

## STUDY OF THE APPLICATION OF WB600-KERT IN UNHAIRING PROCESS

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**Abstract.** Unhairing process is usually considered as the most polluted process in leather production. The conventional method of unhairing which using lime and sodium sulfide produces a large amount of sludge and waste water. In order to reduce pollution, we developed a novel unhairing enzyme and named as Wb600-KerT, which possesses low collagen-degrading ability and high keratin-degrading ability in previous study. The objective of this study is to study the properties and effect of Wb600-Kert to replace traditional chemicals in unhairing process. It found that the protease of Wb600-Kert exhibited optimum keratin activity at 40°C. Compared with commercial unhairing enzyme and conventionally sodium sulfide methods, Wb600 exhibited better unhairing effect and higher efficiency. The results indicated that goat skin unhaird with Wb600 achieve enough softness, shrinkage temperature and tear strength as well as conventionally sodium sulfide methods. Furthermore, if adding a small amount of sodium sulfide, the unhairing process could be accelerated while the unhairing effect was further improved. Generally speaking, this enzyme showed good application potential in unhairing process and was effective for reducing pollution which may promote the development of leather industry.

### 1 Introduction

Leather making is a significant manufacture over the world, however, it is facing the threat of closure because of environmental pollution problems day by day. The problems are mainly occupied by some chemicals utilized during the leather processing, and thence it is important to find cleaner technologies to improve the heavy pollution problems. Generally, unhairing is an indispensable process and it occupies fundamental pollution<sup>1</sup>. The traditional method used lime and sodium sulfide led to solid waste and wastewater contained high levels of sulfur. When referring to this field, the usage of clean technology in unhairing process is urgent.

The hair preserving unhairing process was gradually accepted and replacing the position of conventional lime-sodium sulfide unhairing. Among these, Enzymatic unhairing is the most promising approach to decrease environment problems. Enzymes are specificity, toxic free and safe, it can accelerate the reaction rate by reducing the activation energy of the chemical reaction. As a representative alternative for lime-sulfide process, enzymatic unhairing attracted much attention in recent years. The biggest obstacle for the promotion of enzymes in unhairing was usage and time.

In this paper, a novel genetically modified protease named Wb600-KerT which exhibited low collagen-degrading ability and high keratin-degrading ability was applied in unhairing process. The combination of 0.5% sodium sulfide and 0.04% 2520 U/g Wb600-KerT can gain good unhairing effects. After normal tanning, the shrinkage temperature and tear strength of crust leather can achieve 96 °C and 15.06 N/mm, respectively. These results indicated that enzyme Wb600-KerT has a potential application in enzymatic unhairing.

## 2 Materials and methods

### 2.1 Reagents

Goat salt dry skin (Preparation from Shandong Juncheng Leather Industry Co., Ltd.), Enzyme Wb600-KerT (Preparation from Tianjin University of Science and Technology Microbial Deposit Center), Enzyme X-Zyme 4072 (Preparation from Langsheng Co., Ltd.), Enzyme Dowell UHE (Preparation from). Glacial acetic acid, glycine, tyrosine, trichloroacetic acid, chloramine T and anthrone were all analytical grades and obtained from Sigma-Aldrich (Shanghai, China).

### 2.2 Determination of protease activity

Protease activity was measured by using modified method of Songjian<sup>2</sup>. Casein is used as the substrate. One unit of protease was considered as the amount of enzyme which catalyzes the release from casein to 1 µg of tyrosine per minute under certain temperature and pH conditions.

### 2.3 Determination of collagenase activity

Collagenase activity was estimated by the method of Qiangqiang Zhou<sup>3</sup>, using type I collagen as substrates prepared in 0.1 mol/L PBS buffer (pH=7.5). Reaction systems comprised of 10 mg substrate and 1 mL of appropriately diluted enzyme. Reaction mixtures were incubated at 37 °C for 40 min, stopped using 10% chilled TCA. The amount of released free glycine was measured by the ninhydrin colorimetry method. One unit of collagenase activity was defined as the amount of hydroxyproline enzyme that released 1 µg per minute under the conditions used.

### 2.4 Determination of keratinase activity

Keratinase activity was measured by using modified method of Gradisar<sup>4</sup>. Wool powder used as the substrate was grinded before the experiment. Insoluble residues were removed by centrifugation through TGC-16G high speed benchtop centrifuge (Shanghai, China). Assay mixture containing 10 mg substrate and 2 mL of enzyme was incubated at 40 °C for 1 h, then the reaction was stopped using 10% TCA. One unit of protease was considered as the increase of 0.01 at 280 nm.

