Paper B20

Enzymatic unhairing of cattle hide by protease and α-amylase

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Abstract

Enzymatic unhairing is a potential alternative to conventional unhairing using lime and sodium sulfide, but unhairing of cattle hide using only protease usually cannot remove hairs, especially fine hairs, completely. In order to eliminate this defect, the composite of protease and α-amylase was employed for lime and sulfide free unhairing of cattle hide. The effects of pH and temperature on the activities of alkaline protease and α-amylase were determined, and the unhairing conditions were optimized by analyzing the extent of hair removal and the concentration of hydroxyproline in unhairing liquor. The experimental results indicated that the addition of α-amylase benefits the removal of fine hairs during enzymatic unhairing process. The optimum conditions of unhairing were 0.3% protease, 0.1% α-amylase and 50% water at pH 7 and 25 ^{o}C for 2 h (percentages based on weight of soaked hide), and the performance of complete removal of hair can be achieved by drum method without using beam and blunt knife.

1. Introduction

Conventional unhairing technique using sodium sulfide and lime is an effective and economic approach to hair removing because of the low costs of the chemicals used and high quality of leather products. However, the conventional unhairing process suffers from the high sulfide content, plenty of lime sludge and hair degradation products in the tannery wastewater.[1] Thus, some environmentally acceptable unhairing technologies, such as "Sirolime" unhairing, "Blair" unhairing, enzymatic unhairing, and oxidative unhairing, have been investigated to reduce the pollution loads. [2-7]

The enzymatic unhairing using neutral protease or alkaline protease has been extensively studied in recent decades and is considered to be the most eco-friendly unhairing technology.[4, 5, 8-10] However, some disadvantages of enzymatic unhairing have restricted its practical application. One of the disadvantages is that unhairing using protease alone usually cannot remove hairs completely, especially fine hairs, which will reduce the quality of leather.[1, 11]

Alpha-amylase, which is widely used in food industry, detergents, textile industry and paper industry, has the hydrolytic reaction on carbohydrate containing proteoglycans.[12, 13] It was found that cleavage of proteoglycans is beneficial to the action of protease on hide.[14] Thus, it is reasonable to hypothesize that the introduction of α-amylase during enzymatic unhairing process will obtain a higher extent of hair removal.

In this study, the composite of protease and α-amylase was employed for removing hairs from cattle hide by drum method. The effects of pH and temperature on the activities of protease and α-amylase were determined, and the unhairing conditions were optimized by analyzing the extent of hair removal and the concentration of hydroxyproline (Hyp) in unhairing liquor. In addition, the effectiveness of unhairng using the composite of protease and α-amylase was compared with that of conventional unhairing using lime and sodium sulfide.

2. Materials and method

2.1 Materials

Conventional soaked cattle hides were used for unhairing trials. All the chemicals used for conventional leather processes were of commercial grade. The protease used for unhairing was an alkaline endo-protease by a genetically modified strain of *Bacillus alcalophilus* and of commercial grade. The α-amylase used for unhairing was of biochemical grade. The chemicals used for the analyses were of analytical grade.

2.2 Enzyme activity assay

2.2.1 Effects of pH and temperature on protease activity

The effects of pH and temperature on protease activity were studied by incubating 1 mL of 0.1 mg/mL enzyme solution with 1 mL of 2% (w/v) casein solution prepared in buffers of different pH at different incubation temperatures (25 °C, 30 °C, 35 °C, and 40 °C) for 10 min. Then the incubation was stopped by mixing the enzyme-casein solution with 2 mL of 0.4 mol/L trichloroacetic acid. The mixture was settled at 25 °C for 20 min and successively filtered. The concentration of tyrosine in the filtrate was determined.[15] The buffers were phosphate buffer (pH 7.2, pH 8.0) and boric acid-saline buffer (pH 9.0, pH 10.0, pH 11.0). One unit of protease activity is defined as the amount of enzyme which releases 1 μg tyrosine per minute.

