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Short Communication

Application of Enzymatic Bating Agent on Leather

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Abstract. Protease activity of *Aspergillus niger* was investigated for leather bating by solid state fermentation using wheat bran as substrate at a suitable concentration and was found to be 1200 units. The optimum conditions regarding temperature, time and speed of agitation for enzyme leaching were found to be 37 °C, 2.5 h and 12 rpm, respectively. The leather produced with such materials was compared with that produced using a widely applied commercial bating material with regard to quality, strength and softness. This leather was of good quality having no difference from the leather produced by using imported bating material.

Keywords: protease, *Aspergillus niger*, wheat bran

Tannery effluents are one of the major industrial pollution problems arising mainly from pre-tanning processes. Use of enzymes as a viable alternative has been restored to in pre-tanning operations such as soaking, dehairing (Sirvaityte *et al*., 2006), bating, degreasing and offal treatment. Bating is the process of removing proteins other than collagen using proteolytic enzymes (Atiya, 2008). Failure to remove the non-collagenous proteins causes cementing together of the fibers when the leather is dried and results in firmness and lack of flexibility (Choudhary *et al*., 2004).

The most important effects of bating on leather are an increase of area, improvement of inner softness, more uniform cycling and better physical/mechanical properties (Thanikaivelan *et al*., 2004).

Dunaevsky *et al*. (2000) have reported the use of enzymes from *Aspergillus* species in bating. Solid- substrate fermentation has the potential for higher protease yield (Dunaevsky *et al*., 2000; Pandey *et al*., 1999). Economically this type of fermentation processes is advantageous as reported by Malathi and Chakraborty (1991).

Prior to this century, natural bates consisting of dog and pigeon faeces were used, but they had disadvantages (Suresh and Virithagiri, 2010; Ahmed and Gasmelseed, 2003). The purpose of the present work is to investigate the use of fungal protease from *Aspergillus niger* as an alternative method to the conventional bating method and highlights the importance of this enzyme in minimizing the pollution load.

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Protease production. *Aspergillus niger* was evaluated for protease production by solid state fermentation using wheat bran, an agricultural by-product as a substrate. The conditions favourable for leaching were investigated and the most significant factors were found to be temperature, time and speed of agitation. These were specified and estimated to be 37 °C, 2.5 h and 12 rpm, respectively.

Similar experiments were carried out to specify the optimum time of culture of the wheat bran carrier and the degree of wetability. These were found to be 72 h and 60% water on the grain weight with continuous wetting of the surface to replace water lost by evaporation.

Fungus strain *Aspergillus niger* was collected by Institute of Mycology and Plant Pathology, University of the Punjab, Lahore.

The mycelium from potato dextrose agar PDA slant was used for *Aspergillus niger* growth. Wheat straw obtained from local market was soaked in water overnight. Next day drained and boiled for 30 mins. The moistened material was allowed to cool and dry, a moisture content of the substrate was 60-70%.

Erlenmeyer flask 250 mL containing 50 g wheat straw was sterilized at 121°C (15 lbs/inch^2 pressure), cooled, inoculated and incubated at 25 ± 2 °C for 72 h in laminar flow cabinet. After incubation, 75 mL of distilled water was added to the flask which were shaken on rotary shaker (Digitech Instruments) for 1h at 200 rpm. The contents of flasks were then filtered and the filtrate was used for enzyme assay (Haq *et al*., 2003).

The quantity of alkali consumed in the titration of the filtrate of the mixture after digestion is that required for the surplus acid which represents the measure of the bating agent strength.

Assay of protease. ***Preparation of casein solution.*** Casein solution (5%) was prepared by mixing 50 g of casein (Hamartsten) in an Erlenmeyer flask with 200 mL to 300 mL of water until a thick much was formed. Then NaOH solution (0.1 N) was added and the solution was heated on a water bath until clear solution was obtained. The clear solution was poured into a 1000 mL volumetric flask and diluted 3/4 volume of the flask with distilled water. The pH value of the solution was adjusted to be exactly 8.2 and made up to the mark.

