

Enzymatic Bating Technology for Wet Blue:

I. Characterization of Protease Activities Towards Chrome-tanned Elastin and Collagen Fibers

by

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Abstract

To characterize the reacting performances of proteases against chrome-tanned leather, the methods to quantitatively measure protease activities against chrome-tanned elastin and collagen fibers were established through detecting the produced amount of Desmosine (DES) or Hydroxyproline (Hypro) in unit time. Chrome-tanned elastin and collagen fiber substrates with different Cr content were prepared, and the influence of the Cr content on the enzymatic hydrolysis resistance of tanned substrates was investigated. The activities of several typical proteases against chrome-tanned elastin and collagen fibers were tested based on the methods, and the relationship between the activities and bating effectiveness on wet blue was also preliminarily investigated. The results showed that the two kinds of activities of proteases decreased with the increase of Cr content in the substrates. When the Cr contents in elastin or collagen fiber reached 0.5% or 2.0%, the corresponding activity approached to the lowest level, respectively. Different protease showed different activity decrease extend towards tanned protein fibers. Meanwhile, the bating effectiveness of proteases on wet blue was positively related to their activities towards the chrome-tanned protein fibers measured by the established methods, i.e. proteases with highly chrome-tanned elastin and collagen fiber activities could significantly improve the softness of leather. The new methods can be used as an available tool to correctly select proteases and optimize process parameters for wet blue bating.

Introduction

With more concerns about the environmental protection, more and more tanneries are tending to purchase wet blue as raw materials because of the government restricting the import of

raw hides, such as in China. In order to reduce pollution, control production cost and lower risk of hide damage, wet blue tanneries often adopt conservative technology in beamhouse processing, thus purchased wet blue generally has some obvious defects, such as insufficient opening up of fiber structure, vein marks and scars, pigments, mildew, wrinkles, folds and even harden fibers from long-term transportation and storage, especially, evident quality difference from different tanneries, and so on, which seriously affects the unification of the subsequent processing technology and the performance and use value of the leather. Hence, enzymatic bating operation is usually the first stage of post-tanning process to resolve the problems in most tanneries used wet blue as raw materials.

Enzymatic bating process of wet blue is the process of opening up protein structures, removing grease and inter-fibrillary substances, complementally, by using enzyme preparations. Mainly based on the proteolytic effect on protein components, fiber structure is further opened, defects are alleviated, thus the uniformity, softness, area yield, physical and mechanical performances and sensory properties of finished leather are improved. Consequently, wet blue performance difference from varying sources is reduced, subsequent processing can be standardized, as a result, the production management becomes more easily, through properly selecting bating enzymes and bating conditions according to wet blue situations and the property requirement to finished leather.

There are some but not too many reports about the enzymatic bating technologies of wet blue. Most of the reports only involved in the bating effectiveness of some specific enzyme preparations to wet blue and the optimization of using conditions, but rarely mentioned the mechanism and how to select the correct enzymes. It is well accepted that the use of enzymes in post-tanning process is useful in improving the quality of finished

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leather, such as softness, evenness, dye-levelling, area yield and so on.¹⁻³ Some typical proteases were also used to solve vein marks defects of leather.⁴⁻⁵ But, under the restriction of traditional thinking pattern, the bating technology of wet blue leather is limited to choosing acidic proteases, because post-tanning processing is conducted on acidic condition. However, the acidic protease itself defects, i.e. it has low activity towards chrome tanned protein fibers, especially almost has no effect on the elastic fiber,⁶⁻⁷ leads to enzyme bating required for a long time, large enzyme dosage, and not obvious treatment effect. The well chrome tanned collagen fibers and elastic fibers have higher protease resistance, and protease activities towards tanned protein fibers are affected by the chromium content, moreover, the tolerance of tanned protein fibers to different protease is diverse. Therefore, the results of established methods for assaying protease activity based on untanned protein fiber substrates cannot reflect the actual effectiveness of the protease on chrome-tanned protein fiber.⁶⁻⁹ Consequently, the selection of enzyme and the control of bating conditions are mainly based on the experiential judgment in the present enzymatic bating techniques. In a word, the structures of elastin and collagen fibers have great change after chrome-tanned, hence, to properly select enzymes for bating wet blue, the quantitative methods for characterizing the protease activities on chrome-tanned elastin and collagen fibers should be established at first. Then the relationship between the bating effectiveness, and the degree of enzymatic hydrolysis of chrome-tanned protein fibers should be investigated.

