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Comparative studies of a new microbial bate and the commercial bate 'Oropon' in leather treatment

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An extracellular protease of *Bacillus subtilis* K2, when compared with the pancreatic bate 'Oropon' used at comparable protease activity, produced a finer grain and greater elasticity of goat pelt. Quality of the processed, finished leather after microbial bating showed <41% and <14% increases in tensile and tear strengths, respectively, compared with 'Oropon'-bated leather.

Keywords: microbial bate; alkaline protease; *Bacillus subtilis*; pancreatic bate; leather treatment

Introduction

In converting animal skins into leather, a series of treatments is used to modify the structure of the skin so that the resulting leather is soft, supple and receptive to fast dyes. Proteins provide the major structural component of which 96.5% are the fibrous proteins collagen (98%), elastin (1%) and keratin (1%), whereas only 3.5% are albumin and globulin [2,4].

The first stages in leather making involve soaking the salted hide before dehairing and bating. Dehairing is normally achieved by the chemical lime-sulphide process, and although proteolytic enzymes can be used they have not proved economically viable [5,7]. A significant contribution to environmental pollution (especially in Pakistan) is the release of salt, lime and sulphide into effluent water. If an economic one-step microbial process could be developed to replace chemical treatment for dehairing and bating, this would reduce and possibly eliminate the use of lime-sulphide in processing.

For high quality leather, bating is one of the most important procedures, in which non-collagenous protein is degraded, collagen fibres are separated, and unwanted debris (scud) removed from the hide. It softens the hide for tanning, renders the grain silky, slippery, smoother and more porous, increases its width and diminishes the wrinkles. The usual bating agents are pancreatic proteases, such as 'Oropon', although some microbial bating enzymes, eg Pyrase 250 (Novo Nordisk, Bioindustries UK Ltd, Farnham, Surrey, UK), are available. In Pakistan the latter are considered too expensive to replace existing technology. Although microbial alkaline proteases have been isolated from *Bacillus subtilis* [4,6,8] and *Aspergillus flavus* [3], their use in leather making has so far been confined to dehairing and limited to tanneries in the developed world. No data are available on their bating proficiency compared with using pancreatic proteases. However, microbial proteases suitable for bating are an alternative to pancreatic protease and could enable development of a one-step dehairing and bating process using microbial enzymes. Recently we described an alkaline protease produced by a strain of *B. subtilis* [1] isolated from tannery wastes in Pakistan. This paper compares the efficacy of the microbial protease in bating with the commercial bate 'Oropon'.

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Materials and methods

Microbial bate

Bacillus subtilis K2 (identified by the National Collection of Industrial and Marine Bacteria Ltd, Aberdeen, UK) was grown in a 6-L biofermenter (Eyela, Tokyo, Japan) in 3 L of a casein-gelatin medium (0.48% casein, 6% gelatin) at pH 7 to which 36 ml of 20% sterile glycerol was added [1]. Culture filtrate containing 220 protease units ml^{-1} (PU) was separated from cells (cell number 2.8×10^9 CFU ml^{-1}) after 48 h incubation at 37°C, with a dissolved oxygen tension of 40% air saturation. Crude culture filtrate containing extracellular alkaline protease was used in varying volumes (depending on proteolytic activity) as the microbial bate, using equivalent proteolytic units to that of 'Oropon' in efficacy trials.

Commercial bate

'Oropon' (BASF, Germany) was obtained from the Institute of Leather Technology, Gujranwala, Pakistan. The proteolytic activity of 'Oropon' was determined after stirring 10 g in 100 ml of distilled water for 1 h. After filtration proteolytic activity in the filtrate was measured.

Assay for proteolytic activity

Crude enzyme solution (1 ml) was incubated with 1 ml of 1% casein (pH 8.5) at 40°C for 30 min. Protein was precipitated with 3 ml trichloracetic acid (TCA), and was cooled for 30 min before centrifugation at $10000 \times g$ for 30 min. The optical density of the supernatant phase was measured at 280 nm. Blanks were prepared in the same way for each sample, except that 3 ml of TCA was added prior to incubation. All assays were made in triplicate. One unit of activity is defined as that amount of enzyme releasing 1 μg

of tyrosine under the assay conditions used (pH 8.5, 40°C, 30 min).

Application of bate to delimed goat pelts

Delimed goat pelt which had been soaked and dehaired by the lime-sulphide process was used for bating trials. Twelve 40-g pieces of pelt were cut from the completely delimed pelts. Each piece was kept in deliming liquor in a separate bottle which was shaken in a mechanical swinging shaker at 10 rpm. Bating trials used 900, 1800 or 2700 PU of microbial bate or 'Oropon' both at pH 8.5, for different periods of time (1–1.5 h). All experiments were performed in triplicate.

Evaluation of bating efficacy on finally prepared leather

After bating with the microbial bate or with 'Oropon', goat pelts were processed to prepared leather. The leather was cut into pieces either perpendicular or parallel to the backbone orientation, and pieces were tested for tensile strength, percentage elongation at break, tear strength and bursting strength. Tensile strength was measured with a Priifen and Messon (Hamburg, Germany) type 85654 tensile machine with a uniform speed of separation of the jaws (100 ± 20 mm min^{-1}). A section around the central 50-mm portion of a piece of leather (110 mm long), 10 mm from the centre, 25 mm from the outer margins, was tested. The average thickness of the piece was determined and the load at break noted giving the tensile strength (kg cm^{-2}). Percentage elongation at break was measured on the tensile machine using a similar-sized sample. The jaws of the machine were set 50 mm apart and the percentage elongation at break measured [(difference in length of sample/original length) × (100)]. The tear strength of the leather (50 × 25 mm of measured thickness) with a 20-mm long cut in the centre was assessed by running the tensile machine until the leather tore apart. The highest load at break was recorded (kg cm^{-1}). Bursting strength was measured with a Lastometer (STD-104).

