Collagen Fiber Opening of Cattle Hides in Urea/Calcium Hydroxide Solutions

by
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Abstract

In the beamhouse, liming might directly affect the structure and performance of collagen, as well as the quality of resultant leather. However, the influences of composition and content of liming agents on liming mechanism are quite complicated. In this study, calcium hydroxide and urea were utilized in liming. The solution pH, hide swelling ratio and non-collagenous protein removal were quantitatively analyzed. The morphologies of both limed and fresh hides were studied by optical microscopy. The reaction mechanism of fiber opening up of cattle hides was analyzed and speculated by the combination of thermogravimetric analysis and Fourier transformation infrared spectroscopy. It was found that the fiber bundles of hides limed by urea/calcium hydroxide have a better opening up effect than that by pure calcium hydroxide. The mechanism of liming in an urea/calcium hydroxide solution system was proposed.

Introduction

Animal hides are transformed into leathers through a series of leather making processes including soaking, fleshing, degreasing, unhairing, liming, deliming, bating, pickling and tanning.[1, 2] Animal hides are mainly composed of three layers of grain surface/epidermis, dermis, and subcutaneous tissue. The dermis between the epidermis and subcutaneous tissue is the main component of leather. Leather making includes a lot of complicated processes affecting the structure of collagen, in which liming is one of the most critical steps that can determine the final style and properties of leather.[3-8] It is widely accepted that after liming, the partial secondary bonds in collagen can be destroyed and some glycoproteins among the collagen fiber bundles are also removed. As a result, the collagen fiber bundles are opened to expose enough active sites for the penetration and reaction of subsequent leather chemicals.

Traditional liming is done in a complex aqueous system with lime, sulfide, alkaline, neutral salt, enzyme, liming auxiliaries and surfactant.[9] However, most of these reagents cannot be fully utilized in the water medium, resulting in a waste of chemical regents and environmental pollution.[10,11] Marsal et al. studied the composition and properties of wastewater from a tannery and proposed the recovery of nitrogen from wastewater in the beamhouse.[12] Mohamaed et al. studied the composition and change in liming wastewater.[13] The recycling of liming wastewater was realized by adding chemicals to adjust its composition for 8 times of cycling. However, due to insufficient research on the contribution of each component to the liming and the interaction discipline among the components, it is still difficult to reveal the reaction process to reuse the liming agent.

With the increasing need for environmental protection, attention has been paid to greener and cleaner processes, such as biological treatments.[14-19] For example, Ranjithkumar et al. used enzymes from solid-state fermentation for unhairing.[20] Leather samples unhaired by an enzymatic method showed better smoothness, finer grain pattern than those unhaired by conventional method. Sivasubramanian et al. used a bacterial protease based a commercial unhairing enzyme for enzyme-assisted unhairing.[21] From the microscopic structure of goatskins, it was found that the hairs were not destroyed totally and the roots of the removed hairs were observed. Liu et al. developed a novel liming agent based on sodium silicate and enzyme to replace lime.[22] The dosage of the liming agent was 1.5%, and the swelling ratio, fiber bundle opening, shrinkage temperature and mechanical properties of the leather were comparable to those of leather made by the conventional liming method.

In order to find a suitable liming condition to moderately disperse the collagen fibers, Liu et al. studied the effect of liming time on collagen fibers.[23] They determined the opening up degree of collagen fibers at different liming times by acoustic emission and found that the opening up degree of dermal collagen fibers was related to the liming time. Cheng et al. explored the changes of hides limed with alkali and neutral salts and found that a high concentration of sodium sulfate depressed the swelling of collagen fibers in alkali solutions, while no obvious effect was found in sodium chloride solution.[24] Tang et al. studied the changes in thermal degradation activation energy of collagen fibers and discussed the influence of alkali solution and urea solution treatment on the dry heat shrinkage properties.[25-27]

Currently, increasing effort has been focused on cleaner production in leather making. It is of great importance to know the liming

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mechanism to find a more suitable liming system. In this work, the mechanism of liming in calcium hydroxide and urea/calcium hydroxide solutions was studied. In general, calcium hydroxide is a commonly used liming agent for collagen fiber bundle opening. The morphology of collagen fibers in different concentrations of calcium hydroxide was investigated using optical microscopy as well as scanning electron microscopy (SEM). The swelling ratio of cattle hides and the total protein, proteoglycan and hydroxyproline in wastewater were measured. Thermogravimetric (TG) and Fourier transformation infrared (FTIR) analyses were used to analyze the thermal properties and chemical structure of limed hides. Furthermore, as a good auxiliary agent for liming, urea was used instead of sodium sulfide for the purpose of reducing sulfide use and pollution.

Experimental

Materials

Wet-salted cattle hides were provided by Prosper Skins & Leather Enterprise Co., Ltd. (Jiaozuo, China). Sodium carbonate (Na_2CO_3), calcium hydroxide ($Ca(OH)_2$), urea, ammonium sulfate ($(NH_4)_2SO_4$), and sulfuric acid were purchased from Civi Chemical Technology Co., Ltd. (Shanghai, China). Sodium chloride, tannic acid and dispase (50 U/mg) were from Macklin Biochemical Co., Ltd. (Shanghai, China).

