Acknowledgement

Begun with total unconditional submission of myself as the almighty Allah who created me and endowed me the might to be here in this world and produce this work

Again, I acknowledge my sincere gratitude hearty, thanks and indebtedness to my honorable teacher Mahatab sir lectural, Bangladesh college of leather technology for giving me scope to work on this topic and also his active interest in my work despite his pre occupation

I like to express my deepest sense of gratitude, sincere appreciation, indebtedness to my guide teacher Mr. Mahatab sir, lecturer, college of leather technology

I am also grateful to Mr. Hanif Ali, foreman instructor and other who held me to complete my thesis.

I owe to my beloved parents and family members for the completion of this thesis.

Finally, I express my gratitude to others who directly and help me for completion of this work.

Akterozzaman sikder
August 2006.

CHAPTER-1

Introduction

It may be defined as organic catalysts produced by the cell of plants and animals they are capable of bringing about changes in various substances without becoming a part of the final product of the action and are not used up in the using. Enzymes posses the properties of proteins. the rate of enzyme reaction are influenced by [1] temperature [2] ph of solution [3] ultraviolet light [4] concentration of enzyme [5] concentration of substrates,[6] presence of activators and [7] presence of inhibitors.

Enzymes have optimum, maximum and minimum temperatures for their activities and destroyed by the light temperatures. Usually action causes at 0 degree centigrade, the optimum m for most types lie between 35 degree centigrade to 50 c and with few exceptions they are soon destroyed at above 70 c and almost instantly by boiling water. When completely dried, enzymes may withstand higher temperatures.

Some enzymes act best in acid solution, others requires alkaline solution, still others do not function well unless their environment is neutral in reaction. Pepsin is a proteolytic enzyme active only in acid solution. Try sin 19 another proteolitic enzyme active only alkaline solution. Strong acid and alkaline destroys enzymes. Enzymes are becoming increasingly more important in various industrial processes. The enzymes, which are used in leather industry, are proteolitic and therefore can hydrolyze proteins.

Tanning is a process of converting participle outer covering of animals to no participle leathers with definite physical, chemical and biological properties so that they can be used in our daily life and industries. Before going into details, if necessary to discuss a little bit about the composition of hides and skins.
Reasons for using enzyme [1] improve the quality [2] get a better yield [3] environment friendly.

Among these, protein is the only most important material for leather making 64 percent to 94 percent of the solid matters of the hides and skins are tot fibrous proteins known as albumin and musing are found in hides and skins. During soaking liming, bating, most of soluble proteins and fibrous proteins like keratin, reticular etc are removed. Then the white collagen fibers are tanned to usable leather.

Most of the enzymes used in the tannery processing are still of the proteolysis type, required to selectively degrade the peptide bonds of the different non collagen us proteins of hides and skins whilst avoiding any damage to the collagen itself..

OBJECT OF THE PROJECT

The object of the present work is.

[1] To study the comparative action of different types enzymes available from different sources.

[2] To continue this study at different stages of preliminary process of tanning.

[3] To observe the use of enzymatic method to remove wrinkle.

The use of enzymes in leather processing has a long and colorful history and a brief outline of this fascinating subject may perhaps help to put our present preoccupations into some perspective. Enzyme dependent processes have been employed in leather making for thousands of years. The enzymatic degradation of individual components of skins such as derma tin soleplate proteoglycan or elastin that can occur during bating process to achieve specific production objects, for example, the development of rapid unhairing liming processes or the production of more open or relaxed fiber structure.

The enzymatic formulations used in the beam house are [1] soaking sides for salted and dry raw hides and preserved fur skin [2] unhairing formulations for all types of hides and skins.[3]alkaline bates for scud removal and opening up of fiber structure[4]acid bates for blue chrome and fur skins [5 enzymatic degreasing agents for greasy skins and [6] enzymatic formulations for by production utilization

CLASSIFICATION OF ENZYMES

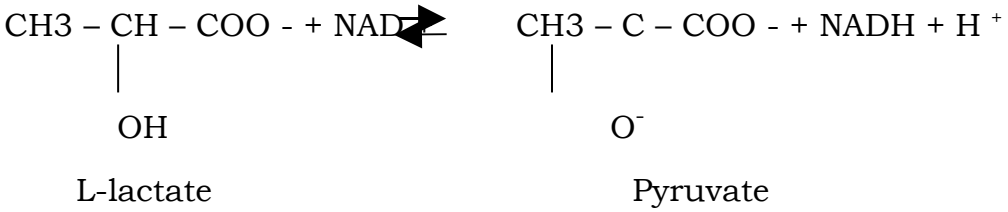
In recent years, the rapid growth different in the science of enzymology and the great increase in the number of enzymes have given rise to many difficulties of terminology. The naming of enzymes by individual workers had proved far from satisfactory in practice. In many cases, the same enzyme become known by several different names, while there were cases in which the same name was given to different enzymes. Many of the names conveyed no idea of the nature of the reaction catalyzed.

All the enzymes are classified into six major categories.

Oxidoreductases

These enzymes catalyses the removal of [dehydrogenises] addition of o2 [oxygenizes]. They catalyzed the ox reduction of =CHOH, =CH-CH=, -CHNH2 and others.

Example-L-lactate; NAD oxidoreductase [travail name lactate dehydrogenate] catalyses.



L-lactate Pyruvate

It is the alcohol group of lactate, which is involved in the reaction, and this is indicated in the above equation.

Transferees

This enzyme catalyzed transfer of group, which is not free during reaction from one substrate to another

Examples of transferred groups:

-CH3, -CH2OH, -NH2.

