FUNGAL LIPASE AND ITS USE IN DEGREASING SHEEP SKINS

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The optimum conditions for the maximum hydrolysis of skin lipid by a fungal lipase have been standardised. The skin lipid is found to be optimally hydrolysed at a pH between 5.8–7.9 and at 30°–37°C for one hour. A process for the manufacture of suede clothing leather using this fungal lipase as the degreasing agent has been developed. Using enzymatic pretanning processes viz. unhairing, bating and degreasing, the total load of tannery effluent has been minimised. Comparative chemical, physical and microscopical studies of the quality of leather thus produced from sheep skins showed the good degreasing efficiency of fungal lipase.

Introduction

The presence of excess natural grease in raw hides and skins, especially in highly greasy wooled sheep skins, leads to various defects in the finished leather such as fatty spues and improper penetration of tan-liquors, dye stuffs, etc. During the beam-house processes viz. liming and bating, the normal fat present in hides and skins is removed either as soluble lime soap or hydrolysis products like fatty acids. The excess fat present in hides and skins, however, is removed after bating or after pickling by adopting either aqueous emulsification, solvent extraction or mechanical degreasing treatments. To avoid the possibility of damaging wool in wooled sheep skins as a result of the routine mechanical degreasing treatments, they are normally degreased by treatment with soda and a detergent.

The possibility of applying lipolytic enzymes from plant, animal and microbial sources for the degreasing of hides and skins has been suggested by early investigators. The suitability of using a fungal lipase for the satisfactory degreasing of wooled sheep skins in the production of suede clothing leather has been reported in an earlier communication[1]. 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 using a fungal lipase with those produced without it.

Materials and methods

Fine wooled sheep skins were obtained from the Ooty region. The fungal lipase and dehairing enzyme used in this study were supplied by M/s. Sarabhai M. Chemicals, Baroda. Pancreatin product used as bate was prepared in the pilot plant of the Central Leather Research Institute, according to the method developed by Dhar *et al*[2].

The skin lipids were extracted from a fresh wooled sheep skin by the method reported in an earlier communication[1].

The lipolytic activity of the fungal lipase was estimated according to the procedure described in an earlier communication[1]. The lipolytic activity is expressed as units of enzyme activity and a unit is defined as that amount of enzyme which gives a net titre of 1 ml. of 0.1 *N* sodium hydroxide at pH 6.99 and 37°C in one hour.

Hydrolytic action of lipase on different substrates

1 g. portions of tallow, olive oil or skin lipid were each buffered to different pH values with 7 ml. veronal buffers, emulsified and incubated with 0.2 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.

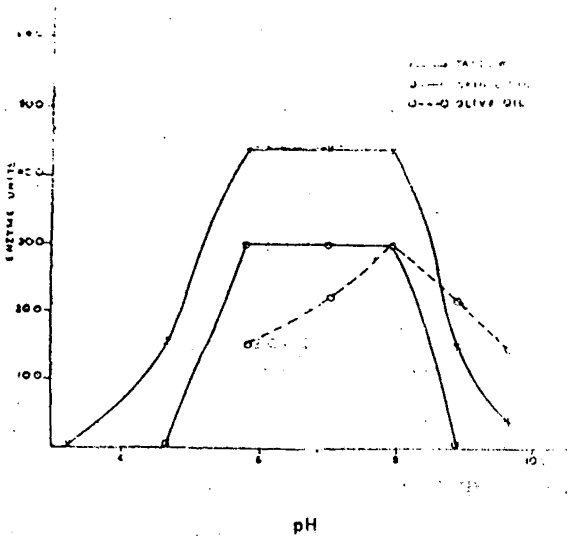

Fig. 1 : pH-activity relationship

Effect of enzyme concentration on the activity of lipase

1 g. portions of skin lipid emulsified in ml. veronal buffer of pH 7.0 and buffered 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.

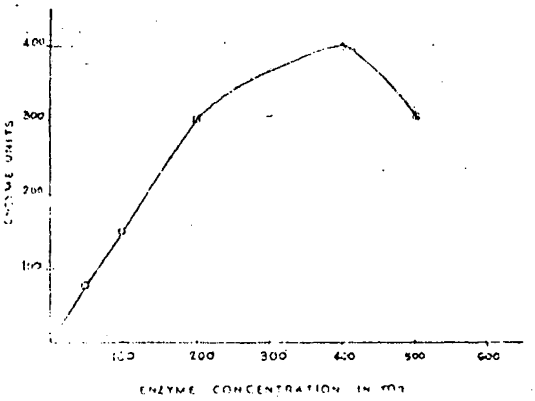

Fig. 2 : Enzyme concentration-activity relationship (Substrate : skin lipid)

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 7.0 were incubated with 0.2 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.

