Calorimetric and Kinetic Analysis of Thermal Behaviors of Chrome-tanned Collagen Fibers Using Isoconversional and Multivariate Non-linear Regression Methods

by
Wentao Liu,[1,2,3]* Yuanzhi Zhang[1,2] and Guoying Li[1,2]
[1]*National Engineering Laboratory for Clean Technology of Leather Manufacture, Sichuan University,* Chengdu 610065, China
[2]*Key Laboratory of Leather Chemistry and Engineering (Sichuan University), Ministry of Education,* Chengdu 610065, China
[3]*Department of Chemical Engineering, University of Michigan,* Ann Arbor, MI 48109

Abstract

To further understand the thermal stability of collagen in hide and leather, the thermal denaturation behaviors of chrome-tanned collagen fibers were studied by non-isothermal differential scanning calorimetry (DSC) using isoconversional and multivariate non-linear regression (Multivar-NLR) methods. The differential (Friedman) and the integral (Ozawa-Flynn-Wall) isoconversional methods as well as the Multivar-NLR method showed that the denaturation (or shrinkage) process could be best described by a three-step model, in which a reversible reaction was followed by a rate-limited irreversible step. The simulation of thermal behaviors of collagen at different temperature conditions indicated that the denaturation kinetics could be approximated to a one-step irreversible reaction at low heating rates or temperatures. For the design of new chrome-free tanning systems, which can endow collagen with high enough thermostability, the decrease in the rate of the irreversible denaturation of collagen might be an important criterion as well, besides the increases in the denaturation (or shrinkage) temperature, enthalpy and effective activation energy.

Introduction

One of the most important changes in properties of animal hides (or skins) induced by tanning is the increase in the thermal stability of collagen fibers, the building blocks of which are collagen molecules having a unique triple helix structure.[1-4] The study of the thermal stability of collagen in tissues (e.g., hide and tendon) and leather is an active field and has attracted the attention of leather chemists and technologists for several decades,[1-17] not only because it can be used to evaluate the efficiency of the tanning process, but also because it can give the information regarding optimal temperature conditions for the safe storage and use of leather. As a protein, collagen is susceptible to heat that can breakdown the hydrogen bonds stabilizing the triple helix, resulting in the thermal denaturation of collagen, of which shrinkage is the macroscopic manifestation.[1,2,11,18,19] Therefore, the knowledge of the thermal behavior of collagen is of great importance to understand its structural stability. In leather industry, the thermal stability of collagen is conventionally measured by observing the shrinkage temperature (T_s) at which a specimen shrinks when heated in water at a certain heating rate,[1,2,13-15] or by determining the denaturation temperature (T_d) of collagen using a differential scanning calorimetry (DSC).[3,4,9-17] Although the thermal denaturation of many different types of collagen and leather has been investigated extensively,[1-25] little information is available on the thermal behavior of hide collagen fibers, especially for those after being tanned, from a kinetic point of view.

Studies on the kinetics of the thermal denaturation of collagen in tissues and leather may date back to the 1940s' pioneering works by Weir.[5,6] Based on the isothermal measurements of the rate of shrinkage of kangaroo tail tendon, the thermal shrinkage of tendon collagen fibers was found to be an irreversible rate process involving first-order reaction kinetics, and the measured T_s was an interval rather than a sharp point on the temperature scale.[5,6] Later, such an irreversible rate process was reported for the thermal denaturation of collagen in rat tail tendon by using DSC isothermally, and there was an rate dependence on temperature obeying the Arrhenius equation.[8] It is obvious that the thermal denaturation (or shrinkage) of collagen is a kinetic process and thus a single value of T_d (or T_s) could not provide an accurate estimate for the thermal stability of collagen. Moreover, both T_d and T_s are highly dependent on the heating rate which might be responsible for the difference between the values of T_d

*Corresponding author e-mails: scu.collagen@gmail.com; wentaol@umich.edu
Manuscript received June 15, 2017, accepted for publication September 2, 2017.

and T_s for the same collagen sample.[13-15] Recently, the kinetics of the thermal denaturation of collagen in sheep skin and three types of tanned leather was investigated using the method of modulated temperature DSC (MT-DSC), and the denaturation reaction was shown to be apparently irreversible under the specific applied conditions.[17] However, the current knowledge of the thermal behavior of hide collagen fibers is far from enough to understand how the denaturation (or shrinkage) occurs, how the denaturation process could be controlled by temperature, and how the denaturation behavior of tanned leather differs from that of untanned hide.

DSC has been the most widely used technique to study the thermal stability and denaturation kinetics of native collagen and leather.[3,4,7-25] For isothermal DSC, the experimentally accessible temperature interval for the absolutely reliable measurements is relatively limited, due to the large uncertainties of DSC signals at the temperatures where the rate of denaturation of collagen is too fast or too slow.[8,26] In contrast, non-isothermal DSC is more convenient to perform and has been highly recommended for the kinetic analysis of thermal degradation of polymers.[26-28] Nevertheless, it should be recognized that conventional DSC intrinsically do not allow the complete isolation of elementary reactions, resulting in its failure to detect the possible multi-step kinetics for a thermal process.[27,29,30] Thus the macroscopic kinetics of the thermal denaturation of collagen might contain information about simultaneously occurring multiple steps,[18,19,24,25] which has been treated approximately as a single step involving first-order reaction kinetics.[5,6,8,17] According to the proposal of the International Confederation for Thermal Analysis and Calorimetry (ICTAC) Kinetics Project, isoconversional and multi-heating rate methods were particularly successful in correctly describing the multi-step kinetics and could be used to explore the kinetics and mechanisms of complex processes.[31] However, to the best of our knowledge, there is no published literature regarding the application of such methods to the kinetic analysis of the thermal denaturation of collagen in untanned hide and tanned leather.

Chrome tanning will be still the most popular tannage for a long time due to the excellent properties of chrome-tanned leather, although various chrome-free tanning systems have been developed to solve the environmental problem regarding the discharge of chromium.[32] In this work, therefore, the thermal denaturation behaviors of chrome-tanned hide collagen fibers were studied by non-isothermal DSC using isoconversional and multivariate non-linear regression (Multivar-NLR) methods, which were highlighted by the ICTAC Kinetics Project.[31] These techniques were applied in combination to evaluate the mechanism and kinetic parameters of the denaturation process, and the denaturation behaviors of chrome-tanned collagen fibers under different temperature conditions were simulated. The fundamental data are expected to further understand the thermal stability of hide collagen fibers and chrome-tanned leather, as well as to inspire the design of chrome-free tanning agents which can endow leather with the desired thermostability.

