Influence of containing moisture on hydrothermal stability of modified collagen thermal characteristics analysis by DSC

Ya-juan Wang · Jun Guo · Hui Chen · Zhi-hua Shan

Abstract The thermal stability of sheepskin collagen cross-linked with chrome sulfate and mimosa (MI)–oxazolidine (OZ), respectively, had been researched in this experiment. All samples' shrinkage temperatures (T_s) are determined by a special T_s-testing-apparatus and denaturation temperatures (T_d) are determined by the differential scanning calorimetry. The relations between the modified collagens containing moisture and their hydrothermal stability, T_s or T_d, were studied. The results show that the cross linking agents can enhance the thermal stability of modified collagen whose T_s are 109.8 and 110.6 °C for collagen treated with chrome and MI–OZ, respectively. When the samples contain 25–71.9% moisture for chrome leather and 20–71.1% for leather treated with MI–OZ, the hydrothermal stability will decrease with the increase of moisture. It was found that the difference between T_s and T_d of collagen modified by chrome is more obvious than that of collagen modified with MI–OZ. And when the moisture of chrome leather exceeds 55%, T_d cannot express thermal stability of modified collagen as a substitute for T_s, and the moisture is 40% for leather tanned with MI–OZ.

Keywords Collagen · Containing moisture · Hydrothermal stability · Shrinkage temperature · Denaturation temperature · Differential scanning calorimetry

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Introduction

As a family of structural protein of extra-cellular matrix, collagens represent about one-third of the total protein content of vertebrate animal. In body, these collagens form insoluble networks of fibrous bundles, which can act as scaffolding, providing shape and form [1, 2]. And naïve collagen, which has a rigid chain structure due to hydrogen bonds among its triple helix, is tough and highly resistant to heat, however, the structure of natural collagen is liable to de-stabilization by collagenase at physiological pH, temperature, and ionic strength, which limits the usage of raw collagen. Its dimensional will change under heat, that is, to say the collagen undergoes length reduction at a characteristic temperature to a lever of one-third of its original dimension [3–6].

Therefore, chemists pay a large attention to increase the stability of collagen by using some modified agents. And a tanning process is understood to convert raw hide/skin collagen into leather in leather-making industry, which aims at bringing about thermal and enzymatic stability to the hide matrix by introducing additional cross-links among the native collagen fibers that can be stabilized by the specific arrangement of polypeptide chains with hydrogen bond [7–9]. When the firm links are formed among the collagen, the hydrothermal stability of the modified collagen will be enhanced evidently, which was thought as the change from raw collagen to modified collagen or leather. This can be measured by observing the point temperature at which a specimen shrinks, when the specimen is immersed in water, heated at a rate of 2 °C/min. This is conventionally measure shrinkage temperature (T_s) of modified collagen [10, 11]. Improvement of these procedures was accomplished by development of the analytical techniques, providing information about the

physical and chemical properties of a material by using samples of 1–5 mg. Among these techniques, the thermal analysis methods (thermal gravity analysis (TGA), differential thermal analysis (DTA), differential scanning calorimetry (DSC)) were applied to characterize some samples. DSC curves recorded in static air adosphere can be considered a material "fingerprint," which can give some hints on the degradation degree of the samples. These statements could be explained by above mentioned biphasic amorphous crystalline structure of collagen-based materials, according to which the softening of the crystalline part is hindered by rigidity of the amorphous matrix. The tanning process, by inducing cross-linking, stabilizes the crystalline region [12–16]. The thermal stability of modified collagen can be consulted by the onset temperature in the DSC curve, which is thought of denaturation temperature (T_d) of the sample [17]. That is, to say, the active parameters of the change of collagen in phase can be determined by the DSC, which makes the problem easier [13, 18].

As the matter of fact, the hydrothermal stability is the expression of the collagen's heat-resistant ability in wet phase. The stability of most material has a close relationship with its containing moisture. However, the research about the relation between the two variables is not clear. And it is confused that the relationship between T_s and T_d of the same sample with the same moisture. Therefore, the purpose of this article is to confirm the difference and relation between the stability and containing moisture of modified collagen. In the leather-making industry, the chrome compound is the most useful tanning agent. And with the development of chrome-free technology in recent years, the combing use of two tanning agents is becoming more important. The collagen modified with plant polyphenol mimosa (MI) and oxazolidine (OZ) has a better effect according to the published reports. Therefore, basic chrome sulphate, MI and OZ have been chose in this experiment. And by testing the T_s and T_d of leather with the same moisture, the influence of the containing moisture of leather on its thermal stability can be ascertained.

