

Microbial and Other Enzymes as Depilatory Agents¹

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T. C. CORDON, H. W. JONES, I. D. CLARKE, AND J. NAGHSKI

Eastern Regional Research Laboratory,² Philadelphia, Pennsylvania

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Probably the oldest method known for removing the hair from animal skins is to allow the fresh skins to remain for a day or two in a warm, damp place. In this process called "sweating," bacteria decompose the cells of the Malpighian layer and thus loosen the hair. Because of the difficulties of controlling the process and the constant danger of the destruction of valuable leather-making proteins, this procedure has been largely replaced by safer methods.

For many years, saturated lime water has been the most widely used depilatory. As lime solutions are reused, they become more effective in loosening the hair. This has been attributed to the presence in the used liquors of ammonia, amines, and other protein decomposition products as well as bacteria and enzymes, although it is difficult to conceive of bacteria or enzymes being active at pH values above 12. In very old lime liquors, hide collagen is attacked.

The increasing demand for more rapid processing and conservation of skin collagen has led to the extensive use of "sharpening agents." Such chemicals as sodium sulfide, sodium sulfhydrate, arsenic disulfide, and various amines used in conjunction with lime, reduce the time required to loosen the hair from weeks to days. Within the last few years, some tanners have resorted to the use of sulfides sufficiently strong to dissolve the hair. This is referred to as "hair pulping" or "burning."

The use of these processes has many disadvantages, not the least of which is the disposal of the used liquors. A less drastic, more rapid method of removing the hair is badly needed by the leather industry.

As early as 1910 (Röhm, 1910, 1913), enzyme preparations were suggested for unhairing. Since that time, numerous attempts have been made to utilize enzymes for unhairing animal hides and skins in industry. However, except for some reported use in Europe at the present time, the process has not been commercially acceptable, probably due among other things to the requirement that the hides must first be treated with an alkali and the alkali then neutralized before the enzymes become effective. Green (1952) has reviewed

the literature in this field. The report of Burton *et al.* (1953) that mucolytic enzymes would effectively loosen the hair on fresh animal hides has stimulated considerable interest in the problem. Cordon (1955) was not able to confirm this work but did find enzyme preparations that were effective on salt-cured hide or hide that had been treated with dilute salt solution. Bose *et al.* (1955) attributed the unhairing action of proteolytic or amylolytic enzymes to their action on the mucoids. In order to gain more information concerning the action of hair loosening enzymes and to find those most effective, a survey has been made of a number of commercially available enzyme preparations mostly of microbial origin. The relation of hair loosening action to the proteolytic and amylolytic activities is reported here.

MATERIALS AND METHODS

Hide. The hides used in these tests were the so-called green salted steer hides of commerce obtained from a local slaughterhouse. (In this method of handling, the freshly flayed hides are allowed to cool for 1 or 2 hr, sorted, and placed in packs of about 800 hides. As the hides are spread out in the pack, they are treated with about $\frac{1}{4}$ to $\frac{1}{2}$ of their weight of salt. During a curing period of 30 days, much of the blood and salt-soluble proteins drain away.) On arrival at the laboratory, the fat and flesh were removed and the hides stored in a refrigerator. Before use, hide pieces were soaked overnight in water containing disinfectant, a common tannery practice to soften the hide and to remove dirt and salt.

Prevention of microbial contamination. Since growth of microorganisms during the soaking of the hide and during the enzyme treatment would invalidate the results, disinfectants were used. Phenylmercuric acetate in 0.015 per cent concentration was found to be effective and noninhibitory to the enzymes, and this was used in most of the tests reported here. Another disinfectant, BSM 11,^{3, 4} used by some tanners in the soak waters, was found to be effective at a ratio of 1 part per

³ A formulation sold by the Buckman Laboratories, Memphis, Tennessee. Contains 10 per cent phenylmercuric acetate and 50 per cent potassium-2,4,6,-trichlorophenol in undissolved solvents.