2.2.2 Effect of pH on α-amylase activity

1 mL of 1.0 mg/mL enzyme solution was mixed with 1 mL of Briton-Robinson buffer (pH 5.6, 7.0, 8.0, 9.0, 10.0). The mixture was heated at 25 °C for 15 min and then incubated with 1% (w/v) starch solution at 25 °C for 5 min. 4 mL of 0.4 mol/L NaOH solution was successively added into the mixture to stop the reaction. The mixture was filtered and the concentration of maltose in the filtrate was determined. One unit of α-amylase activity was defined as the amount of enzyme which releases 1 μg maltose per minute at 25 °C.[16]

2.3 Sampling for unhairing trials

The soaked cattle hide was washed and divided into eight groups numbered as Figure 1 for the following unhairing trials. Each group samples include four pieces of soaked hides taken from the neck, back, belly and butt areas, respectively.

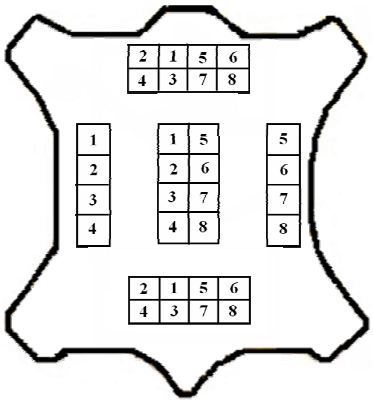


Figure 1 Sampling of soaked cattle hide for unhairing trials

2.4 Optimization of unhairing using protease

An orthogonal experimental design L_{16} (4^5) (Table I) was used to optimize the conditions of unhairing using protease. The effects of temperature, time, initial pH of unhairing liquor, amount of protease and amount of water on unhairing were evaluated by analyzing the extent of hair removal after deharing.

Table I The orthogonal experimental design L_{16} (4^5) [a]

Factor	Temperature (°C)	Time (min)	pH	Amount of protease (%)	Amount of water (%)
Level 1	15	90	7.0	0.2	30
Level 2	20	120	8.0	0.3	50
Level 3	25	150	9.0	0.4	80
Level 4	30	180	10.0	0.5	100

a - Percentage is based on weight of soaked hide.

2.5 Optimization of the amount of α-amylase

Unhairing was performed in the solution of X% α-amylase (X represents amount of α-amylase, which is 0, 0.05, 0.10, 0.15, and 0.20), 0.3% protease and 50% water at 25 °C and pH 7. After unhairing, the concentrations of Hyp in unhairing effluents were analyzed.

2.6 Comparison of enzymatic unhairing and conventional unhairing

One soaked hide was cut along the backbone and made into two sides. One side was unhaired by using the composite of protease and α-amylase for experiment, and the other was unhaired by using sodium sulfide and lime for control. The experimental and control unhairing and liming processes are shown in Table II and Table III, respectively. After liming, the pelts were delimed, bated, pickled and chrome tanned according to the conventional procedures. The limed pelts were freeze-dried and their grains were observed by Scanning Electron Microscope (SEM, JSM-5900LV, JEOL LTD., Japan). After chrome tanning, the shrinkage temperature (Ts) of wet blue was measured by using a shrinkage temperature recording instrument. Then the chrome tanned leather products were piled for 24 h and their chromium contents were analyzed.

Table II Experimental unhairing and liming processes

Process	Offer of agent	Time
Unhairing	50% water, 0.3% protease, 0.1% α-amylase	120 min
Washing		20min
Liming	6% lime, 250% water	30min
Run on automatic-stop 55 min/run 5 min for 10 h, overnight. Next day run 30 min.		

Table III Control unhairing and liming processes

Process	Offer of agent	Time
Unhairing and liming	100% water, 1% NaHS	Run 20 min/ stop 40 min
	1% Na_2S	Run 20 min/ stop 40 min
	1% lime	Run 20 min/ stop 20 min
	0.5% Na_2S, 0.5% lime	Run 20 min/ stop 40 min
	0.5% Na_2S, 0.5% lime, 0.5% wetting agent	Run 20 min/ stop 40 min
	6% lime	10min
	300% water	10min
Run on automatic-stop 55 min/run 5 min for 8 h, overnight. Next day run 30 min		

2.7 Estimation Methods

2.7.1 Determination of Hyp concentration

Unhairing liquors were filtrated by using 100 mesh filter cloth, and their Hyp concentrations were determined by the method described in literature.[17]

2.7.2 Determination of the extent of hair removal
The extent of hair removal was monitored and rated on the basis of the area without hair out of the total area of unhaired pelts.

