STUDIES ON THE DEGREASING OF SKINS USING A MICROBIAL LIPASE*

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A procedure has been standardized for estimating the optimum conditions for the hydrolysis of skin lipid by a fungal lipase. The skin lipid is found to be optimally hydrolysed at a pH between 3.2 and 3.6 at 37°C for one hour. A process has been described for the manufacture of suede clothing leather using fungal lipase as the degreasing agent. Comparative chemical, physical and microscopical studies of the quality of suede clothing leather produced from both coarse wooled and fine wooled sheep skins by using kerosene and fungal lipase degreasing treatments have been carried out. Although critical evaluation of the data showed minor differences in some respects, it would appear from the general trend of the results that fungal lipase showed good degreasing efficiency similar to that of the standard solvent treatment.

Introduction

The presence of natural grease in raw hides and skins, especially wooled sheep skins, results in various defects in the finished leather, viz. fatty spues, uneven dyeing and finishing, waxy patches in alum-dressed leather and pink stains on chrome blues. The liming process was reported to cause the rupture of fat cells present in the epidermis and the saponification of the liberated fatty acids. However, Innes[1] observed that the corium lipids remained relatively unaltered during the liming process and suggested the necessity of special degreasing operations for the processing of greasy hides. The degreasing operations were reported to be carried out after bating or after pickling by using either aqueous emulsification procedures[2,3] or by solvent extraction procedures.[4-7] Since the wool of the wooled sheep skins might get damaged as a result of the mechanical treatments involved in the usual degreasing procedures, such skins were given a scouring treatment, wherein soda and a detergent were used for degreasing. The use of a dishwashing detergent and certain surface active agents in the degreasing of leather and fur skins was also reported.[8,9]

Anon[10] reported the removal of grease, epidermal residue and glands by π combination of protease and lipase. Pancreatic and bacterial lipases were reported to be

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active at the pH range of bating[11,12]. Addition of lipases to the bate containing fungal proteases was suggested by Grimm[13]. Madhavakrishna and Bose[14] studied the degreasing of sheep skins by means of castor seed lipase and suggested the use of an active lipase for the satisfactory removal of grease. The best method of degreasing raw pig and sheep skins based on lipolytic breaking down and emulsification of natural fats present in the raw material by the use of enzyme preparations from *Aspergillus oryzae* and *Aspergillus flavus* was reported by Markin.[15] Dhar[16] suggested the suitability of microbial lipase for the degreasing of greasy skins. An unhairing enzyme from pig's pancreas was reported[17] to reduce the grease content of the skins by about 80% and further degreasing treatment was found unnecessary. In the present study, a systematic investigation was undertaken to standardise the optimum conditions for the hydrolysis of skin lipids by a fungal lipase and to carry out a comparative assessment of the leathers produced by degreasing sheep skins using fungal lipase with those produced by kerosene degreasing treatment.

Materials and methods

Coarse wooled sheep skins were purchased from Bangalore region and fine wooled skins were from Ooty region. The fungal lipase used in this study was a gift from Hindustan Antibiotics Limited, Poona.

Preparation of skin lipids

Skin lipids were extracted from a fresh coarse wooled sheep skin by the procedure described by Madhavakrishna and Bose.[14] To state briefly, the washed skin was unhaired with a razor, cut into small pieces and then minced into a pulp in a meat mincer. The pulp was dehydrated by treatment with alcohol and the residual lipids were extracted with low boiling petroleum ether (B. P. 40-60°C) in a Soxhlet extractor till the material became almost free from grease. The lipids were recovered from both the alcohol and the ether extracts by removing the solvents and were finally dried in vacuum.

Enzyme assay

The lipolytic activity of the fungal lipase was estimated by the method described by Madhavakrishna and Bose[14] with slight modifications. 10 g. portions of skin lipid were mixed with 70 ml. of veronal buffer (pH 3.6) and were emulsified in a magnetic stirrer for ten minutes to obtain a stable and fine emulsion of the lipid. 7 ml. of this lipid emulsion was incubated with 0.1 g. of lipase at 37°C for one hour. At the end of the incubation period, 25 ml. of warm neutral alcohol was added to the digestion mixture, the contents were mixed well and titrated against 0.1 *N* sodium hydroxide using phenolphthalein as the indicator. A control experiment was always run in an identical manner using boiled enzyme solution.

