Biomaterials* **23**, 4827-4832, 2002.
16. Yao, C. H., Liu, B. S., Chang, C. J., Hsu, S. H., and Chen Y. S.; Preparation of networks of gelatin and genipin as degradable biomaterials. *Mater. Chem. Phys.* **83**, 204-208, 2004.
17. Mi, F. L.; Synthesis and characterization of a novel chitosan-gelatin bioconjugate with fluorescence emission. *Biomacromolecules* **6**, 975-987, 2005.
18. Ding, K., Taylor, M. M., and Brown, E. M.; Effect of genipin on the thermal stability of hide powder. *JALCA* **101**, 362-367, 2006.
19. Komanowsky, M.; Thermal stability of hide and leather at different moisture contents. *JALCA* **86**, 269-280, 1991.
20. Ding, K., Liu, J., and Zhang, T.; The composition analysis of organic acid masked aluminum tanning solutions by Ferron assay and GFC. *JALCA* **101**, 260-265, 2006.
21. Ding, K., Liu, J., and Zhang, T.; The composition and structure analysis of organic acid masked aluminum complexes tanning solutions by ^{27}Al NMR. *JALCA* **101**, 381-387, 2006.
22. Brown, E. M., and Dudley, R. L.; Approach to a tanning mechanism: study of the interaction of aluminum sulfate with collagen. *JALCA* **100**, 401-409, 2005.
23. Gervais, M., Commeneges, G., and Laussac, J-P.; 1D- and 2D-NMR study of a nonapeptide, a fragment of collagen, in its free state and complexed with aluminum (III). *Magn. Reson. Chem.* **25**, 594-599, 1987.
24. Constantini, N., Ciardelli F., and Covington, A. D.; Studies on the tanning reactions of zeolite. *JALCA* **95**, 125-137, 2000.
25. Madhan, B., Rao, J. R., and Nair, B. U.; Tanning agent based on mixed metal complexes of aluminum and zinc. *JALCA* **96**, 120-126, 2001.
26. Beebe, C. W., Happich, W. F., Luvisi, F. P., and Hannigan, M. V.; The use of acrylates in aluminum tannning. *JALCA* **52**, 560-565, 1957.
27. Gangopadhyay, S., Lahiri, S., and Gangopadhyay, P. K.; Chrome-free tannage by sequential treatment with synthetic resins and aluminum or titanium. *J. Soc. Leather Tech. Chem.* **84**, 88-93, 2000.

28. Madhan, B., Gunasekaran, S., Narasimman, R., Rao, J. R., and Sadulla, S.; Integrated chrome-free upper leather processing-Part II: Standardarization and evaluation of vegetable-aluminum tanning system. *JALCA* **100**, 373-379, 2005.
29. Luvisi, F. P., and Filachione, E. M.; The effect of glutaraldehyde on vegetable tanning. *JALCA* **63**, 584-600, 1968.
30. Slabbert, N. P.; The basics of practical tanning systems reconciled with vegetable tanning theories. *JALCA* **94**, 1-7, 1999.
31. Lu, Z., Liao, X., Zhang, W., Tao, Y., and Shi, B.; Mechanism of vegetable tannin-aldehyde combination tannage. *JALCA* **100**, 432-437, 2005.
32. Madhan, B., Aravindhan, R., Siva, M. S., Sadulla, S., Rao, J. R., and Nair, B. U.; Interaction of aluminum and hydrolysable tannin polyphenols: An approach to understanding the mechanism of aluminum vegetable combination tannage. *JALCA* **101**, 317-323, 2006.
33. Haslam, E.; Vegetable Tannage-Where Do the Tannins Go? *J. Soc. Leather Tech. Chem.* **81**, 45-51, 1997