Liming Treatment

Wet-salted cattle hides were used as raw materials and pre-treated by conventional leather making process before liming (Figure 1). After pre-treatment, the fresh hides were cut into small pieces sized 0.25 cm^2 for liming. Then the samples were stirred in two sets of liming solutions for 24 hours in beakers. The first set is pure calcium hydroxide solutions with the concentrations of 0.5 %, 2 %, 3.5 %, 5 % and 6.5 % (w/v), and the resulting samples limed by these solutions were named as P-0.5%Ca, P-2%Ca, P-3.5%Ca, P-5%Ca, and P-6.5%Ca, respectively. Another set is urea/calcium hydroxide solutions at weight ratios (urea:calcium hydroxide) of 1:2, 4:2, 7:2, 10:2, 13:2, and 16:2, and the samples limed by these solutions were coded as S-1%urea-2%Ca, S-4%urea-2%Ca, S-7%urea-2%Ca, S-10%urea-2%Ca, S-13%urea-2%Ca, and S-16%urea-2%Ca, respectively. After liming, the hides were delimed, bated, pickled and tanned with tannin acid. The procedure and detailed experimental conditions are illustrated in Figure 1.

pH of the Liming Solutions

The pH of the liming solutions was monitored with a PHS-3G pH meter (Shanghai Yidian Scientific Instrument Company, China) after the various liming times: 0 h, 1 h, 4 h, 8 h, 12 h and 24 h, respectively.

Swelling Ratios of Cattle Hides

Swelling ratios of the samples were calculated according to the following equation:[28]

$$\Delta h\% = (h_i - h_0)/h_0 \times 100\% \quad (1)$$

where h_i is the thickness of the sample after liming for 24 h and h_0 is the thickness of the sample before liming. The thickness of hides was measured by a leather thickness gauge (Randall & Stickney Dial Company, USA). The thickness of leather samples was obtained after 1 min of compression at 50 kPa.

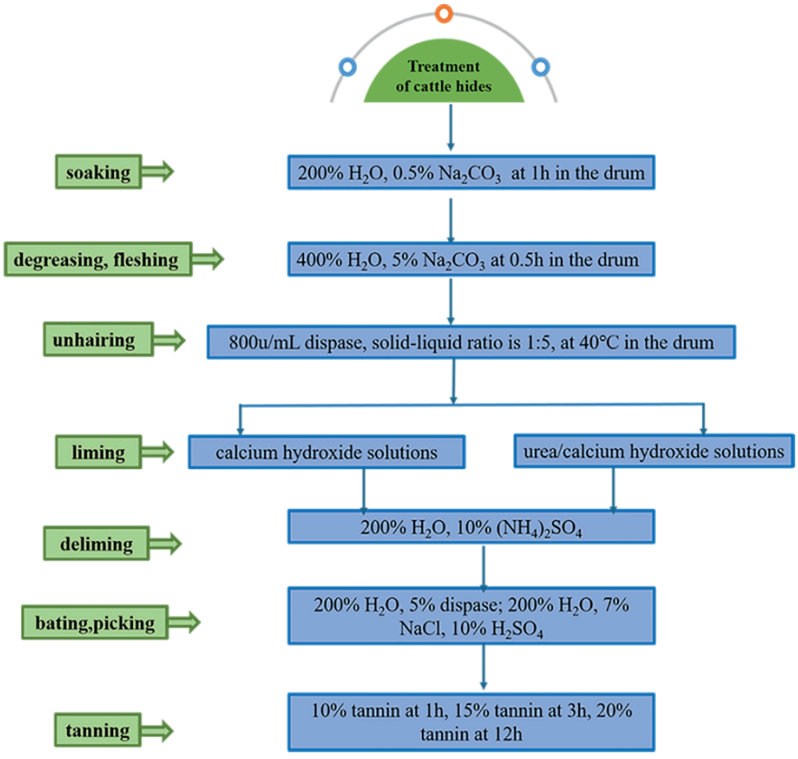

Figure 1. Schematic representation of the overall treatment of cattle hides.

Protein Content in Wastewater

The Bicinchoninic acid reagent was used to test the content of proteins in wastewater, whose mechanism is that Cu^{2+} can be reduced to Cu^{+} by proteins under alkaline condition, and the complex formed by Cu^{+} and bicinchoninic acid presents purple color with an absorption peak at 562 nm. Therefore, spectrophotometer (Thermo Fisher Scientific Oy Ratastie 2, FI-01620 Vantaa, Finland) was employed to characterize the absorbance of the complex at 562 nm, and the protein content in the wastewater was obtained by comparing the absorbance to the standard absorbance curve of protein at 562 nm.

Proteoglycan Content in Wastewater

In a boiling water bath, proteoglycan was dehydrated by sulfuric acid to form furfural or hydroxymethyl furfural and their derivatives through the dehydration condensation of anthrone ($C_{14}H_{10}O$). Furfural or hydroxymethyl furfural has a maximum absorption peak at 620 nm. Based on this principle, proteoglycan content was identified by adding sulfuric acid and anthrone reagents into the wastewater. The absorbance of furfural or hydroxymethyl furfural derivatives at 620 nm was recorded by an ultraviolet spectrophotometer (TU-1950, Beijing Purkinje General Instrument Co., Ltd., China). By comparing the absorbance of samples with the standard absorbance curve, the content of proteoglycan in the wastewater was calculated.

Hydroxyproline Content in Wastewater

Hydroxyproline was measured by Ehrlich methods according to the reported studies.[29,30] Hydroxyproline may be oxidized by an oxidant (Chloramine T), and the oxidation products may react with dimethylaminobenzaldehyde to yield a purple color complex with an absorption peak at 550 nm. Similarly, the absorption peak of distilled water and hydroxyproline standard concentration at 550 nm was measured. The hydroxyproline content in the wastewater was obtained according to Eq. (2).

$$C_H = (OD_T - OD_C)/(OD_S - OD_c) \times C_S \times 10 \quad (2)$$

where is the hydroxyproline content in the wastewater, is the absorbance of test sample in the wastewater, is the absorbance of control sample, is the absorbance of standard sample, and is the content of standard sample of hydroxyproline.