The weighed quantity was put into a stoppered graduated measuring flask, 10 mL of distilled water was added and the mixture was heated at 37 °C with periodical mixing for 15 min. Twenty mL of 5% casein solution was added to the mixture and heated for 1 h at 37 °C. Then, 10 mL of 0.2 N HCl and 10 mL of 20% Na_2SO_4 were added and the undigested casein precipitated. The mixture was then filtered and the filtrate was titrated against 0.1 N NaOH with 4-7 drops of bromothymol blue as an indicator. Meanwhile, a blank experiment was carried out without heating, i.e. at 15-20 °C HCl was added with sodium sulphate, the mixture was filtered and the filtrate solution was titrated against 0.1 N sodium hydroxide.

One mL of 0.1 N NaOH difference between the real titration and the blank test titration corresponds to 0.3 g of digested casein.

Enzymatic value (strength or activity)

$$\frac{(a\ \ b) \times 0.3 \times 100}{C \times 1.725}$$

where;

a = mL of real titration experiment

b = mL of blank titration experiment

c = sample weight

1.725 = empirical constant. One enzyme unit is defined as the amount of enzyme which is able to decompose 1.725 g of casein

Application of bating agent on leather. The mould culture was applied as a bating material to both sheep and goat skins. The skins were processed and assessed after bating and it was shown that the removal of the scud became much easier, soft and smooth grained pelts were obtained. The skins were properly determined as checked by the pH of the cut which was colourless to phenolphthalein (pH 8.0-8.5).

Deliming and bating:

200 % Float at 37 °C on drained lime weight

1.5 % Ammonium sulphate
run for 1 h, pH 8.0 – 8.5

1.5 % Bating agent blend of 1300 strength
run for 2 h.

After bating the pelts showed facility, looseness of the scud and open porosity as was checked by the finger imprint and the air entrapped in the fabled pelt which permitted air to escape easily. The pelts were further processed through normal processing to finishing.

The finished leather were then physically and chemically analysed using official methods of Society of Leather Technologists & Colourists.

The finished leathers were analysed for physical parameters including thickness, tensile strength and elongation (Fig. 1) as well as for chemical parameters (Fig. 2)

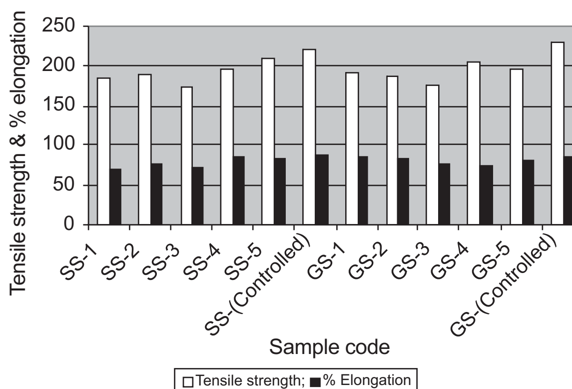

Fig. 1. Physical characteristics of sheep and goat skins treated with mould in comparison with skins treated with locally available bating agent.

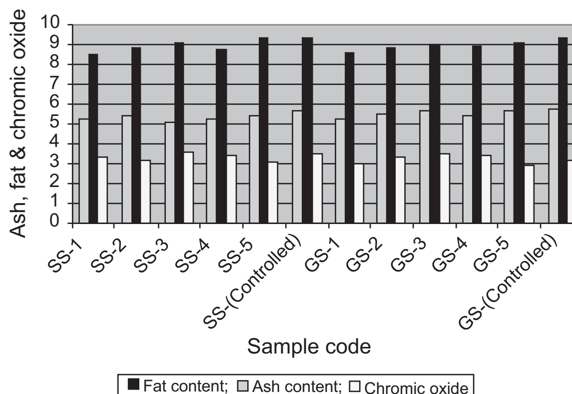

Fig. 2. Chemical parameters of sheep and goat skins treated with mould in comparison with skins treated with locally available bating agent.

including ash content, chromic oxide and fat contents. The results are satisfactory as compared with the standard specifications. The same results were compared with a controlled sample using the market available bating agent and there was no significant difference with regard to visual, physical and chemical tests.

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