2.7.3 Determination of chromium content
2.0 g of chrome tanned leather was added into 100 mL of 2mol/L HCl solution, followed by constant shaking in 130 rpm for 4 h.[18] Then the mixture was filtered and the concentration of chromium in filtrate was determined by using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES, Optima 2100DV, PerkinElmer, USA). The moisture content of chrome leather was initially analyzed and the chromium content was calculated as:

$$\text{chromium content} = \frac{Cr_2O_3 \text{ weight in chrome leather}}{\text{dry weight of chrome leather}} \times 100\%$$

3. Results and discussion

3.1 Optimization of unhairing using protease

The activity of enzyme has a great effect on the effectiveness of enzymatic unhairing. Therefore, effects of pH and temperature on the protease activity were first investigated in this study. As shown in Figure 2, the activity of protease was relatively lower at neutral pH and increased with the increase of pH. The damage of hide collagen will increase significantly if temperature is higher than 40 ^{o}C during unhairing process, so the temperature range employed herein is from 25 ^{o}C to 40 ^{o}C. It was found that the increase of temperature exhibited positive effect on the protease activity, which could be attributed to the fact that the increase of temperature enhances the rate of collision between the protease and the substrate.[19]

The extent of hair removal from pelt is a key parameter to evaluate the effectiveness of unhairing, because the remaining hairs on the surface of pelts have a negative effect on leather quality. The effects of unhairing conditions such as temperature, time, initial pH of unhairing liquor, amount of protease and amount of water on the extent of hair removal are shown in Figure 3. According to the orthogonal experiment, the effects of unhairing conditions were ranked as follows: temperature > time > pH > amount of protease ≈ amount of water. The optimum conditions for unhairing by protease were 0.3% protease and 50% water at 25 ^{o}C and pH 10 for 150 min. However, it was observed that unhairing using only protease by drum method under optimum conditions could not remove hairs completely, especially fine hairs.