Experimental

Materials

Untanned bovine hide collagen fibers (white hide powder) were obtained from Chinese Academy of Forestry. Chromium sulfate hexahydrate (analytical reagent, 50% solution) was purchased from Tianjin SHENTAI Chemical Industry Co., Ltd. of China. All other reagents including sodium chloride, sulphuric acid and sodium bicarbonate were of analytical grade and supplied by Sichuan University.

Preparation of Chrome-tanned Collagen Fibers

Chrome-tanned collagen fibers were prepared according to conventional chrome-tanning procedures with some modifications. 400 mg of white hide powder samples were soaked overnight in a conical flask containing 30 mL of distilled water and 2.4 g of sodium chloride. The pH of the above suspension was adjusted to 3.0 using dilute sulphuric acid and then 200 μL of 50% chromium sulfate hexahydrate was added dropwise with continuous stirring. After shaking for 2 hours at 20°C, the suspension was slowly basified to pH 4.0 with 6% sodium bicarbonate solution, followed by stirring for another 6 hours at 35°C. The suspension was filtered and the resultant chrome-tanned samples were washed thoroughly with distilled water to remove salts and unreacted chromium. The chrome-tanned collagen fibers were lyophilized by a freeze-dryer (FreeZone 6 Liter, Labconco, USA) and the Cr_2O_3 content of the lyophilized samples was determined to be 2.76% by using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES, Optima 2100DV, PerkinElmer, USA).

Differential Scanning Calorimetry (DSC)

Prior to DSC measurements, both the untanned and chrome-tanned collagen fibers were conditioned in a controlled atmosphere (temperature 20 ± 2°C, relative humidity 65 ± 5%) over a saturated sodium chloride solution for more than two weeks. DSC measurements were performed by using DSC 200PC (Netzsch-Gerätebau GmbH, Germany) as previously described with some modifications.[18] The heat and temperature scales, as well as corrections regarding the thermal resistance and the transfer function were calibrated according to the manufacturer's instructions with indium standard. All of the untanned and chrome-tanned collagen fiber samples (3.0 ± 0.1 mg) were sealed in 100 μL aluminum pans and an empty pan was used as the reference. DSC thermograms were recorded by heating the samples from 10°C to 120°C at heating rates of 2, 5 and 10°C/min, respectively. The temperature at the peak of a DSC trace was used as the apparent denaturation temperature (T_d) of

collagen samples, and the dependences of T_d and the enthalpy of the denaturation (ΔH) on heating rate (b) were studied. All measurements were performed for at least thrice until good reproducibility was achieved and the DSC data were evaluated using Proteus® from Netzsch-Gerätebau GmbH.

Kinetic Analysis

The kinetic analysis of the non-isothermal DSC data was performed according to the general algorithm suggested by Budrugeac,[28] being in accordance with the proposal of the ICTAC Kinetics Project.[31] To begin with, the isoconversional methods such as their popular representatives, i.e., the method of Friedman[33] and that of Ozawa, Flynn and Wall (OFW),[34,35] were applied to detect whether the thermal denaturation of the untanned and chrome-tanned collagen fibers could be described by single or multi-step kinetics. Next, the method of multivariate non-linear regression (Multivar-NLR) was used to evaluate various kinetic models and their corresponding kinetic parameters.

Isoconversional Analysis

The DSC curves for the untanned and chrome-tanned collagen fibers at 2, 5 and 10°C/min were subjected to the isoconversional kinetic analysis. By using isoconversional methods, the dependence of the effective activation energy (E) on the degree of conversion (α) (a ratio of the heat released between the start of a reaction and the actual time to the total heat), can be estimated without need of any information of the kinetic model.[24] It should be emphasized that a significant variation of E with α unambiguously indicates a multi-step process and the shape of E_α (the value of E corresponding to a given α) dependence could provide important insights into the mechanism of the process.[28-30] The differential isoconversional method according to Friedman[33] is based on the following equation:

$$\ln \beta \frac{\mathrm{d}\alpha}{\mathrm{d}T} = \ln Af(\alpha) - \frac{E}{RT} \quad (1)$$

where $f(\alpha)$ is the differential form of the reaction model, A is the Arrhenius pre-exponential factor, R is the gas constant, and T is the absolute temperature. For a constant α, the plot of $\ln\beta(\mathrm{d}\alpha/\mathrm{d}T)$ *vs.* $1/T$ for the DSC curves at 2, 5 and 10°C/min, should be a straight line whose slope could be used for calculating the value of E_α. In addition, the integral isoconversional method according to Ozawa, Flynn and Wall (OFW)[34,35] is based on the following equation:

$$\ln \beta = \ln \frac{AE}{Rg(\alpha)} - 5.331 - 1.052\frac{E}{RT} \quad (2)$$

where $g(\alpha)$ is the integral form of the reaction model. Similarly, for a constant α, the plot of $\ln\beta$ *vs.* $1/T$ for the DSC curves at 2, 5 and 10°C/min, should be a straight line whose slope allows for calculating the value of E_α.

Multivar-NLR Analysis

The DSC curves for the chrome-tanned collagen fibers at 2, 5 and 10°C/min were subjected to the Multivar-NLR kinetic analysis using the software package Netzsch Thermokinetics 3 as previously described.[19] In this work, the following kinetic models were evaluated: the three-step Lumry-Eyring model[36] (defined as model 't:r,f') containing reversible steps and an irreversible step, which are all of nth-order reaction (Fn: $f(\alpha) = (1-\alpha)^n$) type

$$N \underset{k_2}{\overset{k_1}{\rightleftharpoons}} U \xrightarrow{k_3} D \quad (3)$$

where N, U and D stand for the native state, the partially unfolded state and the denatured state of collagen, respectively, and k_1, k_2 and k_3 are rate constants for the steps. And the one-step irreversible model, defined as model 'F1' or 'Fn', respectively, in terms of the reaction type is first-order reaction (F1: $f(\alpha) = (1-\alpha)$) or Fn

$$N \xrightarrow{k_{app}} D \quad (4)$$

where k_{app} is the apparent rate constant for the single step. During the Multivar-NLR analysis, the experimental DSC data at 2, 5 and 10°C/min were brought together and fitted to the models 't:r,f', 'Fn' and 'F1'. The relevant differential equations of the reaction were solved numerically and the kinetic parameters (e.g., E, A and the reaction order n) were iteratively optimized. The fit quality was evaluated by the minimum sum of least squares (LSQ), the mean value of deviations (MD) and the correlation coefficient (Corr. coeff.). The evaluation of fit-quality for the models was performed by F-test via the experimental F-value (F_{exp}) and critical F-value at a confidence level of 0.95 [$F_{crit}(0.95)$].