The lipolytic activity is expressed as unit of enzyme activity and is defined as the amount of enzyme powder that gives a net titre value of 1 ml. of 0.1 *N* sodium hydroxide at pH 3.6 and 37°C in one hour.

Effect of pH on the activity of lipase

1 g. portions of skin lipid were buffered to different pH values with 7 ml. veronal buffer of varying pH, emulsified and incubated with 0.1 g. portions of lipase at 37°C for one hour. After incubation, the lipolytic activity of the enzyme was measured by the usual procedure. The results are presented in Fig. 1.

Effect of enzyme concentration on the activity of lipase

1 g. portions of skin lipid emulsified in 7 ml. of veronal buffer of pH 3.6 were incu-

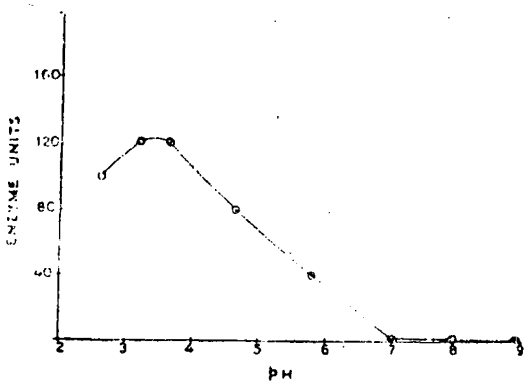


Fig. 1 : pH - Activity relationship

bated with different concentrations of lipase for one hour at 37°C, at the end of which the lipolytic activity was estimated by the usual procedure. The results are presented in Fig. 2.

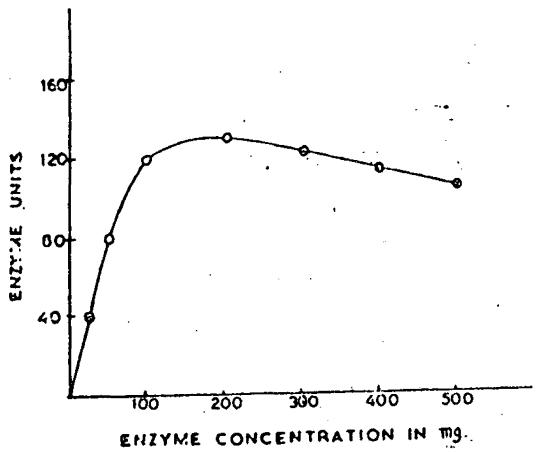


Fig. 2 : Enzyme concentration - Activity relationship

Effect of period of hydrolysis on the activity of lipase

1 g. portions of skin lipid emulsified in 7 ml. of veronal buffer of pH 3.6 were incubated with 0.1 g. portions of lipase at 37°C for different periods. After incubation, the lipolytic activity of the enzyme was measured by the usual procedure. The results are presented in Fig. 3.

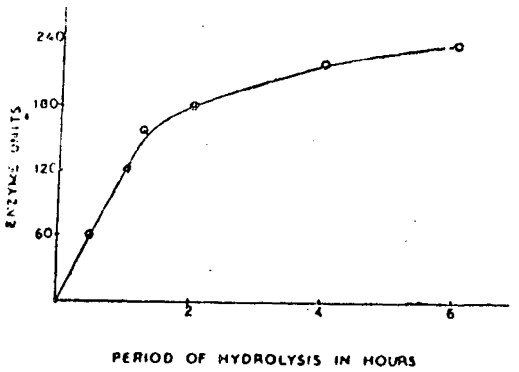


Fig. 3 : Time - Activity relationship

Effect of temperature on the activity of lipase

1 g. portions of skin lipid emulsified in 7 ml of veronal buffer of pH 3.6 were incubated with 0.1 g. portions of lipase at different temperatures for one hour. At the completion of incubation, the lipolytic activity of the enzyme was estimated by the usual procedure. The results are presented in Fig. 4.

