Neutral Protease Assisted Low-sulfide Hair-save Unhairing Based on pH-sensitivity of Enzyme

by

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Abstract

The mass transfer of protease in hide during enzymatic unhairing was first investigated by fluorescent tracer technique. It was found that the penetration rate of protease in hide was quite slow, and protease remained on the grain/papillary layer even after removal of hair, which is the main reason why enzymatic unhairing may cause grain damage or loose grain. But protease could effectively remove epidermis in a short time. From these phenomena, we developed an enzyme assisted low-sulfide hair-save unhairing (EALS unhairing) based on pH-sensitivity of neutral protease activity. To avoid risk of hide damage, soaked cattle hide (pH 8) was first treated with neutral protease (20 units/g hide) for 40 min at 22°C to cleave epidermis but not to unhair, and then 1% lime was immediately added both for inactivation of the neutral protease (pH>12) and for hair immunization. Finally, the hide was completely unhaired by using 0.8% sodium sulfide with intact hair shaft. The smartly controlled action of protease, as well as the synergistic effect of protease, lime and sulfide, ensured the complete removal of hair and epidermis with reduced offer of chemicals, and prevented pelts from defects. The crust leather processed by using EALS unhairing had a cleaner grain surface compared with that using conventional sulfide-lime unhairing. Additionally, the physical properties of the leather processed with EALS unhairing were comparable to those of conventional leather. Sulfide, total solids, suspended solids and chemical oxygen demand in the EALS unhairing effluent were markedly reduced due to a dramatic decrease in the input of sodium sulfide and lime as well as the recovery of hair.

Introduction

Conventional sulfide-lime unhairing system using 3-4% sulfide and 5-8% lime is an economical and effective unhairing technique.[1] However, it results in a high sulfide content in tannery wastewater, a large amount of lime sludge and many degradation products of hair.[2] Therefore, eco-friendly hair-save unhairing systems have attracted widespread interest in leather industry, such as enzymatic unhairing,[3,4] Sirolime unhairing process,[5,6] Blair hair system,[7] oxidative unhairing,[8] and so on. Among them, enzymatic unhairing is considered as one of the most eco-friendly unhairing methods. But this technology has not been widely applied in commercial scale, since it has some problems that are difficult to solve. The main problem is that enzymatic unhairing may cause grain damage and loose grain. This is because proteases also hydrolyze hide/skin collagen when they remove hair from hides/skins during enzymatic unhairing process.[9] Moreover, after enzymatic unhairing, a small amount of fine hair usually remains in hides/skins, especially for cattle hide.[9,10]

Chemical hair-save unhairing systems, such as Sirolime unhairing process,[5,6] Blair hair system[7] and Rohm HS-process,[11] which remove hair from hides by destroying hair root and can recover intact hair shaft by balancing the actions of lime and sulfide on hair,[9] are able to significantly reduce suspended solids and organic substances in unhairing effluent. Compared to enzymatic unhairing, the chemical hair-save unhairing has no potential risk of pelt damage. Nevertheless, they need to use a larger amount of sulfide[5-7] or special materials such as Erhavit HS,[11] or may cause incomplete removal of hair and epidermis when exceeding the hair immunization time or temperature[9].

Both to reduce the sulfide pollution and to completely remove hair, some research focused on the development of enzyme-sulfide synergistic unhairing systems based on alkaline protease and sodium sulfide.[12-14] But these unhairing systems still cannot completely avoid grain damage or loose grain, because hides/skins are also needed to be treated with enough protease for a long time or at a relatively higher temperature. As we known, the so-called substrate specificity of protease refers to that one protease only hydrolyzes a special peptide bond in proteins but not a unique protein, which means that one protease is normally able to hydrolyze various proteins having the suitable peptide

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bond. Hence, proteases used for unhairing have a potential risk to damage collagen to some degree. Furthermore, partial denaturation of hide/skin collagen resulted from sodium sulfide would make the hydrolysis of collagen by protease easier.[15]

In our preliminary experiment, it was found that the damage to hide collagen could become very slight and acceptable by treating soaked hides with less protease and less time at room temperature. In the present study we developed a pH-controlled enzyme assisted low-sulfide hair-save unhairing technique (EALS unhairing) to guarantee high quality of resultant leathers. The EALS unhairing was based on the pH-sensitivity of neutral protease that usually has high proteolytic activity in the pH range of 7-9 and is almost free of proteolytic activity at a pH higher than 12. First, soaked cattle hide (pH 7-9) was treated with a small amount of neutral protease for 40-60 min at room temperature to cleave and remove epidermis. Then, lime was immediately added both for inactivation of the neutral protease by increasing pH to above 12 and for hair immunization. At last, sodium sulfide (less than 1%) was used to obtain complete removal of hair. The extent of hair and epidermis removal from hide, the physical properties of the leather produced by using EALS unhairing and the pollution loads in EALS unhairing effluent were determined and compared with those by using conventional sulfide-lime unhairing.