### 2.5 Goat hide unhairing assays

Before unhairing, the goat skins were soaked with water containing fungicide, degreaser and alkaline protease until they are in good conditions. Then the soaked hide was weighed and put into controllable drum, a variety of enzyme and chemicals was separately added into different drums for unhairing under 40 °C within 4 h. After the function of enzyme, 1.2% and 1.3% calcium hydroxide was added within 2 h in order. Finally, the hide was left overnight in the drum. Next day, the waste liquid was collected for further analysis.

### 2.6 Later process of goat hide

After unhairing, the hides were washed with 300% water for 10 min for twice to remove hair and de-hairing enzyme. The pelts were delimed with 2.5% ammonium sulfate and 150% water, the treatment was for 1 h. In the following pickling process, the pelts were treated with 0.3% formic acid, 1.5% sulfuric acid, 8% sodium chloride and 150% water for 3 h. The eventual pH value was adjusted to 3. Then 4% glutaraldehyde, 8% sodium chloride and 80% water were added together, subsequently 2.5% baking soda was added within 90 min in three times. After that, the skins were neutralized with 150% water and 1% ammonium sulfate for 1 h. When retaining, 100% water, 15% jing bark silicone

were taken for 2 h, then 0.5% diluted formic acid were used for fixing. At last, 4% sulfonated fats, 4% synthetic fats, 4% beef hoof and 150% water used for fatliquoring with running 2 h under 50°C.

## 2.7 Analysis of polysaccharide concentration

The waste liquid of unhairing were collected and filtered as samples. The proteoglycan concentration of samples was measured by using a modified method of Lin.<sup>5</sup> The reaction of proteoglycan and anthrone was performed in a 10 mL test tube; the mixture was place at a boiling water bath for 15 min. After cooling down to room temperature by cold water for 10 min, the absorbance was measured at  $\lambda=628$  nm in order to calculate the proteoglycan concentration of the samples.

## 2.8 Physical properties of crust leather

Samples from crust leathers were cut for various physical tests. Before tests, they were conditioned at  $20\pm 2$  °C and  $65\pm 2\%$  R.H. over a period of 48 h. For certain tests, such as those performed to evaluate tear strength, shrinkage temperature and softness follow standard methods<sup>6</sup>.

# 3 Results and Discussions

## 3.1 Various enzyme activities in protease used in the experiments

**Table 1.** Different protease activities.

Protease activity	Wb600-KerT	X-Zyme 4072
Collagenase activity	260 U/g	7.14 U/mL
Casein activity	3720 U/g	26.86 U/mL
Keratin activity	2520 U/g	48.7 U/mL

As shown in table 1, the enzymes tested in the experiments exhibited a certain vitality. Enzyme unhairing mainly hydrolyze soft keratin and hair root sheath polysaccharide protein, which weakens the relationship between hair bags and hair shafts, and achieves hair removal effect by mechanical action eventually<sup>7</sup>. Therefore, keratin activity is a key point be considered. when under the same keratin activity, the collagenase activity of enzyme Wb600-KerT is inferior to X-Zyme 4072 because that the alternation of Wb600-KerT decrease the activity of collagenase lead to it suitable for unhairing. The post-sequence unhairing experiment was established according to the industrial routine method of X-Zyme 4072, further compared with conventional lime sodium sulfide unhairing to explore the application condition unhairing of Wb600-KerT.

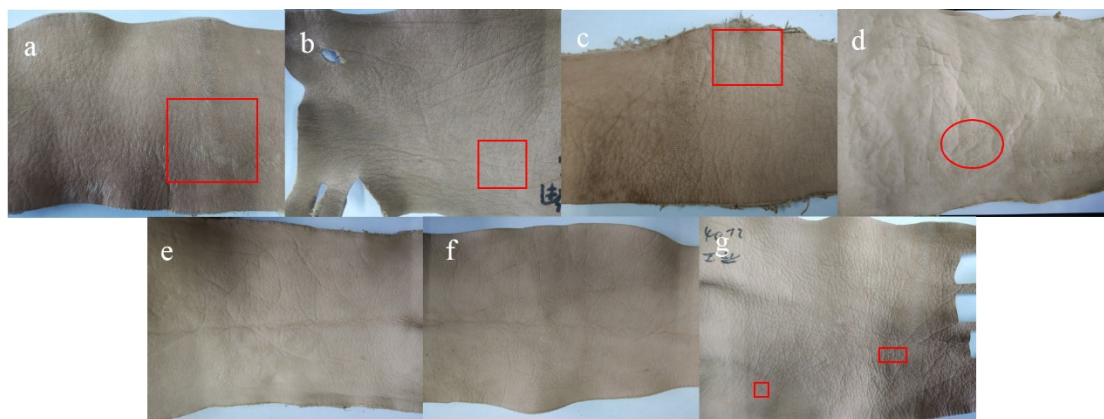
## 3.2 Unhairing methods of different groups