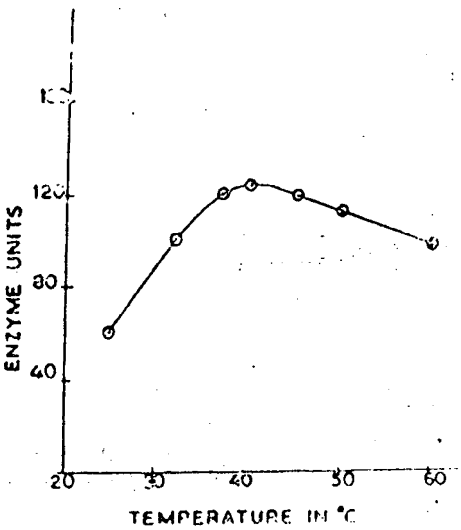


Fig. 4 : Temperature - Activity relationship

Since sodium chloride is normally used for depressing the acid swelling of hides and skins during pickling and since the fungal lipase was found to be highly active at acid pH range, a separate set of analysis was carried out using different concentrations of sodium chloride solutions in the digestion mixture and estimating the lipolytic activity of the digestion mixture in the usual manner as described earlier. It was, however, observed that sodium chloride, upto a concentration of 10%, exhibited no inhibitory effect on the lipolytic activity of the enzyme.

Preparation of suede clothing leather from wooled sheep skins

A lot of 15 wet-salted coarse wooled sheep skins (32" × 36") was used in this experiment.

For the manufacture of suede clothing leather from the wooled sheep skins, normal liming procedure as followed in the tannery practice was applied using a paint composed of 2.5% sodium sulphide, 5% lime and 20% water (on soaked weight) painted on the flesh side of the skin and left overnight, folded on flesh to flesh. Next morning, they were unhaired, relimed with 10% lime and 300% water for three days, fleshed and washed. The washed pelts were delimed in a drum with 1.5% ammonium chloride, 0.5% sodium bisulphite and 200% water for 25 minutes. The pelts were taken out and scudded. They were then pickled with 40% water, 6% common salt and 1% sodium formate for 10 minutes, after which 0.2% formic acid mixed with 5% water was added to the pickle bath and the drum was allowed to run for 10 minutes. After the period, 1.5% sulphuric acid (conc.) mixed with 15% water was added to the drum and the drum was allowed to run for another 2 hours, after which the pelts were taken out and piled up for 3 days.

For comparative studies, each of the five pickled pelts was cut along the backbone line into two halves and numbered. The left halves of all the pelts and five full pelts were subjected to degreasing treatment with kerosene and were considered as control. The remaining right halves and five full pelts were treated with fungal lipase for degreasing.

Degreasing with kerosene

For degreasing by kerosene treatment, the lot of pickled pelts was taken in a drum with 20% kerosene and 0.2% Noigen LS (Dai-Ichi Karcaria) and the drum was allowed to run for one hour. The pelts were then taken out for further processing.

Degreasing with fungal lipase

In order to remove the natural grease from wooled sheep skins, the lot of pickled pelts was immersed in an enzyme bath containing 1% lipase and 200% water adjusted to a pH of 3.6 and left in the same bath overnight at room temperature (28-32°C). After the period, the degreased pelts were removed from the bath.

At this stage, both the kerosene and the enzyme treated pelts were pooled and subjected to salt wash twice with 100% water and 8% common salt for 40 minutes. The washed pelts were repickled in a drum with 20% water, 3% common salt and 0.5% formic acid in 5% water for 30 minutes. The pickled pelts were then tanned in the same drum adding 6% chrome powder dissolved in 6% water, 3% Lipamin Z3 and allowing the drum to run for 30 minutes, after which 1.5% formaldehyde and 1% sodium acetate were added in three instalments and run for 1½ hours. After the period, 2% borax and 20% water were added to the same bath in five instalments and the drum was run for 1 hour. The leathers were taken

in another drum and washed in warm water (50°C) for 15 minutes. They were taken out and piled up.

The leathers were then neutralised, dyed, fatliquored and finished as suede clothing leather by adopting normal procedures.

As it was observed from the microscopical analysis of the coarse wooled sheep skins (raw) that the natural fat in those skins was comparatively less than those of fine wooled sheep skins, a separate set of experiment was carried out in an identical manner using the fine wooled sheep skins. The results of microscopical, physical and chemical analyses of the leathers produced by degreasing both coarse wooled and fine wooled sheep skins by treatment with kerosene and the fungal lipase treatments are presented.

