Mechanism of Collagen Processed with Urea Determined by Thermal Degradation Analysis*

by

Keyong Tang,[a†] Weilin Li,[a] Jie Liu,[a] Cheng-Kung Liu,[b] and Hongbo Pan[a]

[a]*School of Materials Science and Engineering, Zhengzhou University,* Zhengzhou, Henan 450001, China

[b]*Eastern Regional Research Center, United States Department of Agriculture***,* Wyndmoor, PA 19038, USA

*Presented in part at the 30th International Congress of the IULTCS, Beijing, 2009.

Abstract

During the beamhouse process for nappa leather, pelts are usually limed with amino compounds such as urea, ethylenediamine, and triethanolamine. However, the interaction between amino compounds and collagen is not well known. In this work, collagen fibers were soaked in various concentrations of urea and the thermal degradation of collagen fibers were studied by the methods Horowitz-Metzger and Coats-Redfern. The mechanism of the reaction between urea and collagen fibers is discussed, wherein the thermal degradation activation energy first decreases and then increases. The lowest thermal degradation activation energy of urea processed collagen appears at 2-3 mol/L urea, suggesting that the stability of collagen is the poorest when the pelt is processed in the urea solution. At the urea concentration above 6 mol/L, the thermal degradation activation energy of the sample is similar to samples without urea processing and the higher concentrations does not have the same effect as lower concentrations of urea. The collagen fibers with a urea processing history were washed to remove the urea in them, and the samples were studied again for their thermal degradation behavior. The results indicated that the thermal degradation activation energy of the collagen fibers might recover to the unprocessed level. Therefore, it was suggested that the reaction between urea molecules and collagen fibers is reversible. Urea molecules might help to destroy some of the hydrogen bonds between collagen peptides in the urea solution. After the urea is washed out, the structure of the collagen will return to its original state, because the hydrogen bonds might be reconstructed.

Introduction

As an abundant natural polymer in animal skins and bones, collagen has found wide applications in many fields, such as leather, gelatin, glue, food, health products, medicine and the cosmetic industry because of its favorable triple helix structure and biochemical characters.[1] Leather is made from hides or skins through a complicated process, including soaking, degreasing, liming, reliming, deliming, bating, pickling, tanning, retanning, fatliquoring and finishing, in which liming is one of the most important ones in determining the feature and properties of resultant leathers.[2, 3] In liming, pelts are usually limed with amino compounds such as urea, ethylenediamine, and triethanolamine. However, the interaction between amino compounds and collagen is not well known.[3] Urea is a low-cost chemical and an important protein denaturant, which is frequently used in liming. So, the interaction mechanism of urea on collagen deserves a thorough study.

The influence of urea on shrinkage temperature, isometric tension, swelling behavior, tensile strength, and percentage extension of native rat tail tendon (RTT) were examined by *R Usha*.[5-6] They found that the lyotropic swelling increased the width of the fiber and was associated with the action of urea on the collagen fiber. The melting behavior and the swollen fascicles were clearly seen in scanning electron micrographs of 3 and 6M urea-treated RTT. The reduction in the dimensional stability of native RTT collagen fiber on treatment with urea demonstrated the role of secondary structure in the dimensional stabilization of collagen. In the DSC study of urea process on rat tail tendon (RTT) collagen fiber, the peak temperature and enthalpy changes decreased with increasing concentrations of urea, increasing chain length of alcohol and decreasing pH.[7] As to the effect of guanidinium salts on protein, lyotropic activity increased in the series formamide <urea <guanidinium ion, and in the guanidinium salts in the anion order of fluoride< sulphate< chloride< bromide< nitrate< iodide. Low activities of guanidinium fluoride and sulphate were attributable to counter-effects of the anions, which acted as structural stabilizers.[8] Urea might cause a decrease of venous tissue volume over the whole range of concentration with the exception that, beyond 1.0 osM, an increase appeared.[9] The renaturation rates of calfskin collagen were largely determined by the degree of undercooling" irrespective of the particular perturbant present, although perturbants with hydrocarbon structure deviated progressively from the linear trend found for formamide and urea. A direct relationship existed between lyotropic activity and perturbant hydrogen bonding to collagen peptide bonds.[10]

From the thermal behaviors of a material, we may indirectly know some structural information of the material. In our reported

†Corresponding author email: kytang@zzu.edu.cn ; kytangzzu@hotmail.com

Manuscript received May 24, 2020, accepted for publication June 30, 2020.

**Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U. S. Department of Agriculture. USDA is an equal opportunity provider and employer

works, the thermal degradation kinetics of collagen fibers tanned by different tanning agents and soaked in sweat were studied, as well as the interaction between collagen fibers and the tanning agents or sweat soaking was discussed from viewpoints of thermal degradation of collagen fibers.[11-15] It was found that chrome tanning might improve the thermal degradation stability obviously, while no significant changes were found for aldehyde tanning.

In this work, after being soaked in various concentrations of urea, the collagen fibers were washed to completely remove the urea. The thermal degradation kinetics of the samples before and after washing were studied by the thermal degradation activation energy of the samples using the methods of Horowitz-Metzger and Coats-Redfern. The action of urea on the aggregation structure of the collagen fibers and the interaction mechanism between urea and collagen fibers were considered.

Experimental

Materials and apparatus

Cattlehide collagen fibers (CCFs) were obtained from Sichuan University, China. Urea was analytically pure and purchased from Luoyang chemical reagent Co. Ltd, China. The thermal analysis system was from Netzsch Company, Germany. The constant temperature water bath oscillator, THZ-82, was made by Fuhua instrument Co. Ltd., Jiangsu, China.

Preparation of urea solutions

Various weights of urea were dissolved in 100 mL distilled water to yield urea solutions with the concentrations of 0.5 mol/L, 1.0 mol/L, 1.5 mol/L, 2.0 mol/L, 3.0 mol/L, 4.5 mol/L, 6.0 mol/L, 7.5 mol/L, and 9.0 mol/L, respectively.