Example: ATP: D- hexose –6-phosphotransferase [trivial name hexokynase catalyses.

$C_5H_9O^5CH_2 + ATP = C_6H_9O_5CH_2OPO_3H_2$

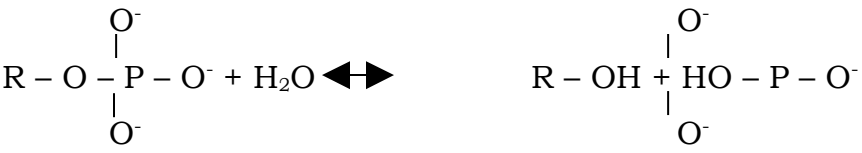
D-Hexose D-hexose-6-Phosphate.

This enzyme will transfer phosphate to variety of D-hexoses.

Hydrolases

These enzymes catalyze the splitting of compounds by addition of water across various bonds e.g.: peptide, glycoside, and ester. Phosphate

Example: orthophosphoric monoester phosphor hydrolase [alkaline. phosphates] catalyses.



(Organic phosphate) (In organic phosphates phosphate)

Alkaline phosphates are non – specific, and act on a variety of substrates at alkaline pH of the hydrolase's proteases are mainly used in the leather industry. Again, proteases are divided into three groups depending upon the origin viz. plants, animal and microbial sources.

Lyases

These enzymes catalyze the non-hydrolytic addition removal of groups, which are free during reaction. Examples of cleaved bonds: C-C in decarboxilation, C-O in removal of water, C-N and C-S.

Example: L - histidine carboxilase (tribal name histidine decarboxilase) catalyses.

$C_2N_2H_3CH_2CHNH_3 + C_3N_2H_3CH_2NH_3+ +CO_2$

COO^-

Histidine Histamine

Isomerases

These enzymes catalyze the condensation of two molecules coupled with the breakdown of a pyrophosphates bond from a nucleotide tri phosphate.

The reaction are represented as

$X +Y +ATP = X -Y +ADP +P_i$

Or

$X + Y + ATP = X -Y +AMP +(PP)_i$

Example: L-glutamate: ammonia ligase (trivial name glutamine synthetics) catalyses.

$O = C\text{-}CH_2–CH_2–CH–COO^- +NH_3 +ATP \leftrightarrow O=C\text{-}CH_2\text{-}CH_2\text{-}CH\text{-}COO^- +ADP +Pi$

O^- NH_3+ NH_2 NH_3+

L-glutamate L-glutamine.

FACTORS AFFECTING ENZYMATIC ACTIVITY

Substrate concentration:

The rate of catalyzed reaction increase with increasing substrate concentration but above a certain substrate concentration, the rate enzyme action ceases to increase [fig-6]. The shape of the curve is commonly explained on the basis of catalically active site on the enzyme that reacts with the substrate maximum velocity, V max of the reaction is reached when all the sides are occupied by the substrate molecules.

MICHAELIS-MENTEN EQUATION:

L. Michaelis and ML Menton in 1973 postulated the existence of an enzyme substrate complex as the basis for a theoretical analysis of enzymatic reactions. Let us consider a single substrate catalyzed reaction where there is just one substrate – binding site per enzyme.

Enzyme + substrate $\leftrightarrow$ Enzyme- substrate - Enzyme + product

E S k-1 Complex ES k2 P

The Michaelis- Menton assumption was that an equilibrium between enzyme substrate and enzyme- substrate complex was instantly set up and maintained and the breakdown of the enzyme substrate complex to product was negligible to disturb the equilibrium. Briggs and haldane extended this idea and derived and equation assuming steady-state condition, I e. the rate of breakdown of the complex is same as the rate of formation during the period of measurement. Using this assumption, the equation is written as :

K1 [E][S] = k-1 - [E][S] +k2 [E][S] =[ES] (k –1 +k2)
Separating the constants from the variables,

$$\frac{[E][S]}{(k_{-1}+k_2)} = K_m$$

[ES] k1
where Km is another constant.
The total concentration of enzyme present [Eo] must be the sum of the concentration of enzyme free [E] and the concentration of bound enzyme [ES].substituting [E] =[Eo] –[ES] in the above equation.

$$\frac{[Eo]-[ES][S]}{[ES]} = Km$$

the term [ES] governs they rate of formation of product[the overall rate reaction] according to relationship :
Vo=k2[ES]
If this is substituted in the above equation,

Moreover ,when the substrate Concentration is very high, all the enzyme is present as the enzyme substrate complex and the maximum velocity, Vmax,is reached. Under this conditions,

Vmax =k2[Eo]

Finally, since the substrate concentration,[So],is usually greater than the enzyme concentration ,

This equation has retained the name Michaelis-Menten equation and Km is called the Michaelis constant

A graph Vo against [So] will have the from of rectangular hyperbola consistent with experimental finding for many enzyme- catalyzed reaction .Vmax, the maximum initial velocity at a particular [Eo] can be obtain from thegraph.

When,Vo =1/2 Vmax, and when this is substituted VIN Michaelis Menten equation,

Therefore, Km is the substrate concentration at which an enzyme reaches half the maximum velocity.

