

Evaluation of unhairing effectiveness of “pure” α -amylase

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Abstract

Researchers have recently shown renewed interest in the use of α -amylase preparations (AP) for non-sulfide or non-lime unhairing systems. However, the mechanism of AP action on hides or skins remains unclear. AP is generally a mixture of α -amylase and concomitant protease(s), and it is not yet known whether “pure” α -amylase or concomitant protease(s) are mainly responsible for unhairing. In order to determinate which components of AP actually have an ability to unhair, a protease-free α -amylase preparation (PFAP, viz. “pure” α -amylase) was obtained by thermal treatment of AP solution at 70°C for 15 min to inactivate protease(s) in AP, while an amylase-free α -amylase preparation (AFAP, viz. concomitant protease(s)) was obtained by oxidation treatment of AP solution with sodium hypochlorite solution to inactivate α -amylase in AP. The effectiveness of unhairing of cattle hide using AP, PFAP and AFAP was evaluated by analyzing the extent of hair removal from hides and the concentration of protein in the effluents. The experimental results indicated that AP and AFAP are both effective in removing hairs and protein from hides, while PFAP can hardly remove hairs. These results demonstrate that the concomitant protease(s) in AP are primarily responsible for unhairing rather than “pure” α -amylase, suggesting that the unhairing mechanism of AP should be consistent with that of protease.

Keywords: unhairing, α -amylase, protease, selective inactivation

1. Introduction

In order to reduce pollution produced by conventional sulfide-lime unhairing system, such as high sulfide content, high COD content in tannery wastewater and a large quantity of lime sludge, some researchers have shown renewed interest in the use of α -amylase for non-sulfide or non-lime unhairing systems in the last decade. They found that unhairing with the composite of α -amylase and protease resulted in a higher amount of hair removal in comparison with unhairing using protease alone (Song et al. 2007; Zeng et al. 2011). Furthermore, α -amylase has fiber opening-up action and is considered to be a potential substitute for lime (Aravindhana et al. 2004; Thanikaivelan et al. 2002).

However, the mechanism of action of α -amylase on hides/skins remains unclear. Burton, Reed and Flint (1953) found that amylase had ability to unhair, and they suggested that this may have been due to the removal of mucoid material. Bose, Madhava Krishna and Das (1955) supported the suggestion of Burton *et al.* and reported that unhairing using amylase depended essentially on the hydrolysis and removal of mucoid. But Gillespie (1953) argued that amylase could neither

unhair nor act on mucoid material. On the basis of Burton's and Gillespie's discussions, Cordon (1955) stated that commercial amylases could effectively remove hair from pretreated hides. However, Cordon also pointed out that commercial amylase preparations are not single enzymes and that the effective components of the amylase preparations for unhairing is not yet known. In fact, α -amylase preparations are generally mixtures of amylases and concomitant proteases, and the concomitant proteases are difficult and expensive to remove. (Hmidet et al. 2009; Leach and Hebeda 1980; Moseley and Keay 1970) In addition, it has been shown that many proteases are useful for unhairing (Choudhary et al. 2004). Therefore, for understanding the action mechanism of α -amylase preparation on hides or skins, it is necessary to first determine whether "pure" α -amylase is effective in unhairing.

In this study, a protease-free α -amylase preparation (PFAP) and an amylase-free α -amylase preparation (AFAP) were first prepared by selectively inactivating protease(s) and α -amylase in α -amylase preparation (AP). In order to evaluate the unhairing effectiveness of "pure" α -amylase, the extent of hair and protein removal from hide using AP, PFAP and AFAP were investigated.

2. Material and Methods

2.1 Materials

Conventional soaked cattle hide was employed for unhairing trials. Commercial bacterial α -amylase preparation (AP) was obtained from Youtell Biochemical Co. Ltd. (Shanghai, China). All the chemicals used for processes in leather manufacture were of commercial grade, and the chemicals used for the analyses were of analytical grade.

