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Eco-Friendly Enzymatic Dehairing on Animal Hides

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Abstract. This study presents the development of enzyme-assisted and enzyme-mediated methods as an alternative and eco-friendly approach to minimize the use of chemicals that will reduce the environmental impact in the manufacturing of leathers. Two types of protease namely alkaline protease 50a and keratinase were used in the enzymatic application in dehairing. Four sets of experiments of cow hides were performed. The sets included the use of chemicals which are 5% calcium oxide (CaO) and 2% sodium sulfide (Na_2S), the use of 2.5% enzymes with 5% CaO, the use of two types of enzymes and the use of the single enzyme. The hides which undergo treatment with enzymes eliminated 85% of hair after 26 hours under shaking condition at 37 °C while the treatment with enzyme together with 5% calcium oxide eliminated 100% of hair under the same conditions. The treatment of Na_2S along with CaO also eliminated 100% of hair faster than the other two treatments. As revealed by Scanning Electron Microscopy (SEM), the surface of the hides that were treated with enzymes was smoother and silkier than hides that were treated by chemicals. Hence, these results revealed that the enzymes-assisted method could be implemented in commercial leather manufacturing to reduce the use of severe Na_2S. The application of enzyme-assisted technique can help in the minimization of pollutions in the environment and can be a good alternative for the production of good quality of leather.

INTRODUCTION

The leather industry is one of the most popular industries in countries. There are many vital processes involved in the manufacturing of leather such as curing, soaking, dehairing, fleshing, deliming, pickling, and tanning. These entire following steps involve the use of enzyme in assisting the processes and enhancing the production of desired quality of leather [1]. Dehairing is one of the essential steps in leather manufacture. In this step, the hair along the epidermis and other unwanted substances are removed from the hides.

In the conventional method of dehairing, it involves the use of harsh chemicals. Thus it will contribute to pollution and generate noxious gases and solid wastes. To overcome this problem, another approach has been made which is by using an enzyme as the alternative ways in the dehairing process. For the reduction of the environmental impact of chemicals, the application of biotechnology in the manufacturing of leather is an excellent and effective alternative.

Protease is the most popular enzyme that is being used in the enzymatic dehairing. Protease is a proteolytic enzyme that catalyses the breakdown of proteins by peptide bonds hydrolysis. Protease is produced from various sources such as plants, animals, and microorganism. Even though protease is produced from numerous sources, bacteria are still the dominating source, and *Bacillus subtilis* is the prominent genus in the production of commercial protease [2]. The classification of protease is an acidic, neutral and alkaline protease. Keratinase is one of the enzymes that is used together with a protease in the dehairing process. Microbial keratinase is a class of proteolytic enzymes that can degrade the insoluble structural protein that exists in feather, hair and it is known as keratin. Instead of sodium sulphides, keratinase has broader applications in the leather industry [3]. Also, lipase is one of the essential enzymes

Materials Characterization using X-Rays and Related Techniques
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that are being used in the leather industry. Lipase is an enzyme which hydrolyse fats, and oil that can be found in the hypoderm [4]. Therefore, in this research, we used chemicals and enzymes approach for dehairing of animal hides.

MATERIALS AND METHODS

Curing and Drying Processes of Cow Hides

The cow hide was washed for several times with tap water. The fats layer was removed with a sharp knife and washed again. Then, the sodium chloride salt was spread all over the hides. Next, the hides were allowed to dry under the sunlight for 3 to 4 weeks. Then, the hide was cut into a 4 cm × 3 cm.

Preparation of Thermostable Alkaline Protease 50a and Commercial Keratinase

Thermostable alkaline protease 50a was locally produced [5]. Meanwhile, commercial keratinase was used in this research. The protease activity was determined by a modification of the method using azocasein as a substrate [6]. Keratinolytic activity was determined based on the method using azocasein as a substrate [7]. The protein content of both enzymes was measured based on the Bradford method using bovine serum albumin as a standard.

Dehairing of Cow Hides Using A Dip Method with Different Treatments

Dehairing of cow hides were using dip method with four different treatments. First, the conventional treatment, the pieces of hides were dipped in flasks containing 93 mL of water, 5% calcium oxide and 2% sodium sulphide. Second, the enzyme-mediated treatment, the pieces of hides was submerged in the water solution containing 2.5 % keratinase (U/mg) and 2.5 % thermostable alkaline protease 50a (U/mg). Third, enzyme-assisted treatment, the pieces of hides were soaked in 5% calcium oxide (w/w of the skin) solution for 6 hours at 37 °C. After treated with calcium oxide, the hide was washed multiple times by tap water until the pH of the skin released near 7.5. Next, the hides were dipped in 100 ml containing 2.5% (U/mg) keratinase and 2.5% (U/mg) thermostable alkaline protease 50a.