Km VALUES OF SOME TYPICAL ENZYME

ENZYME	SUBSTRATE	Km (M)
Chymotropsin	Acetyl-I-L-tryptophamide	5×10^{-3}
Lysozyme	Hexa-n-acetylglutamine	5×10^{-6}
B-galactosidase	Lactose	4×10^{-3}
Trypsin	Benzoyl-l-arginine ethylestar	5×10^{-5}
Pepsin	Acetyl-l-phenylalanyl di-iodotryrosine	7.5×10^{-5}
Papain	Benzoyl-l-arginine ethylestar	1.89×10^{-3}
B-amylase	Amilose	7.3×10^{-5}

LINEWEAVER_BURK PLOT

The graph of the Michaelis-Menten equation showing enzyme activity against substrate concentration, is not entirely satisfactory for the determination of Vmax and Km .Unless there are at least three consistent points on the plate of the curve at different [S]values, an accurate value of Vmax and hence of Km can not be obtained. The graph being a curve, can not accurately exploited upwards from non-saturating values of [S]

Line weaver and bark overcome this problem without making further assumptions. The Michaelis-Menten equation was simply inverted as shown below.

This is form y =mx+c, which is the equation for the straight- line graph a plot of y against has aslope m and intercept c on the Y axis. A plot of 1/ Voagainst 1/[o][Line weaver and bark plot] for system obeying the Michaelis-Menten equation. The graph ,being linear, can be extrapolated substrate concentration and from the extrapolated graph, the values of Km and Vmax can be determined.

ENZYME CONCENTRATION

the relation between enzyme concentration and its activity is demonstrated by maintaining substrate concentration, ,pH and temperature constant while the concentration of the enzyme solution is varied.

The different conditions are expressed A, indicates normal response showing that the activity varies with linearly enzyme concentration. B shows that some activator is present in the enzyme preparation and hence the reaction does not proceeding a linear way "C" shows a condition of substrate exhaustion where there is no increase in reaction velocity once the substrate is depleted.

TEMPERATURE

Enzyme catalyzed reaction are similar to other reaction in that the rate is increased by increasing Temperatures up to a point. Beyond that Temperature, the activity of an enzyme declines sharply. as the Temperature increased beyond 45^0 C-50^0 .the rate decreases. This decreases is caused by the thermal denaturation of the enzymes protein or the inactivation a thermoliabile component in the enzyme system when thermal energy becomes great enough to cause the rupture of a new bond, the neighboring bonds are weakened and the whole molecule becomes denatured .the optimum Temperature of enzyme under physiological is close to 400 c. the maximum velocity of enzyme reaction is obtained around the optimum Temperature.

PH

When the activity is plotted against Ph, a bell shaped curve is obtained. This indicates a marked dependence of an the Ph of the reaction mixture with enzyme.

CHAPTER-2

Enzyme characterization.

Before examining some of the newer applications of enzyme technology in the modern tannery ,I would like to first consider the importance role of enzyme characterization ..As our demand son enzyme become more sophisticated, it essential to ensure that they are accurately characterized .this is particularly necessary at the present stage of development in enzyme technology in the leather industry ,where the most enzyme used in tannery processing are still of the proteolytic type, required to selectively degrade the peptide bonds of the different non collageneous proteins of hides and skins, whilst avoiding any damage to collagen itself .The importance of assaying enzyme activities against representative substrate of skin was clearly pointed out over 60 year ago by john Arthur Wilson in early papers on the evaluation of bating materials.

Assays based on chromogenic substrates such as azo- albumin, hide powder azure ,keratin azure and elastin red both rapid to perform and relevant to leather processing. They provided a convenient way of screening comparatively large number enzymes for their relative activities against representative substrate s of the different components of hides and skin .

The information obtained by this type of approach does provide a comparatively ration basis for improving leather processing .If certainly offers an extremely useful starting point for selecting the most

appropriate enzyme system or set conditions for achieving specific objectives during processing. For example, the Ph activity profiles of the commercial enzyme shown in fig-1 would suggest that this particular protease should prove effective, at both pH 8.5 –9.0 and pH 12.5 at degrading general interfibrillary protein (i.e., significant activity against azo albumin and denatured hide powder azure) but the enzyme might only be expected to have appreciable activity against elastin at the lower pH (elastin red) fig-1 shows azure increase at the higher pH

All the chromogenic substrates illustrates in fig-1 are commonly available, except for elastin red which has been synthesized at BLC from elastin and reactive red. Other workers have also found direct assays on skins samples to be useful for the studies on bating, when factors such as scud removal are under investigation. In our own studies on opening up in the beam house, we have similarly relied on direct analysis of hide or skin to follow the proteolitic degradation of proteoglycan, using sensitive electrophoric techniques develop to distinguish dermatan sulfite from hyaluronic acid.

TABLE-2

PROPERTIES OF THIN BOVINE CLOTHING LEATHER

Name	Pancreatic bate	Bacterial bate
Elastin (degradation%)	0	95
Grain (thickness mm)	0.48	0.38
Tear (strength kg/mm)	3.1	4.0

CHAPTER-3

WHAT IS WRINKLE

Toss wrinkles, often referred to as “fat wrinkles ” and ” neck wrinkles”, or more properly as ” grain wrinkles”, are among the natural characteristics of skin and hides which may be evidenced on finished leather. These wrinkles are encountered in all leathers, the degree of prominence varies. Grain wrinkles according to Roddy associated with the thick muscle, a straight or voluntary muscle which is found on both side of the back bone for a skin or hide, running from the butt area to the shoulder area and extending down about half the distance to the belly edge. The fibers of those sheathed, voluntary muscles are inserted in some areas of the skin and their contraction twitches the skin or produces temporary folds. Removal of this muscular sheath allows the skin to spread out, resulting in less accentuated grain wrinkles.