Effect of temperature on the activity of lipase

1 g. portions of skin lipid emulsified in 7 ml. of veronal buffer of pH 7.0 were incubated with 0.2 g. portions of lipase at different temperatures for one hour. At the completion of incubation, the lipolytic

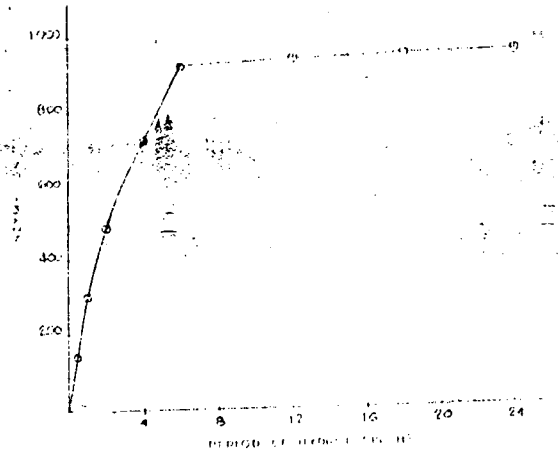

Fig. 3 : Time-activity relationship
(Substrate : skin lipid)

activity of the enzyme was estimated by the usual procedure. The results are presented in Fig. 4.

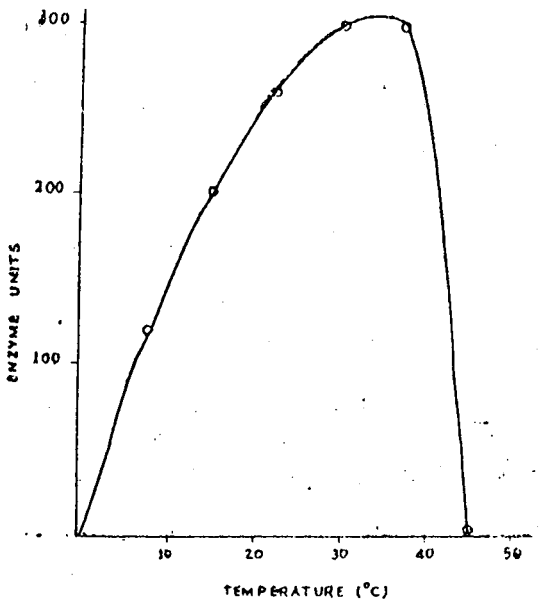

Fig. 4 : Temperature-activity relationship
(Substrate : skin lipid)

Preparation of semi-chrome suede clothing leather from wooled sheep skins

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

Soaking

For the manufacture of semi-chrome suede clothing leather, the skins were soaked in paddle for 4 hours with 500% water (on raw weight of the skin) with two changes of water.

Unhairing

They were then unhaired by applying on the flesh side of the skin a paste composed of 1.5% dehairing enzyme, 5% kaolin and 15% water (on soaked weight of the skin); pH of the paste was adjusted between 8.5 – 9.0 and skins were left overnight. Next morning, they were unhaired, scudded and washed for 15 minutes in plain water.

Bating

For comparative studies, 5 pelts were cut along the backbone line into two halves and numbered. The left halves of all the pelts and five full pelts were bated in a tub containing 1% CLRI pancreatin bate and 150% water (on pelt weight) adjusted to a pH of 7.8 – 8.0 and 37°C for 2 hours and were considered as control.

Bating cum degreasing

To the remaining right halves and five full pelts, 1% lipase (on pelt weight) was used along with the bate and the bating and the degreasing treatments were carried out simultaneously.

Salt wash

All the treated pelts from both the lots were pooled and subjected to salt wash twice with 100% water and 8% common salt

for 40 minutes. The washed pelts were rinsed with luke warm water for 10 minutes, scudded on both sides and finally washed.

Pickling

They were then pickled in a drum with 100% water, 8% common salt, 0.5% sodium formate and 1% sulphuric acid (on pelt weight) for 1 hour at pH 2.5.

Pretanning

The pickled pelts were then pretanned in the same drum by adding 2% Basyntan P powder, 0.5% sodium sulphite and 1.5% China clay (on pelt weight) and running the drum for 40 minutes at pH 4.5.

Tanning

The pelts were then subjected to dry-drum tanning with 5% wattle extract and 0.5% sodium bisulphite (on pelt weight) for 30 minutes. To the same drum was added 1% TRO emulsified with hot water (10% on pelt weight) and run for 15 minutes. To the same bath 5% wattle extract and 0.5% sodium bisulphite (on pelt weight) were added and the drum was run for 3 more hours. Then 50% water was added and the drum was run for 20 minutes and the pelts were left in the bath overnight. Next day, the drum was run for 30 min and the pelts were washed thoroughly and drained.

Bleaching

To the drum was added 1% bleaching syntan and 150% water (on pelt weight) and the drum was run for 20 min.

Chrome tanning

To the same bath, 5% chrome extract (on pelt weight) was added and the drum was run for 30 minutes. An additional 5% chrome extract was added and the drum was run for 1½ hours. Basification was carried out by adding 1% sodium bicarbonate dissolved in 10 times of water in two feeds at 10 minutes interval, running the drum for 30 minutes. The pelts were rinsed and piled overnight.

Neutralisation

Next day, the pelts were neutralised for 1 hour in a drum with 150% water, 0.5% sodium formate, 0.5% sodium sulphite and 0.5% sodium bicarbonate (on tanned weight) added in two feeds at 10 minutes interval.