Preparation of different CCFs

Urea-processed CCFs were soaked in various concentrations of urea for 7 days at room temperature. After being filtrated, the cakes were air-dried and put in a desiccator to constant weight, ready for subsequent thermal analysis.

Water-washed CCFs were soaked in 3.0 mol/L and 9.0 mol/L urea solutions for 7 days. The CCFs were then soaked in 20 times the weight of sample of distilled water for an hour, the samples were then filtrated and soaked in fresh distilled water again for a total of 7 cycles to dissolve out the urea. After the final wash, the samples were filtrated and the cakes were air-dried then put in a desiccator to reach constant weight, ready for subsequent thermal analysis.

TG/DTG analysis and data processing

For TG/DTG analysis, the samples were put in aluminium crucibles. Nitrogen was used as the protective gas, and the samples were heated at a heating rate of 10°C/min from room temperature to 650°C. The thermal analysis system gave the TG and DTG curves automatically. The thermal degradation activation energies of all the CCFs of urea processed and water washed were obtained with the help of the Horowitz-Metzger and Coats-Redfern methods.

Results and Discussion

Thermal degradation behaviors of urea processed collagen fibers

Thermal degradation curves

The TG and DTG curves of the CCFs processed by various concentrations of urea solutions are shown in Figure 1. It demonstrated that when the CCFs were heated, two obvious weight losses appeared. The first one appears before 100°C, which is the water molecules adsorbed by the samples. The other weight loss appeared at a

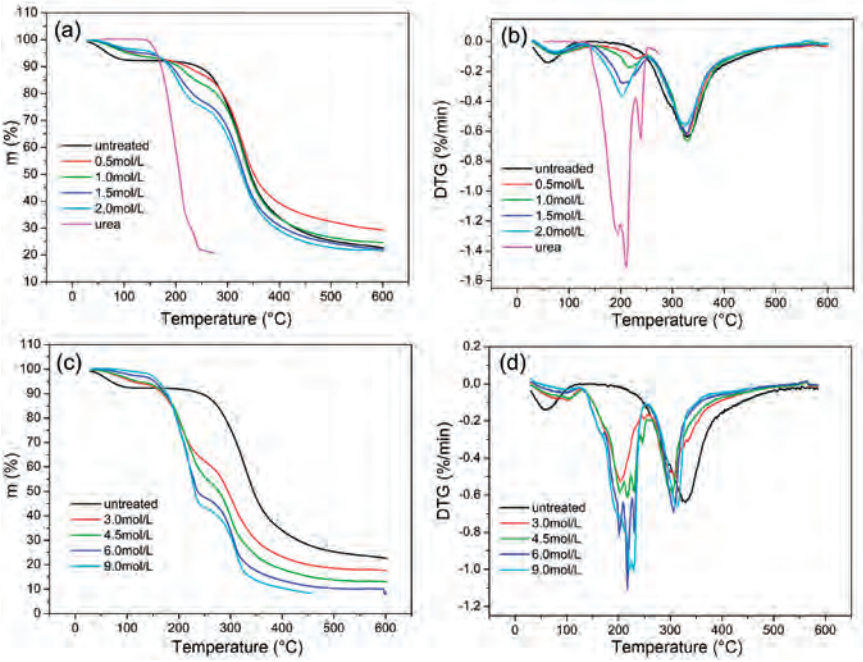

Figure 1. (a, c) TG and **(b, d)** DTG curves of CCFs processed in urea solutions with various concentrations (β=10 K/min)

temperature range from 250°C to 450°C, which is most likely due to the thermal decomposition of the CCFs. The decomposition of pure urea takes place in the temperature range from 130°C to 250°C. After CCFs are soaked in various concentrations of urea, the decomposition peak of urea should appear in the thermogravimetry curves of the samples. With an increase of urea concentration, the weight loss peak continued to grow, which suggested that the urea in the samples contributes to the whole thermal degradation curves. Regarding the weight loss peak of CCFs, it first decreased and then increased.

Thermal degradation activation energy and thermal degradation mechanism

Thermogravimetry (TG), differential scanning calorimetry (DSC) and differential thermal analysis (DTA) are usually used to determine the thermal kinetic parameters of polymers. Among them, thermogravimetry (TG) is the most widely used. Based on the TG data and curves, the thermal degradation activation energy of polymers might be calculated. Isothermal, non-isothermal and high-resolution analysis are the most popular methods to obtain the thermal degradation activation energy of polymers, based on the TG and DTG curves. There are some sub-methods in each one. In the present paper, the non-isothermal method, also known as the dynamic method, was chosen for the study of the thermal degradation behaviors of the samples with the weight loss by linearly heating. Based on the data in the TG curves, the methods of Coats-Redfern and Horowitz-Metzger were used to yield the thermal degradation activation energy (TDAE) of the various CCFs. The meaning of the symbols in this paper are as follows:

E or TDAE—Thermal degradation activation energy, kJ/mol;

A—Pre-exponential factor;

α—Conversion or reactive fracture at time t, %;

R—Universal gas constant, 8.314 J/mol K;

f(α)—Conversion in differential form;

G(α)—Conversion in integral form;

β—Heating rate, 10 K/min;

T_P—Temperature at which reaction rate is the greatest, K;

α_p—Conversion at which reaction rate is the greatest, %;

T_S—Corresponding temperature at $\alpha = \frac{1}{e}$;

T—Reaction temperature, thermodynamics temperature, K;

θ—Difference between T and T_S, $\theta = T - T_S$;

(1) Horowitz-Metzger method[7-8]

According to the theory of Horowitz-Metzger method, we have equation (1) as follows:

$$\ln G(\alpha) = \ln \frac{ART_S^2}{\beta E} - \frac{E}{RT_S} + \frac{E\theta}{RT_S^2} \quad (1)$$

The TDAE (or E here) of the sample might be calculated from the slopes of the plots of $\ln G(\alpha)$ versus θ [16-17].

There are thirty different forms of *G(α)* as shown in Table I.