Grain wrinkles are seen depressed valleys upon the grain surface of skin and hide. They vary in depth and length <generally running a course at a right angle to the backbone. As wrinkles the backbone of the skin or hide, they some times divided many times before terminating. The most prominent wrinkle are along the backbone and the least prominent are in the sides and the belly. Some beam house methods are accentuate wrinkles, other reduces the severity. The bottom piece was treated in lime alone containing 10 percent excess. The next 4 pieces are treated in lime solution containing 10 percent excess with solution 4 containing 1

percent dim ethylamine – arsenic sulfide mixture solution 3 containing 1 percent arsenic sulfide solution 2 containing 1 percent methyl amine and solution 1 containing 1 percent sodium sulfide on the stock weight. The flesh on the hide or skin should be removed before liming as this reduces the degree of wrinkling. Longer and milder beam house methods tend to reduce the severity of wrinkles. Any condition, which prevents "letting out" of the stock, tends to accentuate grain wrinkles.

Many tanneries practices green fleshing because the presence of muscular tissue and flesh during liming operation keeps the stock,

Muscular tissue, which is referred to by the trade as corduroy flesh, is found occasionally on sole lather and appears on the fleshed side of the finished leather. After tannage, muscular tissue becomes much darken color than the surrounding collagenous fibres and is hard and brittle. The tissue breaks readily upon flexing the sole leather and leaves grooves on the fresh it can cause trouble during channeling of soles. Muscle tissue as it appears in cross sectional view.

CAUSTIC SOULITONS

Rapid swelling in the stock or liming operation due to high alkalinity, increase the thickness of hide or skin and reduces the footages. Grain wrinkles are accentuated by this condition. They are less pronounced when milder lime systems are used when there is more control of the plumping action. Longer liming reduces the prominence of such wrinkles>four factors that influences swelling and plumpness and five

ways to modify swelling and plumpness are listed under" over plumping and poor letting out".

POOR DEHYDRATION

When the center layer of a hide or skin has not been properly rewetted in the soak, the footage or area of the hide or skin is not extended to the maximum in the liming operation. This incomplete "letting out" accentuates grain wrinkles.

INCOMPLETE DELIMING, IMPROPER BATING.

One purpose of deliming and bating is to produce a "fallen" condition in the stock which is a type of "letting out" of the tissues. Grain wrinkles are accentuated in stock that is not properly bated or incompletely deli med

CHAPTER-4

ENZYMES IN SOAKING.

Soaking is the first operation in the tannery in which fresh or preserved hides and skin are treated in water for making clean and soft hides and skin received in the tannery in one of four conditions viz

(1)Fresh immediately after flaying

(2)Wet salted.

(3)Dry salted

(4)Dried.

The restoration of moisture lost due curing and preservation and the removal of extraneous matter are of prime importance in soaking where water dehydrates the dried interfibrillary protein and loosens the cementing substance of fibres. The collagen fibres and keratin cells of the hair and epidermis. Also take up water to become more flaccid and flexible. The duration of the soaking period, the number of changes of water and the use of antiseptics are all dependent on the thickness of hide and skins, their curing methods and often greasiness of the skins.

Dry hides absorbs water very slowly and consequently much time is required to make them absorb the required amount of water to be soft, if, some amount of sodium hydroxide or sodium sulfide is added to the soak water, the hides and skins can absorb water more quickly, usually, 1-2 parts of sodium hydroxide and 1-1.5 parts of sodium sulfide are used per

1000 parts of water to make a soak liquor for dry and dry salted hides and skins. After36 hours periods of soaking, if they are not soft enough, they can be made soft by “breaking over the beam” or “dry drumming” methods. It should be borne in mind that they should be made almost as soft as green/fresh hides. When hides are dried, the fibres become hard and horny. The interfibrillary substance between the fibres becomes hard by drying. By soaking, sufficient water should be introduced into the fibres and into the interfibrillary cementing substance to make them soft.

In soaking, penetration of sufficient moisture within the hiders or skins is necessary for solubilisation and elimination of salts and globular proteins contained with in the fibrous structure. The replenished water serves as a vehicle for penetration of the chemicals employed subsequently to effect hair loosening, plumping and alkaline action.

In addition, soaking removes blood, manure and urine, which are nutrients for bacterial growth and they, along with certain salts in soaks, may cause dark stains to the finished leathers. Sand, stones and parasites are also removed, there by avoiding the possibility of damage to machines used in subsequent operations. It has been observed that curing salts, if not removed in soaking but transferred to the lime liquors, have been found to reduce the alkaline plumping action and also the unhairing rate. Flesh and fatty adipose tissue can be easily removed mechanically from the pelts only after proper soaking.

Proteolytic enzymes, especially acid proteases, increase the rate of soaking dried fur skins. The short soaking time and low pH prevent damage to the hair bulb and subsequent hair loss. The advantage include loosening of the scud, initiation of the opening of the fibre structure and production of the leather with less wrinkled grain when used at an alkaline pH of less than 10.5

Protease solubility's and thereby helps in the removal of the interfibrillary cementing substance from the hides and skins. In addition to proteases, if small amount of amylases lipases are added, the glycol proteins as well as lipoproteins are degraded and removed in the soaking operation itself. This facilitates the subsequent unhairing and bating operation also. Use of enzyme preparation in soaking of preserved rabbit skins improved the softness and elasticity and incre3ased area yield of the fur by 3.3% while reducing the processing time by 10-20 hours.