Fourth, dehairing using a single enzyme was done by dipped the pieces of hides in the water solution containing 2.5% protease or keratinase. For the control, the hides were soaked with 100 ml of distilled water. All the pieces of hides were placed in the orbital shaker rotating at 120 rpm for 26 hours at 37 °C. The hair was removed by gentle scraping with a scalpel. The experiment was done in triplicate.

Scanning Electron Microscopy (SEM) for Observation of the Surface of Hides

All samples were directly observed under SEM without any pretreatment. FEI Quanta 200 series environmental SEM unit operated at an accelerating voltage of 12 kV was used to examine the cross and surface view of the hides.

RESULTS AND DISCUSSION

In this study, dry salting method was being used. Cowhides were washed several times with tap water to remove blood, dirt, and ticks. After removing the fats with a sharp knife, the hides were scrubbed for the second times to remove the remaining dirt then the sodium chloride (salt) was sprinkled all over the hides except the hair. Salt was used to lessen the odour of the cowhides and to preserve it. The preservation using salt was mainly to drains and dried the salts. The salt that was being used must be a fresh salt that has not been used before. If the salt was reused, it would affect the hides and the preservation process was not being guaranteed because the reused salt consisted of too many microorganisms. As the hides are excellent media for the bacterial growth, the incomplete bleeding and dirt are some of the aspects that aid the increase of microorganism rate that will cause putrefaction on the hides. When curing and drying process was completed, the hides can be used in the dehairing process under different methods.

A dehairing process was done in four different types of treatments. The methods included conventional, enzyme-assisted, enzyme-mediated and single enzyme treatment. In the conventional treatment, dip method was used. In this treatment, lime or calcium oxide was used because it will cause the hides to swell by hydrolysing asparagine and glutamine in the structure of the collagen that aids the distortion of the fibre bundle to remove hairs [8]. The sodium sulfide was used in dehairing because the sulfide ions help to attack the disulphide bridge that presents between the cysteine residues that exists in the structure of the keratin.

Protease was used in this treatment was locally produced. It was the thermostable enzyme, which able to help in dehairing of hides as same as reported by other researcher [9]. Meanwhile, keratinase used in this treatment was a commercial enzyme. Keratinase also was one of the enzymes that used in the dehairing process. The specific activity of each enzyme was measured and used a range of 120 to 190 U/mg. This specific activity was calculated based on enzymes activity and their protein content. Both enzymes were used in enzyme-assisted, enzyme-mediated and single enzyme treatment. After treatment, the pieces of hides were observed under scanning electron microscopy (SEM).

Electron microscopy was used to examine the smoothness of the grain surface of dehaired hides due to the removal of hair from the epidermis of hides and it was also showed the undamaged grained structure of hides. The scanning microscopy showed that in enzymatically treated hides, the opening of the hair follicles was clearer and smoother compared to the conventionally treated hides. For the control, the hair was clearly seen under the SEM. SEM also showed the surface of hides that undergone enzyme assisted treatment was smoother than hide undergone conventional method. For the hides that were treated with single enzyme either protease or keratinase, the SEM showed that the surface was smooth but there was some hair that still attached on it because not being removed during the treatment.

For enzyme-mediated treatment, the surface of the hides was smooth without any deposition of chemicals on it. Microscopic analysis also showed that the recovered hair from enzyme assisted dehairing was intact in hides and the hair still in good condition without being damaged by the treatment. In addition, it was noticed that the hair was presented in the dislodge hair of the enzymatic method whereas it was absent in the conventional method. This could be explained that in the enzymatic method, the residue hair might be left on the grain surface because the recovered hair from this method had damaged ends.

In the conventional treatment, the hair structure cannot be seen as it was entirely destroyed in the bath. The effluent derived from conventional treatment consisted of a mixture of degraded non-collagenous proteins, reacted and unreacted products of calcium oxide and sulfide, proteoglycans, dislodged intact hair and epidermal keratinous substances [8]. For the enzymatic method, the effluent comprised the same constituent with conventional beside insignificant amount of enzymes and dislodge intact hair. Fig. 1 shows the grain surface from different method under SEM.