⁴ The mention of trade names or companies does not constitute an endorsement by the Department of Agriculture over other products of a similar nature not mentioned.

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² A laboratory of the Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

1000 parts of hide for preventing microbial growth in soak waters and in enzyme solutions.

Estimation of hair looseness. To measure hair looseness, the device shown in figure 1 was used in the absence of a more quantitative method. The blade, under a constant load, was pulled for a distance of 1 in. across the skin; if most (over 90 per cent) of the hair was not removed on the first pull, additional pulls were made up to a total of 10. The number of pulls was recorded and also an estimate of the percentage of the scraped area (1 square in.) that was free from hair. This device helps to reduce the "human factor" but an accurate quantitative method is still needed.

Assay of protease activity. The method used was an adaptation of the Gross-Fuld method (Tauber, 1949).⁵ The method is based on the principle that the degree of digestion of a casein solution by a proteolytic enzyme, conducted under standard conditions, is proportional to the proteolytic activity of the enzyme. The digested casein solution, upon acidification, produces a turbidity which is inversely proportional to the degree of digestion. This turbidity is measured with ease and reliability in a photoelectric colorimeter. One protease value (PV) unit is defined as the quantity of enzyme which digests 1 mg of casein to the "standard turbidity end point" in 1 hr at 37 C and pH 7.0.

Assay of amylolytic activity. The dextrinizing value (DV) of the enzyme preparations was measured photometrically using the Tappi suggested method T643sm-54 (1954). One DV unit represents the enzyme activity equivalent to the dextrinization of 20 mg of Lintner starch in 30 min at 30 C and pH 6.6.

⁵ The authors wish to thank Mr. M. Weber, the Pabst Laboratories, Milwaukee, Wisconsin, for furnishing his adaptation of this method.

DISCUSSION OF RESULTS

Relation of Hair Loosening to Proteolytic and Diastatic Activities of the Enzymes

Some 37 enzyme preparations, mostly of microbial origin, were obtained from several commercial sources and their amylase and protease potencies determined as described above. A few enzymes from plant and animal sources were also included. Experiments with HT Concentrate, a bacterial enzyme from the Takamine Laboratories, had shown that treatment of a green salted, soaked steer hide with a 0.1 per cent solution (that is, containing 17,000 PV units per 100 ml) for 16 hr at 45 C would loosen the hair to such a point that two or three scrapes with the device described in figure 1 would remove 98 to 100 per cent of the hair. For comparison, corresponding solutions of each enzyme were made up by weighing out the appropriate amounts of the individual enzyme to give 17,000 PV units per 100 ml. Pieces of hide were placed in the solutions which were held in a water bath at 45 C. In most cases, the pH was not adjusted but in a few instances where the optimum for the particular enzyme was out of the range of the natural pH of the hide, adjustment was made with acetic acid or sodium bicarbonate. In one instance, a reducing agent was added to activate the enzyme. Determination of hair looseness was made after various periods. The results are recorded in table 1.

As expected, the hair loosening on hide pieces treated in the same solution was not always uniform. It is well known that the difficulty of loosening hair varies with the color, black being the most difficult to loosen, red next, and white easiest. Different hides of the same color may also show considerable variation and certain parts of any hide are notoriously more difficult to un-hair than others. The results presented do give a fair