Structure

After liming, samples of around 0.25 cm^2 were cut from the standard sampling area of the cattle hides. The samples were placed in paraformaldehyde solution for staining. The hide samples were subjected to Verhoeff's Van Gieson (EVG) staining for the observation of histological features using an optical microscope (Eclipse E100, Nikon, Japan). After tanning, the vegetable-tanned leather was freeze-dried in a GT2-Type-8 freeze dryer (LYOTECH, Germany). The samples of the vegetable-tanned leather were cut into thin strips. The strips were sprayed by a thin layer of gold under vacuum conditions and observed by a Quanta 250 scanning electron microscopy (FEI, USA). The orientation of collagen fibers were observed from SEM for the cross-section at the accelerating voltage of 20 kV with different magnifications. After liming, the hides were delimed, washed and freeze-dried. The dried samples were analyzed by a TGA/DSC1 thermogravimetric analyzer (NETZSCH, Germany). Approximately 5~10 mg of samples were exposed to a nitrogen atmosphere at a flow rate of 140 mL/min, and heated in a ceramic sample pan at a heating rate of 10 °C/min from 25 to 600 °C. The Fourier transform infrared (FTIR) spectra of hide samples were recorded using a VERTEX 70 spectrometer (Bruker Optik GmbH, Ettlingen, Germany). The spectrum was obtained ranging from 4000 cm^{-1} to 400 cm^{-1} at a resolution of 4 cm^{-1}, and the spectrum scan was conducted with 8-s intervals.

Results and Discussion

The pH of Liming Solutions

The pH is especially important in liming, which influences the swelling ratio of hides and even the properties of resultant leathers. Since the pH of the bath is higher than the isoelectric point of collagen, collagen molecules are negatively charged due to the dissociation of the carboxyl groups on the side chains, and collagen fiber bundles become shorter and thicker due to the electrostatic interactions with the adjacent protein chains and the hydration of the charged groups.[31] This shows that the swelling of hides depends primarily on the difference between the bath pH and the isoelectric point of the collagen. Therefore, the pH of liming solutions was further studied in the present work and the results are shown in Figure 2. In Figure 2(a), the pH of calcium hydroxide solution is about 12.81, which changes slightly with the increase of calcium hydroxide concentration. It is also observed that the pH of calcium hydroxide solution is decreased after liming, illustrating that the liming agents dissolved in the water and penetrated into the hide. Figure 2(b) shows that the pH of the urea/calcium hydroxide solution increases from 12.83 to 13.01 with increasing the urea concentration, a little higher than that of the pure calcium hydroxide only. However, the pH of 1%~16% pure urea solutions is all about 7.4. Therefore, it was illustrated that urea might act as a solubilizer for calcium hydroxide in liming solution via coordination of Ca^{2+} with $CO(NH_2)_2$ in the urea/calcium hydroxide solutions.[32]

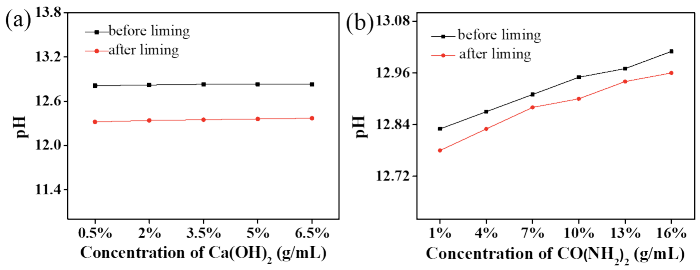

Figure 2. Variation of pH with the increase of **(a)** $Ca(OH)_2$ concentration and **(b)** urea concentration with 2% $Ca(OH)_2$.

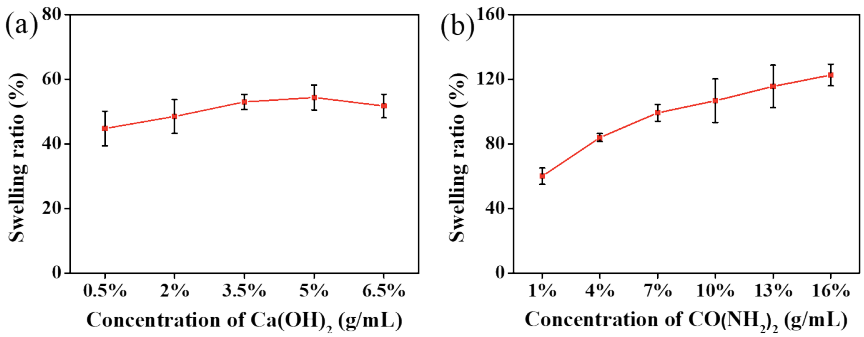

Figure 3. Variation of swelling ratio of hides with concentrations of **(a)** $Ca(OH)_2$ and **(b)** urea (in the presence of 2% $Ca(OH)_2$).

Swelling Ratio of Hides after Liming

Collagen fiber bundles become shorter and thicker as observed by optical microscopy, and a macroscopic swelling of hides can be observed after liming. Therefore, the swelling ratio of hides in liming was further studied. As shown in Figure 3(a), after liming, the swelling ratio of hides increases with increasing the calcium hydroxide concentration. Figure 3(b) shows that the swelling ratio of hides limed by urea/calcium hydroxide solution gradually increases with the increase of urea concentration. The hides limed by urea/calcium hydroxide solution showed a higher swelling ratio than those limed by a pure calcium hydroxide solution. The swelling ratio of hides limed by 2% $Ca(OH)_2$ was 48.5 %, while those limed by urea/calcium hydroxide was in the range of 60.0~122.6%. Herein, the inter- or intra-molecular hydrogen bonding of collagen might be partially destroyed by urea, and then more water molecules might be able to enter into the hides to fully fill in the hides, resulting in a more obvious swelling.