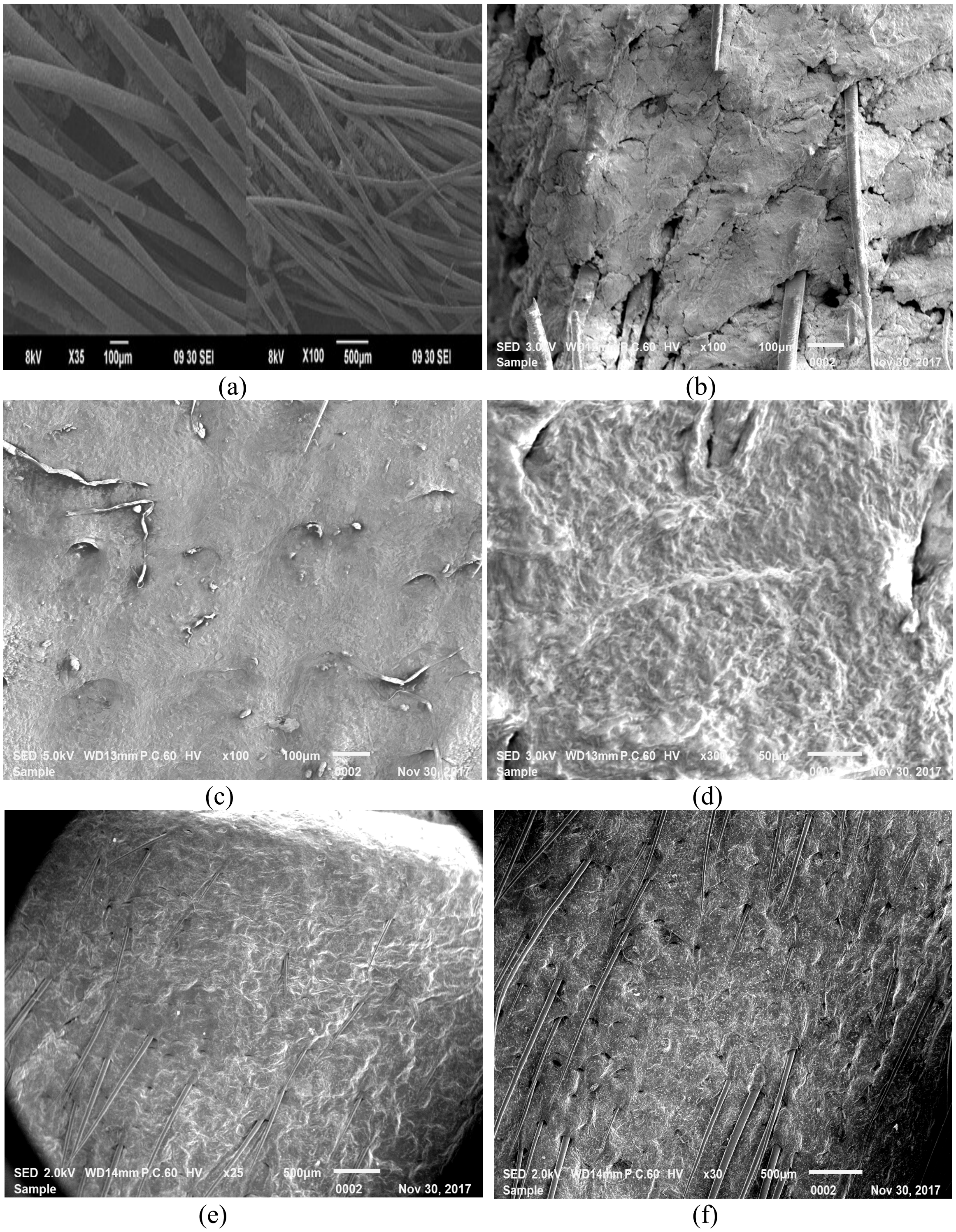

FIGURE 1. SEM showing top surface of the hides for (a) control (×100), (b) conventional (×100), (c) enzyme assisted (×100), (d) enzyme-mediated (×300), (e) protease only treatment (×25), and (f) keratinase only treatment (×30) samples.

CONCLUSION

The present study was conducted to develop the eco-friendly enzymatic dehairing by using the enzyme-assisted method and enzyme-mediated method to decrease thus can eliminate the use of harsh chemicals in the leather industry. The use of enzyme will help to minimize the environment pollutions to the significant level and can become one reasonable alternative to obtain a smoother and silkier grain surface for the production of good quality leather. As the conclusion, the enzyme-assisted method was the best method to be used in dehairing because it can remove almost 100% of hair, plus the surface of the hide was smoother due to the removal of the epidermis and silkier. Hence, it helps to produce a good quality of leather.

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