Fatliquoring

The pelts were then fatliquored at 60°C for 1 hour with 4% Lipoderm liquor I, 5% Lipoderm liquor II and 0.5% raw castor oil. After the period, 0.5% diethylene glycol was added to the same bath and the drum was run for 15 minutes after which 0.3% formic acid mixed with 10 times water was added, and the drum was run for 10 minutes. The pelts were taken out and piled overnight. They were then set, dried, staked and buffed to produce suede nap. They were dry-drummed and brushed off.

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 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 freezing microtome and then stained using the Sudan IV staining technique. The extent of removal of fat was then observed by examining the microscopic slides in the microscope.

All the finished leathers produced after using both degreasing systems were assessed

for quality from the 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[8] 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 discussions

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 hydrolyses skin lipids and tallow over a broad range of pH 5.8—7.9. Olive oil was optimally hydrolysed at pH 7.9. During the hydrolysis of 1 g. of skin lipid at a temperature of 37°C, a linear relationship was maintained between the enzyme concentration and lipolytic activity upto 0.2 g. concentration of lipase (Fig. 2). The optimum concentration was about 0.4 g. 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; but optimum time of hydrolysis was about six hours. The skin lipids were observed to be optimally hydrolysed over a broad range of temperature from 30°C—37°C. At higher temperatures, there was a sharp fall in the lipolytic activity, the

TABLE 1

Physical analyses of semi-chrome suede clothing leather manufactured by degreasing with lipase

Nature of physical tests	*Direction of test*	*Leathers obtained after treatment*	
		Without lipase (Control)	*With lipase (Experimental)*
Tensile strength (Kg./sq. cm.)	Parallel	139.7	150.8
	Perpendicular	111.6	111.6
Elongation at break (%)	Parallel	68.8	75.0
	Perpendicular	81.3	100.0
Double hole stitch tear strength (Kg./cm.)	Parallel	60.4	86.5
	Perpendicular	58.1	83.9
Tongue tear strength (Kg./cm.)	Parallel	14.5	16.0
	Perpendicular	14.5	20.6
Grain crack resistance (Kg./sq. cm./cm.)		120.0	151.5
Bursting resistance (Kg./sq. cm./cm.)		120.0	172.3

TABLE 2

Chemical analyses of semi-chrome suede clothing leather manufactured by degreasing with lipase (On 6% moisture basis)

Chemical tests	Leathers obtained after treatment	
	Without lipase (Control)	With lipase (Experimental)
Oils & fats (%)	16 . 44	13 . 24
Hide Substance (%)	63 . 43	69 . 75
Fixed organic matter (%)	13 . 56	8 . 54
Total ash (%)	6 . 53	7 . 19
Cr_2O_3 (%)	2 . 39	3 . 07

enzyme getting completely inactivated at 45°C (Fig. 4). The skin lipid was found to be best acted upon by the fungal lipase under emulsified condition.

During the process of manufacturing suede clothing leather, pelts were examined under the microscope after bating and after combined bating and degreasing treatments for ascertaining the extent of removal of natural fats from the treated pelts. It was observed that the bating treatment could also remove some of the natural fats present in the fat glands. The combined treatment with lipase was found to be efficient in removing natural fats from the skins and there was only a negligible amount left in the fat glands.

The suede clothing leathers, after finishing, were assessed for quality and the data obtained with respect to the experimental leathers (Pancreatin Bate+Lipase-treated) were compared with those obtained from the control lot (Pancreatin Bate-treated). It was observed from the comparative degreasing experiments that both treatments produced equally good suede clothing leather, with lipase treatment providing a slightly superior feel to the leather.

The data obtained with regard to physical tests (Table 1) show that the suede clothing leather produced from fine wooled sheep skins using a combination of bating and lipase degreasing system had uniformly better physical properties than the corresponding control (without lipase).

The data (Table 2) on the chemical analyses of the finished leathers obtained from fine wooled sheep skins showed minor differences between the lipase-treated and lipase-untreated leathers with regard to all the chemical tests carried out.

From the above study, it may be concluded that the fungal lipase could be used commercially in the degreasing of wooled sheep skins for the manufacture of suede clothing leather. Enzyme preparations from *Aspergillus oryzae* and *Aspergillus flavus* were reported to be efficient in degreasing raw pig and sheep skins.[4] It was also observed that the reported lipolytic activity of pancreas[5] could not bring about a complete hydrolysis of natural fats present and the skins required further treatment with fungal lipase for satisfactory degreasing.

The above process of manufacturing suede leather makes use of all enzymatic pretanning techniques, viz. enzymatic unhairing, bating and degreasing, thereby resulting in a considerable reduction in the total load of the effluent disposal problem.

In conclusion, the advantages of enzymic degreasing can be summarised as below:

(a) Avoidance of the use of expensive, toxic and inflammable organic solvents.

(b) Since the enzymatic degreasing agent was highly active at alkaline pH range, the

process could be carried out simultaneously along with bating at pH 7.8—8.0.

(c) The lipolytic enzymes will find their way into the effluent collection tanks and will help in the biological degradation of the various lipids that are present there, provided pH conditions of the effluent liquors are favourable.

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