Experimental

Materials

Conventional soaked cattle hides (pH 8.0) that had tight hair were used for unhairing trials. Collagen fiber (hide powder) was prepared according to the method described in the literature.[16] Commercial neutral protease produced by culturing *Bacillussubtilis* was purchased from Nanning Pangbo Enzyme Co., Ltd. (China). Trypsin from bovine pancreas purchased from Shanghai Aladdin Biochemical Technology Co., Ltd., fluorescein isothiocyanate isomer I (FITC, ≥90% (HPLC)) and Sephadex G-25 (fine) purchased from Sigma-Aldrich Co. LLC. were used for preparation of fluorescent protease. Commercial sodium sulfide (Na_2S≥60%) was purchased from Kangxin Sichuan Chem Co., Ltd. (China). All the other chemicals used for leather processing were of commercial grade, and the chemicals used for the analyses were of analytical grade.

Visualization and Quantification of Protease in Cattle Hide During Enzymatic Unhairing

Preparation of FITC-labeled Trypsin (FITC-trypsin)

FITC-trypsin (viz. fluorescent protease) with high purity was prepared according to the method described in our previous study with minor modification.[17] Trypsin solution (20.0 mg/mL) and FITC solution (10.0 mg/mL) were prepared by dissolving trypsin and FITC into carbonate-bicarbonate buffer (0.1 mol/L, pH 9.16), respectively. Then, 9 mL of the trypsin solution was mixed with 3 mL of the FITC solution, and the labeling reaction was performed in the dark at 4°C for 10 h. After labeling, the mixture was concentrated to about 3 mL using Amicon Ultra-15 centrifugal filter devices (10 kDa MWCO, Millipore). Subsequently, the concentrated solution was loaded onto a Sephadex G-25 gel-filtration column (3.5 x 85 cm) for removal of unreacted FITC. The column was equilibrated and eluted with ultrapure water at a flow rate of 1.0 mL/min, and fractions containing FITC-trypsin without FITC were collected and freeze-dried. As a result, the purified FITC-trypsin that retained 72% of its original proteolytic activity was obtained.

Observation of FITC-trypsin in Cattle Hides

Four pieces of soaked cattle hides (5 mm in thickness) were treated by 2% protease containing 0.15% FITC-trypsin and 1.85% trypsin (800 units/g hide) and 100% water (based on weight of soaked cattle hide) at 25°C for 60, 150 and 180 min, respectively. After enzymatic treatment, the hides were cut into vertical sections of 20 μm thickness using a freezing microtome (CM1950, Leica, Germany). The sections were observed using a fluorescence microscope (Ti-U, Nikon, Japan) to locate FITC-trypsin in the hides, and then the fluorescence micrographs were processed with Image J software to semi-quantify the relative content of FITC-trypsin in the hides. Additionally, the sections were stained with Weigert's iron hematoxylin and counterstained with Van Gieson's stain to distinguish epidermis, hair roots and collagen fibers. After staining, the sections were observed using an optical microscope (CX41, Olympus, Japan). Moreover, the surfaces of treated cattle hides were captured using a digital camera.

Effect of Enzymatic Unhairing Time on Damage to Hide Collagen

Approximately 500 g of soaked cattle hide was treated in the solution containing neutral protease (20 units/g hide) and water (100%, based on weight of soaked hide) at 25°C for 8 h. To evaluate the damage to hide collagen caused by protease, after enzymatic treating for 0.5, 1.0, 2.0, 4.0 and 8.0 hours, the concentrations of hydroxyproline (Hyp) in the treating liquor were determined as reported in the document.[18] Additionally, the control experiment was conducted by the same method except addition of neutral protease.