Materials and methods

Main material and instrument

Raw collagen was obtained from sheep skin. MI used was LR grade, and came from MingKai Corporation in Zhe-Jiang. OZ used was AR grade, and come from DOW Chemical Company in USA. Chrome sulphate was AR grade, from Kelong Chemical Factory in Chengdu.

Vibrator at constant temperature comes from Fuhua Instrument Limited Company in Jintan. SW-1 Shrinkage Temperature Instrument comes from Fur making Factory in ChangChun. Differential scanning calorimeter comes from PERKIN-Elmyer.

Preparation of sample treated by MI–OZ

Twenty grams raw collagen was weighed and 60 g water with 10% sodium chlorinate was prepared in a 500 mL conical flask. The quality of all the materials was accounted with the raw collagen. Adjust the pH to 5.0, and 15% MI was added into the conical flask divided into two times. It was librated for 10 h at 25 °C, and then the pH was adjusted to 3.8 with formic acid gradually. After 12 h, the modified collagen was washed 20 min with water. Eighty percent water was added and the pH of the solution was adjusted to 6.5. At the same time, 3% OZ was added. The solution was heated to 45 °C 1-h later and vibrated for another 4 h. Twelve hours later, the modified collagen was washed 10 min and then was dried at 50 °C in an incubator for 8 h. And the gained leather contains 20% moisture.

Preparation of sample treated by chrome sulphate

Twenty gram raw collagen was weighed and 60 g water with 10% sodium chlorinate was prepared in a 500 mL conical flask. The quality of all the materials was accounted with the collagen. Adjust the pH to 3.0, and 10% chrome sulphate was added into the conical flask. It was librated for 10 h at 30 °C, and then the pH was adjusted to 4.0 with sodium bicarbonate. Keeping the conical flask for 12 h at 45 °C, then the modified collagen in the flask was washed and was dried at 50 °C in an incubator for 8 h. And the leather sample contains 25% moisture.

Determination of the T_s

Shrinkage temperature was the most immediate factor of hydrothermal stability of leather. The modified collagen tanned with basic chrome sulphate was divided into seven pieces, and every piece was weighed out. Then, wet every piece of sample to make sure that they contain different moisture, hermetically sealed in a container. After 48 h, weighed out every piece of sample, then test their T_s in a hermetic condition. The sample tanned with MI–OZ was treated with the same process.

DSC analysis

The aim of DSC is to determinate the thermal change of samples, the peak of DSC curve is the direct reflection to the phase change, and the onset temperature of the first sharp peak is regarded as the T_d of sample. T_d is the result of the behavior of heated sample, so the thermal stability of leather also can be induced from T_d by DSC method.

Each sample (1–5 mg) with different moisture was analyzed by DSC in which the indium was the standard material [19]. Each leather sample was heated from 25 to 200 °C at a heating rate of 5, 2, 3 K/min, respectively. The onset point of every DSC curve was the phase changing temperature of the sample, which is the T_d of leather tanned with different tanning agent. The extrapolated onset temperatures of the endothermic peaks recorded during DSC analysis of samples were compared with the T_s values of the same sample with the same moisture.

Results and discussion

The T_s and T_d of leather tanned with chrome

T_s and T_d of the modified collagen tanned with chrome sulphate containing different moisture were listed in Table 1.

According to the data in Table 1, Fig. 1 can be got whose x-axis is the moisture value and y-axis is the corresponding temperature. From this figure, it can be found that the relationship between the hydrothermal stability of collagen tanned with chrome sulphate and its containing moisture is very obvious. By comparing the T_s and T_d of the same moisture leather, it can be found that there is an obvious difference between the two temperatures. The curve is very smooth at begin, which means that the containing moisture has little influence on the T_s of chrome leather when the moisture is low. That is, to say, when the moisture less than 55%, the hydrothermal stability has small relation with the moisture, and the T_d is higher than T_s. However, when the containing moisture quantum exceeds 55%, the T_d will decrease sharply with the increase of the moisture, T_s will steady all the process. As the increase of moisture, T_d is even lower than that of the raw skin, which is 62.2 °C [20]. This is different from the traditional change of shrinkage temperature obviously. All of these proved that the onset point in the DSC curve is not the T_d of the leather in the high moisture containing.