**Table 2.** Different unhairing methods in the experiments.

methods of unhairing	the compoments of unhairing
A	0.006% 2520 U/g Wb600-KerT
B	0.01% 2520 U/g Wb600-KerT
C	0.04% 2520 U/g Wb600-KerT
D	0.1% 2520 U/g Wb600-KerT
E	0.04% 2520 U/g Wb600-KerT+0.5% Na <sub>2</sub> S
F	2.5% Na <sub>2</sub> S+2% Ca(OH) <sub>2</sub>
G	0.3% 48.7 U/mL X-Zyme 4072+1% Na <sub>2</sub> S+0.5% NaHS

As presented in table 2, methods from A to D were unhairing by different dosage of keratin Wb600-KerT. In addition, method F was a conventional unhairing in the leather industry, method G was a common enzymatic unhairing used in industries. The keratin activity of method A and G was equality. For the early research, we know that 0.1% 2520 U/g of Wb600-KerT was safety for unhairing. While 1.5% sodium sulfide was used for enzymatic unhairing. Therefore, 0.5% sodium sulfide was used to assist the enzyme in this experiment for exploration the application of Wb600-KerT.

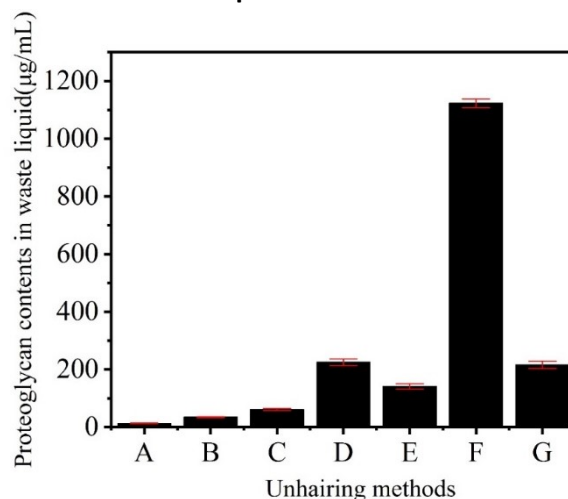
### 3.3 The observation of crust leathers



**Fig. 1.** The observation of crust leathers by different unhairing methods (a. Method A, b. Method B, c. Method C, d. Method D, e. Method E, f. Method F, g. Method G)

As shown in Figure 1, after the same tanning process, the surface of several methods of unhairing seemed to be more visible. In method A, certain hair can be found in the surface of the leather partly due to the lower dosage of Wb600-KerT. Along with the increasing of Wb600-KerT, the remain of hair become less to less even none. However, when the usage of 2520U/g Wb600-KerT gained to 0.1%, the grain surface of leather was damaged mainly because of the high activity of Wb600-KerT which lead to the high degradation of collagen in leathers. Compared with last three crust leather, little residual hair can be found, however, in unhairing method G, hair removal was not complete, it confirmed the possibility of incomplete unhairing by common enzymatic unhairing. Above all, the safety usage of 2520 U/g Wb600-KerT was 0.1%, the combination of 0.5% sodium sulfide and 0.04% 2520 U/g Wb600-KerT can achieve excellent unhairing.

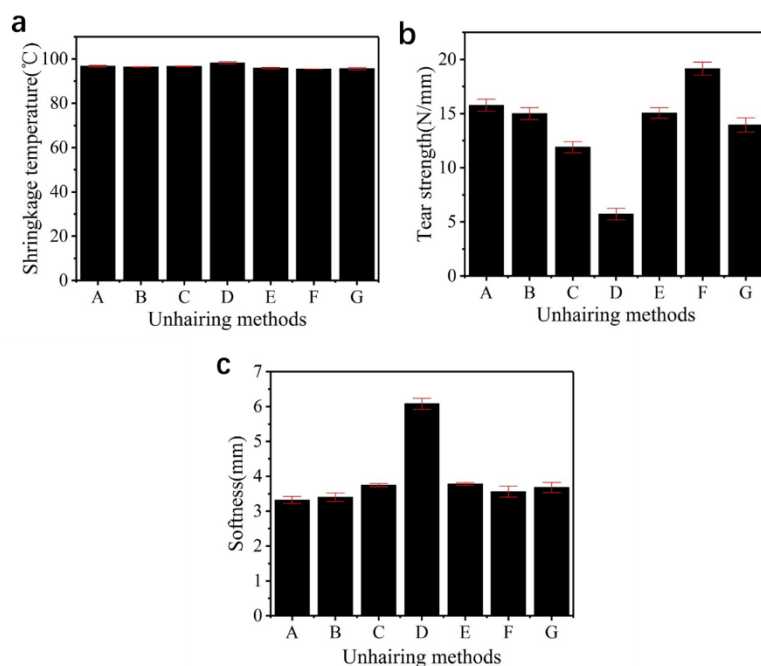
### 3.4 Polysaccharide contents in waste liquid after different unhairing methods