Simulation of Thermal Behaviors of Chrome-tanned Collagen Fibers

The changes in the fraction of the states (N, U and D) during the thermal denaturation of the chrome-tanned collagen fibers were simulated at different heating rates, i.e., 0.001, 0.01, 1, 2, 5, 10, 100, 500 and 1000°C/min, and at different temperatures, i.e., 75, 76, 77, 78, 79, 80, 83, 85, 88, 89, 90, 92, 95 and 100°C, based on the best of evaluated models and the corresponding kinetic parameters.

Results and Discussion

Effects of Heating Rate and Chrome-tanning on the Thermal Denaturation of Collagen Fibers

Figure 1 and Table I show the DSC data for the thermal denaturation of the untanned and chrome-tanned collagen fibers at different heating rates. Although the DSC curves displayed only one endothermal peak, there were obvious

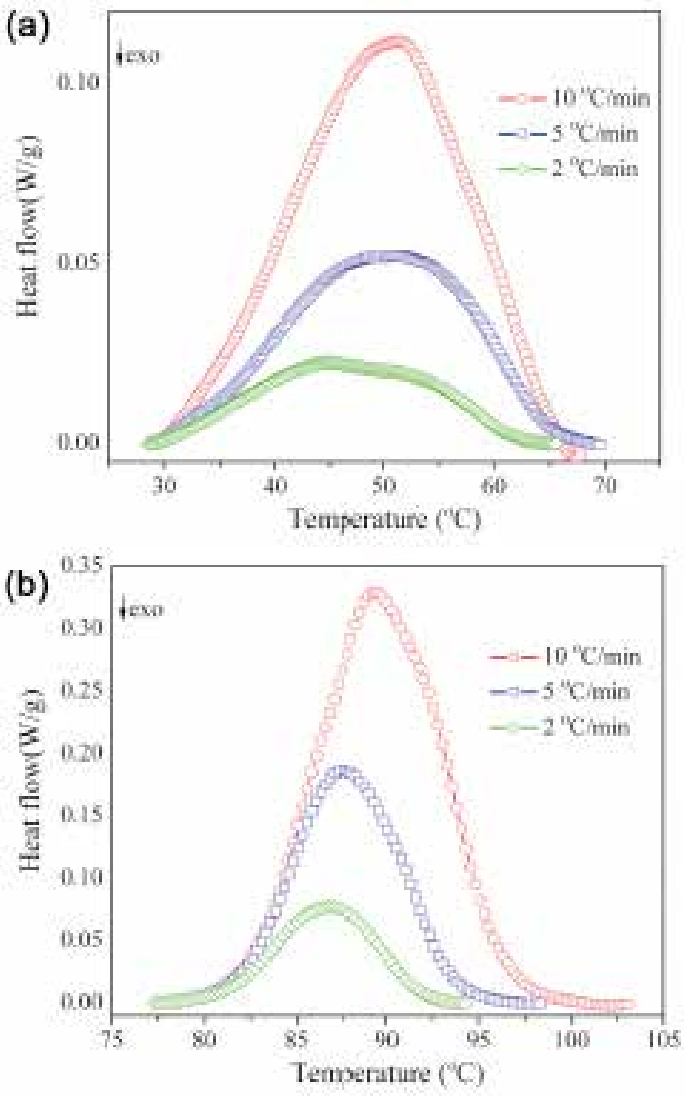

Figure 1. DSC curves for the thermal denaturation of the (a) untanned and (b) chrome-tanned collagen fibers at different heating rates.

differences in both the width and intensity of the peaks. As a whole, the peaks for the untanned collagen fibers were wider but weaker than those for the chrome-tanned samples. The T_d values were significantly dependent on heating rate, indicating that the denaturation (or shrinkage) process was to some extent kinetically controlled.[18,19] Whereas there was no significant difference regarding the ΔH values at different heating rates, being consistent with other studies on the denaturation of collagen.[8,18,19] Moreover, at the same heating rates, the T_d and ΔH values for the chrome-tanned collagen fibers were much higher than those for the untanned samples, indicating an increase in collagen stability endowed by the chrome-tanning. However, the only use of T_d and ΔH are not adequate to evaluate the thermal stability of collagen as mentioned earlier.

Isoconversional Kinetic Analysis

Figure 2(a) and 2(b) show the Friedman and OFW plots for the thermal denaturation of the chrome-tanned collagen fibers, according to Eq. (1) and Eq. (2), respectively. As shown in Figure 2, the straight lines fitting the data are not parallel, which is an indication that the calculated activation energies at various degrees of conversion are nearly different. Besides, the corresponding plots for the thermal denaturation of the untanned collagen fibers displayed similar profiles (data not shown). Figure 2(c) shows the E_α dependences for the thermal denaturation of the untanned and chrome-tanned collagen fibers, calculated for different degrees of conversion from 0.02 to 0.98 in terms of Eq. (1) and Eq. (2), respectively. On the whole, the E_α dependences are almost decreasing and approximately have the concave and convex shapes at lower (about 0.02-0.6) and higher (about 0.6-0.98) degrees of conversion, respectively. It should be recognized that the shapes of the E_α dependences could be associated with the kinetics and mechanisms of thermal processes, i.e., a decreasing and concave E_α dependence indicates

Table I
Values of T_d and ΔH for the thermal denaturation of the untanned and chrome-tanned collagen fibers at different heating rates.[a]

Heating rate (°C/ min)	T_d (°C)		ΔH (J/g)	
	Untanned	Chrome-tanned	Untanned	Chrome-tanned
2	46.6 ± 1.5	87.0 ± 0.3	12.28 ± 0.09	16.04 ± 0.15
5	52.3 ± 0.6	88.3 ± 0.2	12.68 ± 0.06	16.49 ± 0.04
10	55.6 ± 0.9	91.5 ± 0.5	12.27 ± 0.18	16.30 ± 0.12

[a]Values are means ± standard deviations (n = 3). The student's t-test was employed to evaluate statistical differences.

the process with reversible stage, and a decreasing and convex E_α dependence indicates the process with a change of limiting stage.[28-30] Luckily, such a characteristic shape of the E_α dependences in Figure 2(c) was previously established for a three-step model process,[29] in which a reversible reaction was followed by an irreversible one, just as shown in Eq. (3). Therefore, the kinetics of the thermal denaturation of the untanned and chrome-tanned collagen fibers seemed to obey a complex three-step reaction rather than a simple one-step process.