Table I

Different integral forms of kinetics functions for *G(α)* [15-17]

No.	*G(α)*
1	α^2
2	$\alpha+(1-\alpha)\ln(1-\alpha)$
3	$(1-2/3\alpha)-(1-\alpha)^{2/3}$
4, 5	$[1-(1-\alpha)^{1/3}]^n$ (n = 2,1/2)
6	$[1-(1-\alpha)^{1/2}]^{1/2}$
7	$[(1+\alpha)^{1/3}-1]^2$
8	$[(1/(1+\alpha))^{1/3}-1]^2$
9	$-\ln(1-\alpha)$
10-16	$[-\ln(1-\alpha)]^n$ (n = 2/3, 1/2, 1/3, 4, 1/4, 2, 3)
17-22	$1-(1-\alpha)^n$ (n = 1/2, 3, 2, 4, 1/3, 1/4)
23-27	α^n (n = 1, 3/2, 1/2, 1/3, 1/4)
28	$(1-\alpha)^{-1}$
29	$(1-\alpha)^{-1}-1$
30	$(1-\alpha)^{-1/2}$

The original thermogravimetric data of the samples from the TG curves of the different samples in Figure 1 are shown in Table II.

Various functions in Table I were tried for the TG data in Table II. By fitting and comparing, the function best consistent with the data in Table II was chosen for subsequent study. $\ln G(\alpha)$ was plotted against θ, in which the slopes of the fitted lines were used to calculate the thermal degradation activation energy of various samples.

Table II

TG data for samples processed in different concentrations of urea solutions

Urea Concentration (mol/L)	$T_{\alpha=0.20}$ (K)	$T_{\alpha=0.25}$ (K)	$T_{\alpha=0.30}$ (K)	$T_{\alpha=0.35}$ (K)	$T_{\alpha=0.40}$ (K)	$T_{\alpha=0.45}$ (K)
0	562.6	574.4	584.4	593.2	601.2	609.2
0.5	560.0	577.4	587.5	596.3	604.6	613.4
1.0	551.0	569.9	582.3	590.6	599.5	607.2
1.5	505.2	542.2	565.0	578.2	587.8	596.4
2.0	471.2	481.0	492.0	509.2	537.2	556.2
3.0	492.0	524.2	554.2	570.3	582.2	591.8
4.5	472.7	481.4	490.3	499.7	509.9	528.0
6.0	468.7	475.6	483.0	489.2	494.3	501.9
9.0	468.2	475.2	482.1	488.2	493.8	498.9

In this study, $G(\alpha)=[-\ln(1-\alpha)]^4$ best fits the data, and the correction coefficients are reasonable for all the samples as shown in Table III.

Figure 2 was obtained by plotting $lnG(\alpha)$ against θ. The TDAE of the samples processed in various concentrations of urea was calculated based on the slopes of the lines in Figure 2, with the TDAE values in Figure 4.

(2) Coats-Redfern Method [16-17]

According to the theory of Coats-Redfern method, we have equation (2) as follows.

$$\ln [G(\alpha)/T^2] = \ln\left(\frac{AR}{\beta E}\right) - \frac{E}{RT} \quad (2)$$

If $\ln [G(\alpha)/T^2]$ is plotted against $1/T$ to yield straight lines, the thermal degradation activation energy of the different samples might be calculated from the slopes of the lines.[16-17]

Table III

Correlation coefficients of the linear fitting between $\ln G(\alpha)$ and θ by $G(\alpha)=[-\ln(1-\alpha)]^4$

Urea Concentration (mol/L)	0	0.5	1.0	1.5	2.0	3.0	4.5	6.0	9.0
Correlation Coefficient	0.9999	0.9973	0.9953	0.9782	0.9875	0.9639	0.9751	0.9970	0.9995

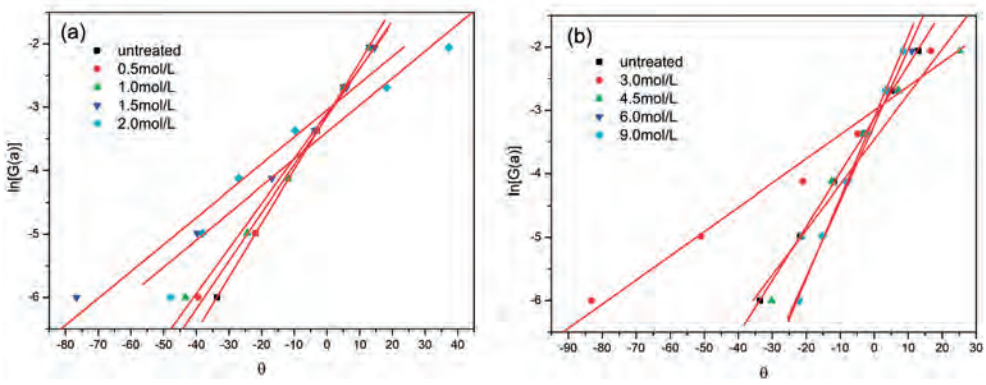

Figure 2. $lnG(\alpha)$ versus θ of samples processed in various concentrations of urea: **(a)** 0.5-2.0 mol/L; **(b)** 3.0-9.0 mol/L.

Table IV

TG data for samples processed in different concentrations of urea solutions

Urea concentration (mol/L)	$1/T_{\alpha=0.20}\times10^3$ (K^{-1})	$1/T_{\alpha=0.25}\times10^3$ (K^{-1})	$1/T_{\alpha=0.30}\times10^3$ (K^{-1})	$1/T_{\alpha=0.35}\times10^3$ (K^{-1})	$1/T_{\alpha=0.40}\times10^3$ (K^{-1})	$1/T_{\alpha=0.45}\times10^3$ (K^{-1})
0	1.777	1.741	1.711	1.686	1.663	1.641
0.5	1.786	1.732	1.702	1.677	1.654	1.630
1.0	1.815	1.755	1.717	1.693	1.668	1.647
1.5	1.979	1.844	1.770	1.729	1.701	1.677
2.0	2.122	2.079	2.032	1.964	1.861	1.798
3.0	2.032	1.907	1.804	1.753	1.717	1.689
4.5	2.115	2.077	2.039	2.001	1.961	1.894
6.0	2.133	2.102	2.070	2.044	2.023	1.992
9.0	2.136	2.104	2.074	2.048	2.025	2.004

The original TG data of different collagen fibers from the TG curves in Figure 1 are shown in Table IV.

