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## A COMPARATIVE STUDY ON CERTAIN UNHAIRING SYSTEMS USING JAWASEE PROTEASE, MICROBIAL PROTEASE AND LIME ON THE QUALITY OF THE FINISHED LEATHERS\*

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A process for the manufacture of grain garment leather using jawasee protease is described. The quality of leathers produced by unhairing with jawasee protease, microbial protease and lime has been comparatively assessed. Chemical analyses, microscopical examination and determination of physical characteristics that have been carried out for all the leathers have indicated that the three unhairing procedures give leathers of similar and comparable characteristics with very minor differences. The advantages of using enzyme unhairing process for solving the effluent disposal problem and for the recovery of good quality hair/wool have been discussed.

### Introduction

A raw hide or a skin undergoes a series of treatments prior to tanning and is finally converted into a finished leather. Dehairing is one of such treatments and is normally done by treatment with lime alone or in combination with sulphides. The process is technically called liming process, which is nothing but a hair-destroying treatment. In the liming process, strength of the hair is very much affected by the high alkalinity of the lime and sulphide. The effluents arising out of the liming process are due to the suspended solids, high alkalinity and toxicity. The disposal of the effluent has been posing a major serious problem in the leather

industry. The seriousness of the tannery effluent problems arising out of liming process and their solution using enzymes in place of lime has been adequately discussed in a recent communication.<sup>1</sup> Viewed from this angle of environmental pollution and effluent disposal, we concentrate our efforts to hair-saving processes using enzymes in place of lime and sulphide.

Ever since the discovery of the enzymic unhairing process as early as 1910, a considerable amount of literature is available on the subject. A few review articles have also been published.

It is well recognised now that a strong proteolytic enzyme is required for the proper unhairing of hides and skins. These enzymes can be derived from plant, animal and microbial sources. Protease from each source has its own advantages and disad-

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vantages for use as an unhairing agent. Of all these sources, attention is drawn towards the use of a plant protease as a dehairing agent in view of the work that has been done in the recent past.

Proteolytic enzymes from various plant sources, viz. papaya latex<sup>2, 3</sup>, sprouting soya beans,<sup>4</sup> *Solanum Campylacanthum Hochst*<sup>5</sup> and madar latex<sup>6</sup> have been reported to effectively unhair hides and skins.

The powdered leaves and barks of the jawasee shrub (*Alhagi Pseudalhagi* (Dieb) Devsyn *A camelorumfisch*), which contain rich amounts of a proteolytic enzyme, are used for unhairing hides and skins in Gujarat, Rajasthan and Madhya Pradesh. The leathers made out of the unhairing pelts using jawasee process of unhairing were found to possess certain characteristic properties which attracted the attention of a few investigators who worked out a few processes for the manufacture of different types of leathers.<sup>11-13</sup> Studies have also been made on the effect of jawasee unhairing on the physical properties of leathers.<sup>14-15</sup> The optimum conditions for extracting maximum amount of the proteolytic enzyme from jawasee have been standardised and reported in an earlier communication.<sup>11</sup> In the present study, a systematic investigation has been undertaken to develop a suitable process of unhairing using jawasee enzyme and to compare the physical, chemical and microscopical characteristics of leathers produced by the jawasee process of unhairing with those of leathers produced by a standard enzyme unhairing system. The traditional liming process has always been taken as control.

#### Materials and methods

Fresh jawasee powder was obtained from Gujarat, sieved and the fine powder was

used; the microbial protease was a product of 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.*<sup>10</sup>

#### Preparation of grain garment leather

A lot of 25 well soaked red hair sheep skins (30" x 36") was divided into three groups and used in this experiment

For strict comparison, each of ten skins was cut along the backbone line into two halves and numbered. The left halves of all the skins and five full skins were unhaird by the liming process. Out of the right halves, five right halves and five full skins were unhaird with jawasee enzyme. The remaining five right halves and five full skins were unhaird with microbial protease.

#### Unhairing by liming

For unhairing sheep skins, normal liming procedure as followed in the tannery was applied using a paint composed of 2.5% sodium sulphide, 5% lime and 20% water (on soaked weight of the skin). The skins were painted on the flesh side and left overnight, folded flesh to flesh. Next morning, they were unhaird, relimed with 10% lime and 300% water for three days, fleshed and washed. The well washed pelts were bated in a paddle with 1.5% Pancreatin product and 400% water at 35°C for 2-3 hours. The bated pelts were then pickled in a drum with 8% common salt, 1.25% sulphuric acid (conc.) and 80% water (on fleshed weight of the skin) for 2 hours. The pickled pelts were then chrome tanned in the usual manner.