2.2 Methods

2.2.1 Preparation of PFAP and AFAP

In order to obtain PFAP (viz. "pure" α -amylase), the protease(s) present in AP were inactivated according to the method described by De Stefanis and Turner (1981) with minor modification. Briefly, 10 g/L AP solution was at 70°C for 15 min and then immediately cooling the solutions to room temperature. As a result, PFAP was obtained. The α -amylase activity of PFAP was assayed by the method described in literature (Bernfeld 1955). One unit of α -amylase activity was defined as the amount of enzyme which releases 1 mg maltose per minute at pH 7.0 and 25°C. The protease activity was assayed by the method described in standard (SB/T 10317-1999). One unit of protease activity was defined as the amount of enzyme which releases 1 μ g tyrosine per minute at pH 7.0 and 25°C. The residual relative α -amylase activity and protease activity were calculated as:

$$\text{residual relative enzyme activity} = \frac{\text{enzyme activity after inactivation}}{\text{enzyme activity before inactivation}} \times 100\%$$

For achieving AFAP (viz. concomitant proteases), the α -amylase in AP was inactivated according to the method established by Hoerle (1978). AFAP was obtained by adding sodium hypochlorite solution (20% of AP weight) into 10% (w/v) aqueous solution of AP, followed by shaking at 25°C for 2 h. After inactivation, the residual relative α -amylase activity and protease activity were analyzed.

2.2.2 Evaluation of unhairing effectiveness of AP, PFAP and AFAP

Four pieces of soaked cattle hide without hair slip were prepared for the following unhairing trials. Enzymatic unhairing was performed in the solution containing X% enzyme (X represents amount of enzyme, as listed in Table 1) and 50% water at 25°C for 4 h. After unhairing, the unhairing liquors were sampled and centrifuged at 8000 rpm for 10 min. Then, the supernatant liquors were taken for measurement of protein concentration as reported in the document (Lowry 1951). The grain of unhaired pelts was captured using a digital camera. Furthermore, samples cut from unhaired pelts were fixed in 10% neutral buffered formalin for 48 h. Subsequently, the samples were cut into sections of 15 µm thickness using a freezing microtome (CM1950, Leica, Germany). The sections were stained with Weigert's iron hematoxylin and then counterstained with Van Gieson's stain. After staining, the histological sections were observed using a biologic microscope (CX41, Olympus, Japan).

Table 1 Enzymes used for unhairing trials ^a

Number of group	Offer of enzyme
1 (control)	no enzyme
2	0.25% AP
3	0.25% PFAP
4	0.25% AFAP

a - Percentage is based on weight of soaked hide.

3. Results and Discussion

3.1 PFAP and AFAP

Since α -amylases are mostly derived from microorganisms, especially bacteria (Choudhary et al. 2004; de Souza and Magalhaes 2010), a commercial bacterial α -amylase preparation (AP) was employed in this study. It was found that AP is a mixture of α -amylase and protease(s), where the α -amylase activity and the protease activity were 2147 U/g and 4276 U/g, respectively. The result is consistent with previous studies (Leach and Hebeda 1980; Moseley and Keay 1970).

As mentioned previously, the purpose of this study was to determinate which components of AP have an ability to unhair. For this purpose, we first need to obtain “pure” α -amylase and “pure” protease(s) from AP. Therefore, a thermal treatment and an oxidation treatment of AP solution were used for selectively inactivating protease(s) and α -amylase in AP, respectively. As shown in Figure 2, after heating at 70°C for 15 min, the AP solution was almost free of protease activity while simultaneously full of α -amylase activity. In addition, the relative α -amylase activity remained about 5% and the residual relative protease activity was nearly 60% after oxidation with sodium hypochlorite. The residual α -amylase activity was negligible and the residual protease activity was acceptable. These results suggested that PFAP and AFAP were obtained by inactivating protease(s) and α -amylase in AP respectively.