Since the isoconversional methods according to Friedman and OFW have their own characteristics, the synchronous use of the two methods would be beneficial for a more reliable isoconversional kinetic analysis.[18,19] For the chrome-tanned samples, the E_α values evaluated by Friedman were lower than those by OFW at the same degree of conversion, whereas it was not the case for the untanned ones. The differences in the E_α values calculated by the Friedman and OFW methods might be due to the fact that the former was very sensitive to experimental noises while the latter could introduce systematic errors.[18,19] Anyway, for the same isoconversional method and a given degree of conversion, the E_α values of the chrome-tanned samples were higher than those of the untanned ones during most of the denaturation process, indicating an enhanced collagen stability induced by the chrome-tanning. For Eq. (3), the E_α values are limited by the sum of the enthalpy (ΔH^*) of the reversible reaction ($N \leftrightarrow U$) and the activation energy of the irreversible reaction ($U \rightarrow D$) at low conversions (i.e., $\alpha \rightarrow 0$), and by the activation energy of the irreversible reaction at high conversions (i.e., $\alpha \rightarrow 1$).[29] As shown in Figure 2(c), for the chrome-tanned sample, $E_{\alpha \rightarrow 0}$ is about 800-950 kJ/mol, and $E_{\alpha \rightarrow 1}$ is about 230-320 kJ/mol, thus the calculated value of ΔH^* is about 480-720 kJ/mol. Although the isoconversional analysis was powerful to uncover the complex kinetics of the thermal denaturation of collagen, we also performed the Multivar-NLR analysis, which could be more universal than the isoconversional analysis,[37] to further explore the denaturation mechanism of the chrome-tanned collagen fibers.

Multivar-NLR Kinetic Analysis

Figure 3 shows the fits of the experimental DSC curves for the thermal denaturation of the chrome-tanned collagen fibers based on the kinetic models F1, Fn and t:r,f by Multivar-NLR. As shown in Figure 3, the fitting with model F1 leads to the worst fit quality, and the fit of model Fn is just a bit better than that of model F1; model t:r,f (Eq. (3)) indicates the best fit quality among the three models. Table II shows the evaluation of fit quality of the kinetic models by F-test and statistical analysis. It is clear from Table II that model t:r,f (Eq. (3)) is unambiguously the best model for the thermal denaturation of the chrome-tanned collagen fibers, conforming to the results of the isoconversional analysis. Table II also shows the kinetic parameters for the above

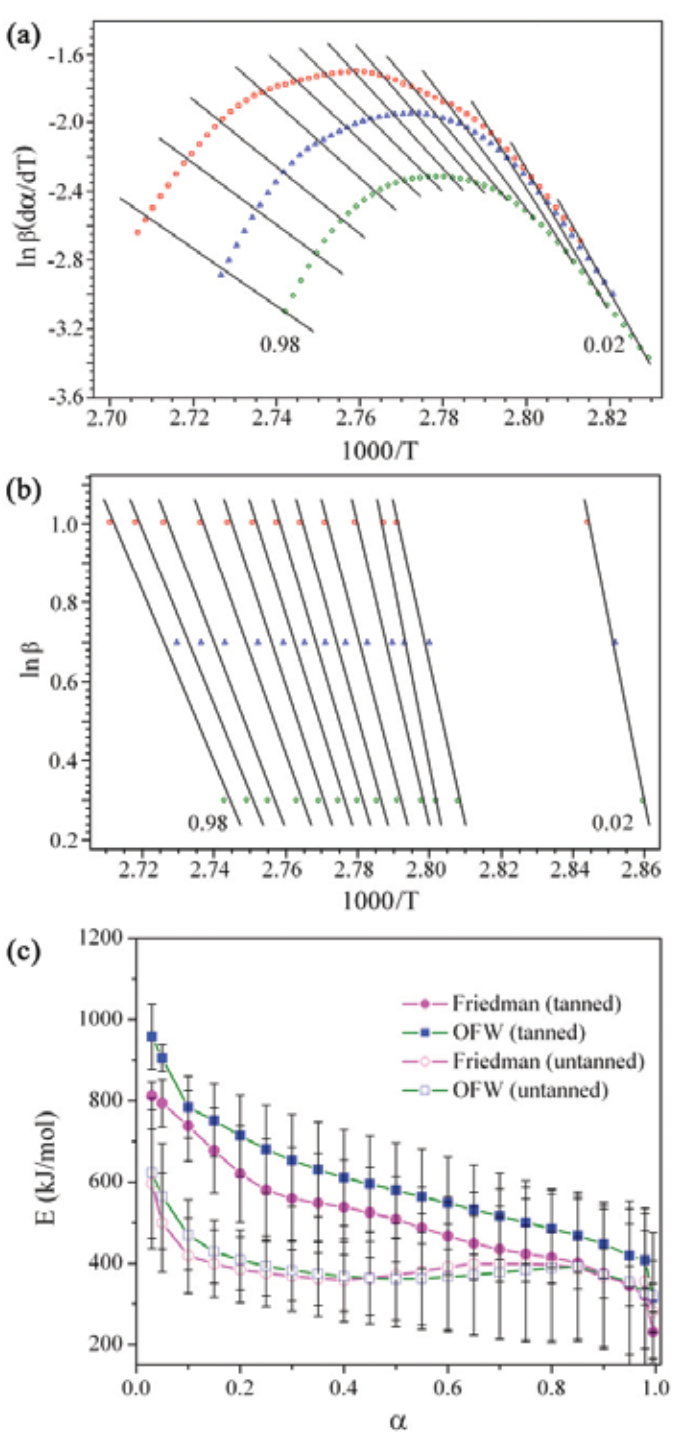

Figure 2. The (a) Friedman plots and (b) OFW plots for the thermal denaturation of the chrome-tanned collagen fibers according to each isoconversional method, where 0.02 and 0.98 are the degrees of reaction for the first and the last isoconversional line, respectively. The green, blue and red data points were calculated from the DSC curves at 2, 5 and 10°C/min, respectively. (c) Dependences of the apparent activation energy on the degree of conversion for the thermal denaturation of the untanned and chrome-tanned collagen fibers according to the method of Friedman and OFW, respectively.

evaluated models. For Eq. (3), in terms of $\Delta H^* = E_1 - E_2$,[29] the calculated value of ΔH^* for the chrome-tanned sample was about 520 kJ/mol, which fell within the range of the ΔH^* values (480-720 kJ/mol) estimated by the isoconversional methods. Combining the results of both the isoconversional and Multivar-NLR methods, it could be concluded that model t:r,f (Eq. (3)) is the most probable mechanism to describe the thermal denaturation of the chrome-tanned collagen fibers.