#### Unhairing by jawasee enzyme

In order to unhair sheep skins using jawasee enzyme, well soaked skins were

immersed in a tub containing 15% jawasee powder and 200% water (on soaked weight of the skin), pH of which was adjusted to 6.0 with a little alkali. After 24 hours, the skins were taken out, unhaired as far as possible and were put back in the same bath adding 1% dimethylamine sulphate and 1% sodium hydroxide. After another 24 hours, the skins were taken out, unhaired completely, washed well and relimed in a separate bath containing 400% water, 5% lime, 0.25% sodium hydroxide and 0.2% Noigen LS (Dai-Ichi Karkaria) for two days. The pelts were fleshed and scudded. They were then washed with 200% water and 0.5% lactic acid for 15 minutes. The washed pelts were delimed with 1.5% ammonium chloride, 0.5% bisulphite and 200% water for 30 minutes. The delimed pelts were scudded, washed, pickled and chrome tanned as described in the liming process earlier.

#### *Unhairing by microbial protease*

For unhairing sheep skins with microbial protease, the same procedure as adopted earlier<sup>21</sup> was followed. To state briefly an outline of the procedure, well soaked skins were painted on the flesh side with a paste consisting of 1.5% microbial protease, 4% kaolin and 15% water (on soaked weight of the skin), the pH of which was adjusted between 8.5 and 9.0. They were kept in the folded condition at room temperature (28-32°C) for sixteen hours, after which they were unhaired completely and washed. They were then relimed, fleshed, scudded, washed, delimed, pickled and chrome tanned as described in the jawasee process of unhairing.

All the chrome tanned leathers obtained after the three unhairing systems were put together, neutralised, dyed, fatliquored and finished as grain garment leather in the usual manner.

The unhaired pelt of each group was subjected to microscopical examination. For this, samples were cut out from identical positions of the corresponding halves and were fixed in formol saline. After washing for 30 minutes in running tap water, sections were cut at a thickness of 80 $\mu$  in a Leitz's freezing microtome, washed in distilled water, mounted on glass slides using glycerin jelly and studied under the microscope. A few typical photomicrographs of the pelts are presented in Fig. 1. The results obtained with respect to the microscopical assessment of the fibre structure of the pelts unhaired by adopting three different systems are presented in Table I.

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

For physical tests, the finished leathers were conditioned for more than a week in the physical testing room in an atmosphere of 80° ± 4°F temperature and 65% ± 2% relative humidity. After proper conditioning, test samples were cut from identical positions of the two counterparts. For each set of experiments, test specimens were taken both parallel and perpendicular to backbone line except in the case of grain crack resistance and bursting resistance where one specimen leather piece was used for each. The sampling positions of the specimen as described by Ramanathan and Subbalakshmi<sup>22</sup> were followed here. A Scott Tensile Strength Tester (type J) was used for the determination of tensile strength, elongation, double hole stitch tear strength and tongue tear strength. Grain crack resistance and bursting resistance were determined by Good Brand Bursting

TABLE 1

Microscopical assessment of the fibre structure of the pelts obtained after different unhairing treatments

<i>Lime-sulphide</i>	<i>Jawasee protease</i>	<i>Microbial protease</i>
Epidermis and its appendages are fully removed except a few fat glands; opening up and splitting of collagen fibres in the corium are comparatively better; merging of grain layer into corium is fair.	Epidermis completely removed; fibre bundles in corium are opened up and well separated. They are full and are horizontally woven in the lower portion of the corium proper. Merging of the grain layer into the corium is fair.	Epidermis completely removed except for a small amount of epidermal appendages especially the fat glands and hair roots with bulbs; opening up and splitting of the collagen fibres in the corium are less than medium amount; merging of grain layer into corium is fair.

TABLE 2

Physical analyses of grain garment leather manufactured by unhairing with lime-sulphide mixture, jawasee protease and microbial protease

<i>Nature of Physical Tests</i>	<i>Direction of Test</i>	<i>Unhairing system followed</i>		
		<i>Lime-sulphide</i>	<i>Jawasee protease</i>	<i>Microbial protease</i>
Tensile strength (Kg./sq. cm.)	Parallel	162	298	171
	Perpendicular	124	169	137
Elongation at break (%)	Parallel	60	34	63
	Perpendicular	78	43	69
Double hole stitch tear strength (Kg./cm.)	Parallel	68	100	71
	Perpendicular	68	85	71
Tongue tear strength (Kg./cm.)	Parallel	26	22	23
	Perpendicular	27	27	22
Grain crack resistance (Kg./sq.cm./cm.)		260	312	243
Bursting resistance (Kg./sq.cm./cm.)		270	384	243

Strength machine following standard procedures. The results of the physical analyses are presented in Table 2.