The degreased pelts after salt washing were subjected to microscopical analysis to find out the extent of fat removed by the treatments. For this, identical samples were cut out from the same skin (processed by the two different treatments), fixed in formol saline and then washed in running tap water for 30 minutes. The sections were cut at a thickness of 80 μ in a Leitz's freezing microtome and then stained by following the Sudan IV staining technique.

Sudan IV staining technique

70 ml. of a saturated solution of sudan IV in absolute alcohol was diluted to 100 ml. with distilled water and then filtered. The frozen skin sections were dehydrated by passing through 50% alcohol for 3 minutes and then through 70% alcohol for another 3 minutes. Dehydrated skin sections were stained with Sudan IV stain for 30 minutes and then washed in 50% alcohol for 3 minutes and finally transferred to distilled water. The sections were mounted on glass slides using glycerin jelly and studied under the microscope. A few typical photomicrographs of the pelts are presented in Fig. 5.

All the finished leathers produced after using both the degreasing systems were assessed for their quality from tanners' point of view with special reference to general appearance, feel, fullness, tearing strength, etc.

For the physical and chemical analyses of finished leathers, the same procedures as described in an earlier communication[11] were followed. The results of the physical and chemical analyses are presented in Tables 1 and 2.

For physical analysis, an average value of at least five tests was taken for each item of the tests carried out.

Results and discussion

In order to find out the hydrolytic action of the fungal lipase on hides and skins, the action was first studied against the isolated skin lipids. It can be seen from Fig. 1 that the fungal lipase is found to hydrolyse skin lipids only at acid pH, its optimum activity being between pH 3.2 and 3.6. The optimum concentration of fungal lipase which is required for the hydrolysis of 1 g. of skin lipid at a temperature of 37°C has been found to be 0.1 g. (Fig 2). As can be seen from Fig. 3, upto a maximum period of one hour, the linear relationship between time and degree of hydrolysis is maintained. The optimum temperature for hydrolysis of the lipids has been found to be 40°C. At higher temperatures, a sharp fall in the lipolytic activity has been observed (Fig. 4). The skin lipid was found to be best acted upon by fungal enzyme under emulsified condition.

TABLE 1

Physical analyses of suede clothing leather manufactured by degreasing with kerosene and fungal lipase

Nature of physical tests.	*Direction of test*	*Degreasing experiment with sheep skins*			
		Coarse wooled		*Fine wooled*	
		Kerosene degreased	*Lipase degreased.*	*Kerosene degreased.*	*Lipase degreased.*
Tensile strength (Kg./sq.cm.)	Parallel	160 . 3	164 . 4	122 . 2	133 . 9
	Perpendicular	152 . 3	172 . 1	97 . 8	110 . 1
Elongation at break (%)	Parallel	62 . 5	62 . 5	50 . 0	62 . 2
	Perpendicular	100 . 0	112 . 5	45 . 0	57 . 5
Double hole stitch tear strength (Kg./cm.)	Parallel	70 . 4	99 . 7	55 . 5	83 . 9
	Perpendicular	61 . 0	90 . 7	56 . 3	81 . 7
Tongue tear strength (Kg /cm.)	Parallel	22 . 6	21 . 3	16 . 1	15 . 9
	Perpendicular	17 . 0	18 . 8	16 . 1	16 . 2
Grain crack resistance (Kg./sq.cm./cm.)		171 . 4	200 . 0	77 . 8	75 . 0
Bursting resistance (Kg./sq.cm./cm.)		171 . 4	200 . 0	77 . 8	75 . 0

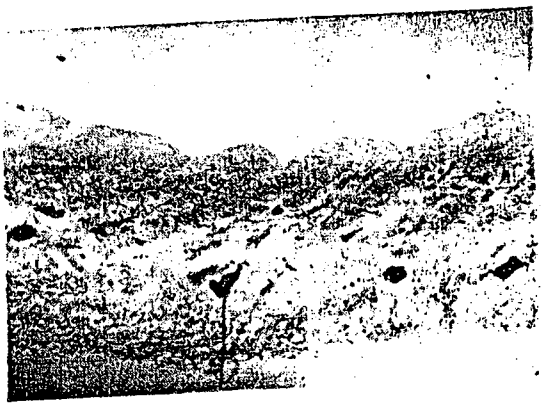
TABLE 2

Chemical analyses of suede clothing leather manufactured by degreasing with kerosene and fungal lipase (on 0% moisture basis)