To counter the problems above, this paper tries to establish the methods to quantitatively characterize the properties of proteases hydrolyzing chrome-tanned elastin and collagen fibers through measuring the produced amount of Desmosine (DES) and Hydroxyproline (Hypro) in the reaction liquor. Establishing this will provide a useful tool to correctly select proteases and optimize process parameters for wet blue bating.

Materials and Methods

Materials

Hydroxyproline (Hypro) and Desmosine (DES) standards were obtained from MembraPure GmbH (Germany). Shaved cowhide wet blue leather was supplied by Tongtianxing Group Co. Ltd, China. All the other chemicals used for the analysis were of analytical grade. All proteases (see Table I) were purchased from the market. Elastin fiber powder was prepared by our laboratory through a hot alkaline process from bovine ligamentum nuchae.¹⁰⁻¹¹ Nature collagen fiber powder was prepared by our laboratory from the reticular layer of bovine hide.

Methods

Primary Purification of Enzyme

The ultrafiltration method was used for the desalting of industrial protease preparations as the following procedure.

First, 1.00 g of an enzyme preparation was dissolved in 50 mL distilled water and centrifuged at 3500 rpm for 10 min. Then, the supernatant was transferred into an ultrafiltration membranes tube (3000 Da) and centrifuged at 3000 rpm for 30 min under 4°C. The enzyme solution was stored in a freezer, and the caseinolytic activity was determined by Folin Method.¹²

Preparation of Chrome-tanned Elastin and Collagen Fiber Substrates

10 g±0.1 g of elastin fiber powder or collagen fiber powder was soaked in 100 g of 8% NaCl solution at room temperature in a 250 mL Erlenmeyer flask and stirred for 3 h. Then the pH of the liquor was adjusted to 3.2 by using a sulfuric acid solution (1:10, v/v). Then varying dosage of chrome powder was added to the suspension and stirred for another 2 h. A certain amount of sodium bicarbonate solution (1:10, w/v) was added into the suspension to adjust the pH to about 4.0. The mixture was stood for another 5 days, and the chrome-tanned fiber powder was thoroughly washed with distilled water for 5 times and dried at room temperature. The dried powder was milled and passed through a 120 (collagen fiber powder) or 40 (elastin fiber powder) mesh screen. The contents of Cr in the substrates were tested according to the steps described in the following measuring method section.

Influence of Cr Content on Protease Activity

Test tubes were charged with 100 mg±1 mg of chrome-tanned elastin or collagen fiber and 5 mL of Britton-Robinson buffer (0.1 mol/L, pH 3.5 or 6.5), and stirred in an incubator at 40°C for 10 min. Then, 1 mL of purified enzyme solution (diluted into a certain concentration by Britton-Robinson buffer) was added and stirred for another 4h under 150 rpm, then the concentration of DES or Hypro in the digested reaction liquor was tested to represent the performance of the protease hydrolyzing elastin or collagen fiber, respectively.⁶⁻⁷

Assay of Protease Activities on Elastin and Collagen Fiber Substrates

The protease activity was measured as the above testing procedure. The chrome-tanned elastin with 0.53% of Cr content and collagen fibers with 2.11% of Cr content were chosen to be the substrates. The amount of DES or Hypro in the digested reaction liquor was tested. The definitions of elastin fiber activity and collagen fiber activity were given and calculated as follows.⁶⁻⁷

One unit (1 U) of elastin fiber activity was defined as the amount of enzyme capable of digesting the elastin fiber substrate to produce 1 ng of DES per 1 hour under the certain conditions.⁶

One unit (1 U) of collagen fiber activity was defined as the amount of enzyme capable of digesting the collagen fiber substrate to produce 1 ng of Hypro per 1 hour under the certain conditions.

