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DEHAIRING OF CATTLE HIDE BY KERATINASE ENZYME OF *ASPERGILLUS FLAVUS* S125

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ABSTRACT

The keratinase enzyme produced by the fungus *Aspergillus flavus* S125 for depilation of skin was investigated. Dip method was adopted for enzymatic dehairing in which dried hide pieces were incubated with freshly extracted enzyme. Chemical processing was also performed simultaneously. During enzymatic dehairing the complete dehairing was observed after 12 hr at pH 9, while in chemical process, dehairing took 12 hr, at a high pH 11. Scanning electron micrographs of the dehaired skins showed the superiority in the quality of the enzymatically dehaired skin in comparison with the chemically treated one. Collagenolytic activity of the enzyme was also tested, as collagen is an important component of the hide and it was interesting to note that the keratinase produced by *Aspergillus flavus* S125 was highly keratnolytic and while showing no collagenolytic activity. Dehairing at pH 9 was economical and environment friendly as it avoids the dumping of toxic effluents into the environment. The results of the present study revealed that keratinase isolated from *Aspergillus flavus* S125 has the potential to replace chemical dehairing process, in addition to producing superior quality leather.

KEYWORDS: ***keratinase, Aspergillus flavus S125, depilation, dehairing and collagenolytic.***

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INTRODUCTION

Leather industry is one of the highly polluting industries contributing large amount of pollutants to the environment. Major chemicals used are lime, sodium sulphide, salt, solvents, etc. produced from pre-tanning operations such as soaking, dehairing, bating & degreasing. Soaking is the first step in pre-tanning operation, where the hide are subjected to water treatment followed by dehairing. This is done by mild putrefaction leading to hair loosening. Dehaired skin is then introduced into alkaline solution of lime milk, where swelling occurs and the nonfibrillar protein dissolves. After the mechanical removal of the subcutaneous tissue, deliming is performed in order to remove the adsorbed lime from the hide and to eliminate the lime swell. Bating, is the only step in leather processing where enzymatic process cannot be substituted by chemical process. Earlier, the process was carried out using dog dung or manure which was unhygienic. The fat present in the hide skins is removed using soluble lime soap, kerosene, chlorinated hydrocarbons, and white spirit which make the environment toxic. The leather processing industry generates a considerable amount of chemical wastes as effluents and has been causing serious environmental pollution. In the conventional pre-tanning process, depilation of animal hide is done by employing lime and sodium sulphide. It accounts for 70% of the total pollution in terms of biological oxygen demand, chemical oxygen demand, total dissolved solids and total suspended solids[1]. The alkaline nature of tannery effluents and the high sulphide content directly pollute the surface water and also the ground water sources, causing serious health problems to the people living in the vicinity of leather processing industries[2], in addition to affecting the health of tannery workers. A number of attempts have been made to find alternative methods for depilation of animal hide. Though a number of bacterial and fungal strains are known to grow on hides, only a few of them produce extracellular proteases with depilatory activity[3,4]. Collagen is the principle protein that gives strength, durability and flexibility to leather and its processing requires removal of non-collagenous constituents, partially or completely. In principle, the proteases having high depilatory properties with mild or no collagenolytic activity are considered to be the best for dehairing animal hide[5 6 7 8]. Until recently, most proteases used in enzymatic dehairing studies were of bacterial origin. The two major setbacks being encountered with the use of bacterial proteases are the cost of the enzyme and the inability to remove the fine hairs from the hide[9]. Filamentous fungi, such as *Aspergillus*, have been reported as the organism of choice for large scale production of industrial enzymes, due to the factors like relatively inexpensive media like agricultural waste, for growth and the ability to secrete bulk quantities of enzymes[10]. The use of microbial enzymes as an alternate technology to the conventional methods and the importance of microbial enzymes in minimizing the pollution load are gaining importance in recent time as we have turned to be more environmentally conscious. Enzymes are important in reducing energy consumption also. The advantages of enzymatic dehairing includes the recovery of good quality leather with a good saleable value and creation of an ecologically conducive atmosphere for the workers. The present investigation was designed to study the keratinolytic and collagenolytic activity of the enzymes produced by *Aspergillus flavus* S125 and also to compare the enzymatic and chemical processing of dehairing and their results.

MATERIALS AND METHODS

Cattle skin.

Pieces of cattle skin were collected from a slaughter house. The skin pieces were washed thoroughly with soap water and air dried.