Various functions in Table I were tried for the data in Table IV. By fitting and comparing, the function best consistent with the data in Table IV was chosen for the study. It was found that $G(\alpha)=[-\ln(1-\alpha)]^4$ is the most consistent with the data and with reasonable correction coefficients, as shown in Table V.

From Table V, we observed that the function of $G(\alpha)=[-\ln(1-\alpha)]^4$ presents a reasonable linear relation between $\ln[G(\alpha)/T^2]$ and $1/T$, so $G(\alpha)=[-\ln(1-\alpha)]^4$ was chosen for the study.

$\ln[G(\alpha)/T^2]$ was plotted with $1/T$ to yield Figure 3. The thermal degradation activation energy (TDAE) of the samples processed in different concentrations of urea was calculated with the slopes of the lines in Figure 3, with the results shown in Figure 4.

Table V

Correlation coefficients of the linear fitting between $\ln[G(\alpha)/T^2]$ and $1/T$ by $G(\alpha)=[-\ln(1-\alpha)]^4$

Urea Concentration (mol/L)	0	0.5	1.0	1.5	2.0	3.0	4.5	6.0	9.0
Correlation Coefficient	0.9998	0.9950	0.9914	0.9620	0.9677	0.9752	0.9798	0.9981	0.9998

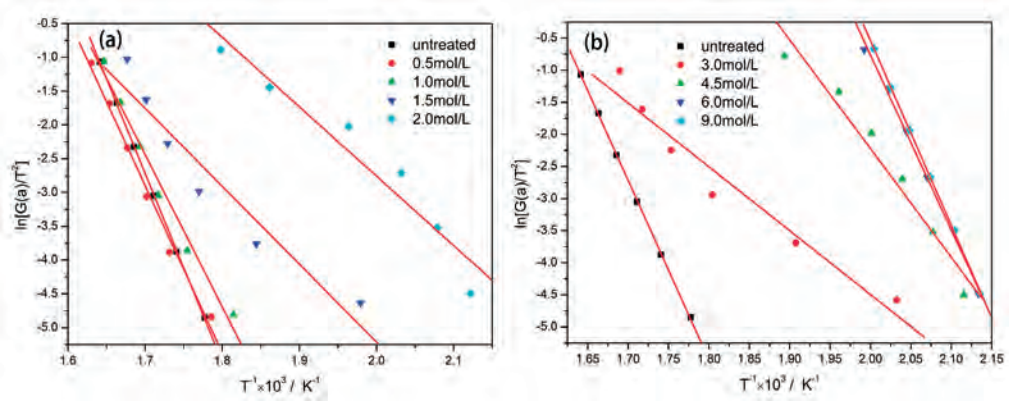

Figure 3. $\ln[G(\alpha)/T^2]$ versus $1/T$ of different samples.

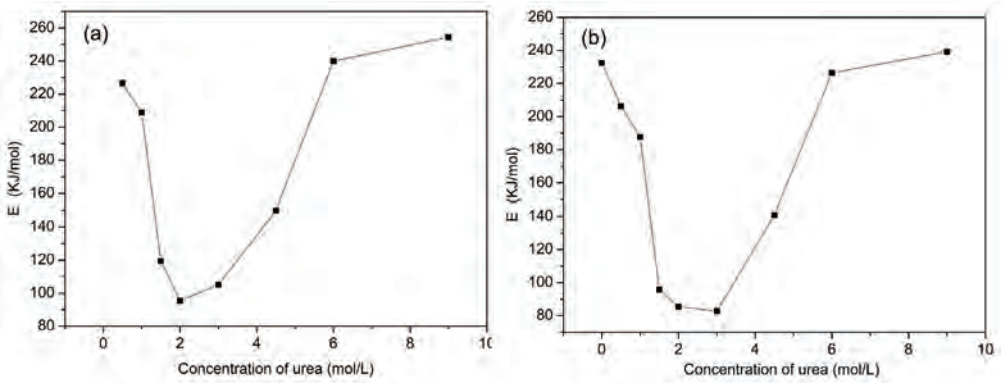

Figure 4. TDAE of different samples versus urea concentrations by the methods of **(a)** Horowitz-Metzger and **(b)** Coats-Redfern

Influence of urea concentration on the TDAE of collagen fibers

The relationship between the thermal degradation activation energies of CCFs processed in different concentrations of urea solutions are shown in Figure 4. In Figure 4, it is apparent that the changing trend in TDAE of CCFs is the same, no matter which method is used. The difference in the TDAE values by different methods is not obvious. With an increase in urea concentration, the TDAE of the CCFs firstly decreases and then increases. At the urea concentration ranged from 2.0 mol/L to 3.0 mol/L, the minimum value of the thermal degradation activation energy for the CCFs appeared. When the CCFs are soaked in a low concentration of urea solution, the hydrogen bonds inter- and intra- collagen peptides might be partly destroyed by the attacking of amino groups in the urea molecules, which will weaken the interaction between collagen peptides. So, the structure stability of the collagen will be decreased, resulting in poor heat resistance and lower thermal degradation activation energies for the urea processed collagen fibers. However, further increase of urea concentration from 3.0 mol/L to 9.0 mol/L, great damage might be caused for the CCFs by destroying the hydrogen bonds between adjacent collagen peptides. Some of the structure of CCFs might even be destroyed. Before the thermal degradation takes place, dry heat shrinkage will appear, causing the adjacent collagen peptides to get closer. In this case, the interaction between adjacent collagen peptide chains will be enhanced because of the mutual reaction between the reaction groups on the collagen chains such as carboxyl, hydroxyl, and amino, resulting in an increase in the thermal degradation activation energy of the CCFs (239.2 kJ/mol at 9.0 mol/L). Therefore, in liming, appropriate urea concentrations (lower than 1.0 mol/L) should be applied in order to get an excellent liming effect. In the reported works, the melting behavior and the swollen fascicles were clearly seen in scanning electron micrographs of 3 M and 6 M urea-treated RTT, and the reduction in the dimensional stability of native RTT collagen fiber on treatment with urea was demonstrated by the role of secondary structure in the dimensional stabilization of collagen.[6] Urea might cause a decrease of venous tissue volume over the whole range of concentration with the exception that, beyond 1.0 osM, an increase appeared.[9] The present work further proved the interaction of urea on the structure of collagen from viewpoint of thermal degradation activation energy.