Effect of pH on Proteolytic Activity of Neutral Protease

The effect of pH on proteolytic activity of the neutral protease was investigated by the method described in the literature.[2] A series of 1.0 mg/mL neutral protease solutions and 2% (w/v) casein solutions were prepared by using Briton-Robinson buffers at pH 7.0, 8.0, 9.0, 10.0, 11.0 and 12.0, respectively. One mL of neutral protease solution was mixed with 1 mL of casein solution (both at the same pH) and then incubated at 25°C for 10 min. Subsequently, the enzymatic hydrolysis reaction was stopped

with trichloroacetic acid, and the amount of tyrosine released during enzymolysis was measured with Folin-Ciocalteu reagent. One unit of proteolytic activity was defined as the amount of protease that releases 1 μg tyrosine per minute under the assay conditions. The relative proteolytic activities of the neutral protease at different pH values were calculated as:

$$\text{relative proteolytic activity} = \frac{\text{proteolytic activity at a certain pH}}{\text{proteolytic activity at pH 7.0}} \times 100\% \quad (1)$$

Effect of pH on Hydrolysable Action of Neutral Protease on Collagen Fiber

Fifty mg of collagen fiber was suspended in 25 mL of 0.15 mg/mL neutral protease solution, where the pH values of the protease solutions were 7.0 and 12.0, respectively. The mixtures were constantly shaken in 130 rpm at 25°C for 1 h. Then, the mixtures were filtered and the filtrates were taken for measurement of Hyp concentration as reported in the document.[18] Moreover, the control experiments were conducted simultaneously by the same procedures except addition of neutral protease.

Comparison Between Low-sulfide Hair-save Unhairing (LS Unhairing) and Enzyme Assisted Low-sulfide Hair-save Unhairing (EALS Unhairing)

Two pieces of soaked cattle hide, approximately 1 kg for each, were treated by using LS unhairing and EALS unhairing, respectively, and hide/pelt samples (No. 1-6) were collected as shown in Table I. The samples from the hides (No. 1 and 2) and the pelts (No. 5 and 6) were cut into sections of 15 μm thickness using a freezing microtome. The sections were observed using an optical microscope after staining with Weigert's iron hematoxylin and Van Gieson's stain. Moreover, the surfaces of pelts (No. 3, 4, 5 and 6) were captured using a digital camera.

After unhairing, the pelts were both processed by using 2% lime, 2% swelling agent (containing sodium silicate (30 wt.%), magnesium chloride (5 wt.%), sodium hydroxide (5 wt.%) and water (60 wt.%)) and 250% water for 18 h. Subsequently, the limed pelts were delimed, bated, pickled and chrome tanned by using common procedures. Then, the chrome tanned leathers were piled for 24 h and their grain was observed with stereomicroscope (SZX12, Olympus, Japan).

Comparison Between Conventional Sulfide-lime Unhairing and EALS Unhairing

Four soaked cattle hides (approximately 100 kg) were cut along the backbone in halves. The left sides were treated with the conventional sulfide-lime unhairing and liming procedures (control process) listed in Table II, and the right sides were treated by using the EALS unhairing and low-lime liming procedures (experimental process) given in Table III. After liming, the limed pelts were captured using a digital camera. As shown in Table II and Table III, effluent samples (No. 1-3) were collected for analyses of concentrations of S^{2-}, total solids (TS), suspended solids (SS), chemical oxygen demand (COD), total organic carbon (TOC) and total nitrogen (TN) according to the methods reported in documents.[20, 21] Loads of these pollutants were calculated by multiplying their concentrations by volumes of the effluents per ton of raw hides and expressed as kg/t of raw hides. The control and experimental limed pelts were then further processed to obtain crust leathers by using the common techniques of leather processing. The crust leathers were sampled for analyses of physical properties such as tensile strength,[22] percentage elongation at break,[22] tear load[23] and softness.[24] Moreover, their grain was observed with stereomicroscope.

Table I
Procedures of LS unhairing and EALS unhairing.[a]

Process	Offer of agent[b] and remarks[c]	
	LS unhairing	**EALS unhairing**
Enzymatic treatment	–	+100% water, neutral protease (20 units/g hide) Run 40 min, pH 8
Hair immunization /protease inactivation	+100% water, 1% lime Run 90 min, pH>12 Hide sampling (No. 1)	+1% lime Run 90 min, pH>12 Hide sampling (No. 2)
Hair removal	Drain 50% water +0.8% Na_2S Run 10 min +0.8% NaCl[d] Run 15 min, pelt sampling (No. 3) Run 45 min, pelt sampling (No. 5)	Drain 50% water +0.8% Na_2S Run 10 min +0.8% NaCl[d] Run 15 min, pelt sampling (No. 4) Run 45 min, pelt sampling (No. 6)

a -The offer of neutral protease and other chemicals was the optimized amount according to our preliminary trials.
b - Percentage of chemicals was based on weight of soaked hide.
c - All the processes were performed at 22°C.
d - NaCl could make it easier for Na_2S to remove hair.[19] A slight dehydration of hide surface by using 0.8% NaCl could reduce penetration rate of Na_2S in hide and, to some extent, increase the concentration of Na_2S in grain, so that Na_2S could more effectively destroy hair root.