Bating Wet Blue with Typical Protease Preparations

Pieces of wet blue (25×25 cm) samples were symmetrically taken along the backbone in a piece of shaved cowhide wet blue leather. The samples were wetted and adjusted the pH to 3.5 or 6.5 by using formic acid (1:10, v/v) or sodium bicarbonate solution (1:10, w/v) and stood overnight. Samples were treated by different protease with the dosage of enzyme was 500 U/g (caseinolytic activity, based on wet blue leather) at 40°C, pH 3.5 (acidic protease) or 6.5 (neutral protease and alkaline protease) for 4 h, and the dosage of water was 150%. After bating, the concentration of DES and Hypro in the digested effluents were tested according to the steps described in the following measuring method section. The leather samples were horizontally sliced up and the elastin fiber was stained per the aldehyde-fuchsin method.¹³ The residual of elastin fiber was observed with an optical microscope. The subsequent operations were conducted as the same conventional post-tanning process, and the softness of the crust leather was determined with a Leather Softness Tester (GT-303, Gotech Testing Machines Inc., China).

Determination of Cr Content

100 mg±1 mg of chrome-tanned protein fiber was mixed with 1 mL of concentrated HCl and 3 mL of concentrated HNO₃ and digested at 120°C for 4 h. Then the digested liquor was cooled to room temperature and diluted, then the concentration of chromium (Cr) was detected with AES-ICP method.¹⁴ The Cr content in the chrome-tanned protein fiber was calculated.

Determination of the Concentrations of DES and Hypro in the Reaction Liquor

After the end of the reaction, the reaction liquor was centrifuged immediately for 5 min at 3500 rpm and 2 mL of the supernatant was mixed with 2 mL of concentrated HCl in a 10 mL digestion tube. The mixture was digested at 120°C for 12 h and 2 mL of EDTA-Na₂ (20 g/L) solution was added to avoid the interference of chromium.¹⁵ Then the hydrolytic liquor was evaporated to dryness with a Vacuum Concentrator (TC-8F, TAITEC, Japan). A certain amount of amino acid analysis sample dilution buffer was added to dissolve the dry sample to ensure the concentration of DES and Hypro are in the range of 50-250 nmol/mL. The dissolved liquor was filtrated with an aqueous filter head (D=0.22 µm). The concentration of DES was tested with an Amino Acid Analyzer (A300, membraPure GmbH, Germany).

Results and Discussion

Combined Cr Amount in Tanned Elastin and Collagen Fiber Substrates

The enzymatic bating of wet blue can further open the fiber structure of leather through proteases hydrolyzing the chrome-tanned elastin and collagen fiber. The treatment effectiveness is related to the content of Cr in leather, i.e. the tanning degree of

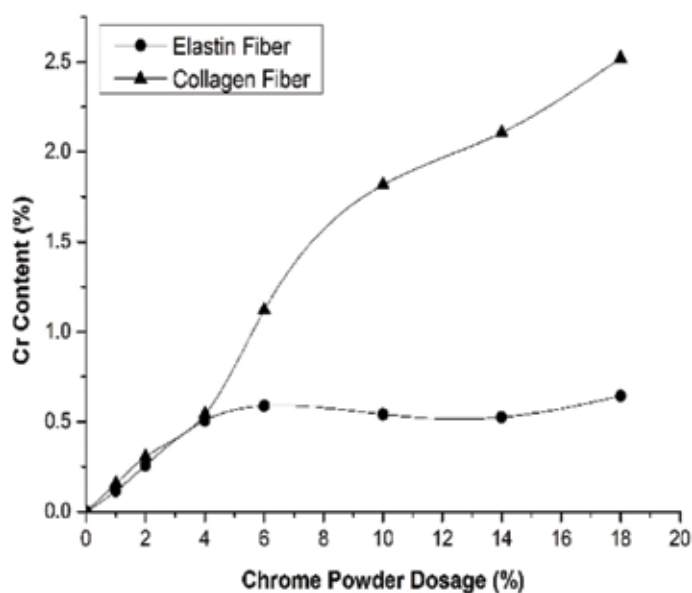


Figure 1. Changing of Cr contents in tanned elastin and collagen fibers with Cr dosage.

protein fibers. Therefore, to evaluate the influence of Cr content on the protease activities towards tanned protein fibers, a series of chrome-tanned elastin and collagen fiber substrates with varying content of Cr were prepared.