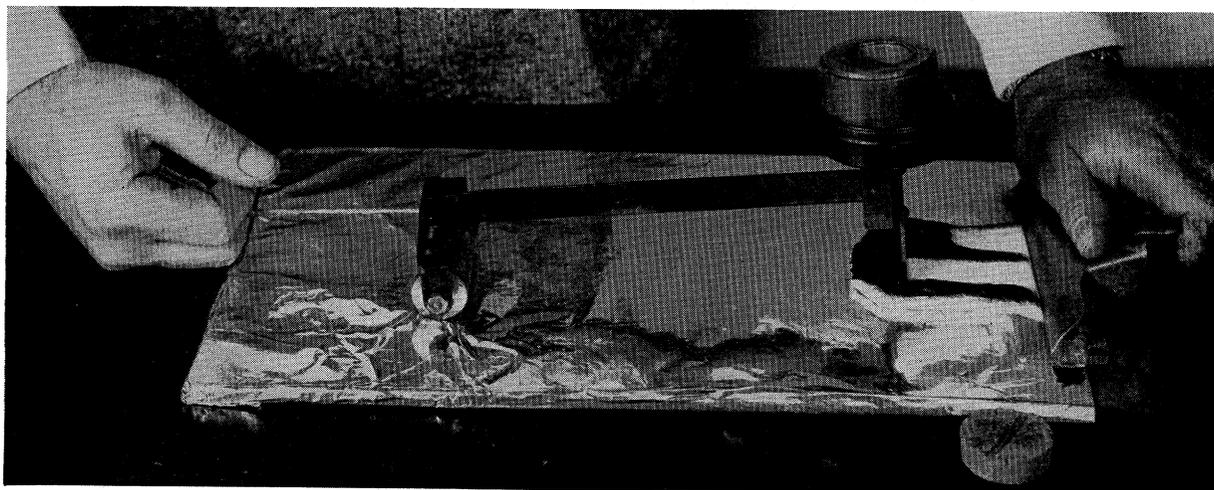


Figure 1. Device for testing the degree of hair loosening of enzyme treated hides

TABLE 1

The depilatory action of various enzymes as related to their proteolytic and amylolytic activities

Enzyme and Supplier	Source	PV Units	DV Units	Amount Used ^a	Hair Looseness After					
					16 hr		24 hr		40 hr	
					P ^b	R ^c	P	R	P	R
<i>Takamine Laboratories</i> ¹										
H. T. Concentrate	Bacterial	170,740	61,700	0.10	3	99 ^d	3	99		
					3	99	3	99		
H. T. Proteolytic	Bacterial	80,470	29,750	0.21	3	95	3	95	3	99 ^e
					3	95	3	95		
H. T. 440	Bacterial	17,475	9,460	1.00	10	90	10	99	2	99 ^e
					10	80	10	95		
H. T. 44	Bacterial	1,600	1,000	10.10	10	0	10	70	3	95 ^e
					10	0	10	70		
Clarase 300 ^f	Fungal	1,960	3,490	8.67	10	25	10	75	3	95 ^e
					10	25	10	75		
Special Diastase 160	Plant and animal materials	6,720	7,200	2.59	3	99	2	99		
					3	98	2	99		
Pancreatin 3 USP	Pancreas	66,870	16,300	0.25	10	75	10	99	3	98 ^e
					10	75	10	99		
<i>Pabst Laboratories</i> ¹										
Protease	Fungal	56,000	1,830	0.30	4	95	3	98	4	90
					10	70	10	95		
Amylase	Fungal	800	83,200	21.25	10	0	10	0	4	80 ^e
					10	0	10	0		
Protease L-56-D	Bacterial	110,400	4,530	0.15	10	70	3	99		
					10	70	3	99		
Amylase L-254	Bacterial	14,180	49,300	1.20	4	90	5	95	4	80
					10	70	10	95		
<i>Rohm and Haas</i> ¹										
Rhozyme A4	Fungal (<i>Aspergillus oryzae</i>)	14,550	3,450	1.18	10	95	3	98	4	90
					10	25	10	80		
Rhozyme DX ^g	Bacterial	600	925	28.40	10	0	10	0	10	25
					10	0	10	0		
Rhozyme B6	Fungal	16,300	1,060	1.05	10	95	3	95	3	95
					10	90	3	95		
Rhozyme H39	Bacterial	19,150	29,675	0.89	10	95	10	98	3	98
					10	95	10	98		
Rhozyme P-11	Fungal	21,775	220	0.78	10	95	3	98		
					10	98	3	98		
Rhozyme S	Fungal (<i>A. oryzae</i>)	9,125	2,660	1.87	10	25	10	50	4	85
					10	50	10	75		
Rhozyme 51	Fungal (<i>A. oryzae</i>)	<500	450	35.00	10	0	10	0	10	25
					10	0	10	0		
Diastase 32	— ^h	5,100	1,350	3.35	6	90	7	98	4	85
					10	0	10	95		
Lipase B	Microbial	1,750	1,330	10.00	10	0	10	0	10	25
					10	0	10	0		
Oropon N ⁱ	Pancreas	2,250	<250	7.59	10	0	10	0	10	95
					10	0	10	0		
Oropon N-O ⁱ	Fungal	2,300	<250	7.42	4	98	3	98		
					3	98	3	98		
Oropon N-2 ⁱ	Bacterial	3,000	400	5.69	4	90	3	98		
					4	80	3	98		
Pectinol 100D	Microbial	500	185	34.15	10	0	10	0	10	25
					10	0	10	0		
Protease 15	Bacterial	14,750	715	1.16	3	98	3	98		
					3	98	3	98		
Cellulase Enzyme 19	Fungal (<i>A. niger</i>)	1,160	400	14.71	10	0	10	0	10	60
					10	0	10	0	10	0
<i>Wallerstein</i> ¹										
Mylase L1	Fungal	1,560	3,300	10.94	10	90	5	90	3	95
					10	98	3	95		
Mylase SA	Fungal (<i>A. oryzae</i>)	1,736	3,300	9.80	10	80	10	90	3	98
					3	98	3	98		