**Fig. 2.** Changes in polysaccharide contents in waste liquid after different unhairing methods.

As we all known, the proteoglycans are the main components of fibrous interstitial and were widely distributed in the hides. In addition, proteoglycans were also the core substance which connect the hair and the epidermis. Hence, the removal of proteoglycans was beneficial for unhairing and dispersion of enzymes. After different methods of unhairing, the polysaccharide of waste liquid was shown in Fig 2. It indicated that from Method A to D (increase the usage of enzyme Wb600-KerT), more and more proteoglycans were degraded by enzyme Wb600-KerT owing to the high activity which is consistent with former theory. In the method F, for one thing proteoglycans consist of several disulfide bonds, for another the sodium sulfide can hydrolyze the disulfide bonds. These factors lead to the highest content of proteoglycan. When the combination of 0.5% sodium sulfide and 0.04% 2520 U/g Wb600-KerT used for unhairing, sodium sulfide can dissolve part epidermis then accelerate the promotion of enzyme, degrade more plasmin, his method can get higher unhairing efficiency. Similarity, method G achieve unhairing by degrade plasmin. In the end, the combination of 0.5% sodium sulfide and 0.04% 2520 U/g Wb600-KerT for unhairing can gain good efficiency.

### 3.5 The physical analysis of crust leather



**Fig. 3.** The physical properties of crust leather (a. shrinkage temperature b. tear strength c. softness).

The most quickly and convenient way to determine the shrinkage temperature was to record the temperature when the leathers first shrinks in hot water. The skin after tanning, the tanning agent binds to reactive group in skin to form new crosslink, thereby the shrinkage temperature of leather increased. As shown in Fig.3.a, after seven methods of unhairing, the crust leather seemed little difference in shrinkage temperature. In method D, the shrinkage temperature of it higher than others. It possibility that excessive enzyme Wb600-KerT expose more binding sites, then combine tinctures to gain higher shrinkage temperature. However, this method degraded much collagen even caused the danger of losing the surface. The skin of unhairing by 0.5% sodium sulfide and 0.04% 2520 U/g Wb600-KerT, after tanning, it's shrinkage temperature up to 96°C revealed good thermal stability.

It's well known that tear strength is one of the important physical and mechanical properties of light leather. The higher the tear strength of the sample, the better quality of the crust leather. As

shown in Fig.3.b, with the usage of 2520 U/g Wb600-KerT increasing (from method A to D), the tear strength of crust leather decreased. It revealed that more enzyme can degrade more collagen which reduced the combined collagen and longer the distance between fibers. Therefore, the tear strength of crust leather declined. Method F and G attained normal effect. Apart from this, in method E, 0.5 % sodium sulfide assisted the enzyme Wb600-KerT, part of the epidermis was dissolved, the enzyme infiltrated into the skin which led to the amount of enzyme function on the epidermis decreased. The combination of collagen and tinctures become more and firm. The softness is an important hand feeling of leather, it revealed quality of crust leather. It was worth to pay attention to Fig.5, on the one hand, the softness of method D beyond normal level. It may be corresponding to previous theory, excessive enzyme Wb600-KerT degraded collagen and increase the distance between fibers eventually caused abnormal softness. Above all, the leather unhairing by 0.5% sodium sulfide and 0.04% 2520 U/g Wb600-KerT can acquire common requirements.

## Conclusions

In this paper, the combination of 0.5% sodium sulfide and 0.04% 2520 U/g Wb600-KerT for unhairing can gain excellent unhairing efficiency without damaging the properties of leather. The sodium sulfide made contributions to the penetration of enzyme Wb600-KerT in skin by dissolving part epidermis. What's more, Na<sup>+</sup> has a certain activation on the activity of Wb600-KerT. These factors led to the good results. The unhairing skin after normal tanning, it's shrinkage temperature can attain 96 °C and other properties near the leather unhairing by conventional lime-sodium sulfide. In addition, the amount of sodium sulfide used in this method is only one-third of the common dosage. It revealed enzyme Wb600-KerT has a potential application in unhairing.

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