Influence of water washing on the urea-processed CCFs

TG and DTG curves of urea-processed CCFs before and after water washing

Both TG and DTG curves of CCFs before and after water washing were shown in Figure 5 and Figure 6. Here, the urea concentrations were 3.0 mol/L and 9.0 mol/L, respectively. In Figure 5 and Figure 6, the TG and DTG curves of the CCFs processed with urea solutions are greatly different from those of the untreated ones. After being fully washed with water, the curves changed greatly again, which nearly

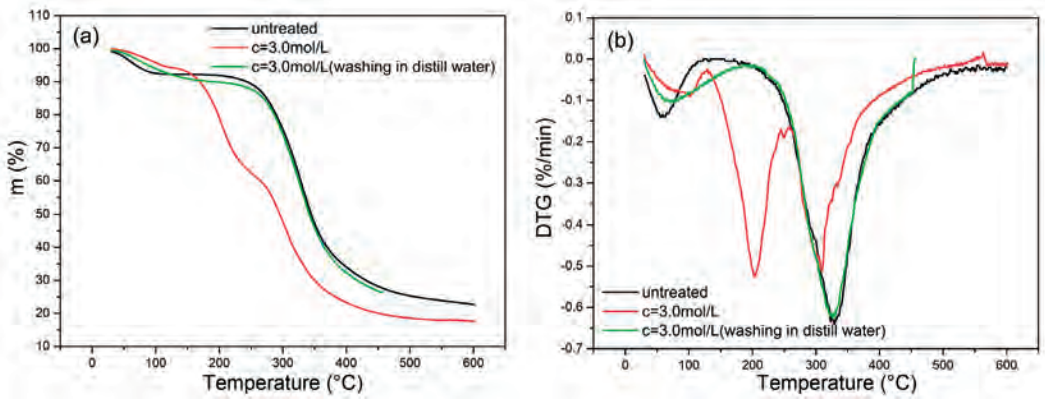

Figure 5. (a) TG and **(b)** DTG curves of urea-processed CCFs before and after water washing (c=3.0mol/L, β=10 K/min)

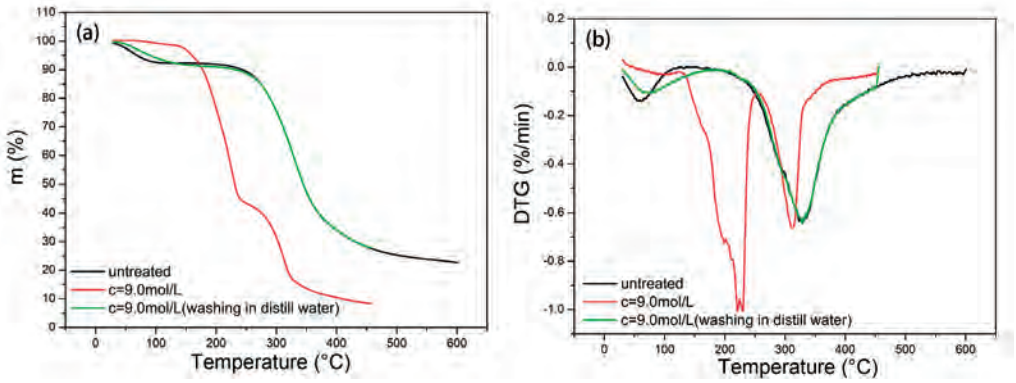

Figure 6. (a) TG and **(b)** DTG curves of urea-processed CCFs before and after water washing (c=9.0mol/L, β=10 K/min)

overlapped those of the untreated ones. Before urea processing, there are two weight losses in the TG and DTG curves of the CCFs. After the samples were processed in urea, a new weight loss profile was found at the temperature ranged from 130°C to 250°C, which should be due to the decomposition of urea in the samples. After being repeatedly washed with distilled water, the urea decomposition peak disappeared, which suggested that the urea in the samples was completely removed. It is interesting to note that the TG curves and DTG curves of the CCFs washed with distilled water nearly overlap those untreated with urea, which suggests the reversibility of the reaction between urea and collagen fibers. So, it could be concluded that no chemical reactions might take place here. In liming, the purpose to use urea is to open the collagen fiber bundles in order to help other chemicals such as protease and calcium hydroxide to be transferred into the hides and react with the collagen fibers. When the collagen fiber bundles are open, the urea is not needed anymore, and should be washed from the hides.

Thermal degradation activation energy and thermal degradation mechanism

(1) Horowitz-Metzger Method

The original thermogravimetric data in the TG curves for the urea-processed samples before and after water washing in Figure 5 and Figure 6 are shown in Table VI.

The functions in Table I were applied to the data in Table VI one by one. By fitting and comparing, the function that is best consistent with the data in Table VI was chosen for subsequent study. Here, $G(\alpha)=[-\ln(1-\alpha)]^4$ best fits the original data, with reasonable correction coefficients for all the CCFs as shown in Table VII. ln$G(\alpha)$ was plotted against θ to yield Figure 7.