Table 1 T_s/T_d of chrome modified collagen containing moisture

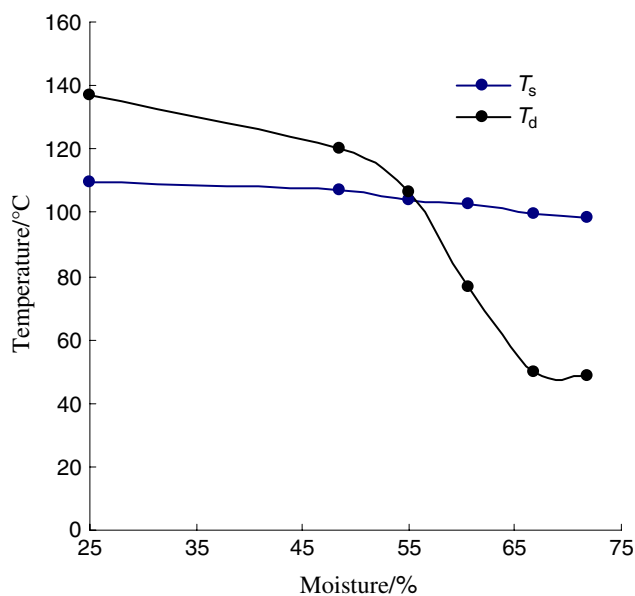| Sample no.	Moisture/%	T_s/°C	T_d/°C
1	25.0	109.8	137.2
2	48.6	107.1	120.4
3	55.0	103.8	106.2
4	60.6	103.0	76.8
5	61.9	101.6	53.4
6	66.8	99.7	50.1
7	71.9	98.1	48.6

Fig. 1 Hydrothermal stability and containing moisture of leather modified chrome leather treated with chrome sulfate was heated in water, whose heated rate was 2 K/min. Leather samples contains different moisture as shown in Table 1

The shrinkage temperature of modified collagen can be noted by the change of macroscopically pattern, such as the shortening of length, the decrescence of volume and the induration of leather. Using the special-T_s-testing instrument, the temperature of heating medium at which the specimen begin to shrink was considered as T_s of sample. In fact, the shrink of modified collagen is the dynamic and transilient process, and the heating rate also affects its value. During the modern measuring and testing techniques, denaturation temperature and energy of oxidation can be gained from DSC curve, which also indicate the transform of phase state of detecting sample. The onset temperature in DSC curve is the T_d of sample, which can simulate T_s of leather, especial for the powdery collagen. And there are more and more reports demonstrate using T_d as representation of leather's thermal. That is to say, both T_s and T_d of modified collagen can indicate its thermal stability. However, because of the heating rate, the difference between T_s and T_d is indubitable. From this experiment, the relation between T_s used in the industry and T_d most used in the laboratory had been discussed.

The T_s and T_d of leather tanned with MI–OZ

Table 2 represented the T_s and T_d of the modified collagen treated with MI–OZ containing different moisture.

According to the data in Table 2, Fig. 2 can be got whose x-axis is the moisture value and y-axis is the corresponding temperature, T_s and T_d. From this figure, it can be concluded that the relationship between the hydrothermal stability of

leather tanned with MI–OZ and its containing moisture is very obvious. By comparing the two temperatures, it can be found there is an obvious difference between T_s and T_d of the same moisture leather. Being similar to the chrome leather, the containing moisture has much less influence on the T_s than on the T_d of combined tanned leather, and when the containing moisture is less than 40%, the hydrothermal stability has weak relation with the moisture, and T_d is higher than T_s. What is important, the changing trend is similar to the two temperatures. However, when the containing moisture quantum exceeds 40%, T_d will decrease sharply with the increase of moisture; when the containing moisture exceeds 55%, T_d is steady. By contraries, T_s would not break during the whole course. That is to say, the onset point in the DSC curve is not the T_d of the leather in the high moisture containing.