Elastin fiber powder and collagen fiber powder were tanned with varying dosage of chrome powder as the conventional tanning method. The changing of Cr content of tanned fibers with chrome dosage is illustrated in Figure 1.

Figure 1 shows that the maximum capacity of elastin fiber to combine Cr is about 0.53%, and the Cr content in elastin fiber does not rise higher when the dosage of chrome powder exceeds 6%. The content of Cr in collagen fiber rises with the increase of chrome dosage. The capacity of elastin fiber binding Cr is lower than that of collagen fiber. The reason is that the amino acid composition of elastin contains a very small percentage of carboxyl containing amino acid.¹⁶ The acidic amino acid content of the elastin fiber powder is about 0.089 mmol/g, and the theoretical value of Cr binding capacity is about 0.463%, and it is like the experimental value. The acidic amino acid content of the collagen fiber powder is about 0.839 mmol/g, and the theoretical value of Cr binding capacity is about 4.36%, about 10 times than elastin fiber. Generally, when the content of Cr in leather is beyond 2%, its shrinkage temperature can reach to above 100°C, and it is well tanned.

Influence of Cr Content in Substrate on Protease Activity

Our research found that most of the commercial proteases have high activity to untanned collagen fiber; but to untanned elastin fiber, alkaline proteases exhibit rather high activity, neutral proteases exhibit lower activity and acidic proteases almost have no activity.¹¹⁻¹² Acidic proteases are often selected in wet blue

bating because the post-tanning process is conducted under acidic condition. But, neutral and alkaline proteases may still have some degree of activity towards elastin and collagen fibers under weak acidic conditions, pH 6-7, hence, they can also be potentially chosen to be used for wet blue bating.

To investigate the influence of Cr content in substrate on the protease activity to elastin fiber, three kinds of alkaline proteases with higher elastinolytic activity, MP, AX and 2709, were chosen. The enzymatic hydrolysis of elastin fiber was carried out at pH 6.5, 40°C for 4 h, and the concentration of DES in the digested reaction liquor was tested to represent the protease activity to elastin fiber. The results are shown in Figure 2.

Figure 2 indicates that the activities of all those selected alkaline proteases reduce with the increasing of Cr content in the substrate, and it reaches to the minimum value, when the Cr content approaches to 0.5%, near to the maximum binding capacity of elastin fiber to Cr.

Further, the influence of Cr content in substrate on the protease activity to collagen fiber was investigated. Three kinds of proteases with higher collagenolytic activity, including acidic protease L80A, neutral protease LKT-N and alkaline protease MP, were chosen. The enzymatic hydrolysis of collagen fiber was carried out at pH 3.5 (acidic protease L80A) or 6.5 (neutral protease LKT-N and alkaline protease MP), 40°C for 4 h, and the concentration of Hypro in the digested reaction liquor was tested to represent the protease activity to collagen fiber. The results are shown in Figure 3.

Figure 3 shows that the activities of all kinds of selected proteases to collagen fiber reduce with the increasing of Cr content in the substrate, and it reaches to the minimum value, when the Cr content approaches to 2.0%, where the collagen fibers are well tanned.

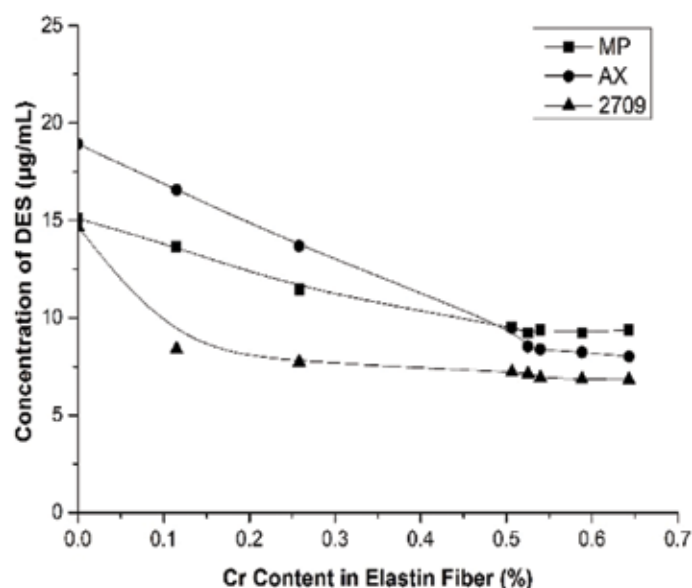


Figure 2. Influence of Cr content in elastin fibers on protease activity.