Table VI

Thermogravimetric data for urea-processed and washed samples

Urea Concentration (mol/L)	$T_{\alpha=0.20}$ (K)	$T_{\alpha=0.25}$ (K)	$T_{\alpha=0.30}$ (K)	$T_{\alpha=0.35}$ (K)	$T_{\alpha=0.40}$ (K)	$T_{\alpha=0.45}$ (K)
0	562.6	574.4	584.4	593.2	601.2	609.2
3.0*	492.0	524.2	554.2	570.3	582.2	591.8
3.0**	559.3	571.1	581.1	590.0	598.1	606.2
9.0*	468.7	475.6	483.0	489.2	494.3	501.9
9.0**	562.9	574.2	584.1	593.1	601.1	609.2

*Before water washing, **After water washing

Table VII

Correlation coefficients of linear fitting between ln$G(\alpha)$ and θ by $G(\alpha)=[-\ln(1-\alpha)]^4$

Urea Concentration (mol/L)	0	3.0*	3.0**	9.0*	9.0**
Correlation Coefficient	0.9999	0.9639	0.9999	0.9795	0.9999

*Before water washing, **After water washing

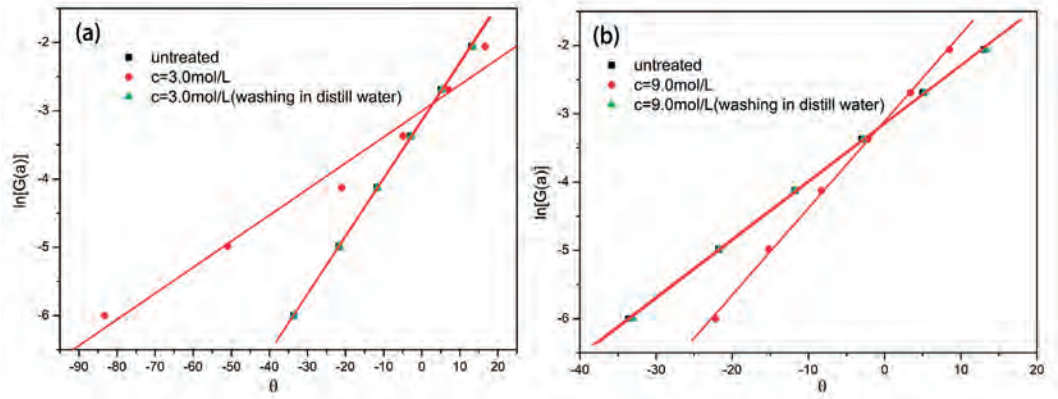

Figure 7. *lnG(a)* versus θ for different samples

Table VIII

TDAE of the various CCFs by Horowitz-Metzger method

Urea concentration (mol/L)	0	3.0*	3.0**	9.0*	9.0**
E(kJ/mol)	251.2	105.1	246.7	254.4	251.9

*Before water washing, **After water washing

With the slopes of the lines in Figure 7, the thermal degradation activation energy (TDAE) of the various CCFs was obtained as shown in Table VIII.

(2) Coats-Redfern method

The original thermogravimetric data in TG curves for the urea-processed samples before and after water washing in Figure 5 and Figure 6 is shown in Table IX.

The various functions in Table I were applied to the data in Table IX. By fitting and comparing, the one best consistent with the data in Table IX was chosen for subsequent study. In this study, the one of $G(\alpha) = [-\ln(1-\alpha)]^4$ was chosen with reasonable correction coefficients for all the CCFs, as shown in Table X. ln$G(\alpha)$ was plotted against θ to yield Figure 8.

Based on the slopes of the fitted straight lines in Figure 8, the thermal degradation activation energy (TDAE) was obtained with the results in Table XI.

As shown in Table VIII and Table XI, it was indicated that, if the CCFs are fully washed in distilled water after soaked in urea solutions, the thermal degradation activation energy of CCFs will return to the original level of CCFs without a urea processing history, no matter what urea concentration was used to process the samples. Soaking in 3.0 mol/L urea solution remarkably decreases the TDAE of CCFs, which might return to the original level with no

Table IX

Thermogravimetric data for urea-processed and washed samples

Urea Concentration (mol/L)	$1/T_{\alpha=0.20}\times10^3$ (K^{-1})	$1/T_{\alpha=0.25}\times10^3$ (K^{-1})	$1/T_{\alpha=0.30}\times10^3$ (K^{-1})	$1/T_{\alpha=0.35}\times10^3$ (K^{-1})	$1/T_{\alpha=0.40}\times10^3$ (K^{-1})	$1/T_{\alpha=0.45}\times10^3$ (K^{-1})
0	1.777	1.741	1.711	1.686	1.663	1.641
3.0*	2.032	1.907	1.804	1.753	1.717	1.689
3.0**	1.788	1.751	1.721	1.695	1.672	1.649
9.0*	2.136	2.104	2.074	2.048	2.025	2.004
9.0**	1.776	1.741	1.712	1.686	1.663	1.641

*Before water washing, **After water washing

Table X

Correlation coefficients of the linear fitting between $\ln[G(\alpha)/T^2]$ and 1/T by $G(\alpha) = [-\ln(1-\alpha)]^4$

Urea Concentration (mol/L)	0	3.0*	3.0**	9.0*	9.0**
Correlation Coefficient	0.9999	0.9639	0.9999	0.9995	0.9999

*Before water washing, **After water washing

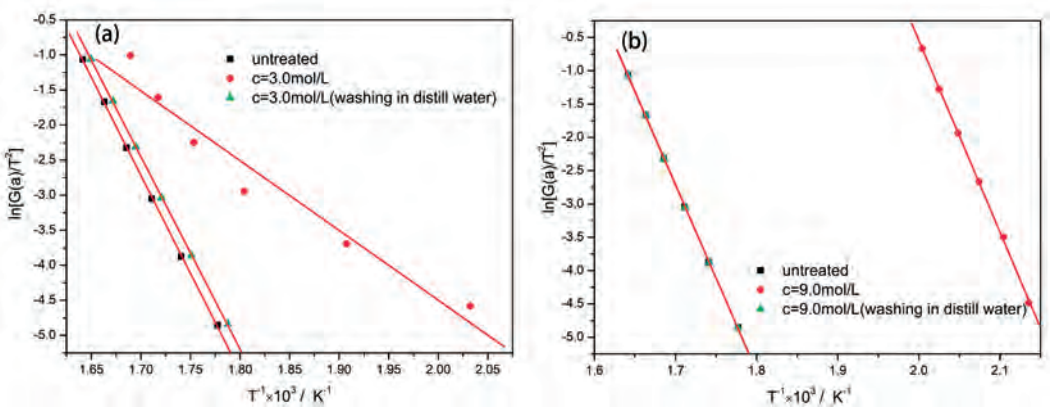

Figure 8. $\ln[G(a)/T^2]$ versus 1/T for various CCFs.