For chemical analysis of the finished leathers, leathers were cut out from the official sampling positions and analysed by using the standard methods. The results are presented in Table 3.

For physical, chemical and microscopical analyses of the pelt and leather obtained after unhairing with jawasee protease and microbial protease, separate controls were taken for each of the two enzyme unhairing systems and were analysed. However, it was observed that differences obtained with respect to two controls were very insignificant and hence data pertaining to only one control are presented in all the tables. In the case of physical analyses, an average value of at least five tests was taken for each item of the tests carried out.

#### Results and discussion

As mentioned in the introduction, a few leather manufacturing processes have been developed using jawasee powder as the

unhairing agent. But, in all cases, the processes are restricted to cow or buffalo hides only and the investigators have adopted mostly vegetable tanning technique. In almost all cases, they have used common salt in the unhairing system to reduce bacterial putrefaction, since the duration of unhairing period is comparatively long. It was observed earlier<sup>19</sup> that high concentration of common salt inhibited jawasee enzyme to a great extent and hence the use of salt in our unhairing process is completely avoided. In the processing of sheep skins (red hair) using jawasee enzyme as a sole unhairing agent, with or without preservatives, we have always encountered with either putrefaction or short hair remaining; hence, in our jawasee process of unhairing, the use of any preservative was avoided and the process was shortened and made trouble-free by treatment with dimethylamine sulphate. We have attempted to manufacture a typical grain garment leather by unhairing local sheep skins (red hair) with jawasee protease and adopting chrome tanning procedure.

TABLE 3  
Chemical analyses of grain garment leather manufactured by unhairing with lime-sulphide mixture, jawasee protease and microbial protease  
(On 0% moisture basis)

Nature of Chemical Tests	Unhairing system followed		
	Lime-sulphide	Jawasee protease	Microbial protease
Oil and fats (%)	15.95	13.21	15.05
Hide substance (%)	45.37	51.45	53.55
Fixed organic matter (%)	25.71	26.40	23.06
Total ash (%)	8.49	9.21	8.12
Cr <sub>2</sub> O <sub>3</sub> (%)	5.39	5.65	5.43

In all previous cases, the leather produced after jawasee process of unhairing were compared with those obtained after liming. In the present case, however, apart from the liming process, another enzymic unhairing process using microbial protease was also taken as control.

Preliminary experiments carried out with enzymic unhairing system revealed poor splitting and opening up of fibre structure and hence, in the case of both the enzymic unhairing systems, swelling treatment was given to get optimum splitting of fibre bundles. The microscopical assessment of the fibre structure of the pelt was obtained after swelling treatment and before pickling. The findings of the microscopical assessments indicated (Table 1) that quite a good quality grain garment leather could be produced by adopting all the three unhairing systems. In the case of microbial protease, splitting of collagen fibres in the corium was found to be less predominant as compared to their control counterparts. In the case of jawasee protease, however, epidermis was found to be completely removed including epidermal appendages and fat glands, whereas in the cases of microbial protease and lime-sulphide, fat glands were found to be not completely removed. On the whole, the jawasee protease is quite comparable with microbial protease in their unhairing characteristics.

As a measure of satisfactory unhairing, the unhaird pelts were judged with respect to their removal of hair including short and fine hair immediately after unhairing and proper unhairing was observed in all cases.

The finished leathers were observed for their quality from tanners' point of view and the data obtained with respect to the experimental lots were compared with those

obtained from the control lots. It was observed from the comparative assessment that both jawasee protease unhairing system and microbial protease unhairing system produced equally good grain garment leather similar to those obtained by lime-sulphide unhairing system. The minor differences observed in some respects between the experimental and control lots were found to be insignificant.