Results and discussion

The efficacy of bating of the microbial bate and the commercial bate 'Oropon' was compared. Proteolytic activity of the two bates was adjusted to the same level as used in the commercial process with 'Oropon' to permit direct comparison. Six separate trials to assess the qualitative effects of the two bating enzymes on a delimed goat pelt were prepared using 900, 1800 and 2700 PU of each. Qualitatively, the same intensity of thumb imprint, similar ease of scud loosening (as judged by scraping with fingernails) and comparable feel of the goat pelt were observed between microbial and commercial bate treatments with all concentrations of bate and length of bating time. The longer the bating time for both bate treatments, the greater was the intensity of thumb imprint, ease of scud loosening and slipperyness of the pelt. The major differences in effect of the two types of bate were that the microbial bate increased the time of reshaping of the thumb impression and produced a finer texture to the grain compared with 'Oropon' treatment. This suggested that the microbial bate was producing more activity than 'Oropon' with respect to elasticity and grain texture. In no respect did the microbial bate perform less well than the commercial bate.

When the processing of the goat pelts treated with the two bates was completed to produce finished leather, quantitative tests for tensile strength, percentage elongation at break, tear strength and bursting strength were made. Pieces of leather were cut both perpendicular and parallel to the grain to assess strength and elasticity throughout the leather. Table 1 shows that optimal treatment was produced with both bates when 1800 PU were used for 90 min. Leather treated with microbial bate had a tensile strength greater than that treated with 'Oropon'. The maximum increase in tensile strength recorded was 35% in a parallel direction and 41% in a perpendicular orientation of the leather. Similarly, the percentage elongation at break was also greater, namely 43% and 56% increases in parallel and perpendicular directions, respectively, when compared with 'Oropon'-treated leather under the same conditions.

Greater strength of the microbial bate-treated leather was also indicated by tear strength, showing 14% and 8% increases in parallel and perpendicular directions. This was again demonstrated by bursting strength with microbial bate-treated leather having 33% more strength than that of 'Oropon'-treated.

These data indicate that the microbial bate produced a higher quality of finished leather, as determined by increased tensile strength and strength on stretching. Elasticity as demonstrated by tear and bursting strengths was also significantly greater in leather treated with the microbial bate. These increases in quality of the leather were not maximised in this study in terms of proteolytic units and times for bating, as a direct comparison would be required with leather treated with the amounts of 'Oropon' used commercially. Further trials will determine these parameters for production of specific leathers such as for hard shoe soles, medium soft shoe uppers, and very soft garments.

Improved quality depends on the nature of the proteolytic activities in the bate. Greater efficiency in degrading albumin and globulin, and the interfibrillary proteins keratin and elastin, without degrading the collagen fibres, enables more effective removal of scud formed by hair roots, sebaceous and sweat glands, and cell debris. The resulting leather has a finer grain and wrinkles are diminished. The drive to develop a new microbial bating agent is partly economic to replace a relatively expensive commercial bate in producing leather of the highest quality, and partly as a step in providing a cleaner environment for tanneries. A one-step dehairing and bating process would replace the use of chemicals. For tanners to be persuaded to modify the processes in leather treatment, alternatives must ideally be both more effective in treatment and provide financial benefits.

Preliminary assessment of the cost of production of microbial bate from *B. subtilis* suggested that costs may be half of that currently spent in using 'Oropon' or importing 'Novo' bating enzymes. In commercial use 'Oropon' is immobilized on sawdust, and this method of application can be used with the microbial bate to avoid the need to develop a different process. In order to produce sufficient

Table 1 Evaluation of microbial and pancreatic bates in finally prepared leather

Test	Cut of leather	Time of bating = 60 min						Time of bating = 90 min					
		Microbial bate (PU)[a]			Oropon (PU)			Microbial bate (PU)			Oropon (PU)		
		900	1800	2700	900	1800	2700	900	1800	2700	900	1800	2700
Tensile strength (kg cm^{-2})	Perpendicular	140.9	169.3	189.5	132.5	152.0	154.2	164.6	233.3	221.5	140.3	165.3	160.8
	parallel	152.4	175.2	199.1	139.4	155.1	157.5	172.8	234.0	226.2	145.8	173.0	164.8
Elongation at break (%)	Perpendicular	10.0	15.4	21.0	9.8	14.8	19.0	14.8	41.0	32.0	13.6	26.2	25.9
	parallel	12.6	19.3	28.0	12.5	16.9	23.6	17.3	43.0	37.0	15.3	30.1	28.5
Tear strength (kg cm^{-1})	Perpendicular	35.8	39.2	40.2	35.3	38.8	39.0	38.5	48.9	41.6	37.8	45.5	41.57
	parallel	38.1	41.0	42.1	37.5	40.1	40.4	39.8	54.0	47.2	39.1	47.3	43.6
Bursting strength (kg cm^{-1})	Perpendicular	217	242	254	186	200	212	232	312	280	198	233	224

[a]PU = proteolytic units ml^{-1}.

bate for industrial use, scale-up of production will be required. This is currently under investigation.

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