Results and Discussion

Penetration of Protease in Hide During Enzymatic Unhairing

As mentioned earlier, the purpose of this study was to develop an enzyme assisted low-sulfide hair-save unhairing technique that can completely prevent leather from grain damage and loose grain, which may occur when using enzymatic unhairing or existing enzyme-sulfide synergistic unhairing. For this purpose, we first investigated the penetration of protease in cattle hide during enzymatic unhairing by fluorescent tracer technique. Trypsin (biological reagent), a kind of protease with satisfactory unhairing effect,[25] was employed as a research model of commercial nuetral protease, since it can be easily labeled by fluorescent agent. Soaked cattle hide without hair slip was treated by using 0.15% FITC-trypsin and 1.85% trypsin at 25°C. The digital photos of enzymatic treated hide surfaces, the photomicrographs of Van Gieson stained vertical sections cut from enzymatic treated hides and the fluorescence micrographs of FITC-trypsin (green) in the vertical sections are shown in Figures 1(a), 1(b) and 1(c), respectively. Furthermore, after processing Figures 1(c-1), 1(c-2) and 1(c-3) with Image J software,

Table II
Procedures of conventional sulfide-lime unhairing and liming (control process).[a,b]

Process	Chemical	Remark
Unhairing	100% Water, 0.8% Sodium sulphide, 0.3% Degreasing agent	Run 30 min
	1.0% Sodium sulfide	Run 30 min
	1.0% Sodium sulphide, 0.5% Lime	Run 20 min, stop 40 min
Liming	7.0% Lime	Run 90 min
	50% Water	Run 15 min
	50% Water	Run 30 min
	50% Water	Run 60 min, pH 13.3; Run 5 min per hour for another 12 hours; Overnight.
Next day, run 30 min. Effluent sampling (No. 1).		

a - Percentage of chemicals was based on weight of soaked hide.
b - All the processes were performed at 22°C.

Table III
Procedures of EALS unhairing and low-lime liming (experimental process).[a,b]

Process	Chemical	Remark
Enzymatic treatment	100% Water, Neutral protease (15 units/g hide)[c]	Run 40 min, pH 8.5
Hair immunization / Protease inactivation	1.0% Lime	Run 20 min, stop 20 min, run 20 min; pH 12.9
Drain 50% water. Effluent sampling (No. 2).		
Hair removal	0.8% Sodium sulphide, 0.3% Degreasing agent	Run 10 min
	0.8% Sodium chloride	Run 50 min. Hair was completely removed.
Hair was filtered out, dried and weighed. Run 30 min, stop 30 min.		
Liming	2.0% Lime	Run 60 min
	1.0% Swelling agent	Run 15 min, stop 30 min
	1.0% Swelling agent	Run 15 min, stop 30 min
	50% Water	Run 30 min
	50% Water	Run 30 min
	50% Water	Run 60 min, pH 13.3; Run 5 min per hour for another 12 hours; Overnight.
Next day, run 30 min. Effluent sampling (No. 3).		

a - Percentage of chemicals was based on weight of soaked hide.
b - All the processes were performed at 22°C.
c - Here, the amount of the neutral protease was reduced from 20 units/g hide to 15 units/g hide because a larger weight of hides (50 kg) can lead to a stronger mechanical action of hides in drum, which is beneficial to the removal of epidermis with protease and the decrease in the amount of protease.

the quantitative distribution of FITC-trypsin in the enzymatic treated hides was obtained as shown in Figure 1(d). It was obvious that protease penetrated very slowly in hide and could not penetrate through the whole cattle hide even after most of the hair had been removed. The fact that protease remains on the grain/papillary layer and the lower reticular layer for a long time in enzymatic treating process, should be the main reason why enzymatic unhairing may cause grain damage or loose grain. From these phenomena, we infer that shortening enzymatic treating time is necessary to avoid risk of grain damage or loose grain. Besides, as shown in Figures 1(a) and 1(b), epidermis was removed from hide prior to removal of hair. Additionally, little protease penetrated into grain when hide still had tight hair (see Figures 1(a-1), 1(c-1) and 1(d)). These results suggest that inactivation of protease, after cleavage of epidermis but before removal of hair, might be crucial for guaranteeing quality of resultant leather.

Enzyme Assisted Low-sulfide Hair-save Unhairing (EALS unhairing)

As shown in Figure 2, an increase in enzymatic treatment time of hide caused an increase in Hyp concentration in liquors, indicating that the treatment of soaked cattle hide with neutral protease for a longer time resulted in a more considerable damage to hide collagen. But, fortunately, when treating soaked hide with neutral protease (20 units/g hide) at 25°C for less than 1 h, the damage to hide collagen was very slight, and the hair of hide was still tight. Based on the results, we consider that grain damage or loose grain can be prevented if hides are treated with mild conditions, for example, with neutral protease (20 units/g hide) for less than 1 h at room temperature.