RECENT RESEARCHES ON THE ENZYMATIC SOAKING AGENTS

Grimm (1966) described a soaking method using proteolytic enzymes and carbohydrates in the pH range of 5.5 to 10.0 Enzymes from asperillus parasitcous, A flavus, A orazae and B. subtilis were used alone or in mixtures and the resulting leathers were found to be full, supple and showed no loose grain. Rokhvarger and zubin (1971) suggested the use of carbohydrates from the mould culture A.awamori in soaking. This enzyme was used at pH 4.5 to 5.0 at 30° C. for 18 to 24 hours whish improved the wetting of hides. Subsequently, the use of rhizopine at 38° C .pH, 4.0 to 5.0 for 8 hours to soften woolskins was advocated (chebotareba et al. 1975). This treatment affectively removed 50 % of the mucopoly saccharides, separated the bundles of collagen fibres and generally softened the fur.

Non salted and preserved sheep skins were wet back with the enzymes of A.oryzae in 4 to 5 hours (Toshev and Esaulenko, 1972). Optimum for extracting nitrogenous components and carbohydrates by the use of

Biofern (Pancreatic amylase) and sodium B1 sulfite at pH 5.0 at 35° C,. this was described and it was shown that by reducing the temperature 225° C., the extraction of mono saccharides was reduced markedly (Esaulenko et al 1975) Botev et al 1976. Showed the use of bacterial amylase for soaking dried wool lamb skin. Bacterial amylase has strong amylolytic activity, much weaker proteolitic activity and no lipolytic activity. At a concentration of 0.5 to 0.6 gm/litre and pH around 5.0 in the presence of 1.5 gm/litre sodium hydrosulphate, the enzyme activity was increased by about 35 percent. Hence, bacterial amylase could be used at 1/3 rd concentration necessary for amylase derived from animal sources.

Monsheimer and Pflieiderer carried out good amount of researches on enzymatic soaking. The advantages of using mold proteases at pH 5 or lower for soaking, unhairing and bating were discussed (pfleider, 1968) and in a patent, soaking with pepsin and papain at a pH of 3.0-4.5 was described (monsheimer and pfleider, 1970). Subsequently, use of alkaline proteases of bacterial and fungal origin was recommended and this reduced the need for the liming chemical by 30-60 % In later patent formula for soaking hides, skins and fur skins using a proteolytic enzyme at a pH of 10.5. After 4 hour the hides were uniformly soaked and showed no sticking of the fibre structure, whereas the fur skins were also well soaked with no evidence of hair slip.

To increase area yield and to improve the quality, furs were first treated with the water followed by a two step of pickling took place in the presence of 0.1 g/L polygalacturonase while the first step of pickling took place in the presence of Lactic acid and sulfuric acid (Dianova et al. 1977).

Soaking of hides in 1% solutions o protogidrolitin (a proteolytic enzyme preparation) or in actinomitset 11 (a lypotic-proteolytic enzyme preparation) tended to remove non-protein constituents thus facilitating further processing. A typical procedure was to teat washed hides in 1.5 vol. (V/W) of solution containing sodium sulfite 0.3%, surfactant 0.3% and enzyme preparation 0.5% at 360º C. for 1 Hr, then to add 3.0% of the enzyme preparation and continue the treatment for 12 Hrs (Moiseeva and Shestakova, 1979).

According to Moiseeva et el (1980), softening and unhairing of dried hides could be improved using protosubtilin G 10 km (alkaline protease) in pre-soaking operation. The optimum pre-soaking conditions were : pH 11.0, temperature 50º enzyme 0.75% on the hide weight and sodium acetate 10 gm/litre for 6 to 8 hours. The pH and temperature were important factors, Raising of temperature from 30ºC to 50ºC increased the proteolytic activity by 20%. It is difficult to understand why presoaking was carried out at such a higher temperature, since the proteins of the raw hides will get denatured at that temperature and the quality of the final leather will be poor quality

Asbeck et al (1980) showed that the dried furs were soaked in an acidic aqueous bath containing 1% acid protease from rhizopus rhizopodiformis and sodium by sulfite at 25º C. for about 20 hour and the soaked furs were finished in the visual way . However, exact pH of soak bath was required to be maintained to get optimum results.

Orlita and Beseda (1985) tested three commercial bacterial alkaline protease preparations for the soaking of salted cowhide. Use of enzyme

preparations resulted in a decrease in soaking time. The relaxation of cow hides soaked in the presence of enzymes increased with prolonged soaking time compared to virtually no changes in the relaxation of hides soaked in pure water or water containing a surfactant. All enzymatic preparations increased significantly the amount of nitrogenous compounds in the liquor after soaking

Enzymes are employed in many tanneries to assist in soaking back of hides and skin and for the initial removal of interfibrillary protein.

We have found that protease based products can be especially useful when used during the soaking / scouring of wool on sheepskins for the production of lighter, softer types of double-faced shearing required for garment manufacture. Lambskin's in particular, tend to produce firmer leathers, having very high levels of endogenous non –collagenous protein and proteoglycan (table-01) removal is limited by the comparatively mild conditions required for wool-on sheepskin processing such as limited mechanical action and alkalinity restricted to below pH 9-10.