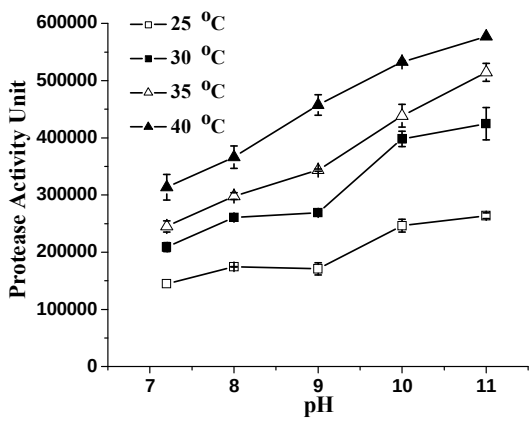


Figure 2 Effects of pH and temperature on protease activity

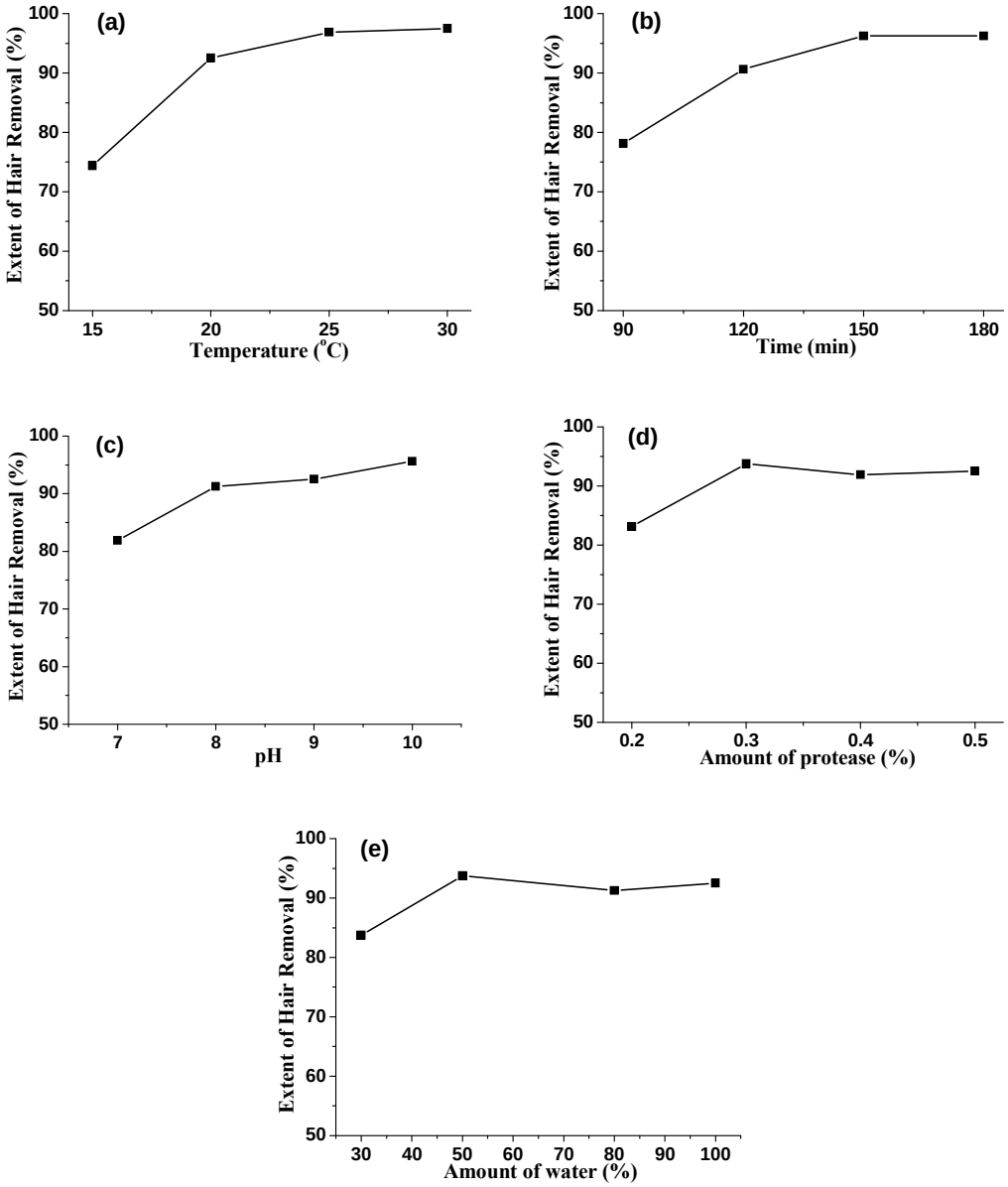


Figure 3 Effects of (a) temperature, (b) time, (c) pH, (d) amount of protease and (e) amount of water on the extent of hair removal

3.2 Optimization of unhairing using the composite of protease and α-amylase

The effect of pH on the α-amylase activity is presented in Figure 4. The activity was significantly inhibited when the pH is higher than 8.0. So cattle hides were unhaired in the solution of 0.3% protease, a certain dosage of α-amylase and 50% water at pH 7 and 25 °C to achieve a higher extent of hair removal. It was observed that hairs were removed completely by drum method for only 2 h, when the dosage of α-amylase was 0.1% or larger. The result suggests that the composite of protease and α-amylase has an obviously synergistic action for removing hairs, especially fine hairs, and therefore, could effectively eliminate the defect of unhairing using protease alone. This phenomenon can be explained by the fact that α-amylase can result in the cleavage of proteoglycans in the hide, which is beneficial to the action of protease on the hide.[13, 14] As shown in Figure 5, the concentration of Hyp in unhairing liquors was almost unchanged with the increase of the dosage of α-amylase. Thus, the

optimum dosage of α-amylase is 0.1% in consideration of the cost. Further experiments indicated that the optimum deharing conditions were the composite of 0.3% protease and 0.1% α-amylase, 50% water and pH 7 at 25 °C for 2 h. These unhairing conditions are very acceptable, where the initial pH of unhairing liquor is neutral and eco-friendly, and the temperature is lower than those of other enzymatic unhairing processes. In addition, unhairng in the drum for only 2 h can save much time and labor compared with commonly enzymatic unhairing.[10, 20]