Fungal culture

The fungus *Aspergillus flavus* was cultured in Feather Keratin medium soaked with mineral salt solution in the ratio 1:2. After inoculation the flasks were incubated at room temperature (30±4°C) for 5 days in a humidified chamber having relative humidity of 70%.

Extraction of enzyme

100 ml of Phosphate buffer at pH 8 was added to the culture flask and agitated on a shaker incubator (Labline) at 200 rpm for 1 hr at 30°C, for extracting the enzyme and after which the contents were filtered & squeezed using muslin cloth and the filtrate was used as crude enzyme.

Enzymatic dehairing process

The dehairing activity of the enzyme was assessed by applying the same on the cattle skin and the method employed was dip method of enzymatic dehairing. Clean pieces of hide, wiped dry with tissue paper and having a size of 3 cm x 5 cm were incubated in 5% enzyme, at a rate of 1 ml per gram of the hide, obtained by diluting the freshly extracted crude enzyme in distilled water, at room temperature (30±4˚C). Observations were made in every 2 hrs to record the changes.

Effect of pH

In order to study the effect of pH on dehairing, the above procedure was repeated at each pH from pH 7.5 to pH 10 and an interval of 0.5.

Chemical process of dehairing

Cattle hide was soaked in 10% NaCl for 15 min, and then washed twice with commercial detergent. Liming was done by dipping in 10% $Ca(OH)_2$ for 30 min. Later 3% Na_2S was added and kept at room temperature (30±4˚C).The observations were done every 2 hrs and the hairs were attempted to be removed from the skin using sterile forceps, to estimate the time required the loosening of hair from hair follicle.

Scanning Electron Microscopy

The dehaired skin pieces were cut into small cube of 2 mm length and washed thoroughly in distilled water and wiped dried. The material was fixed in glutaraldehyde (2%) for 2 hours, osmium tetraoxide (1%) for 4 hours and washed with phosphate buffer. After dehydration in a graded ethanol series (40%, 70%, 80%, 90% and 100%) and acetone, the material was dried using CO_2 dryer (Mini Galaxy E 600 Serial No: 11216), sputter

coated with a thin layer of gold (30 nm) using EMS550X Sputter Coater (Joel, Japan) and mounted on an electron microscope (Jeol,Japan) to take the electron micrographs.

Collagenolytic activity

Collagenase activity was measured as described by Van Wart and Steinbrink in 1981[15]. To 2.90 ml of FALGPA (N-(3-[2Furyl]acryloyl)-Leu-Gly-Pro-Ala) solution, 0.10 U/mL of enzyme was added and incubated for 5 minutes after mixing properly. The decrease in A_{345} for ~5 minutes was recorded. Maximum linear rate (ΔA345/minute) for both the test and blank by using at least a one minute interval and a minimum of 4 data points were obtained. One unit of collagenase hydrolyzes 1.0 µmole of FALGPA per minute at 25°C at pH 7.5 in the presence of calcium ions.

RESULTS

Enzymatic dehairing

The enzymatic dehairing process was completed in 12 hrs. at pH 9. The image of the skin piece before experiment and the enzymatically dehaired skin and are shown in Plates 1 and 2 respectively. Plate 3 shows the image of chemically dehaired skin.

Plate 1
Skin before experiment

Plate 2
Enzymatically dehaired skin

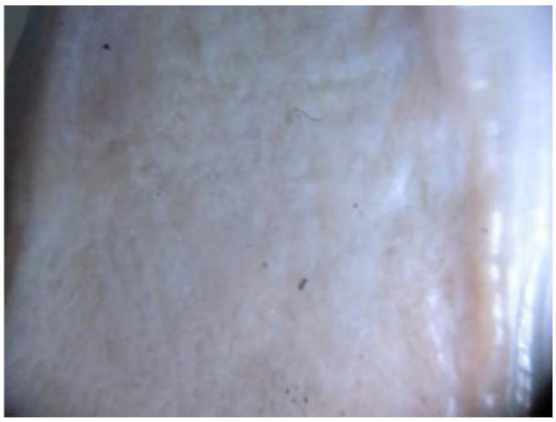
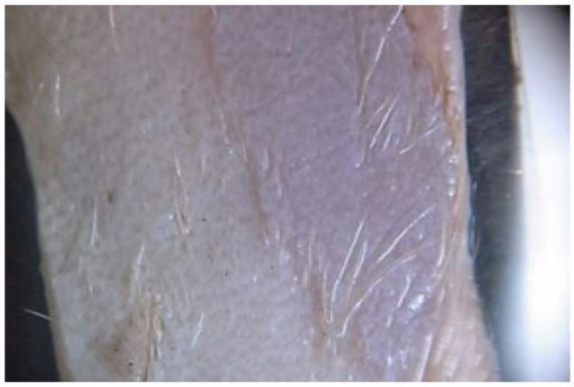


Plate 3
Chemically dehaired skin



By applying the enzyme produced from *Aspergillus flavus* on cattle hide, the dehairing process was completely accomplished without any deformations in the grain layer of skin. The results revealed that the hide retained its natural features without any deformations in the grain layer as evident from the SEM images.