Table 2 T_s/T_d of combined modified collagen containing moisture

Sample no.	Moisture/%	T_s/°C	T_d/°C
1	20.0	110.6	115.3
2	26.7	100.5	104.1
3	35.3	96.8	101.4
4	41.9	96.8	100.9
5	40.9	95.0	100.1
6	50.1	94.3	57.7
7	71.1	88.1	52.4

Figures 3 and 4 are the DSC curves when the break point appears for the leathers tanned with chrome sulphate and MI–OZ respectively. And the data in the Table 3 are the T_d of the DSC curves with different heating rate, which are 2, 3, and 5 K/min, respectively.
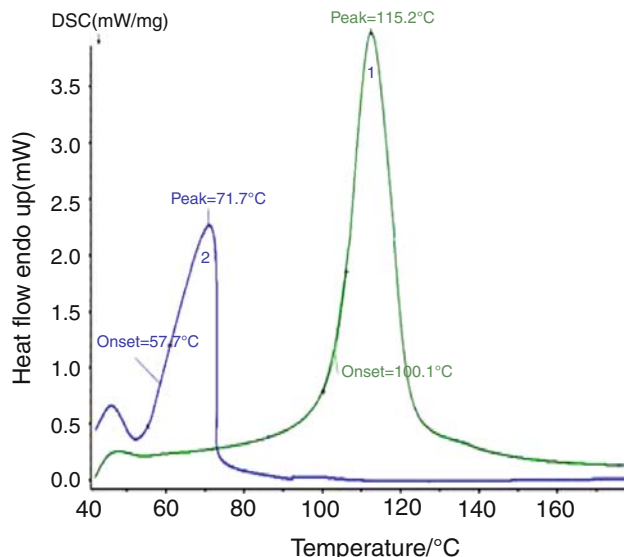
Fig. 3 DSC curve of chrome treated collagen chrome leather sample with different moisture was tested by differential scanning calorimetry. And the containing moistures were 55 and 60.6%, respectively, which was the break point to the leather's hydrothermal stability. The onset temperature point was leather's T_d. *Curve 1* is the sample containing 55% moisture, and *curve 2* is 60.6%
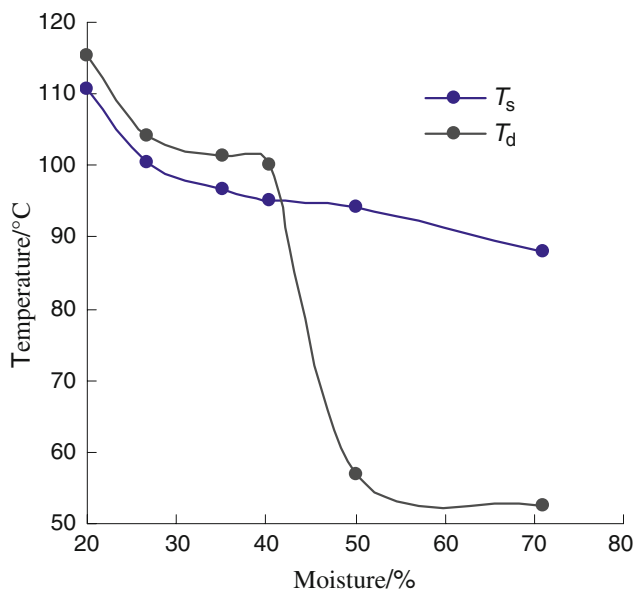

Fig. 2 Hydrothermal stability and containing moisture of combined tanned leather treated with mimosa–oxazolidine was heated in water, whose heating rate was 2 K/min leather samples contains different moisture as shown in Table 2
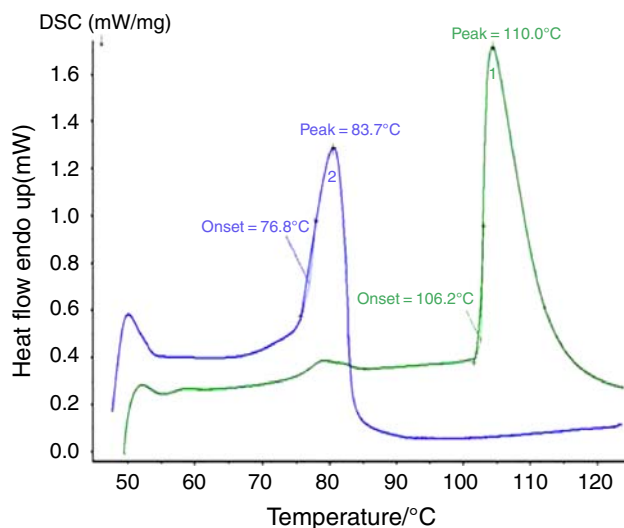