It is well known that the proteolytic activity of protease is closely related to pH. For neutral protease, as shown in Figure 3(a), an increase in pH from 7 to 12 led to a negative effect on proteolytic activity. When pH was above 12, the neutral protease was nearly free of proteolytic activity (Figure 3(a)) and almost lost ability to damage collagen (Figure 3(b)). Quite interestingly, soaked cattle hides are in the pH range of 7-9, where neutral protease has a high proteolytic activity and thus can effectively react with hide. On the other hand, the proteolytic activity of neutral protease can be easily inactived as soon as pH is increased to 12 or higher by addition of lime.

Based on this pH-dependent property of neutral protease activity, we developed a "pH-controlled" enzyme assisted low-sulfide hair-save unhairing system (EALS unhairing). In this system, to aviod pelt defects, soaked cattle hide (pH 7-9) was treated with neutral protease (10-20 units/g hide) for less than 1 h at room temperature to cleave epidermis but not to remove hair. Then, the hide was immediately processed with 1% lime both for inactivation of the neutral protease and for hair immunization. Finally, the hide was completely unhaired by using barely 0.8% sodium sulfide with intact hair shaft.

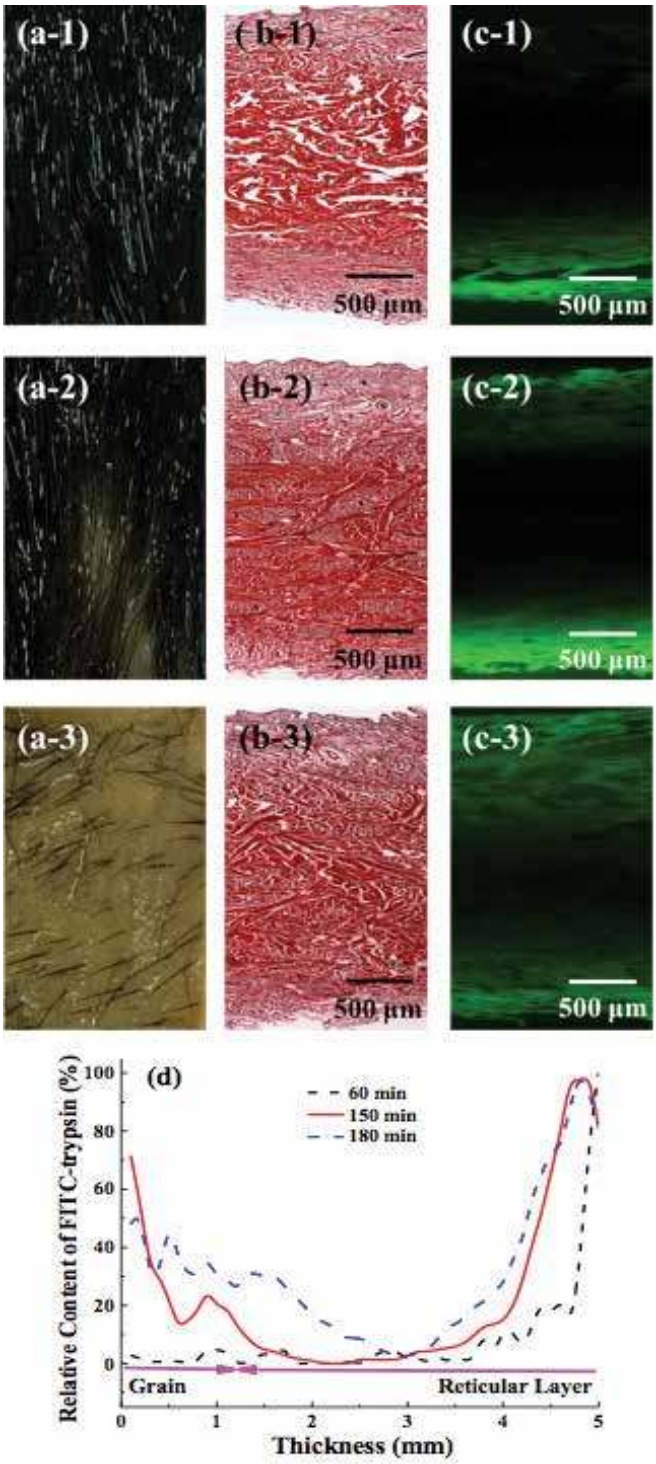

Figure 1. (a) Surfaces of hides captured by digital camera after enzymatic treating for 60 min (a-1), 150 min (a-2) and 180 min (a-3); (b) Photomicrographs of vertical sections (Van Gieson stain) from hides that were treated by enzyme for 60 min (b-1), 150 min (b-2) and 180 min (b-3); (c) Fluorescence micrographs of FITC-trypsin (green) in the vertical sections of cattle hides that were treated by enzyme for 60 min (c-1), 150 min (c-2) and 180 min (c-3); (d) Distribution of FITC-trypsin in the enzymatic treated cattle hides (the relative content of FITC-trypsin in hide was quantified by analysis of Figures 1(c-1), 1(c-2) and 1(c-3) using Image J software).