Table-03

Endogenous levels of dermatan sulfate and hyaluronic acid in ovine skin

Skins	Region	Number of skins	**Dermatan sulfate** (as percentages collagen)		Hyaluronic acid As percentages collagen)	
			Mean level	(Range)	Mean level	(Range)

Sheep skin	Butt	21	0.54	(0.38-0.67)	0.24	(0.13-0.45)
	Belly	8	0.45	0.37-0.60)	0.26	(0.13-0.45)
Lambskin	Butt	3	1.28	1.25-1.31	0.86	(0.77-0.86)

Removal of non –collageneous protein and endogenous dermatan sulfate (table-02) from the skins can be greatly increased by the use of proteolytic enzymes during the soak/scour process. Opening up is improved and the double-faced shear lings produced are softer and lighter in substance (table-02). Under practical tannery conditions, shear ling manufacturers have reduced the weight of standard crust productions by up to 30% by judicious use of enzyme soaking procedures.

TABLE-04

Removal of interfibrillary protein from lambskin during soak/scour

Process conditions (pH 10.35° 4 hour)	Removal (as percentage of endogenous level)		Organoleptic assessment of Double-faced shear lings (0 bad-10 good)	
	Non collagenous protein	Dermatan Sulfate	Softness	Lightness of Substance

No enzyme (control)	9 16	13 38	5.0 7.0	4.6 5.9
Subtilisin				

ENZYME CONTROL

It is probably true that proteolytic enzymes require more care for effective exploitation than most other beamhouse chemicals, but potential benefits far out weigh the risks. In this area above all, knowledge is the key to success. In carefully controlled studies. PH and temperature accurately defined, we have found that potentially adverse effects, such as looseness grain defect, generally only occur at, respectively, 6 times and 20 times optimum levels of enzyme offer . This highlights the importance of correctly determining the optimum enzyme concentration, but the situation is perhaps put into some perspective if one considers the effect of using 6times or 20 times excess of some of the other chemicals used in beamhouse processing, whether acid of alkali.

Chapter-5

Enzyme in dehairing

Dehairing is one of the main operations in the beamhouse. The hair or wool is usually collected in the tannery after treating the skins with lime or lime sulphide mixture. This treatment is liable to damage the hair. Hair and wool are the valuable by products of leather industry. Proper collection of hair or wool is an important problem facing the leather industry. Yet another problem relates to the disposal of tannery effluents. The tannery effluents from the beamhouse contain a lot of toxic and sludge forming chemicals and possibly some pathogenic organisms. These can easily penetrate the soil strata and pollute the ground water. Dissolved sulphide and pulped hair contribute to high chemical oxygen demand (C.O.D.) and Biological Oxygen Demand of the effluent. The atmospheric pollution due to the foul odor emanating from organic amines, H^2S and protein degradation products causes a serious health hazard to beamhouse workers. Viewed from the angle of environmental pollution, effluent disposal and optimal collection of hair nor wool, sustained affords are necessary to rationalize the dehairing process using enzymes and enzymatic products in the place of caustic chemicals.

Methods of dehairing:

The process of unhairing is achieved by one or the other of two general methods viz. (1) by attacking the hair and reducing it to a pulp and (2) by destroying or modifying the epidermal tissue surrounding the hair bulb, so that the hair is loosen and can be removed mechanically (Merrill, 1956). Hair destruction methods involve the rupture of then disulphide

and other bonds which stabilize the hard keratin of the epidermis while hair loosening methods have been observed to involve only a softening of the tissues that hold the hair in place.

Five methods of dehairing are generally adopted viz.

1. Clipping process,
2. Scalding process,
3. Chemical process,
4. Sweating process &
5. Enzymatic dehairing process.

Enzymatic dehairing

Proteolytic enzymes find widespread application in many industries (Under kofler, 1976).
A recent report shows that approximately 50% of the enzymes used as industrial process aids are proteolytic enzymes (Godfrey and Reichelt, 1983) . Proteolytic and amylolytic enzymes derived from various sources Viz. Microbial, animal and plant sources have been applied individually or in combinations to produce efficient in enzymatic unhairing than amylolitic enzymes and hence find wider application.

Advantages of enzymatic deplitation:

The major advantages of the enzymatic unhairing process are that it is useful as a hair saving method by providing both pelt and hair/wool in good condition, helps in easy handling of the pelts avoiding discomfort to tannery workers, reduces the tannery effluent disposal problem and simplifies the pre tanning processes by cutting down process like bating

(Pepper and Wyatt, 1936). Dahr and Bose (1959) have suggested that the pelts unhaired by using an enzymatic depilant containing microbial proteases don't require further liming, deliming and bating. Whitely (1962) has observed that the defects of the painted and sweated wool's could be completely removed by the use of enzymes. It has been reported that one of the major advantages of enzymatic unhairing is the undistributed nature of the original hide structure(Kardos, 1962).

Olita (1963) has observed that the effluent resulting from the enzymatic unhairing methods is found much better adopted to biological purification, mainly because of its lower pH value and even though enzymatic unhairing might have been followed by alkaline swelling, the total amount of waste are much lower that in normal liming, use of enzyme preparation instead of unhairing agents has resulted in a decreased B.O.D. of the effluents from the beamhouse. It has been shown that the difficulty arising from the gelatinization of the sludge derived form the hair could be avoided by the use of enzymes, Thereby greatly simplifying the effluent problem.

Investigations on the composition of the effluents from nitrite unhairing , Enzymatic unhairing, enzymatic unhairing plus re liming, sodium hydroxide and sulphydrate unhairing and lime sulphide unhairing have revealed that the degree of contamination is elastin the case of enzymatic unhairing plus reliming, taking into consideration the quality of the resulting leathers by the use of enzymes in unhairing the concentration of lime and sharpeners could be minimized, thereby lowering the C.O.D. values of the effluent further, up to 50% reduction in the B.O.D. levels of the effluent could be achieved, while converting from a hair destruction to an enzyme hair saving process. He has also observed that by careful adjustment of the enzyme and sharpeners levels it would be possible to

reduce the total quantity of time and sulphide use, there by producing a significant reduction in the chemical load of the effluent.