Simulation of Thermal Behaviors of Chrome-tanned Collagen Fibers

Figures 4 and 5 show the results of simulation for the thermal denaturation of the chrome-tanned collagen fibers at different temperature conditions using model t:r,f and its kinetic parameters. Figure 4 shows the dependences of the fraction of states (*N*, *U*, and *D*) on temperature at different heating rates. At low heating rates (0.001 and 0.01°C/min), the fractions of *U* were very low compared with those of *N* and *D*, so the denaturation process looked like the irreversible process $N \rightarrow D$ and could be approximately described by a one-step model as Eq. (4). At medium heating rates (1, 2, 5 and 10°C/min) and even 100°C/min, there were significant amounts of *U* together with *N* and *D* during the denaturation processes, appearing just as the three-step model (Eq. (3))[38]. At high heating rates (500 and 1000°C/min), most of *N* transformed quickly into *U* followed by the conversion from *U* to *D*, thus the denaturation process might be approximately described by a two-step consecutive process $N \rightarrow U \rightarrow D$ as that of bovine collagen in solution.[18] Furthermore, it can be inferred from Figure 4 that the conventionally measured T_d or T_s at 1~10°C/min will always be much higher than the intrinsic denaturation temperature which should be measured at $\beta \rightarrow 0$. As shown in Figure 4(a), $T_{d\,(\beta=0.001°C/min)}$ is about 76°C, which is approximately 11°C lower than $T_{d\,(\beta=2°C/min)}$ (about 87°C) (see Table 1).

Figure 5 shows the time dependence of the fraction of states (*N*, *U*, and *D*) at various temperatures, where the changes of states are in different forms compared with those shown in Figure 4. At low temperatures (e.g., 80°C), the presence of *N* and *D* were similar to the situation at low heating rates and could also be approximately described by a one-step model as Eq. (4). Moreover, at lower temperatures (75~79°C), the lifetime values for different degrees of thermal denaturation of the chrome-tanned collagen fibers are listed in Table III, indicating that the denaturation would occur at a temperature lower than that conventionally measured. At higher temperatures (83~100°C), the fractions of *N* and *U* changed within an extremely short of time at the start of the denaturation process, respectively. Although the simulations were based on theoretical calculations, the data shown in Figures 4 and 5 will be helpful to understand the thermal behaviors of the chrome-tanned collagen fibers at different temperature conditions.

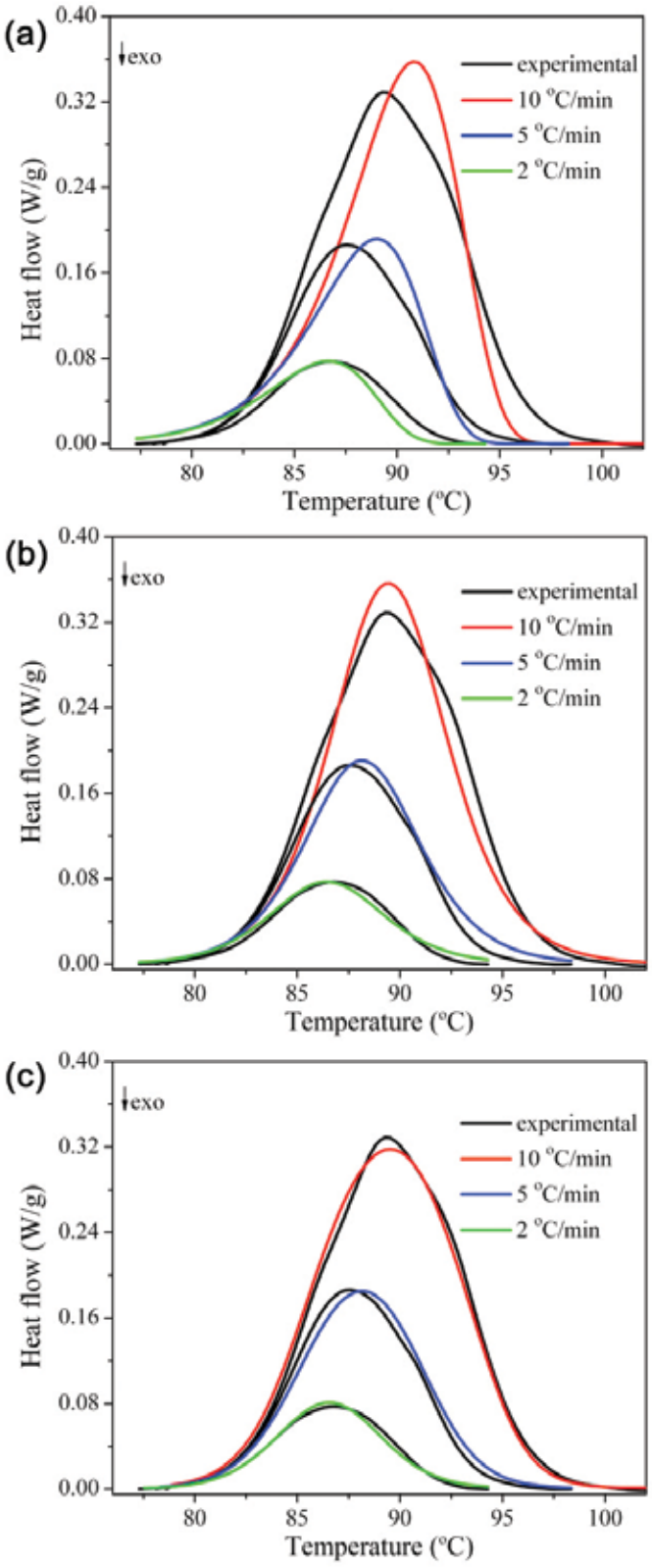

Figure 3. Multivar-NLR kinetic analysis of the thermal denaturation of the chrome-tanned collagen fibers, showing the fits of the experimental DSC data with (a) model F1, (b) model Fn and (c) model t:r,f, respectively.

According to a theoretical analysis of the Lumry-Eyring model (Eq. (3)),[38] the thermal denaturation of the chrome-tanned collagen fibers could be strongly rate limited and the rate-determining step was determined by the unfolding equilibrium constant K ($K = k_1/k_2$) and the relative values of k_2 and k_3. When $k_2 >> k_3$ and $K << 1$, the rate-determining step was $U \rightarrow D$ and the denaturation process can be approximately described by $N \rightarrow D$ (Eq. (4)), whose apparent rate constant was Kk_3; when $k_3 >> k_2$, the rate-determining step was $N \rightarrow U$ and the denaturation process can also be approximately described by $N \rightarrow D$ (Eq. (4)), but its rate constant was k_1. Based on the partly convex E_α dependence shown in Figure 2(c), it can be inferred that there is a change in the rate-determining step from $U \rightarrow D$ to $N \rightarrow U$, at higher (about 0.6-0.98) degrees of conversion for the thermal denaturation of the chrome-tanned collagen fibers. Therefore, the delay of the occurrence of the irreversible step $U \rightarrow D$ might be essential for an enhanced thermal stability induced by a tanning method such as chrome tanning.