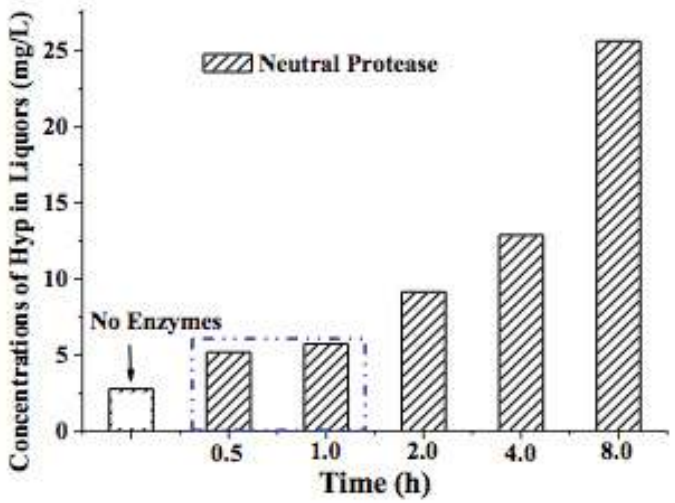

Figure 2. Effect of treatment time of neutral protease on damage to hide collagen (20 units/g hide, 25°C)

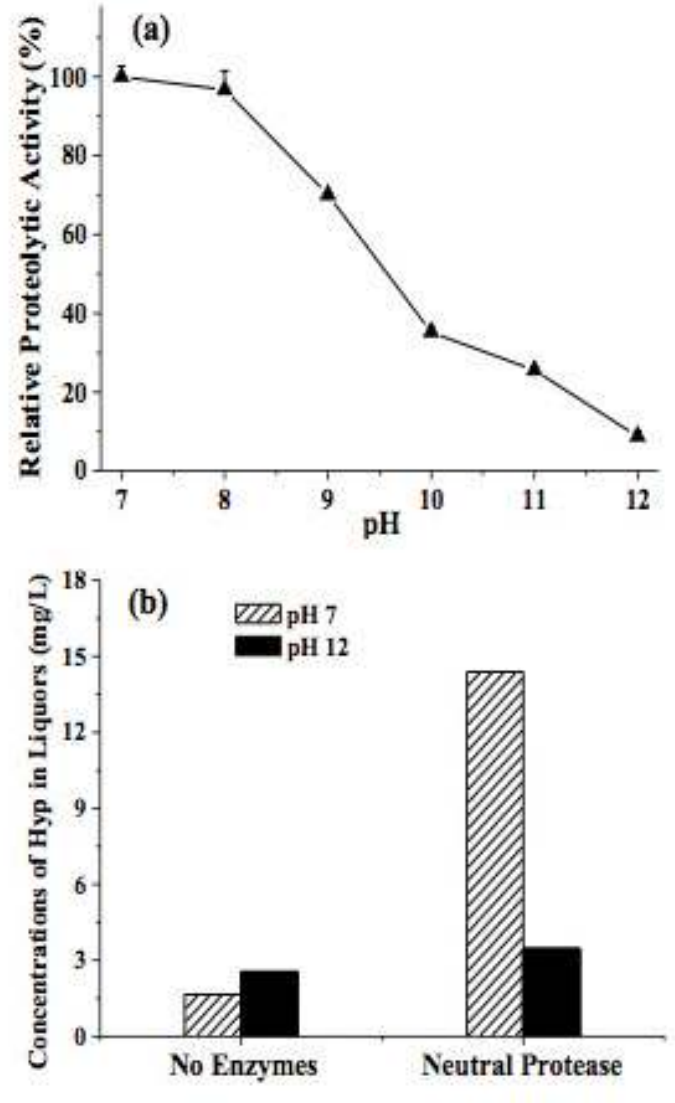

Figure 3. Effect of pH on the proteolytic activity of neutral protease (a) and the damage to collagen fiber caused by neutral protease (b)