Contents of Total Protein, Proteoglycan and Hydroxyproline in Wastewater

The contents of total protein, proteoglycan and hydroxyproline in liming wastewater might indicate the effect of liming on hides.[3] The total protein and hydroxyproline content was determined by measuring the degree of collagen hydrolysis and the removal extent of non-collagenous protein content in the wastewater. The ability of liming agents to hydrolyze collagen fibers and dissolve the interstitial fibers is indirectly estimated by the content of proteins in the wastewater. As revealed in Table I, with the increase of calcium hydroxide concentration, the protein content in liming wastewater increases firstly and then decreases at a concentration higher than 3.5%. Here the protein may be coagulated with the precipitation of calcium hydroxide in the wastewater due to suspension of calcium hydroxide.[33] In addition, compared to that with the pure calcium hydroxide liming solution, the protein in the wastewater is increased by the addition of urea. Thus, more non-collagenous proteins of hides were removed in the urea/calcium hydroxide solution. The removal of proteoglycan aids in the collagen fiber bundle opening. The proteoglycan contents in liming wastewater are shown in Table I. The proteoglycan contents in urea/calcium hydroxide liming wastewater did not decrease obviously, compared to that limed in pure calcium hydroxide solutions. Since hydroxyproline is a unique amino acid in collagen, the degree of collagen hydrolysis might be analyzed by the hydroxyproline contents in the wastewater.[28] As shown in Table I, the hydroxyproline content in the wastewater gradually increases with the calcium hydroxide concentration increasing. This reflects the fact that the degree of collagen hydrolysis increases with the calcium hydroxide concentration. However, the presence of urea in liming solution could restrict the hydrolysis of collagen, as manifested by the lower content of hydroxyproline in urea/calcium hydroxide liming wastewater when compared to those in pure calcium hydroxide liming wastewater. Therefore, it can be concluded that liming in urea/calcium hydroxide solution might be helpful for promoting collagen fiber bundles opening as well as keeping the integrity of collagen molecules.

Table I

Quantitative evaluation of beamhouse processes.

Samples	Total protein content[a] mg/mL	Proteoglycan content[a] μg /mL	Hydroxyproline content [a] μg/mL
P-0.5%Ca	0.21±0.01	199.8±7.1	1.35±0.06
P-2%Ca	0.22±0.04	225.0±15.4	2.90±0.06
P-3.5%Ca	0.23±0.05	288.6±40.4	3.27±0.09
P-5%Ca	0.17±0.01	135.7±15.2	3.54±0.07
P-6.5%Ca	0.17±0.00	163.3±11.1	3.91±0.08
S-1%urea-2%Ca	0.23±0.05	110.2±13.2	0.10±0.00
S-4%urea-2%Ca	0.24±0.01	214.2±13.2	0.26±0.02
S-7%urea-2%Ca	0.30±0.03	192.4±9.5	0.49±0.06
S-10%urea-2%Ca	0.26±0.03	279.9±18.2	0.84±0.03
S-13%urea-2%Ca	0.23±0.06	274.0±39.8	1.44±0.06
S-16%urea-2%Ca	0.20±0.03	313.6±45.4	1.25±0.04

[a]Average value of three test data.

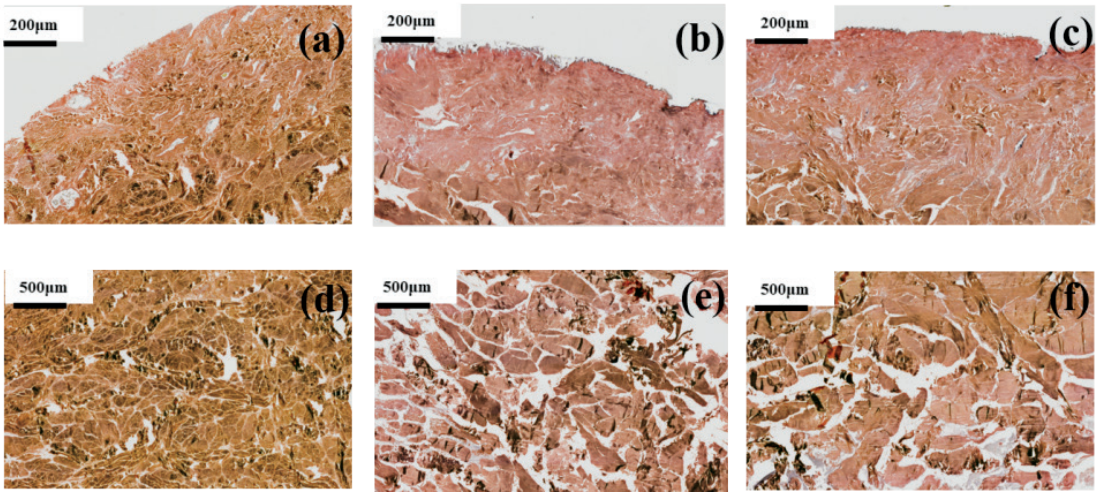

Figure 4. Histologically stained cross-section micrographs of **(a, d)** fresh hide, **(b, e)** P-2%Ca and **(c, f)** S-7%urea-2%Ca samples.