urea process if sufficient washing is conducted to completely remove the urea. From the viewpoint of thermal degradation activation energy, the reaction between urea and CCFs should be reversible, rather physical than chemical. Once the urea in the collagen fibers is completely removed by water washing, no indication of the urea process in the thermal degradation activation energy is detected. Therefore, the action of urea on CCFs should be a physical one, by forming new hydrogen bonds between urea molecules and collagen molecules instead of the original hydrogen bonds between adjacent collagen molecules. Therefore, the opening up of the collagen fiber bundles by urea process results in decreasing the thermal stability of the collagen fibers, with no chemical reaction.

Conclusions

Soaking in low concentrations of urea solution does not significantly change the thermal degradation activation energy of collagen fibers. Increasing the concentration of the urea solution, the thermal degradation activation energy will first decrease and then increase. When the urea concentration is in the range from 2 mol/L to 3 mol/L, the lowest thermal degradation activation energy for the collagen fibers appears. Further increasing the urea concentration to above 6.0 mol/L, however, urea soaking does not affect the thermal degradation activation energy, which is similar to the non-urea processed collagen fibers. The reaction between urea molecules and collagen fibers is temporary and reversible, from the viewpoint of the thermal degradation activation energy. When the urea in the collagen fibers is completely removed, the thermal degradation activation energy might return to the values without urea treatment history. The reaction of the urea process on CCFs should be a physical one, by forming new hydrogen bonds between urea molecules and collagen molecules instead of the original hydrogen bonds between adjacent collagen molecules.

Table XI

TDAE of various CCFs by Coats-Redfern method

Concentration of Urea (mol/L)	0	3.0*	3.0**	9.0*	9.0**
E(kJ/mol)	232.5	82.8	228.4	239.2	233.5

*Before water washing, **After water washing

Acknowledgments

The financial supports of this work from the National Natural Science Foundation Commission of China (No. 51673177, 51373097) and National Key R & D Program of (No.2017YFB0308500) are greatly appreciated.

References

1. Dai, H., Zhang, X.S., Zhang, Z.C., Tian, Z. and Zhang, H.; The separation of hydrolysate of enzyme-soluble pigskin by chromatography and sodium dodecyl sulphate-polyacrylamide gel electrophoresis. *Journal of the Society of Leather Technologists and Chemists* **87**(3), 110–112, 2003.
2. Tang, K. Y. Collagen Physics and Chemistry, Beijing, Science Press, 2012.
3. Liu, H., Yin, Z. H., Zhang, Q., Li, X. M., Tang, K. Y., Liu, J., Pei, Y., Zheng, X. J. 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**, 1–9, 2019.
4. Huang, J. B., Liu, J., Tang, K. Y., Yang, P. Y., Fan, X. L., Wang, F., Du, J. and Liu, C-K.; Effect of cyclic stress while being dried on the mechanical properties and thermostability of leathers. *JALCA* **113**(10), 318–325, 2018.
5. Abernethy, J. L.; Lyotropy-With particular application to collagen. *Journal of Chemical Education* **44**(6), 364-370, 1967.
6. Usha, R. and Ramasami, T.; Effect of hydrogen-bond-breaking reagent (urea) on the dimensional stability of rat tail tendon (RTT) collagen fiber. *Journal of Applied Polymer Science* **84**, 975–982, 2002.
7. Usha, R. and Ramasami, T.; Influence of hydrogen bond, hydrophobic and electrovalent salt linkages on the transition temperature, enthalpy and activation energy in rat tail tendon (RTT) collagen fibre. *Thermochimica Acta* **338**, 17–25, 1999.
8. Russell, A. E. and Cooper, D. R.; Effect of compounds of the urea–guanidinium class on renaturation and thermal stability of acid-soluble collagen. *Biochemical Journal* **127**, 855–863, 1972.
9. Winkler, R.; On the existence of a lyotropic and osmotic type of collagen swelling produced by representative electrolytes and nonelectrolytes. *Pflugers Archiv-European Journal of Physiology* **345**, 37–42, 1973.
10. Russell, A. E. and Cooper, D. R.; Structural and functional factors in the lyotropic activity of amides and alkyl-substituted amides on acid-soluble collagen, *Biochemistry* **9**(14), 2802–2806, 1970.
11. Tang, K. Y., Wang, F., Liu, J. and Wang, Q. J.; Preliminary studies on the thermal degradation kinetics of cattlehide collagen fibers. *JALCA* **99**(10), 401–408, 2004.
12. Yang, P. Y., He, X. C., Zhang, W. J., Qiao, Y. X., Wang, F. and Tang, K. Y.; Study on thermal degradation of cattlehide collagen fibers by simultaneous TG-MS-FTIR. *Journal of Thermal Analysis and Calorimetry* **127**(3), 2005–2012, 2017.
13. Tang, K. Y., Wang, F., Jia, P., Liu, J. and Wang, Q.; Thermal degradation kinetics of sweat soaked cattlehide collagen fibers. *JALCA* **102**(2), 52–61, 2007.
14. Liu, J., Brown, E. M., Liu, C-K. and Tang, K. Y.; Characterization and thermal properties of polygenipin-crosslinked hide powders. *JALCA* **113**(4): 96–104, 2018.
15. Liu, J., Brown, E. M., Uknalis, J., Liu, C-K., Luo, L. and Tang K. Y.; Thermal stability and degradation kinetics of vegetable-tanned collagen fiber with in-situ precipitated calcium carbonate. *JALCA* **113**(11): 358–370, 2018.
16. Hu, R. and Shi, Q.;, Thermal analysis dynamics. Science Press, Beijing, China, 2001.
17. Chen, J. and Li, C.; Thermal analysis and its application. Science Press, Beijing, China, 1985.