Proteases activities to both tanned elastin and collagen fibers are negatively impacted with increasing Cr content in substrates. There are mainly two reasons why the tanned protein fibers have good protease-resistance ability. On the one hand, the stability of the protein structure is improved due to the cross-linking effect between protein chains after tanned, moreover the stability and protease-resistance are improved with the increasing of the tanning degree, i.e. Cr content. On the other hand, the change of some side groups of protein molecules with the combing of tanning agents, mainly carboxyl groups for chrome tanning, may lead to the transformation of the protein molecular conformation; this will possibly result in the protease cannot correctly identify the action sites, wherefore the catalysis ability is reduced.

Characterization of Protease Activity Towards Chrome-tanned Elastin and Collagen Fibers

As mentioned above, well-tanned elastin and collagen fibers exhibit high protease-resistance ability, in addition, the same tanned protein fiber has different tolerance to different proteases, because different proteases have different mechanism of action on the substrate. To choose the suitable bating proteases for wet blue leather, the method of quantitative characterization the efficiency of proteases in hydrolyzing chrome tanned protein fibers should be established.

To guarantee the good tanning results, the dosage of tanning agent is large in the tanning process, and the Cr content in leather is above 2.0%. Therefore, the protein fibers in wet blue have strong protease-resistance. According to the results of Figure 2 and 3, chrome-tanned elastin fiber with 0.53% of Cr content and collagen fiber with 2.11% of Cr content were chosen as the substrates, which were close to the actual tanning status of the protein fibers in wet blue leather.

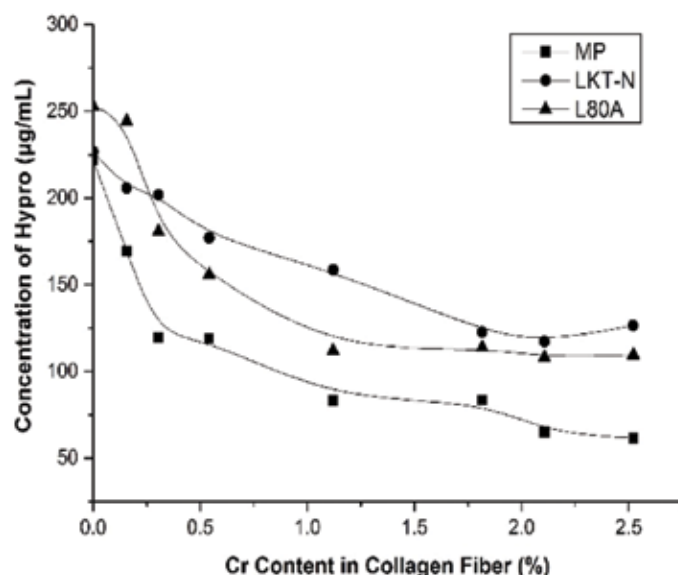


Figure 3. Influence of Cr content in collagen fiber on protease activity.

Some typical protease preparations were chosen, including acidic protease 537 and L80A, neutral protease WB, AS1.398 and GB, and alkaline protease AX, 2709 and MP. Under the conditions simulating the normal wet blue bating, i.e. pH 3.5 (acidic protease) or 6.5 (neutral and alkaline protease) and 40°C, the substrates were treated with different proteases for 4 h. The produced amount of DES or Hypro in the digested reaction liquor was tested, and the activities to elastin fiber and collagen fiber were calculated. The results are shown in Table I.

Industrial protease preparations always contain active enzyme protein, inactive protein and other nonprotein components, and the content of enzyme protein is different for different protease preparations. Hence, the activity value of unit mass of enzyme preparation cannot really reflect the difference of the actual hydrolysis ability of different active enzyme protein molecule to a substrate. The actual content of active enzyme protein in a protease preparation is difficult to be correctly determined. Considering that caseinolytic activity of a protease preparation is always determined per Folin Method and it is often be used for providing the benchmark of protease dosage in leather manufacture process, hence, in Table I, the elastin or collagen fiber activity of a protease is characterized by the relative activity to 1 unit of casein hydrolysis activity (equivalent to using the amount of the active enzyme protein component as the

benchmark), namely the ratio of the elastic or collagen fiber activity to the caseinolytic activity of a protease preparation.