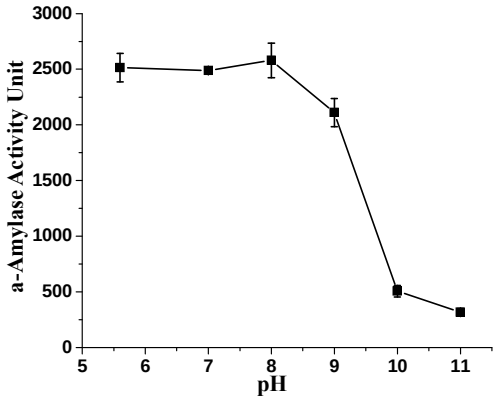


Figure 4 Effect of pH on α-amylase activity

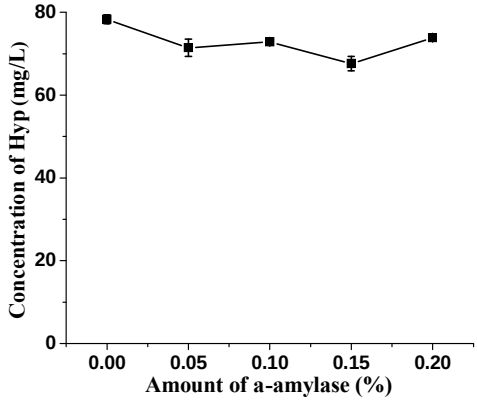
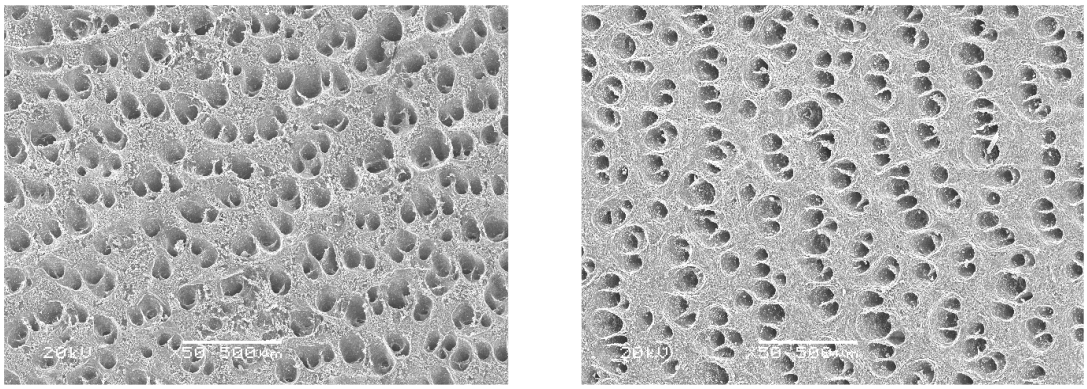


Figure 5 Concentration of Hyp in unhairing liquors

3.3 Comparison of control and experimental unhairing

As shown in the SEM photos (Figure 6a and 6b), hair pores of both the control and experimental unhaired pelts were visible and clear, which indicates that unhairing using protease and α-amylase did not damage the grain. The chromium content and Ts of chrome tanned leathers using control and experimental unhairing processes are shown in Table IV. The chromium content and Ts of the experimental chrome tanned leather were higher than those of the control, which means that unhairing using protease and α-amylase benefits the absorption of chromium. These facts indicated that unhairing using the composite of protease and α-amylase can improve the degree of fiber opening, which enhances the uptake of chromium.[21]



(a) (b)

Figure 6 Grains of unhaired pelts observed by SEM at a magnification of 50: (a) experiment and (b) control

4. Conclusions

Alpha-amylase is effective in helping protease remove fine hairs because of its hydrolytic reaction on proteoglycans. The composite of protease and α-amylase exhibits outstanding ability to remove hairs completely from cattle hide when the drum method was used for unhairing. Protease in combination with α-amylase can be used to replace conventional lime and sodium sulfide for unhairing process.

Table IV Chromium content and Ts of chrome tanned leathers using control and experimental unhairing

Samples	Cr_2O_3 content (%)	Ts (^oC)
Experimental	2.68	112
Control	2.35	105

5. References

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6. Acknowledgements

This work was supported by the National High Technologies R&D Program (2011BAC06B11).