TABLE 1—Continued

Enzyme and Supplier	Source	PV Units	DV Units	Amount Used ^a	Hair Looseness After					
					16 hr		24 hr		40 hr	
					<i>P</i> ^b	<i>R</i> ^c	<i>P</i>	<i>R</i>	<i>P</i>	<i>R</i>
Prolase 40	Fungal	20,496	500	0.83	10	95	3	98	3	98
					10	98	5	98		
Enzyme W3F	Bacterial	840	1,660	20.30	10	0	10	0	10	25
					10	0	10	0		
Enzyme 4511-3	Bacterial	46,300	17,000	0.37	3	99	3	99		
					2	99	3	98		
Enzyme 4511-6	Bacterial	20,800	5,300	0.82	3	98	3	98		
					3	98	3	98		
Enzyme MT7820 ^d	Fungal	6,700	435	2.55	10	90	7	98	3	95
					10	50	7	95		
Invertase	Yeast	<200	<250	85.37	10	0	10	0	10	10
					10	0	10	0		
<i>Viobin Corp.</i> ¹ Viokase ^m	Pancreas	45,000	5,000	0.38	10	90	10	95	6	95
					10	90	10	95		
<i>Mann Research Laboratories</i> ¹ Papain ^k	Plant	23,450	<300	0.73	10	98	6	98	3	98
					10	95	6	95		
<i>Nutritional Biochemicals Corp.</i> ¹ Trypsin 4xUSP Pancreatin	Pancreas	66,450	3,150	0.26	5	95	3	95	3	95
					5	95	3	95		

^a To give 17,000 PV units per 100 ml, equal to the PV potency of 0.1 per cent HT concentrate.

^b Number of pulls with scraper (see figure 1).

^c Estimation of per cent of hair removed.

^d The two sets of figures are from different trials on the same piece or from duplicate pieces.

^e After 4 days' incubation.

^f Unhairing solution adjusted to pH 5.2.

^g Unhairing solution adjusted to pH 5.6.

^h Not disclosed.

ⁱ Unhairing solution adjusted to pH 8.2.

^j Unhairing solution adjusted to pH 5.0.

^k Sulfitc added as activator.

¹ Takamine Laboratories, Clifton, New Jersey; Pabst Laboratories, Milwaukee, Wisconsin; Rohm & Haas Company, Philadelphia, Pennsylvania; Wallerstein Company, Inc., New York, New York; Viobin Corporation, Monticello, Illinois; Mann Research Laboratories, New York, New York; Nutritional Biochemicals Corporation, Cleveland, Ohio.