Table I shows that different proteases exhibit various elastin and collagen fibers activities based on the same caseinolytic activity. On the testing conditions, three alkaline proteases exhibit higher elastin fiber activity than neutral proteases to untanned elastin fiber, and neutral protease GB and acidic proteases does not have elastin fiber activity. The residual activities of three alkaline proteases to the chrome-tanned elastin fiber are higher than 44% comparing to the untanned substrate, and MP is 60.2%, while two neutral proteases have also rather high residual activities, WB is 40.8% and AS1.398 is 33.2%.

All those kinds of proteases exhibit high collagen fiber activity to untanned collagen fiber substrate. The residual collagen fiber activities of the proteases with both elastin and collagen fiber activity, WB, AS1.398, 2709, MP and AX, are lower than 30% comparing to the untanned substrate comparing to the untanned substrate, is lower than that of chrome-tanned elastin fiber, because of higher binding amount of Cr and crosslinking degree in tanned collagen fibers.

The activity of a protease to chrome-tanned protein fiber significantly reduces comparing to the untanned substrate.

Table I
Relative activities of proteases towards elastin and collagen fibers substrates.

	Acidic Protease		Neutral Protease			Alkaline Protease		
	537	L80A	WB	AS1.398	GB	2709	MP	AX
Elastin fiber activity								
$E_0^{*1}(U)$	0.0	0.0	4.9	19.0	0.0	30.5	30.4	36.8
$E_{Cr}^{*3}(U)$	0.0	0.0	2.0	6.3	0.0	14.2	18.3	16.2
$E_{Cr}/E_0(\%)$	-	-	40.8	33.2	-	46.6	60.2	44.0
Collagen fiber activity								
$H_0^{*2}(U)$	407.0	549.7	582.4	521.9	487.3	412.2	639.8	629.6
$H_{Cr}^{*4}(U)$	218.4	193.0	80.7	77.0	196.3	121.8	134.2	59.5
$H_{Cr}/H_0(\%)$	53.7	35.1	13.9	14.8	40.3	29.5	21.0	9.5

^{*1}: E_0 represents the activity of protease per 1 unit of caseinolytic activity to untanned elastin fiber

^{*2}: H_0 represents the activity of protease per 1 unit of caseinolytic activity to un-tanned collagen fiber

^{*3}: E_{Cr} represents the activity of protease per 1 unit of caseinolytic activity to chrome-tanned elastin fiber

^{*4}: H_{Cr} represents the activity of protease per 1 unit of caseinolytic activity to chrome-tanned collagen fiber

Different protease has different decrease degree, and the reason may be ascribed to that different protease has different catalytic mechanism. As mentioned before, some of the side groups of protein molecule will change after tanned, and the conformation of protein will also change, therefore the protease cannot correctly identify the action sites. Different proteases have different catalytic hydrolysis sites, thus the changes of protein substrate after chrome tanning have different effects on different enzymes. Furthermore, it can be speculated that the tolerance of the tanned protein substrate with different tanning method to the same protease will be different, because different tanning agent combines with different groups of protein, and tanning effect is also different.

In conclusion, there are large differences in the activity of a protease between untanned and tanned protein fiber substrates, therefore, the results of characterizing protease performances based on chrome tanned protein fiber substrates have more guiding significance in the aspects of selecting proteases and optimizing process parameters for wet blue bating.

Relationship between Proteases Activities to Chrome-tanned Protein Fibers and Bating Effect of Wet Blue

For verifying the consistency of the measuring results of protease activities to chrome tanned protein fiber substrates with their wet blue bating effects, eight different types of proteases were chosen for bating wet blue as the following conditions: 40°C, pH 3.5 (acidic protease) or 6.5 (neutral protease and alkaline protease), 4 h. Although the dosages of all protease were the same based on the caseinolytic activity, 500 U/g wet blue leather, but the concentrations of the protease activities to chrome-tanned elastin and collagen fiber in the bating liquor for each

protease are significantly different. The concentrations of DES and Hypro in bating liquors were measured, and the relationship between the proteases activities concentrations in bating liquors and the produced DES and Hypro concentrations are illustrated in Figure 4 and Figure 5.