CHAPTER-6

Enzymes in Bating and Degreasing:

Bating is the only step in leather processing where chemical processes can not substitute enzymatic processes. The process of bating gives certain desired characteristics in the finished leather. Modern bating producers employ pancreatic enzymes or proteolytic enzymes of bacterial origin. Enzyme bate is one of the essential auxiliaries and its use is absolutely essential for the manufacture of leathers like glove, shrunken grain softie, nappa, garment and glace kid. The concept of softening hides by treating them in a warm infusion of animal dung has been termed as “bating” and the product used for such process is known as a ‘bate’.

ACID BATING

Normally, bating is carried out in the alkaline pH range, since the enzymes present in pancreatic and microbial bates are optimally active at that pH range, Studies have indicated that comparison to the enzymatic bating process in alkaline or neutral pH range , the pelts treated with proteolytic enzymes in acidic pH range derive special characteristics. The main features of bating in acidic conditions are (a) bating operation can be carried out at a temperature between 25° C and 32°C. (b) deliming, bating , pickling and chrome tanning can be carried out in the same drum avoiding the frequent removal of pelts from the drum (c) reduces the operational cost (d) manual scudding can be avoided and (e) the grain remains tight after finishing.

ENZYMES IN DEGREASING

The presence of excess amount of natural grease in sheep skins results in a number of defects in the finished leather viz. fatty spues, uneven dyeing and finishing, waxy patches in alum dressed leather and pink stains in chrome blues. The removal of natural grease from the interfibrilliary spaces facilitates more even penetration of tanning materials, fat liquors, dyes etc.

The grease of fat should be removed during the pre tanning operation in order to obtain sufficiently soft pliable leather for garment manufacture. The process of removal of excess natural fat from hides and skins is known as degreasing and it is an essential step in the production of glove and clothing leather.

ENZYMATIC DEGREASING

Early investigators have suggested the possibility of applying lipolytic enzymes from plant, animal, and microbial sources for the degreasing of hides and skins. Lipases are enzymes, which hydrolyze the carboxyl ester bonds in triglycerides at an oil water interface. Hydrolysis of triglycerides by lipase results in the liberation of free fatty acids, di glycerides, substantial amounts of monoglycerides and sometimes-free glycerol.

Acid lipases obtained from various sources have been found to hydrolyze glycerol esters optimally in the pH range of 4-6.

The advantages of using enzymes for degreasing are the elimination of solvents, reduction surfactants and possible recovery of valuable by products. The disadvantages are that the lipase do not remove all types of fats in the same way that solvents do and the process cost is a little escalated.

CHAPTER-7

MANUFACTURING PROCESS

Sample-1 + Sample-2 (Cow Hide)

Sample-1 enzyme used in beamhouse.

Sample-2 enzyme not used in beamhouse.

Beamhouse operation

Process for Cow Hide:

Wash:	500 water	
Soaking:	Busan880-	60’
<u>Sample-1</u>		
	400% water	
	0.3% LD-600(wetting agent)	30’
	0.2%busan880	
	0.3%soda ash	
	0.8%pellvit	
	SPH	
	Rest for 12 hours	
	Then drain.	
Liming:	300% water	10’
	2 .0% sodium sulphite	15’
	2,0% lime	10’
	2.0% sodium sulphite	20’
	2.0% lime	10’

	2 .0% sodium sulphite	20’
	2,0% lime	10’
	leave o/n	

total 48 hours. Check pH- 13

drain drain=unhairing: BY hand sliker

washing-

fleshing: By M/C

Weight-(pelt weight) --- gms

Washing – 20

Deliming:

	50 % water	
	1.0% ammonium sulphate	
	0.5% sodium Meta bisulphate	45’

Bating:-

	Deliming with ½ bath drain	
	Add 1.0 % bate –EG –98	
	1.0%LD-600.	
	Check pH -8.0	90’

Drain, scudding by hand sliker

Washing for 30

Pickle:	80% water	
	7% salt	15’
	0.2%Co	
	0.4% formic acid-	15’
	1.1% sulphuric acid	
	15’+15’+15’ – 2 hours	
	Check pH-2.6	
	0.5% hypo-30’	

Tanning :	1/3 bath drain	
	Add 4.0% chrome powder	60’
	Add 4.0% chrome powder	
	1% Relugan RF	60’
	2% Busan 330L-	30’
	100% Water	
	0.5% Bi carb	30’
	0.5% Bi carb	30’
	0.5% Bi carb	30’
	5 hrs rest	
	Check pH-3.06	
	Drain pile	
	Ageining- 7 days	

<u>Sample-2</u>

	400% water	
	0.3% LD-600(wetting agent)	30’
	0.2%busan880	
	0.3%soda ash	
	0.8%pellvit	
	SPH	
	Rest for 12 hours	
	Then drain.	