^m Whole pancreas, desiccated and defatted after being activated to the highest possible proteolytic activity.

indication of the relative effectiveness of the different preparations.

The evidence is very strong that the enzyme assays used for proteolytic potency did not measure the hair loosening power of the enzyme preparations.

If the hair loosening activity were due to the proteolytic action measured by the Gross-Fuld procedure, the same degree of loosening should have been obtained in all cases since all solutions were of equal PV concentration. This is obviously not the case. The first four preparations listed in table 1 are from the same organism, a thermophilic bacterium, yet though the same number of protease units was present in each case the hair loosening activity was very different. However, when the preparations were ranked according to proteolytic activity there was a rather high correlation with hair loosening even though adjustment had been made to give solutions of equal activity. This would seem to indicate that there is a hair loosening compo-

nent that increases more rapidly than the total proteolytic activity as the latter increases.

The dextrinizing values (DV) show even less correlation with hair loosening power than protease values. The preparation having the highest DV potency, a bacterial amylase, had very poor hair loosening power; conversely, some relatively effective hair loosening preparations were very low in dextrinizing power, for example Rhozyme P-11 (table 1).

That bating activity of enzymes is not equivalent to hair loosening power is shown by the fact that the Oropons, which are bates used by the leather industry and are approximately equivalent as far as the bating action on hides and skins is concerned, did not give the same degree of hair loosening. Oropon N, prepared from pancreas, was decidedly inferior to the fungal and bacterial products as hair loosening agents.

Under conditions of these experiments, which did not involve alkaline pretreatment of the hide, none of the

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preparations from pancreas were outstanding depilatories. This is interesting because most of the previous attempts to use enzymes for unhairing were based on pancreatic enzymes.

Whatever the actual component or components which hold the epidermis to the corium, the ability to loosen the bonds seems to be rather widespread, for plant, animal and microbial preparations all possess the activity to some degree. If incubated long enough, all preparations showed some hair loosening activity. In some, however, particularly the nonproteolytic enzyme, such as lipase B, Pectinol 100D, cellulase Enzyme 19, Invertase, Enzyme W3F, Rhozyme DX, and Rhozyme 51, it was very weak.

From these results, it appears that several of the preparations tested could possibly be developed for commercial use. The final criterion, however, is whether enzyme unhairing hides and skins can be converted into salable leather, and that is yet to be determined.

ACKNOWLEDGMENTS

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SUMMARY

Studies with bacterial, fungal, and other enzymes revealed varying degrees of depilatory action. This activity could not be definitely correlated with the proteolytic or dextrinizing power as measured by hydrolysis of casein and starch.

REFERENCES

- BOSE, S. M., KRISHNA, W. M., AND DAS, B. M. 1955 Mechanism of unhairing skins and hides by means of certain proteolytic or amylolytic enzymes. *J. Am. Leather Chemists' Assoc.*, **50**, 192-199. (See also Indian patents numbered 50806 and 52013.)
- BURTON, D., REED, R., AND FLINT, F. O. 1953 The unhairing of hides and skins without lime and sulfide. The use of mucolytic enzymes. *J. Soc. Leather Trades' Chemists.*, **37**, 82-87.
- CORDON, T. C. 1955 Unhairing of hides and skins by amylase preparations. *J. Am. Leather Chemists' Assoc.*, **50**, 270-274.
- GREEN, G. H. 1952 Unhairing by means of enzymes. *J. Soc. Leather Trades' Chemists.*, **36**, 127-134.
- RÖHM, O. 1910 Dehairing and cleaning of skins and hides, German Patent No. 268,873.
- RÖHM, O. 1913 A new unhairing process. *Collegium*. 374-377. [Abst. *J. Am. Leather Chemists' Assoc.*, **88**, 408 (1913)].
- Tappi Suggested Method T643sm-54 1954 Dextrinizing value of enzymes (Enzyme activity). *Tappi*, **37**, 113A.
- TAUBER, H. 1949 *The chemistry and technology of enzymes*, p. 181. John Wiley & Sons, New York, New York.