Figure 4 and Figure 5 show that the higher the protease activity to chrome-tanned elastin fiber or chrome-tanned collagen fiber, the larger the concentration of DES or Hypro in the reaction liquor, correspondingly. Furthermore, both concentrations of DES and Hypro increase linearly with the increase of the associated activity. It can be calculated that the produced amount of DES by 1 unit chrome-tanned elastin fiber activity is

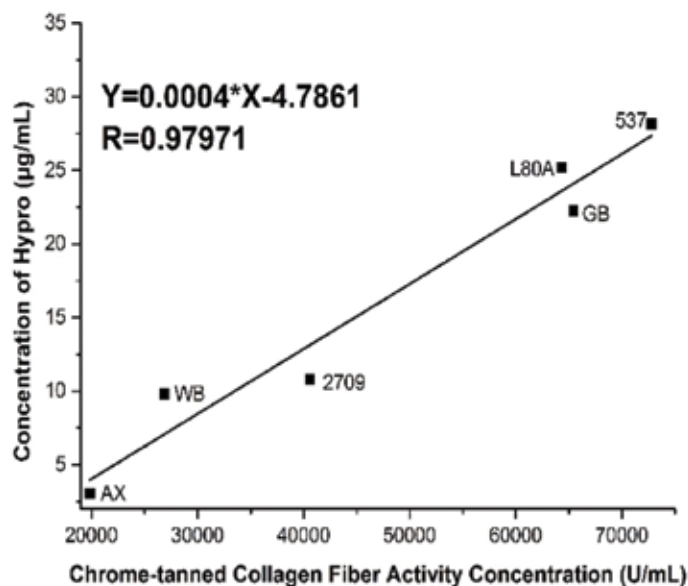


Figure 5. Concentrations of Hypro in bating liquor vs. chrome-tanned collagen fiber activity concentration.

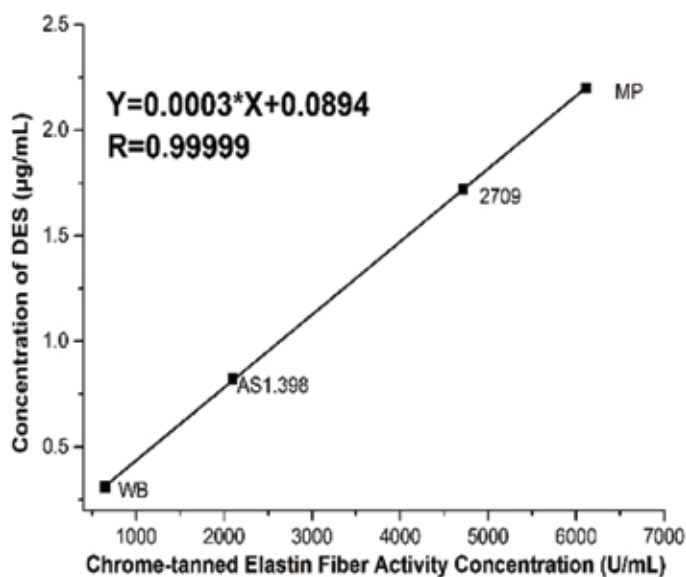


Figure 4. Concentrations of DES in bating liquor vs. chrome-tanned elastin fiber activity concentration.

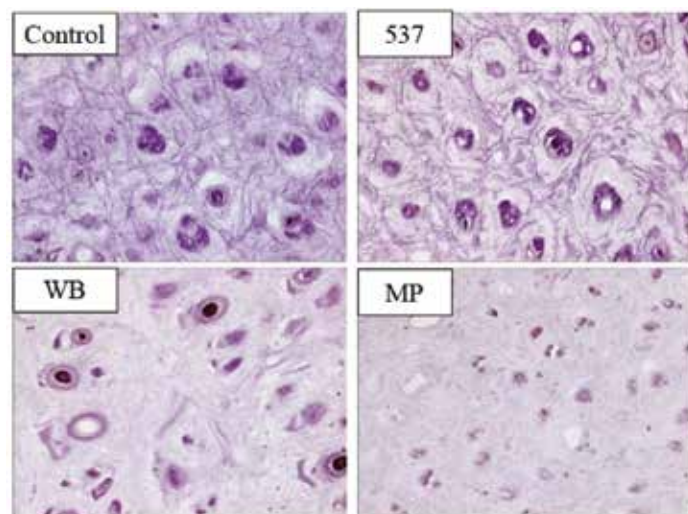


Figure 6. Residual of elastin fiber in wet blue treated by different proteases (40× hor.sec).