Nature of chemical tests	*Degreasing experiment with sheep skins*			
	Coarse Wooled		*Fine Wooled*	
	Kerosene degreased	*Lipase degreased*	*Kerosene degreased*	*Lipase degreased*
Oils and fats (%)	9 . 40	10 . 40	16 . 92	15 . 95
Hide substance (%)	61 . 87	62 . 09	58 . 30	59 . 70
Fixed organic matter (%)	20 . 77	19 . 24	17 . 13	15 . 73
Total ash (%)	7 . 27	7 . 40	7 . 20	7 . 36
Cr_2O_3 (%)	3 . 19	3 . 25	3 . 53	3 . 92

FIG 5: PHOTOMICROGRAPHS OF COARSE WOOLED SHEEP SKINS

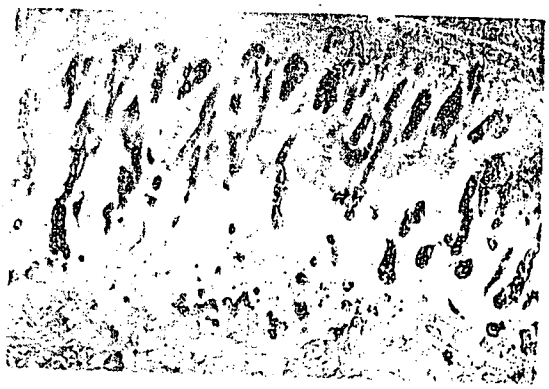
Raw sheep skin before degreasing (× 56.0)



Pelt of sheep skin after degreasing with kerosene (× 33.6)

Pelt of sheep skin after degreasing with lipase (× 33.6)

PHOTOMICROGRAPHS OF FINE WOOLED SHEEP SKINS



Pelt of sheep skin before degreasing (× 56.0)

Pelt of sheep skin after degreasing with lipase (× 56.0)

During the process of manufacturing suede clothing leather using coarse wooled sheep skins, pelts were examined under microscope after degreasing and salt washing for ascertaining the extent of removal of natural fats from the treated pelts. It was, however, observed that in the case of both kerosene and lipase treated pelts, fats were found to be not completely removed and traces of fats were present in the fat glands (Fig. 5). From the microscopical examination of the fine wooled sheep skins, it is also observed (Fig. 5) that the lipase treatment could remove major portion of fats from the pelts compared to the untreated sample.

The suede clothing leather, after finishing, were assessed for their quality from tanners' point of view and the data obtained with respect to the experimental (lipase treated) were compared with those obtained from the control lot (kerosene treated). It was observed from the comparative degreasing experiments that both degreasing treatments produced equally good suede clothing leather with minor differences between the experimental and control lots. The same trend of results was found in both coarse wooled and fine wooled sheep skins.

The data obtained with regard to physical tests (Table 1) show that the suede clothing leather produced from coarse wooled sheep skins using lipase degreasing system had uniformly better physical properties than the corresponding control (kerosene degreased) in all the tests carried out except in the case of tongue tear strength, where the values were found to be almost similar. In the case of fine wooled sheep skins, however, the same trend of results was observed except in the cases of both grain crack and bursting resistances where the values obtained with respect to the lipase-degreased samples were found to be slightly lower than those of their control counterparts. Regarding tongue tear strength, no significant differences were observed between the experimental and the corresponding control counterpart.

The data (Table 2) on the chemical analyses of the finished leathers obtained from both coarse wooled and fine wooled sheep skins showed no significant differences between both lipase degreased and kerosene degreased leathers. However, significant differences were observed when compared between the coarse wooled and fine wooled sheep skins with regard to all the chemical tests carried out.

From the above mentioned study, it may be concluded that fungal lipase could be used commercially in the processing of wooled sheep skins for the manufacture of different types of leather, replacing the more expensive solvent degreasing treatments.

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