Comparing Figures 4(a-LS) and 4(a-E) shows that, after hair immunization by lime, epidermis (black or brown) was nearly entirely remained in hide in LS unhairing system, while a majority of epidermis was removed from hides in EALS unhairing system. These results demonstrated that a mild treatment of neutral protease (20 units/g hide, 40 min, 22°C) was quite effective in hydrolyzing and removing epidermis from soaked hides (pH 7-9) before hair immunization. For both of the systems, hair (yellow) was well remained in hides. According to Figures 4(b-LS), 4(b-E), 4(c-LS) and 4(c-E), it is obvious that the cleavage of epidermis by using neutral protease was very beneficial to a rapid and complete removal of hair and epidermis by using a small amount (0.8%) of sodium sulfide. This proves that the cleavage of epidermis in hide by neutral protease can largely improve the unhairing function of sodium sulfide, so as to prevent synergistic effect between protease and sulfide.

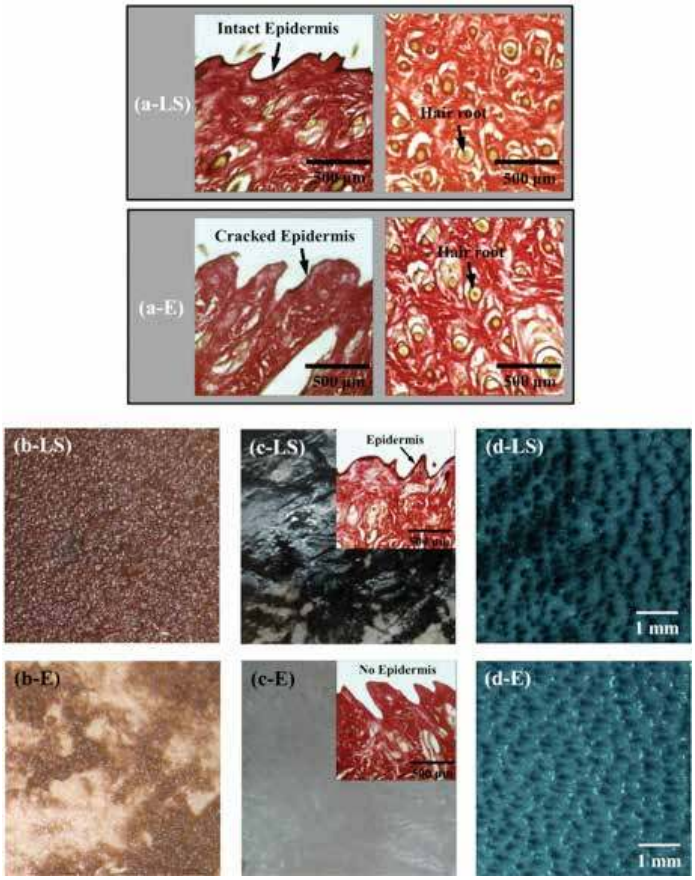

Figure 4. (a) Photomicrographs of vertical sections and horizontal sections (Van Gieson stain) from cattle hides after hair immunization with 1% lime for 90 min: (a-LS) LS technique; (a-E) EALS technique. (b) Digital photos of surfaces of the hides after treating by 0.8% NaCl for 15 min: (b-LS) LS technique; (b-E) EALS technique. (c) Digital photos of surfaces and photomicrographs of vertical sections (Van Gieson stain) from unhaired pelts after treating by 0.8% NaCl for 60 min: (c-LS) LS technique; (c-E) EALS technique. (d) Grain of chrome tanned leathers observed by stereomicroscope: (d-LS) LS technique; (d-E) EALS technique.

Moreover, the undesired case of excessive hair immunization can be avioded by using EALS unhairing technique. Our experiments demonstrated that, in EALS unhairing system, hair and epidermis could be completely removed by using 0.8% Na_2S, even though hair of soaked hide was immunized with lime for 90 min at 35°C (data not shown). From Figures 4(d-LS) and 4(d-E) it can be seen that the grain surface of chrome tanned leather processed by EALS unhairing was considerably cleaner compared with that processed by LS unhairing. This suggests that the EALS unhairing can guarantee good quality of resultant leather based on complete removal of hair and epidermis from hide and timely inactivation of protease.

Comparison of Conventional Sulfide-lime Unhairing and EALS Unhairing

The main purpose of this study was to develop an eco-friendly unhairing system that can be applied on a commercial scale. Therefore, in this section, the unhairing effectiveness, the morphology and the physical properties of leathers produced by using EALS unhairing, and the pollution loads in the EALS unhairing effluent were compared with those by using conventional sulfide-lime unhairing.