Histological Photographs and Scanning Electron Microscopy

In Figure 4, the constituents of the hair follicles and the tissues were stained as dark brown. The elastic fibers were stained as dark purple, and the collagen fibers were stained as dark red. Compared to the hides without liming, less non-collagenous proteins were revealed in hides limed by calcium hydroxide and urea/calcium hydroxide solutions, indicating more opening up of collagen fibers by liming. The cross-sectional SEM micrographs of vegetable-tanned leathers are shown in Figure 5. It can be observed that the vegetable-tanned leather limed by 7%urea/2%$Ca(OH)_2$ solution (Figure 5(c,d)) shows better collagen fiber bundle opening than that of limed in 2% $Ca(OH)_2$ solution (Figure 5(a,b)). These results are consistent with the above-discussed protein contents in liming wastewater (Table I).

TG Results

The thermogravimetric (TG) and differential thermogravimetric (DTG) curves of various limed hides are given in Figure 6. Similar curves were found for all the samples. The evaporation temperature of unbound water is around 100°C. The thermal decomposition

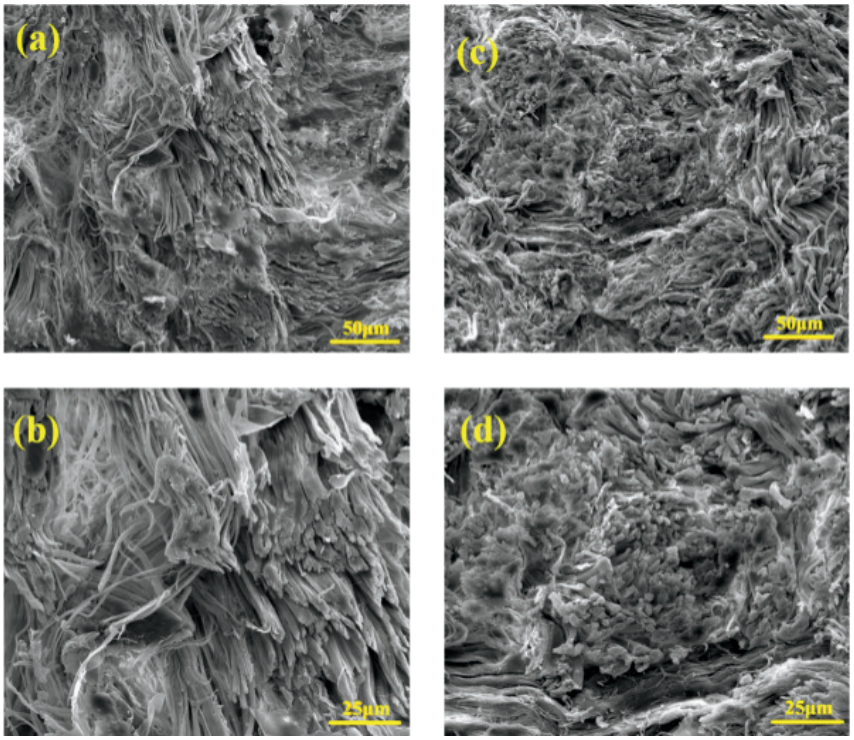

Figure 5. SEM micrographs of the cross-section of vegetable-tanned leather limed in **(a, b)** 2% $Ca(OH)_2$ and **(c, d)** 7% urea/2% $Ca(OH)_2$ solutions.

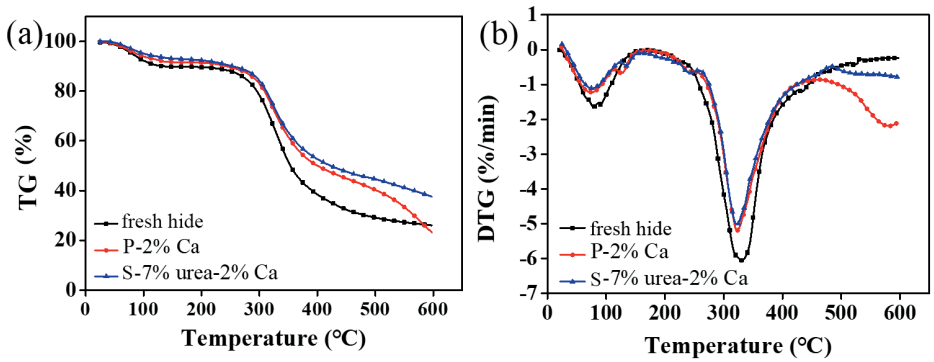

Figure 6. (a) TG and **(b)** DTG curves of hides limed by different liming systems.

temperature of collagen begins at around 200°C and is completed at around 450°C in both hides before limed and limed with urea/$Ca(OH)_2$.[34,35] Table II shows the onset temperature and weight loss percentage at 600°C. The onset temperature of hides limed by 7% urea/2% $Ca(OH)_2$ is higher than that of the fresh hide, suggesting a better thermal stability of hides limed by 7% urea/2% $Ca(OH)_2$ solution than the fresh hide. However, the onset temperature of hides limed by 2% $Ca(OH)_2$ solution is lower than the fresh hide, indicating that liming by calcium hydroxide decreases the thermal stability of hides. Interestingly, the weight loss percentage of hides limed by 2% $Ca(OH)_2$ is more than that of fresh hide, while that of hides limed by 7% urea/2% $Ca(OH)_2$ solution is the lowest of the three samples.

Table II

The onset temperature and weight loss ratio of hides limed with different liming systems.