Table II

F-test and statistics on the fit quality of the kinetic models as well as the kinetic parameters in the models evaluated for the thermal denaturation of the chrome-tanned collagen fibers using Multivar-NLR.

Model	F-test		Statistics			Kinetic parameters[a]		
	F_{exp}	F_{crit} (0.95)	LSQ	MD	Corr. coeff.	Ig A_1(s^{-1})	E_1(kJ.mol)	n_1
t:r.f	1.00	1.27	15.8249	0.2516	0.9971	126.490	865.327	1.882
Fn	2.57	1.26	42.0748	0.4102	0.9882	81.813	575.282	1.983
F1	9.18	1.26	151.3143	0.7780	0.9591	58.525	416.154	1

[a]Kinetic parameters for the other two steps of model t:r.f included IgA^2(s^{-1}), E_2(kJ/mol), n_2, lgA_3(s^{-1}), E_3(kJ/mol) and n_3, the values of which are 51.458, 347.337, 0.697, 22.690, 165.833 and 0.752, respectively.

Table III

The lifetime values for different degrees of thermal denaturation of the chrome-tanned collagen fibers at various temperatures.

Temperature (°C)	Lifetime for different degrees of thermal denaturation (min)					
	5%	10%	20%	30%	40%	50%
75	109.204	230.863	520.477	894.105	1394.232	2097.663
76	53.148	112.418	253.511	435.534	679.182	1021.87
77	25.961	54.973	124.034	213.124	332.371	500.086
78	12.716	26.988	60.957	104.769	163.404	245.86
79	6.235	13.295	30.094	51.752	80.726	121.46

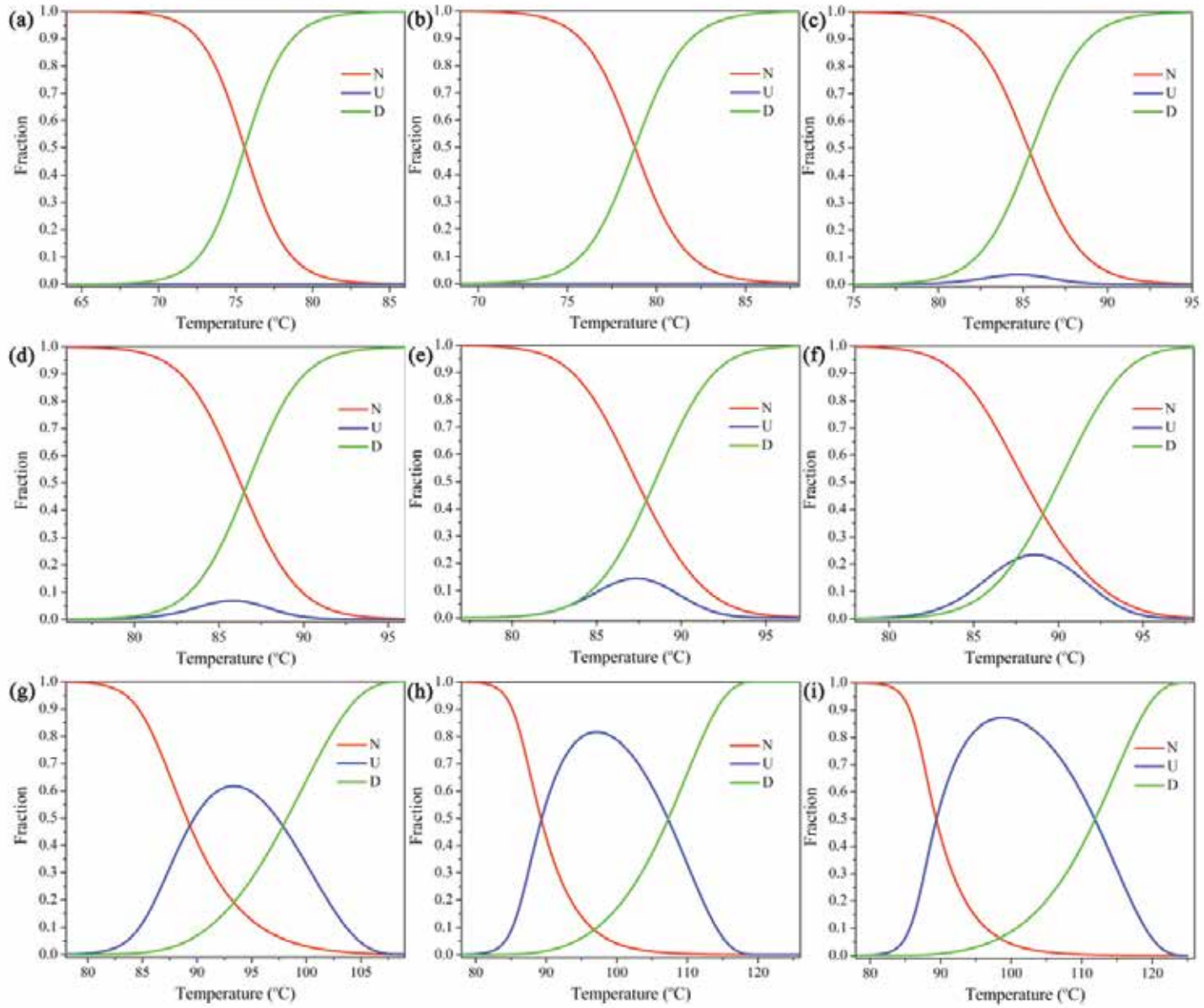

Figure 4. Fraction of states (*N*, *U* and *D*) of collagen *vs.* temperature profiles for the thermal denaturation of the chrome-tanned collagen fibers simulated at various heating rates of (a) 0.001°C/min, (b) 0.01°C/min, (c) 1°C/min, (d) 2°C/min, (e) 5°C/min, (f) 10°C/min, (g) 100°C/min, (h) 500°C/min and (i) 1000°C/min, respectively. *N*, *U* and *D* stand for the native state, the partially unfolded state and the denatured state of collagen, respectively.