With regard to the physical tests, the results (Table 2) show that grain garment leather obtained by using jawasee protease system had uniformly better physical properties than control (lime-sulphide) in all the tests carried out except in the cases of elongation at break and tongue tear strength, where the values were found to be lower than those of the control samples. The tensile strength, double hole stitch tear strength, grain crack and bursting resistances of the grain garment leather produced by using microbial protease unhairing system were found to be lower than those produced by jawasee process of unhairing, whereas elongation at break was found to be higher. The data obtained with respect to microbial protease, when compared with those of lime-sulphide, showed that tensile strength, elongation at break and double hole stitch tear strength of leathers produced by microbial protease unhairing system were higher, whereas grain crack and bursting resistances were lower than those of their control counterparts (lime-sulphide). In the case of tongue tear strength, however, no significant differences were observed in all the three cases.

The data (Table 3) obtained on the chemical analyses of the grain garment leathers produced by the three unhairing systems were found to fall within the standard specifications laid down fo-

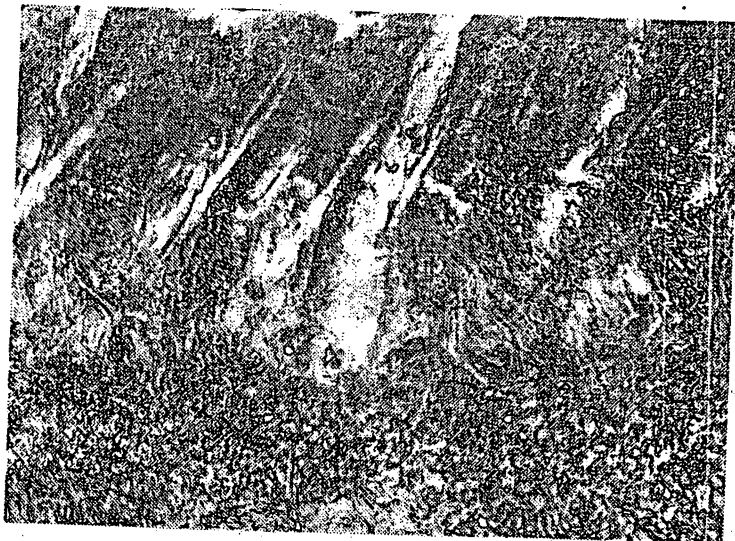
Fig. 1: PHOTOMICROGRAPH OF UNHAIRED PELT



Unhaired with lime-sulphide (x56.0)



Unhaired with microbial protease (x56.0)



Unhaired with jawasec protease (x56.0)



protective clothing leather. No significant difference was observed between the results of chemical analysis of grain garment leather produced by these unhairing systems.

The results obtained with respect to chemical analyses, microscopical examination and determination of physical characteristics for all the leathers indicated that the three unhairing procedures would produce leathers of similar and comparable characteristics with very minor differences. Similar trend of results was also reported by Airoldi,<sup>9</sup> while working on enzymic unhairing with fruits of *Solanum campylacanthum*. Based on the comparative assessments of the quality of leathers produced by two enzymic unhairing processes, viz. (i) the latex of madar plant (*Calotropis gigantea*), (ii) the water extract of germinated ragi (*Eleusine coracana*) and by the liming process, Madhavakrishna and Bose<sup>10</sup> concluded that the leather produced by enzymic unhairing processes could compare favourably with those produced by the traditional liming process.

One distinct advantage secured by both enzymic unhairing processes was that both seed and hair roots were found to be removed completely and hence, separate bating was found unnecessary. During the jawasee process of unhairing, neither the bacterial damage nor the undesirable odour was observed and hence, no bactericide was used.

When the unhairing systems are compared, it has been observed that both the enzyme unhairing systems have certain advantages over the lime-sulphide unhairing system with regard to the effluent disposal problems. In the liming process, the effluent problems are due to suspended solids arising from lime and toxic hydrogen

sulphide liberated from sodium sulphide. Dissolved sulphide and pulp hair contribute to high biological oxygen demand (B.O.D.) of the effluent. The effluent problem can be minimised by applying enzymic unhairing system, where no toxic chemicals are used and hair/wool could be recovered full length without the slightest damage to strength and quality. Another advantage of using a proteolytic enzyme in unhairing is that these enzymes will find their way in the effluent collection tank along with a good deal of proteinous substances. These proteinases will hydrolyse such proteins into their degradation products having lower molecular weights, enabling microorganisms to degrade further more easily liberating carbon dioxide, nitrogen and other volatile end products. This will indirectly help in reducing the high B.O.D. value of the effluent. Further, the enzymic unhairing process simplifies the pretanning operations and helps in the easy handling of pelts without any discomfort to tannery workers.

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