Fig. 4 DSC curve of combined treated collagen combined leather sample with different moisture was tested by differential scanning calorimetry. And the containing moistures were 40.5 and 50.1%, respectively, which was the break point to the leather's hydrothermal stability. The onset temperature point was leather's T_d. *Curve 1* is the sample containing 40.5% moisture, and *curve 2* is 50.1%

Table 3 T_d of DSC with different heating rate

Heating rate (K/min)	T_d/°C (chrome leather)	T_d/°C (combined tanned leather)
2	99.8	107.5
3	116.9	111.7
5	137.2	115.3

By contrasting Figs. 1 and 2, it can be concluded that the hydrothermal stability of leather tanned with the two agents and leather tanned with solo chrome salt will decrease with the increase of the containing moisture. Both T_s and T_d have the same changing trend. It proved that the moisture has an obvious influence on the hydrothermal stability of the leather. But as for the influence is on the tanning agent or on the stability of collagen is not clear. When the moisture is low, T_d is higher than T_s, which can be explained that the hysteresis effect of the leather phase change. And the direct reason is supposed that the heating rate is 2–3 °C/min for T_s testing instrument, 5 °C/min for DSC. And the data in Table 3 also confirm the conclusion that the heating rate can affect the T_d of the leather. And the faster the heating rate is, the lower the T_d will be. Therefore, it can be concluded that the leather have the ability of heating endurance. What is interesting, before the break point, T_s of the two leathers is nearly equal, but there is an obvious disparity between the two T_d, especially for the two T_d whose heating rate is 2 °C/min. From Figs. 1 and 2, it can be resulted that the anti-heating ability of chrome tanned leather is stronger than that of combined tanned leather. When the containing moisture is low, T_s of the two leathers has little difference (T_s is 110.6 °C for combined tanned leather, and T_s is 115.3 °C for chrome leather). And with the increase of moisture, T_d will decrease sharply. In this case, the influence is more obvious on the chrome leather than on the combined tanned leather. It is similar for the T_d of the two leathers. For chrome leather, its T_s is 109.8 °C and T_d 137.2 °C when it is dry (contain 25% moisture). There is an obvious difference between the two temperatures for the leather tanned with chrome sulphate. That prove T_d cannot replace T_s for chrome leather. However, for the combined tanned leather, its T_s is 101.0 °C and T_d is 105.0 °C when its moisture is 25%. That means T_d can take place of T_s for combined leather when the moisture is low. These conclusions can not promise absolutely right for all the leather.

Because of the limit of laboratorial equipment and condition, only the chrome and combined leather were studied. From these results, the relation between moisture of leather and its hydrothermal stability is becoming explicit. However, the difference between the two token ways about leather's stability is still not clear for the leather tanned with other tanning agents. Further research should be carried out about leather tanned with other inorganic and organic and combined tanning agents.

Conclusions

From the shrinkage temperature of modified collagen, it is obvious that both chrome sulfate and MI–OZ can enhance the thermal stability of raw collagen. And within the range of containing moisture of leather in this experiment, the hydrothermal stability of the leather decreases with the increases of the containing moisture. When the moisture is under the certain containing moisture, which is 55% for chrome leather; 40% for combined tanned leather, its denaturation temperature, T_d is higher than the shrinkage temperature, T_s. If its containing moisture exceeds the given value, its T_d, or onset temperature, will decrease sharply, and should not be thought of the phase change of leather. All of these mean that there are different change rules of T_s and T_d of two kinds of leather varying with containing moisture. It can be resulted that leather tanned with chrome sulphate has a more obvious difference between its T_s and T_d than that of leather tanned with MI–OZ. In this experiment, it is also found that the disparity would be evident in test T_d by DSC with different heating rate. As the conclusion of this article, the relationship between the hydrothermal stability of leather and its containing moisture is very important. Especially, to express T_d as hydrothermal stability of collagen or leather is inappropriate for two kinds of leather.

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