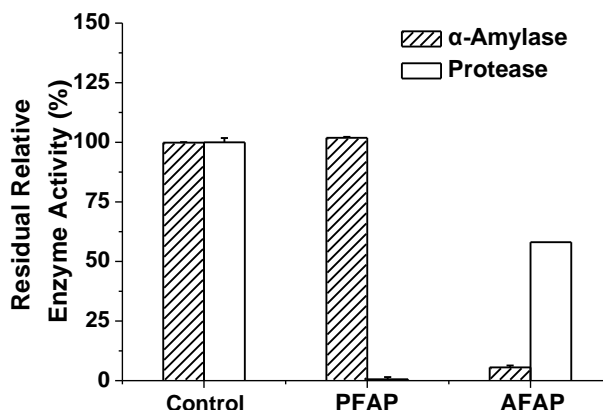


Figure 1 Residual relative enzyme activity of AP, PFAP and AFAP

3.2 Unhairing effectiveness of AP, PFAP and AFAP

Because the main purpose of an unhairing system is to remove hair and non-collagen proteins from hides (Cantera et al. 2003), the unhairing effectiveness of AP, PFAP and AFAP was evaluated by analyzing the extent of hair and protein removal to determine whether “pure” α-amylase or concomitant protease(s) are useful for unhairing.

3.2.1 Extent of hair removal

The digital photos of grain of unhaired pelts and the photomicrographs of Van Gieson stained horizontal sections from unhaired pelts are shown in Figure 2. It can be observed that no hair was removed from the control hide (Figure 2(1)), which indicates that microorganisms on hides cannot cause loosening of hair after unhairing for 4 h at 25°C. According to Figure 2(2), it is obvious that AP removes a majority of hair from the soaked hide, which was consistent with the results obtained in previous studies (Burton et al. 1953; Cordon 1955). The extent of hair removal from the pelt unhaired by using PFAP for 4 h is negligible and extremely similar with that of the control (Figure 2(3)). The result suggests that “pure” α-amylase cannot remove hair. Unhairing with AFAP achieves a satisfactory extent of hair removal (see Figure 2(4)), suggesting that concomitant protease in AP, like some other proteases used in leather manufacture (Choudhary et al. 2004), has an ability to remove hair. Comparing the extent of hair removal from pelts unhaired with PFAP and AFAP, it can be inferred that rather than “pure” α-amylase, concomitant protease(s) are the effective components of AP for removing hair.