Effect of different pH of enzyme on dehairing

The following table shows the effect of varying the pH on dehairing .

Table 1

Effect of varying the pH on enzymatic dehairing

pH	Dehairing time (hr)
7.5	24
8.0	20
8.5	18
9.0	12
9.5	12
10.0	12

At pH 7.5, dehairing took longer time. The shortest span of (12 hrs.) was observed at pH 9, 9.5 and 10. At pH 9 & 10 hair loosening was observed after incubation for 8hrs. With increase in pH, the time duration for dehairing was seen decreasing and the complete removal of hair from the skin were observed at the pH 9, 9.5 and 10.

Chemical dehairing

Results of study using chemical process is shown in Table 2.

Table 2

Time required for chemical dehairing at different pH

pH	Dehairing time (hr)
7.5	24
8	22
8.5	20
9	16
9.5	15
10	14
10.5	13
11	12

In chemical depilation using lime and sulphide method, complete dehairing occurred in 12 hrs at pH 11 and between pH 7.5 and 10 longer duration of time was noted.

Scanning electron microscopy

The animal hide subjected to chemical and enzymatic dehairing were observed under scanning electron microscope. The scanning electron micrographs of grain surface of skin showed that the opening of hair follicles in enzymatically treated animal hides was clearer and smoother than that of conventional chemical treated skin. The SEM images of untreated (Plates 4 and 5), chemical (Plates (6) C, (7) C &(8) C) and enzymatically treated cattle hides (Plates (6) E, (7) E &(8) E) are given below.

SEM images of control (untreated) skin

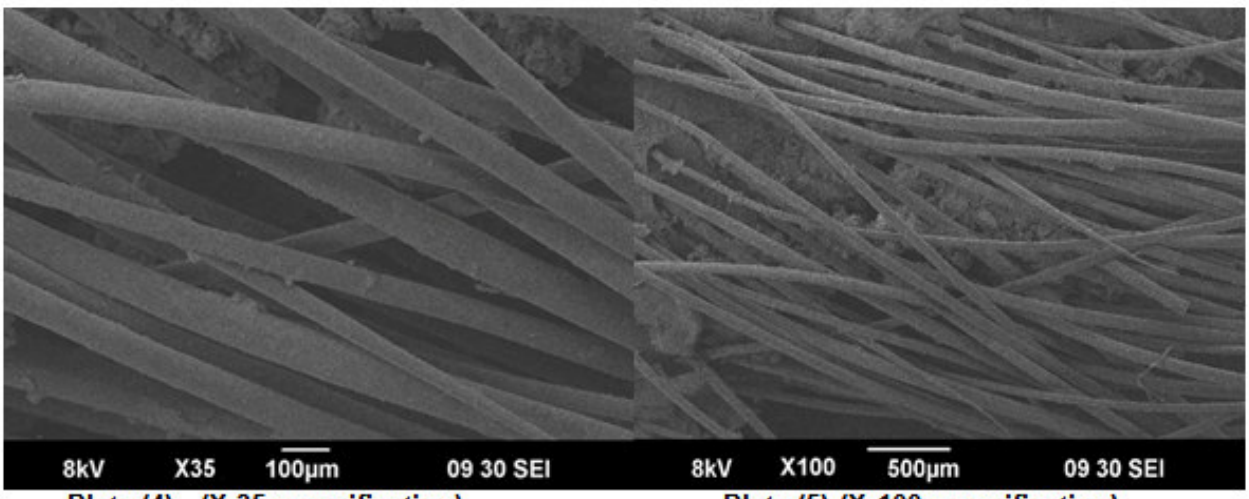


Plate (4) (X 35 magnification) Plate (5) (X 100 magnification)