The limed pelts processed by conventional sulfide-lime unhairing and liming (control process) and EALS unhairing and low-lime liming (experimental process) are shown in Figures 5(a) and 5(b), respectively. It can be seen that the EALS unhairing system completely removed hair and epidermis from cattle hide and resulted in a cleaner grain surface compared with conventional sulfide-lime unhairing system. Therefore, the crust leather processed from EALS unhaired pelt had more uniform color (see Figure 6). These results also indicated that protease was more useful to remove epidermis and pigment from hide than soudium sulfide. As listed in Table IV, the physical properties of the experimental crust leather, such as tensile strength, percentage elongation at break, tear load and softness, were comparable to those of the control crust leather. These results demonstrated that an appropriate replacement for sulfide soudium and lime by neutral protease and swelling agent, along with a smart process control in the experimental process, could guarantee a better quality of resultant leather.

The pollution loads in the control and experimental unhairing and liming effluents are given in Table V. The S^{2-} load in the experimental effluents was reduced by about 82%, which was attributed to the dramatic decrease in the input of sodium sulfide. The TS and SS loads in the experimental effluents were reduced by 43% and 93%, respectively, because approximately 20 kg of dry hair was recovered, and the input of lime was reduced from 75 kg to 30 kg (see Tables II and III) when 1.0 ton of raw hides were processed. Moreover, due to the recovery of hair, the COD, TOC and TN loads in the experimental effluents were reduced by 53%, 50% and 20%, respectively.

The costs of chemicals used for processing 1.0 ton of raw hides were calculated to evaluate the economic feasibility of the experimental process. As shown in Table VI, neutral protease, sodium chloride and swelling agent were employed in the experimental process to partially replace sodium sulfide and lime used in the control process. The chemical cost of the experimental process was slightly higher than that of the control process. But the reduction of pollution loads by using the experimental process was considerable, which brings about a decrease in effluent treatment cost. So overall, the experimental process has economic feasibility.

Figure 5. Limed pelts captured by digital camera: (a) control; (b) experiment.

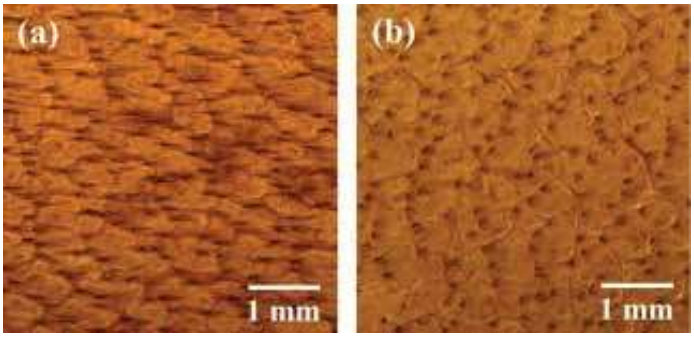

Figure 6. Grain of curst leathers observed by stereomicroscope: (a) control; (b) experiment.

Table IV
Physical properties of control (C) and experimental (E) crust leathers.

Sample	Tensile strength (N/mm^2)	% Elongation at break	Tear load (N)	Softness (mm)
C	12.7	44.5	81.0	6.3
E	13.6	40.8	73.7	6.2

Table V
Pollution loads in control (C) and experimental (E) unhairing and liming effluents (Unit: kg/ton of raw hides).

Sample	S^{2-}	TS	SS	COD	TOC	TN
C (No. 1)	3.64	96.05	46.75	70.00	18.54	3.85
E (No. 2+No. 3)	0.66	54.31	3.06	33.14	9.31	3.07

Table VI
Cost of the chemicals used in control (C) and experimental (E) unhairing and liming processes.

Chemicals	Unit price (RMB/kg)	Chemical consumption (kg/ton of raw hides)		Chemical cost (RMB/ton of raw hides)	
		C	E	C	E
Sodium sulfide	2.5	28	8	70.0	20.0
Lime	0.7	75	30	52.5	21.0
Degreasing agent	7.0	3	3	21.0	21.0
Neutral protease	10.0	-	4	-	40.0
Sodium chloride	0.5	-	8	-	4.0
Swelling agent	2.0	-	20	-	40.0
Total				143.5	146.0

Conclusions

Based on pH-sensitivity of neutral protease and the synergistic effect of protease, lime and sulfide, the enzyme assisted low-sulfide hair-save unhairing technique was developed, which favors production of high quality leather and a remarkable decrease in S^{2-}, TS, SS and COD in effluent. The removal of epidermis from soaked hide by neutral protease is the key step to improve unhairing by sulfide with a reduced offer. The rational and timely control of protease activity in the processing is very important to prevent leather from defects.

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