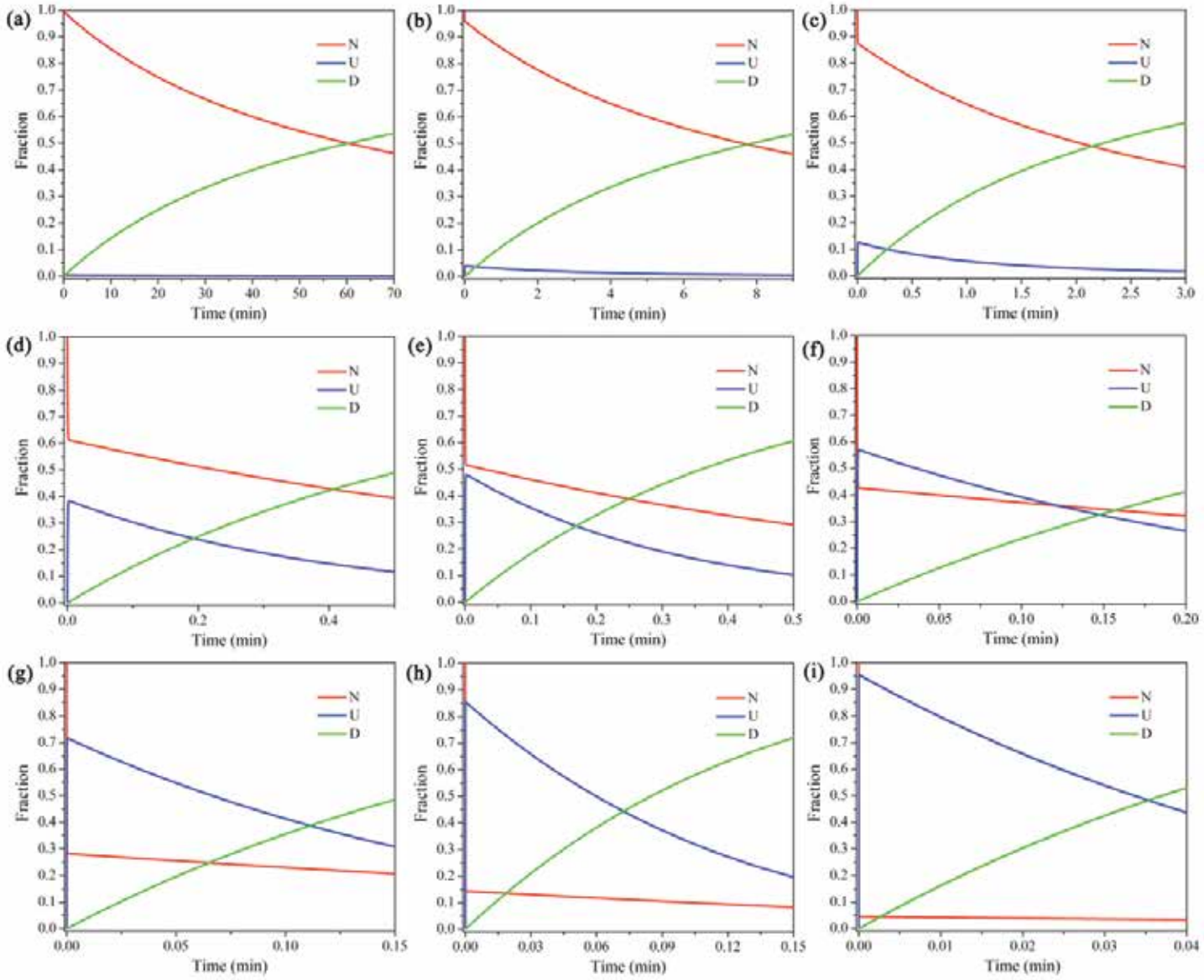

Figure 5. Fraction of states (*N*, *U* and *D*) of collagen *vs.* time profiles for the thermal denaturation of the chrome-tanned collagen fibers simulated at various temperatures of (a) 80°C, (b) 83°C, (c) 85°C, (d) 88°C, (e) 89°C, (f) 90°C, (g) 92°C, (h) 95°C and (i) 100°C, respectively. *N*, *U* and *D* stand for the native state, the partially unfolded state and the denatured state of collagen, respectively.

Conclusions

The thermal denaturation (or shrinkage) of the chrome-tanned collagen fibers is a complex kinetic process, which can be best described by a three-step model $N \leftrightarrow U \rightarrow D$ rather than a simple one-step irreversible process. At low heating rates or temperatures, the denaturation kinetics can be approximated to a one-step reaction $N \rightarrow D$. For a typical DSC trace, there is a change in the rate-determining step from $U \rightarrow D$ to $N \rightarrow U$. Besides the increase in the values of T_d (or T_s), enthalpy and apparent activation energy, hindering the irreversible denaturation of collagen might also be very important when a new tanning system is developed to replace the conventional chrome tanning.

Acknowledgements

The authors gratefully acknowledge the financial supports from the National Natural Science Foundation of China (No. 21606156), the National High Technology Research and Development Program of China (No. 2013AA065701), and the Scholarship Council of Sichuan University.