SEM images of chemical & enzyme treated skin

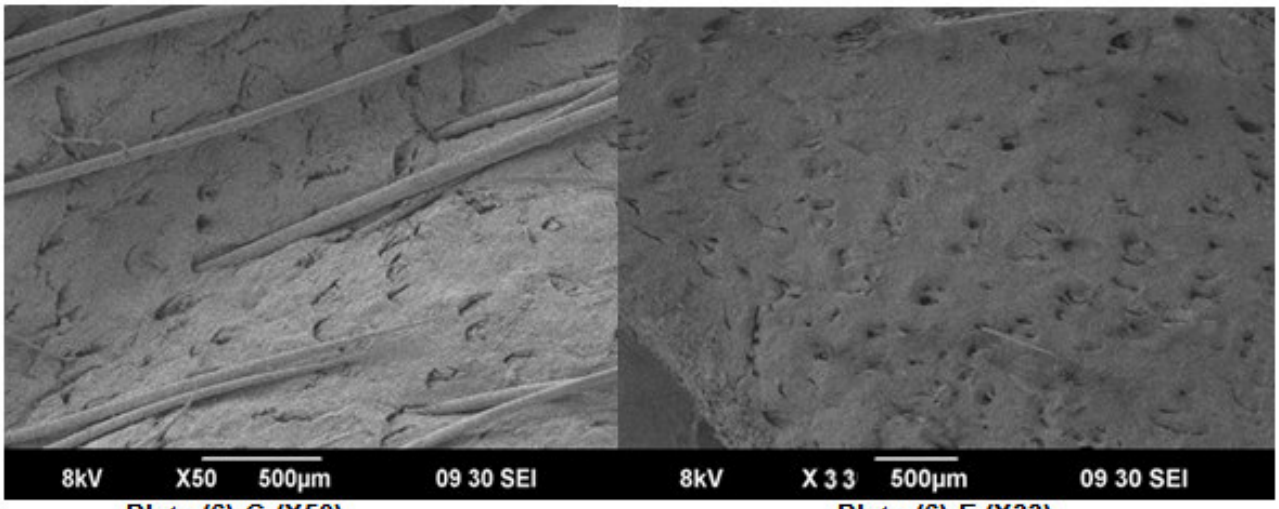


Plate (6) C (X50) Plate (6) E (X33)

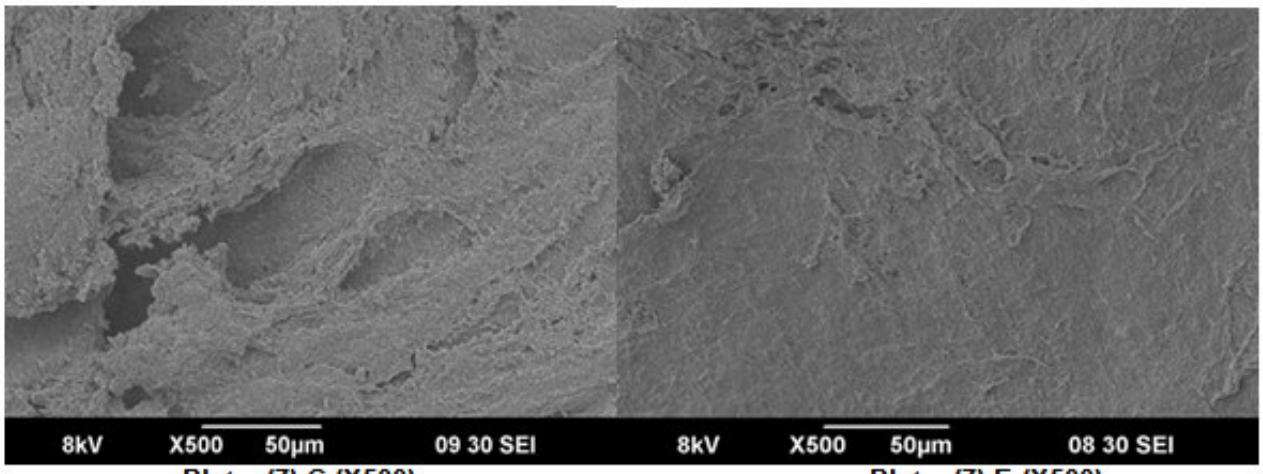


Plate (7) C (X500) Plate (7) E (X500)