Table II
Relation of the softness of crust leather to activities of proteases.

	537	L80A	WB	AS1.398	GB	2709	MP	AX
E_{Cr} (U)	0.0	0.0	2.0	6.3	0.0	14.2	18.3	16.2
H_{Cr} (U)	218.4	193.0	80.7	77.0	196.3	121.8	134.2	59.5
Softness(mm)	7.24	7.22	6.65	6.95	7.36	7.30	7.90	7.46

about 0.18 μ g, and the produced amount of Hypro by 1 unit chrome-tanned collagen fiber activity is about 0.20 μ g under the bating conditions. Therefore, the established methods for quantitatively characterizing the performances of protease towards chrome tanning protein fibers is feasible, and the results of the determination of proteases activities are consistent with their effect on bating wet blue leather.

The damage of the elastin fiber in the grain layers of bated wet blue were observed through histologic staining method. The results are shown in Figure 6.

Figure 6 shows that alkaline protease MP with higher chrome-tanned elastin fiber activity destroyed most of the elastin fibers in the grain layer and around hair follicles. Neutral protease WB with lower chrome-tanned elastin fiber activity broke most of the elastin fibers of the grain layer, but there is still elastin fiber residual around the hair follicles. Acidic protease 537 with no chrome-tanned elastin fiber activity almost has no effect on elastin fiber. The higher chrome-tanned elastin fiber activity the more serious the elastin fiber broken.

One of the main functions of proteases used in wet blue bating process is to further open the protein fiber structure and improve the softness of leather. The softness of crust leather after treated by different proteases are shown in Table II.

Table II shows alkaline protease MP with higher both chrome-tanned elastin fiber and collagen fiber activities gives the crust leather the highest softness; the softness of the crust leather treated by neutral proteases WB and AS1.398 with lower chrome-tanned elastin and collagen fiber activities is the lowest; hence, the degree of opening protein fiber structure of wet blue leather is related to the activities of protease to tanned protein fibers. The softness of the crust leather is increased with the chrome-tanned collagen fiber activity increasing for the acidic protease and neutral proteases, which have lowest chrome-tanned elastin fiber activity. Neutral protease AS1.398 has slightly lower chrome-tanned collagen fiber activity and significantly higher

chrome-tanned elastin fiber activity than WB, and the softness of the crust leather treated by AS1.38 is rather high than WB; alkaline protease MP has similar chrome-tanned collagen fiber activity to protease 2709 and higher chrome-tanned elastin fiber activity, and the softness of the crust leather treated by MP is higher than 2709; which means that the protease with higher chrome-tanned elastin fiber activity can also give leather higher softness. Alkaline proteases with high chrome-tanned elastin fiber activity can improve the softness of leather but make the grain layer loose. Hence, the selecting of bating proteases for wet blue bating should consider the demand of the style of finished leather.

Conclusions

Based on the investigation of the relationship between the Cr content in chrome-tanned elastin and collagen fibers and their protease-resistance ability, the methods of quantitatively determining the protease activities towards chrome-tanned elastin and collagen fibers through measuring the produced amount of DES or Hypro in the reaction liquor was established. The relationship between the activities and bating effectiveness on wet blue was also preliminarily investigated. The results showed that different protease exhibited different activity decrease extend towards tanned protein fiber. The bating effectiveness of proteases on wet blue was positively related to their activities towards the chrome-tanned protein fibers measured by the established methods, i.e. proteases with highly chrome-tanned elastin and collagen fiber activities could significantly improve the softness of leather. The new methods can be used as an available tool to correctly select proteases and optimize process parameters for wet blue bating.

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