References

1. Covington, A. D.; Modern tanning chemistry. *Chem. Soc. Rev.* **26**, 111-126, 1997.
2. Covington, A. D., Lampard, G. S., Hancock, R. A., and Ioannidis I. A.; Studies on the origin of hydrothermal stability: a new theory of tanning. *JALCA* **93**, 107-120, 1998.
3. Bosch, T., Manich, A. M., Carilla, J., Cot, J., Marsal, A., Kellert, H. J., Germann, H. P.; Collagen thermal transitions in chrome leather - thermogravimetry and differential scanning calorimetry. *JALCA* **97**, 441-450, 2002.
4. Carşote, C., Badea, E., Miu, L., and Gatta, D. G..; Study of the effect of tannins and animal species on the thermal stability of vegetable leather by differential scanning calorimetry. *J. Therm. Anal. Calorim.* **124**, 1255-1266, 2016.
5. Weir, C. E.; Effect of temperature on the volume of leather and collagen in water. *J. Res. Nat. Bur. Stand.* **41**, 279-285, 1948.
6. Weir, C. E.; Rate of shrinkage of tendon collagen: heat, entropy, and free energy of activation of the shrinkage of untreated tendon; Effect of acid, salt, pickle, and tannage on the activation of tendon collagen. *J. Res. Nat. Bur. Stand.* **42**, 17-32, 1949.
7. Komanowsky, M.; Thermodynamic analysis of thermal denaturation of hide and leather. *JALCA* **87**, 52-66, 1992.
8. Miles, C. A., Burjanadze, T. V., and Bailey, A. J.; The kinetics of the thermal denaturation of collagen in unrestrained rat tail tendon determined by differential scanning calorimetry. *J. Mol. Biol.* 245, 437-446, 1995.
9. Ding, K., Taylor, M. M., and Brown, E. M.; Effect of genipin on the thermal stability of hide powder. *JALCA* **101**, 362-367, 2006.
10. Brown, E. M., Latona, R. J., Taylor, M. M., and Gehring, A. G.; Powdered hide model for vegetable tanning. *JALCA* **109**, 8-13, 2014.
11. Chahine, C.; Changes in hydrothermal stability of leather and parchment with deterioration: a DSC study. *Thermochim. Acta* **365**, 101-110, 2000.
12. Brown, E., Latona, R., and Taylor, M.; Powdered hide for research on tanning mechanisms. *JALCA* **105**, 116-120, 2010.
13. Krishnamoorthy, G., Sadulla, S., Sehgal, P. K., and Mandal, A. B.; Green chemistry approaches to leather tanning process for making chrome-free leather by unnatural amino acids. *J. Hazard Mater.* **215**, 173-182, 2012.
14. Wang, Y. J., Guo, J., Chen, H., and Shan, Z. H.; Influence of containing moisture on hydrothermal stability of modified collagen thermal characteristics analysis by DSC. *J. Therm. Anal. Calorim.* **99**, 295-300, 2010.
15. Onem, E., Yorgancioglu, A., Karavana, H. A., and Yilmaz, O.; Comparison of different tanning agents on the stabilization of collagen via differential scanning calorimetry. *J. Therm. Anal. Calorim.* DOI: 10.1007/s10973-017-6175-x, 2017.
16. Schroepfer, M., and Meyer, M.; DSC investigation of bovine hide collagen at varying degrees of crosslinking and humidities. *Int. J. Biol. Macromol.* DOI: 10.1016/j.ijbiomac.2017.04.124, 2017.
17. Edmonds, R. L.; Application of modulated temperature differential scanning calorimetry (MTDSC) on leather. *J. Soc. Leather Technol. Chem.* **100**, 283-288, 2016.
18. Liu, W. T., and Li, G. Y.; Non-isothermal Kinetic Analysis of the Thermal Denaturation of Type I Collagen in Solution Using Isoconversional and Multivariate Non-linear Regression Methods. *Polym. Degrad. Stab.* **95**, 2233-2240, 2010.
19. Liu, W. T., Tian, Z. H., Li, C. H., and Li, G. Y.; Thermal denaturation of fish collagen in solution: A calorimetric and kinetic analysis. *Thermochim. Acta* **581**, 32-40, 2014.
20. Engel, J., and Bächinger, H. P.; Cooperative equilibrium transitions coupled with a slow annealing step explain the sharpness and hysteresis of collagen folding. *Matrix Biol.* **19**, 235-244, 2000.
21. Miles, C. A., and Bailey, A. J.; Response to Engel and Bachinger's critique of collagen denaturation as a rate process. *Matrix Biol.* **20**, 263-265, 2001.
22. Bächinger, H. P., and Engel, J.; Thermodynamic vs. kinetic stability of collagen triple helices. *Matrix Biol.* **20**, 267-269, 2001.
23. Leikina, E., Mertts, M. V., Kuznetsova, N., and Leikin, S.; Type I collagen is thermally unstable at body temperature. *Proc. Natl. Acad. Sci. USA* **99**, 1314-1318, 2002.
24. Vyazovkin, S., Vincent, L., and Sbirrazzuoli, N.; Thermal denaturation of collagen analyzed by isoconversional method. *Macromol. Biosci.* **7**, 1181-1186, 2007.
25. Budrugeac, P., and Cucos, A.; Application of Kissinger, isoconversional and multivariate non-linear regression methods for evaluation of the mechanism and kinetic parameters of phase transitions of type I collagen. *Thermochim. Acta* **565**, 241-252, 2013.
26. Flammersheim, H. J., and Opfermann, J. R.; Kinetic evaluation of DSC curves for reacting systems with variable stoichiometric compositions. *Thermochim. Acta* **388**, 389-400, 2002.
27. Vyazovkin, S., and Wight, C. A.; Isothermal and non-isothermal kinetics of thermally stimulated reactions of solids. *Int. Rev. Phys. Chem.* **17**, 407-433, 1998.
28. Budrugeac, P.; Some methodological problems concerning the kinetic analysis of non-isothermal data for thermal and thermo-oxidative degradation of polymers and polymeric materials. *Polym. Degrad. Stab.* **89**, 265-273, 2005.
29. Vyazovkin, S., and Linert, W.; Kinetic analysis of reversible thermal decomposition of solids. *Int. J. Chem. Kinet.* **27**, 73-84, 1995.
30. Vyazovkin, S., and Sbirrazzuoli, N.; Isoconversional kinetic analysis of thermally stimulated processes in polymers. *Macromol. Rapid Commun.* **27**, 1515-1532, 2006.

31. Brown, M. E., Maciejewski, M., Vyazovkin, S., Nomen, R., Sempere, J., Burnham, A., Opfermann, J., Strey, R., Anderson, H. L., Kemmler, A., Keuleers, R., Janssens, J., Desseyn, H. O., Li, C. R., Tang, T. B., Roduit, B., Malek, J., and Mitsuhashi, T.; Computational aspects of kinetic analysis. Part A: The ICTAC kinetics project-data, methods and results. *Thermochim. Acta* **355**, 125-143, 2000.
32. Cai, S. W., Zeng, Y. H., Zhang, W. H., Wang, Y. N., and Shi, B.; Inverse chrome tanning technology based on wet white tanned by Al-Zr complex tanning agent. *JALCA* **110**, 114-120, 2015.
33. Friedman, H. L.; Kinetics of thermal degradation of char-forming plastic from thermogravimetry. Application to a phenolic plastic. *J. Polym. Sci. C* **6**, 183-195, 1964.
34. Ozawa, T.; A new method of analyzing thermogravimetric data. *Bull. Chem. Soc. Jpn.* **38**, 1881-1886, 1965.
35. Flynn, J. H., and Wall, L. A.; General treatment of the thermogravimetry of polymers. *J. Res. Natl. Bur. Stand.* **70A**, 487-523, 1966.
36. Lumry, R., Eyring, H.; Conformation changes of proteins. *J. Phys. Chem.* **58**, 110-120, 1954.
37. Opfermann, J. R., Kaisersberger, E., and Flammersheim, H. J.; Model-free analysis of thermoanalytical data-advantages and limitations. *Thermochim. Acta* **391**, 119-127, 2002.
38. Sanchez-Ruiz, J. M.; Theoretical analysis of Lumry–Eyring models in differential scanning calorimetry. *Biophys. J.* **61**, 921-935, 1992.