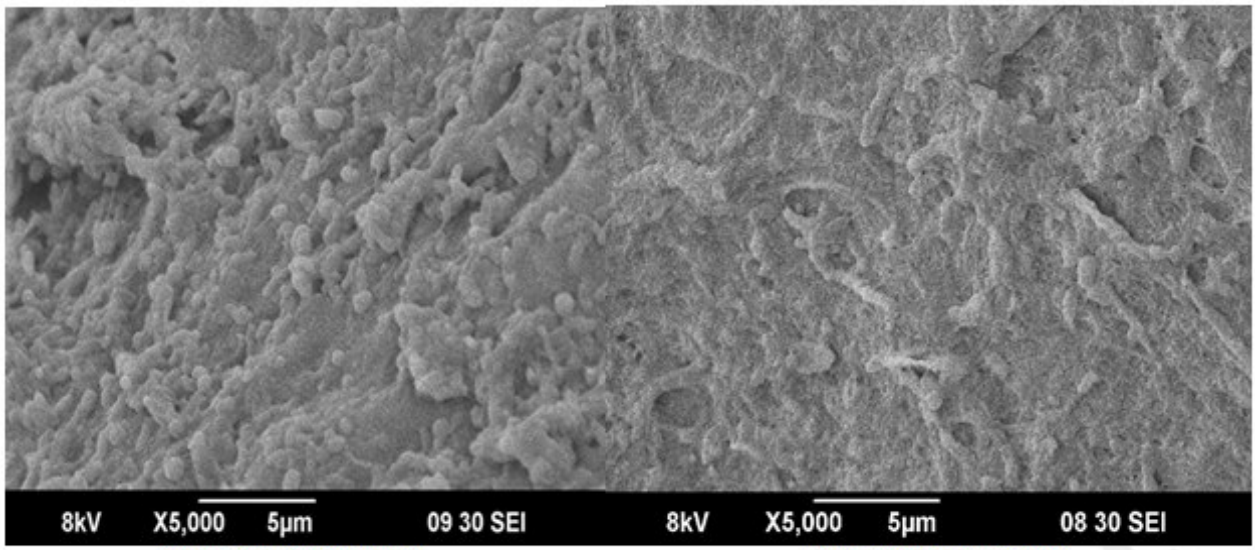


Plate (8) C (X5,000) Plate (8) E (X5,000)

Collagenolytic activity

The results of collagenolytic activity of the enzyme was found to be negligible in the assay that was carried out. Hence it can be concluded that the enzyme produced by the strain of *Aspergillus flavus* does not have any collagenolytic activity.

DISCUSSION

Enzymatically dehaired leathers have shown better strength, properties and greater surface area[12 13]. In the present study, the optimum pH for the enzymatic dehairng, was found to be pH 9, as the dehairing took

place with in the shortest span of time of 12 hrs. In chemical depilation using lime and sulphide method, complete dehairing was observed in 12 hrs at pH 11. At the lower range of pH studied, longer periods of time were required. Some enzymatic preparations need sodium sulphide[14] while others are more effective with addition of lime or detergents[15]. In the present study complete removal of hair was obtained in 12 hrs using the fungal enzyme. The scanning electron micrographs revealed the advantage of enzymatic dehairing process on the quality of the grain layer of the hide. Chemically treated pelt sample showed a white surface with the deposition of lime and higher precipitation of Na and S ions. In contrast, the enzyme-treated sample showed a smooth surface without any deposition of chemicals. Chemically treated skin with hair roots and lime particles on the hair pore, indicates that the lime-based dehairing process badly affect skin. The result of collagenase activity of the enzyme was negligible. It was reported that keratinolytic proteases lacking collagenase activity are more suitable for dehairing process[8]. Since collagens are the leather forming proteins, keratinolytic enzymes without collagenolytic activity can render durability to the finished leather. These results paves way for the use of this enzyme in leather industry for production of good quality leather.

CONCLUSION

The present investigation corroborated a high keratinolytic with zero collagenolytic activity for the enzyme produced by *Aspergillus flavus* S125, which indeed are the characteristic traits of an ideal depilatory enzyme. pH 9 has been found to be the optimal pH for *Aspergillus flavus* S125. Simultaneous comparative study of the enzymatic and chemical dehairing has revealed that enzymatic dehairing was more effective than conventional process, as dehairing at pH 9.0 has less impact on the environment than chemical process. The effectiveness of this enzyme in reducing the strength of hair is also evident from the result. The present study revealed that keratinase produced by *Aspergillus flavus* S125 has the potential for industrial application in dehairing process, rendering good quality leather.

CONFLICT OF INTEREST

Conflict of interest declared none.

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