Samples	Onset temperature (°C)	Weight loss percentage at 600°C (%)
Fresh hide	288.8	74.0
P-2%Ca	286.1	77.3
S-7%urea-2%Ca	292.7	62.4

Possible Liming Mechanism

According to the results discussed above, the possible liming mechanism in different liming solutions can be proposed. In pure $Ca(OH)_2$ solution, the hydrogen bonding in collagen may be disrupted by the hydroxyl ions in calcium hydroxide solution, and some Ca^{2+} may combine with carboxyl groups in collagen by cooperation interactions (Figure 7(A)). Urea is a well-known hydrogen-bond-breaking reagent. The schematic illustration of the disruption of the hydrogen bonds in collagen matrix by urea is shown in Figure 7(B). Moreover, both the pH of urea/$Ca(OH)_2$

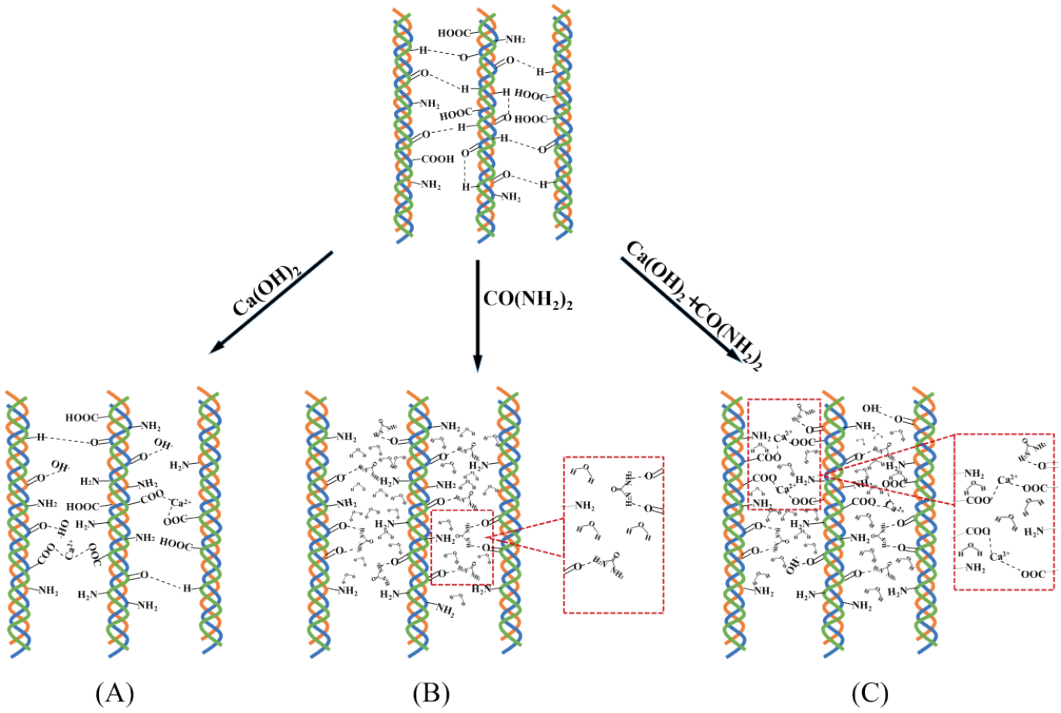

Figure 7. The mechanism of liming in **(A)** calcium hydroxide and **(C)** urea/calcium hydroxide solution; **(B)** The disrupting mechanism of hydrogen bonding in collagen matrix by urea.

solution (Figure 2) and swelling ratio of hides limed by urea/$Ca(OH)_2$ (Figure 3) are higher than those of the samples limed only by $Ca(OH)_2$. Based on these facts, it can be speculated that the inter- and intra-molecular hydrogen bonding of collagen molecules may be disrupted by urea and calcium hydroxide synergistically, and thereby exposing more sites on the collagen to lime. To be specific, hydrogen bonding in collagen may be disrupted collaboratively by the amino groups (from $CO(NH_2)_2$) and hydroxyl ions (from $Ca(OH)_2$) in urea/$Ca(OH)_2$ solution. Consequently, the Ca^{2+} in the solution may combine with more carboxyl groups on collagen by coordination, resulting in a higher thermal stability of hides limed by urea/$Ca(OH)_2$ than that of other two samples (Figure 7(C)). The changes in thermal stability of the hides can be seen from Table II, and the weight loss at 600°C of the hide limed by urea/$Ca(OH)_2$ is the lowest among the three samples. This may be attributed to the formation of more coordination compounds within the collagen matrix after liming with urea/$Ca(OH)_2$ solution.

In this work, the complexation mechanism of Ca^{2+} and carboxyl groups in collagen was investigated by FTIR spectra in the range of 500-4000 cm^{-1}. The FTIR spectra of hides (Figure 8) revealed the typical characteristic peaks of collagen.[36-38] The absorbance bands of the amide A of collagen are located at 3304 cm^{-1}, mainly due to the stretching vibration absorption peak of N–H and O–H. The band near 3078 cm^{-1} responds to the amide B of collagen, which should be assigned to the asymmetric and symmetric stretching vibration absorption peak of $-CH_2$. Besides, the characteristic absorbance bands of amide I, amide II and amide III modes of collagen were all observed at 1633 cm^{-1}, 1540cm^{-1} and 1235 cm^{-1}, respectively. Formation of amide I is mainly by stretching vibration absorption peak of C=O. Especially, the vibration peak of C=O at 1750 cm^{-1} indicated the existence of carboxyl group. Compared with that of the fresh hide, the peak intensity of amide A, amide I and C=O of the hides limed with $Ca(OH)_2$ and urea/$Ca(OH)_2$ is weakened, indicating that the amount of carboxyl group is reduced by liming. Hence, the carboxyl group of collagen may react with Ca^{2+} by coordination.