Lifelines

Anatolii Danylkovych (orcid.org/0000-0002-5707-0419) is a professor at Kyiv National University of Technologies and Design (Department of Biotechnology, Leather, and Fur) where he has been working since 1980. He published more than 470 scientific works, among them 9 monographs, 4 textbooks, and 11 tutorials, and has 72 Ukrainian patents. His current scientific interests include physicochemical fundamentals and modeling of the technological processes of leather and fur production. The main areas of scientific research are focused on the optimization and development of new resource-saving ecologically-oriented technologies for leather and fur production and mathematical modeling of technological processes.

Olena Korotych (orcid.org/0000-0002-0835-2305) is a visiting scientist at the University of Tennessee (Department of Biochemistry & Cellular and Molecular Biology) and at the University of Florida (Department of Chemical Engineering). She received her PhD in Colloid Chemistry from Ovcharenko Institute of Biocolloid Chemistry of the National Academy of Sciences of Ukraine in 2014 and MS degree in Physical Chemistry and BS degree in Chemistry from the Taras Shevchenko National University of Kyiv in 2009 and 2008, respectively. Her current research interests are focused on eco-friendly technologies and biotechnologies; nanoparticles and nanocomposites; biomembranes, membrane protein and supramolecular protein complexes; and synthetic and natural polymers.

Oksana Romaniuk (orcid.org/ 0000-0001-9774-9875) is an associate professor at Kyiv National University of Technologies and Design (Department of Heat Power Engineering, Resource-Saving, and Technogenic Safety) where she has been working since 2000. She published over 70 scientific works, including 1 textbook and has 9 Ukrainian patents. Her current scientific interests are aimed at the physicomechanical properties of leather and fur and on heat and mass transfer processes in leather materials and products.

Didem Berber graduated from Marmara University Atatürk Faculty of Education, Biology Department in 2001. She received MSc. degree from Pediatric Allergy-Immunology Department of Marmara University Medical Faculty in 2003 and PhD degree from Department of Biology, Faculty of Arts and Sciences of Marmara University in 2010. She has been studying as post doctorate researcher in Marmara University, Department of Biology, Faculty of Arts and Sciences from 2016 up to date. She contributed in international projects (COST and other bilateral collaboration projects) on bacterial quorum sensing and biofilm inhibition. Her research topics are hide microbiology, environmental microbiology, antimicrobial agents, fungi, quorum sensing, and biofilm formation.

İpek Türkmenoğlu graduated from Marmara University Atatürk Faculty of Education, Biology Department in 2012. She is continuing to the master program and she is studying as scholarship researcher with the support of TUBITAK on determination and utilization of species-specific allosteric inhibition zones in glycolytic enzymes in pharmaceutical design. Her research topics are hide microbiology, environmental microbiology, antimicrobial agents, quorum sensing, and biofilm formation.

Nüzhet Cenk Sesal graduated from Marmara University, Atatürk Faculty of Education, Biology Department. He has been working at Marmara University, Faculty of Arts and Sciences, Department of Biology since 2001. His research area is molecular microbiology. He has been working as a principal investigator, researcher and consultant in national and international projects, especially about molecular diversity, environmental microbiology, antimicrobial agents, quorum sensing, and biofilm formation.

Yi Zhang received his B.S. in chemistry from Fudan University in 2014 and his B.S.(Hons) from the University of Auckland in 2015. Since 2016, he has worked in Dr Sujay Prabakar's group at the Leather and Shoe Research Association of New Zealand, studying collagen structure using synchrotron SAXS.

Bradley W. Mansel is a Postdoctoral researcher at National Tsing Hua University in Taiwan. He received his PhD from Massey University in New Zealand in 2016. He is currently focused on researching the physical properties of biopolymers.

Jenna K. Buchanan has been working in Dr Sujay Prabakar's research group at the Leather and Shoe Research Association of New Zealand in Palmerston North since 2018. Her research is focused on developing novel crosslinking agents to replace or minimize chrome in leather processing. She received her BSc in chemistry and statistics in 2015 and her MSc in chemistry in 2017 from Massey University in Palmerston North.

Jiasheng Su see JALCA, 113 (12), 424, 2018

Zhuangdou Zhang see JALCA, 113 (12), 424, 2018

Geoff Holmes see JALCA, 110 (11), 379, 2015

Sujay Prabakar see JALCA, 111 (5), 200, 2016

Keyong Tang received his PhD degree in 1998 from Sichuan University, Chengdu, China. He is working for Zhengzhou University as a professor in the College of Materials Science and Engineering, Zhengzhou, China. His research interests include the leather structure and properties. He has published more than 100 papers, co-authored 4 books and edited 1 book in the field of leather chemistry and engineering.

Weilin Li received his Master's degree in 2005 from Henan University of Technology, China. He obtained his PhD degree in material physics and chemistry from Zhengzhou University, China, in 2020. His current research interests include support design for enzyme immobilization, transesterification with immobilized lipase for bio-diesel, collagen-based hydrogels with drug release applications, development of natural fibers from both plants and animals.

Jie Liu received his PhD degree in 2007 from Institute of Mechanics, Chinese Academy of Sciences, Beijing, China. He currently is an associate professor of School of Materials Science and Engineering at Zhengzhou University, Zhengzhou, China. From 2016 to 2017, he worked as a visiting scientist at ERRC, USDA in Cheng-Kung Liu's group. His current research interests focus on green composite materials based on natural polymers and their applications in packaging, leather making, biomedical and environmental fields.

Cheng-Kung Liu, Ph.D. see *JALCA* 94, 158, 1999

Hongbo Pan received his Master degree from Zhengzhou University, Zhengzhou, China, in Leather Chemistry and Engineering in 2010. His research interests mainly focus on effect of force on leather property and has contributed 5 publications in this field.