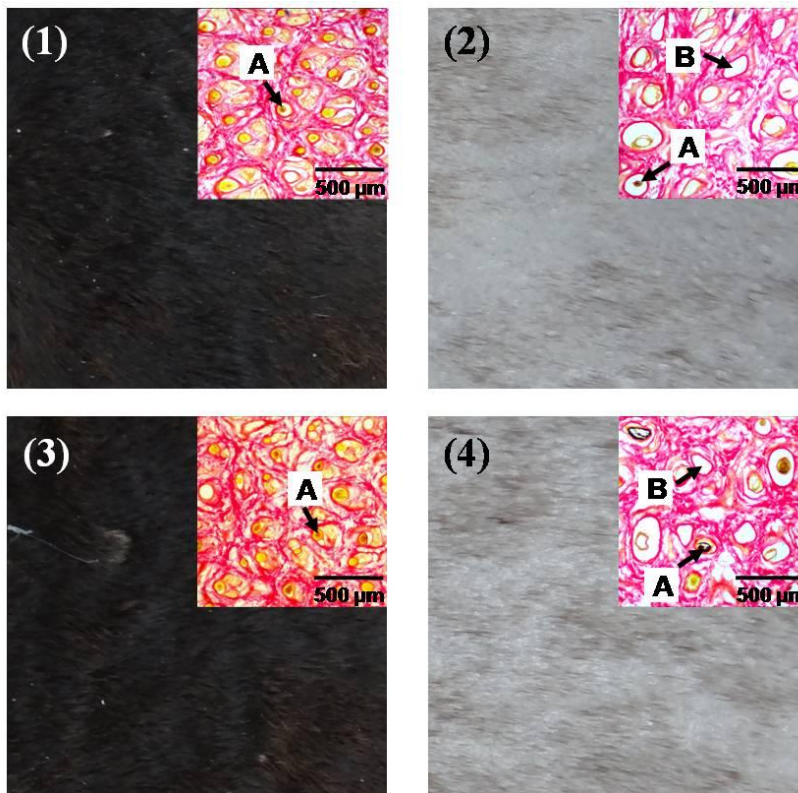


Figure 2 Grain of unhaired pelts captured by digital camera and horizontal sections (Van Gieson stain) from unhaired pelts observed using a biologic microscope: (1) control, no enzymes; (2) 0.25% AP; (3) 0.25% PFAP; (4) 0.25% AFAP. A – hair root; B – no hair.

3.2.2 Extent of protein removal

The concentration of protein in unhairing liquors are shown in Figure 3. It was found that the concentration of protein in PFAP unhairing liquor is higher than the control. This could be mainly attributed to the addition of enzymes which themselves are proteins. The concentrations of protein in AP and AFAP unhairing liquors were much higher than that in PFAP unhairing liquor, which indicated that protease(s) in AP are more beneficial to the removal of protein than “pure” amylase.

According to the results and discussions above, it can be found that the removal of hair is related to the removal of protein. This is in agreement with the conclusion of Bose *et al.* (1955) that enzymatic unhairing depended on the removal of mucoid materials. However, it is affirmative that rather than “pure” α -amylase, protease(s) in AP are the effective components for the removal of proteins including mucoid as well as the removal of hair. These results support the theory of Yates (1972) that the unique type of enzyme activity related to the unhairing effectiveness is a proteolytic activity. The mechanism of action of α -amylase preparation on hides should be consistent with that of unhairing using a protease. It is known from the literature that α -amylase preparations obtained from different suppliers usually have various ratios of α -amylase activity to protease activity (De Stefanis and Turner 1981). Therefore, it is reasonable to speculate that the differences in the unhairing effectiveness of amylase preparations observed by Burton *et*

al.(1953), Gillespie (1953) and Cordon (1955) should be due to the varying protease activities of amylase preparations used by them.

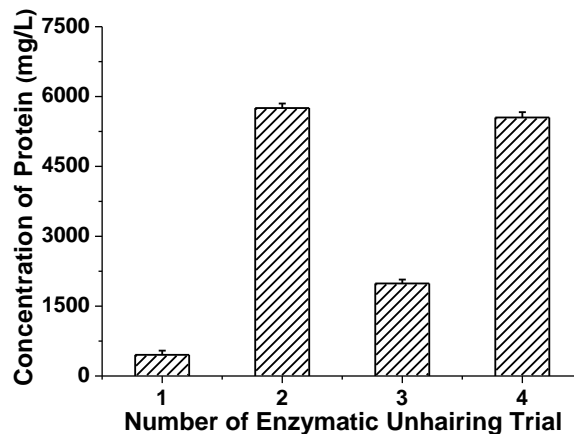


Figure 3 Concentration of protein in enzymatic unhairing liquor.
1 - control, no enzyme; 2 - 0.25% AP; 3 - 0.25% PFAP; 4 - 0.25% AFAP.

4. Conclusion

According to the effectiveness of removing hair and protein from hides using α -amylase preparation, “pure” α -amylase, and concomitant protease(s) in α -amylase preparation, it is clear that the concomitant protease(s) in α -amylase preparation are mainly responsible for unhairing rather than “pure” α -amylase. The action mechanism of α -amylase preparation on hides/skins should be consistent with that of proteases.

5. Acknowledgements

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6. References

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