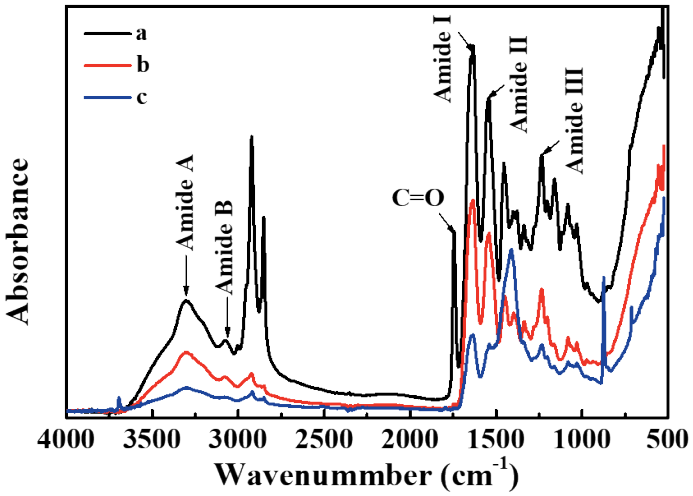

Figure 8. FTIR spectra of **(a)** fresh hide, **(b)** P-2%Ca, **(c)** S-7%urea-2%Ca.

Conclusions

In this study, the effect of urea/calcium hydroxide on the collagen fiber bundles in liming is studied by histological and SEM analyses. The results indicated that the 7% urea/2% $Ca(OH)_2$ solution is the best for liming from viewpoints of collagen fiber bundle opening. Urea/calcium hydroxide has greater ability to remove the inter-fibrillar substance, compared with that of pure calcium hydroxide. The combination of urea with calcium hydroxide has a good opening up effect for collagen fiber bundles. A possible mechanism of liming in calcium hydroxide and urea/calcium hydroxide solution system was proposed based on the results obtained. The inter- and intra-molecular hydrogen bonding of collagen molecules may be destroyed by urea, making the penetration of molecular water into the collagen networks easier.

Acknowledgements

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References

1. Wu, J., Zhao, L., Liu, X., Chen, W. and Gu, H.; Recent progress in cleaner preservation of hides and skins. *Journal of Cleaner Production* **148**, 158-173, 2017.
2. Zhang, Y., Liu, H., Wang, F. and Tang, K.; KCl-assisted enzyme in unhairing and collagen fibre bundle opening for cleaner leather making. *Journal of the Society of Leather Technologists and Chemists* **104**(5), 235-239, 2020.
3. Li, S., Li, J., Yi, J. and Shan, Z.; Cleaner beam house processes trial on cattle sofa leather. *Journal of Cleaner Production* **18**(5), 471-477, 2010.
4. Tang, K., Li, W., Liu, J., Liu, C.-K. and Pan, H.; Mechanism of collagen processed with urea determined by thermal degradation analysis. *JALCA* 2020, **115**(10): 380-389, 2020.
5. Liu, H., Li, X., Li, M., Zhang, Y., Tang, K., Liu, J., Zheng, X. and Pei Y.; A simple and sustainable beamhouse by the recycling of waste-water from KCl-dispase synergistic unhairing in leather making. *Journal of Cleaner Production* **282**, 124535, 2020.
6. Saravanan, P., Renitha, T. S., Gowthaman, M. and Kamini, N.; Understanding the chemical free enzyme based cleaner unhairing process in leather manufacturing. *Journal of Cleaner Production* **79**, 258-264, 2014.
7. Kanagaraj, J., Senthilvelan, T., Panda, R. C. and Kavitha, S.; Eco-friendly waste management strategies for greener environment towards sustainable development in leather industry: A comprehensive review. *Journal of Cleaner Production* **89**, 1-17, 2015.
8. Kanagaraj, J., Panda, R., Senthilvelan, T. and Gupta, S.; Cleaner approach in leather dyeing using graft copolymer as high

performance auxiliary: related kinetics and mechanism. *Journal of Cleaner Production* **112**, 4863-4878, 2016.

9. Liu, H., Yin, Z., Zhang, Q., Li, X., Tang, K., Liu, J., Pei, Y., Zheng, X. and Ferah, C. E.; Mathematical modeling of bovine hides swelling behavior by response surface methodology for minimization of sulfide pollution in leather manufacture. *Journal of Cleaner Production* **237**, 117800, 2019.
10. Sathish, M., Madhan, B., Sreeram, K. J., Rao, J. R. and Nair, B. U.; Alternative carrier medium for sustainable leather manufacturing-A review and perspective. *Journal of Cleaner Production* **112**, 49-58, 2015.
11. Yan, T., Luo, X., Zou, Z., Lin, X. and Yu, H.; Adsorption of Uranium(VI) from a simulated saline solution by slkali-activated leather waste. *Industrial and Engineering Chemistry Research* **56**, 3251-3258, 2017.
12. Marsal, A., Bautista, E., Cuadros, S., Reyes, M., Rius, A. and Font, J.; Recovery of organic nitrogen from beamhouse wastewater in a hair recovery process. *Journal of the Society of Leather Technologists and Chemists* **93**, 176-182, 2009.
13. Mohamaed, K. E. A. and Gasmelseed, G. A.; Recycling of unhairing lime liquors. *Journal of the Society Leather Technologists and Chemists* **87**, 116-118, 2003.
14. Shi, B., Lu, X. and Sun, D.; Further investigations of oxidative unhairing using hydrogen peroxide. *JALCA* **98**, 185-192, 2003.
15. Valeika, V., Beleska, K., Valeikienė, V. and Kolodzeiskis, V.; An approach to cleaner production: from hair burning to hair saving using a lime-free unhairing system. *Journal of Cleaner Production* **17**, 214-221, 2009.
16. Cheng, J., Yue, C., Jiang, S., Gao, Y., Nie, J. and Fang, S.; Synthesis and performances of UV-curable polysiloxane-polyether block polyurethane acrylates for PVC leather finishing agents. *Industrial and Engineering Chemistry Research* **54**, 5635-5642, 2015.
17. Yi, Z., Ingham, B., Cheong, S., Ariotti, N. and Prabakar, S.; Real-time synchrotron small-angle X-ray scattering studies of collagen structure during leather processing. *Industrial and Engineering Chemistry Research* **57**, 63-69, 2017.
18. Madhan, B., Rao, J. R. and Nair, B. U.; Studies on the removal of inter-fibrillary materials. I. Removal of protein, proteoglycan, glycosoaminoglycans from conventional beamhouse process. *JALCA* **105**, 145-149, 2010.
19. Dettmer, A., Cavalli, É., Ayub, M. A. Z. and Gutterres, M.; Optimization of the unhairing leather processing with enzymes and the evaluation of inter-fibrillary proteins removal: an environment-friendly alternative. *Bioprocess and Biosystems Engineering* **35**, 1317-1324, 2012.
20. Ranjithkumar, A., Durga, J., Ramesh, R., Rose, C. and Muralidharan, C.; Cleaner processing: a sulphide-free approach for depilation of skins. *Environmental Science and Pollution Research* **24**, 1-9, 2017.
21. Sivasubramanian, S., Manohar, B. M., Rajaram, A. and Puvanakrishnan, R.; Ecofriendly lime and sulfide free enzymatic dehairing of skins and hides using a bacterial alkaline protease. *Chemosphere* **70**(6), 1015-1024, 2008.
22. Liu, Y., Chen, Y., Fan, H., Hu, Z., Luo, Z. and Shi, B.; Environment-friendly lime-free liming. *Journal of the Society Leather Technologists and Chemists* **93**, 56-60, 2009.
23. Liu, C.-K., Latona, N. and Dimaio, G. L.; Degree of opening up of the leather structure characterized by acoustic emission. *JALCA* **96**, 367-381, 2001.
24. Cheng, H., Chen, M. and Li, Z.; Properties of collagen fibre in alkali and neutral salt solutions. *Journal of the Society Leather Technologists and Chemists* **94**, 65-69, 2010.
25. Tang, K., Wang, F., Jia, P., Liu, J. and Wang, Q.; Thermal degradation kinetics of sweat soaked cattlehide collagen fibers. *JALCA* **102**, 52-61, 2007.
26. Hu, Y., Liu, J., Luo L., Li X., Wang F. and Tang, K.; Kinetics and mechanism of thermal degradation of aldehyde tanned leather, *Thermochimica Acta* **691**, 178717, 2020.
27. Tang, K., Liu, J., Wang, F., Liu, J. and Zhang, J.; Influence of urea process on thermal degradation behavior of collagen fibers. *China Leather* **33**, 25-27, 2004.
28. Ding, Z. and Zhang, M.; Linear relationship between collagen degradation and degree of swelling in the beamhouse. *Journal of the Society Leather Technologists and Chemists* **85**, 164-166, 2001.
29. Bergman, I. and Loxley, R.; Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. *Analytical Chemistry* **35**, 1961-1965, 1963.
30. Stegemann, H. and Stalder, K.; Determination of hydroxyproline. *Clinica Chimica Acta* **18**, 267-273, 1967.
31. Winkler, R.; On the existence of a lyotropic and osmotic type of collagen swelling produced by representative electrolytes and anelectrolytes. *Pflugers Archiv-european Journal of Physiology* **345**, 37-42, 1973.
32. Fast, J., Hakansson, M., Muranyi, A., Gippert, G. P., Thulin, E., Evenas, J., Svensson, L. A. and Linse, S.; Symmetrical stabilization of bound Ca^{2+} ions in a cooperative pair of EF-hands through hydrogen bonding of coordinating water molecules in calbindin D9k. *Biochemistry* **40**, 9887-9895, 2001.
33. Dickinson, E.; Milk protein interfacial layers and the relationship to emulsion stability and rheology. *Colloid Surface B* **20**, 197-210, 2001.
34. Luo, L., Liu, J., Liu, C.-K., Brown, E. M., Wang, F., Hu, Y. and Tang, K.; Thermogravimetric analysis and pyrolysis kinetics of tannery wastes in an inert atmosphere. *JALCA* **115**, 123-131, 2020.
35. Liu, J., Luo, L., Zhang, Z., Hu, Y., Wang, F., Li, X. and Tang, K.; A combined kinetic study on the pyrolysis of chrome shavings by thermogravimetry. *Carbon Resources Conversion* **3**, 156-163, 2020.
36. Meng, Z., Zheng, X., Tang, K., Liu, J., Ma, Z. and Zhao, Q.; Dissolution and regeneration of collagen fibers using ionic liquid. *International Journal of Biological Macromolecules* **51**(4), 440-448, 2012.
37. Lazarev, Y. A., Grishkovsky, B. and Khromova, T.; Amide I band of IR spectrum and structure of collagen and related polypeptides. *Biopolymers* **24**, 1449-1478, 1985.
38. Liu, C.-K., Latona, N. P., Taylor, M. and Aldema-Ramos, M. L.; Biobased films prepared from collagen solutions derived from un-tanned hides. *JALCA* **110**, 25-32, 2015.