Liming:	300% water	10’
	2 .0% sodium sulphite	15’
	2,0% lime	10’
	2.0% sodium sulphite	20’
	2.0% lime	10’

	2 .0% sodium sulphite	20’
	2,0% lime	10’
	leave o/n	
	Total 48 hours. Check pH- 13	

drain drain=unhairing: BY hand sliker

washing-

fleshing: By M/C

Weight-(pelt weight) --- gms

Washing – 20

Deliming:		
	50 % water	
	1.0% ammonium sulphate	
	0.5% sodium Meta bisulphate	45’

Drain, scudding by hand sliker

Washing for 30

Pickling:	80% water	
	7% salt	15’
	0.2%Co	
	0.4% formic acid-	15’
	1.1% sulphuric acid	
	15’+15’+15’ – 2 hours	
	Check pH-2.6	
	0.5% hypo-30’	

Tanning :	1/3 bath drain	
	Add 4.0% chrome powder	60’
	Add 4.0% chrome powder	

	1% Relugan RF	60’
	2% Busan 330L-	30’
	100% Water	
	0.5% Bi carb	30’
	0.5% Bi carb	30’
	0.5% Bi carb	30’
	5 hrs rest	

Check pH-3.06

Drain pile

Ageining- 7 days

MANUFACTURING OF DIED CRUST WET BLUE CALF SKIN

Sammying:

Splitting: By machine

Shaving: By machine Thickness 1 mm

Percentage: calculated on shaved weight

Acid wash:	300% water at 40° c	
	0.7% acitic acid	20”
	pH- 2.8	

	Drain out	
Rechroming:		
	Add 2% Chromesyntan	
	1% Relugan RF	60’
	0.8% Bi Carbonate-10×2-60’	
	pH-4.0	
	Drain, horseup	
Next day:		
Neutralization:		
	100% water at 45°c	
	2% BS3	30’
	1% Sodium formate	60’
	pH-4.7	
Retannage:		
	Add 3% relugan RE	20’
	+3%relugan GTW	
	+3%DFS3	
	+8%mimosa	
	+2% tanigan OS	60’
	drain out	
Dyeing:		
	100%water at 50 c	
	1%neosyn	5’
	3%black B1	45’

	check penetration	
Fatliqoring:		
	Add 5%LLSC	
	2%LLSN	
	0.5%LLSK	60’
	Drain the bath	
Top dyeing:	2%formic acid	15×2-30’
	100%water at 45°c	
	1%bacic black (BM)	
	1% acetic acid	30’
	add 1% formic acid	30'
	0.5%SO	
	Drain, Horse up	
	Leave overnight	

Next day

Set out Prevacuum at 70° c.-1,

Hangdry , stake,Toggle, trimming.

Some Chemical description and nature:

LD 600

Nonionic wetting agent,

Product of BASF,Germany.

Erhavit MB

Sulphide free liming agent,

Ph Value :-11

Product of TFL, Germany

Pancreal Bate PBWI

Pancreatic Bating Enzyme

Activity CIRCA 600 LVE /gm

Products of Hodgson.UK.

IMPRAPEL CO

Bleaching and Oxidizing agent (Chlorine containing)

pH of 10% solution 13-14

Product of Clarient, Spain

CHROMITAN B

Basic chromiumn sulphate

pH 2.4-2.6,

Chromic Oxide 25-26%

Basicity 33-35%

Product of BASF, Germany.

NEOSYN N

Naphthalene Auxiliary dye leveling syntan,

Total solids 92%,

pH of2% solution 6.5-8.0,

Products of Hodgson.UK.

RELUGAN GTW

A Modified Glutaral Dehyde

Light fast Retannig agent with dispersing effect on Tanning agent and fat liquors

pH –7.0

Product of BASF,Germany.

RELUGAN RE

Light fast Polymer Retanning agent for Chrome Leather ,It is an anionic type co polymer

pH –6.5

Product of BASF , Germany

RELUGAN D

Weakly anionic light fast resin tanning agent for full grain and corrected grain chrome leathers, charge weakly anionic.

PH-6.5-7.5,solid content of 75% approx.

Product of BASF,Germany

TANIGAN OS

Replace tanning, materials, concentration 96-98%

pH- approx 3.5

LIPODERM LIQUOR IC

Sulfated oxidized special fish oil

Fat content 90%

pH-7.0

Product of BASF, Germany

SYNTHOL O

Asynthetic oil which is insoluble in water with excellent cold resistance,

Active matter 100%

Product smith and Zoon, Holland.

TENSILE STRENGYH AND ELONGATION AT BREAK:

Tensile strength indicates the overall strength of the leather, which is ascertained by the 'Tensile strength tester' . The process was followed by official method analtsis 1965, 4th-revised edition SLP 6.
Tensile strength of the specimen was calculated as follows

$$\text{Tensile strength} = \frac{\text{Breaking load}}{\text{Thickness (cm)} \times \text{width(cm)}}$$

Breaking load mainly upon the number of collagen fibre acting in the direction of applied load.
The extent elongation of the leather specimen at the time or its breaking , while applying the tensile force, expresses the percentage of the original length said specimen is the elongtion at break. The elongation at breakis taken by the difference between the initial strength and the length at break. Expressed thes difference as a percentage of the initial length.

Final distance between the jaw-initial

$$\text{Elongation at break,percent} = \frac{\text{Distance between the jaws}}{\text{Initial distance between jaws}} \times 100$$

Grain cracking and bursting strength,

The pressure required to cause the first sign of crankiness on the grain, is the grain crack strength. The test information on the degree of resistance the grain offer to the pressure.

$$\text{Grain crack kg/cm} = \frac{\text{Load in kg.}}{\text{Leather thickness in cm.}}$$

Bursting strength is the pressure beyond the grain crankiness till the leather burst. The bursting strength is an index of the overall strength of the leather and is generally determined along with the determination of grain crack strength .

$$\text{Bursting strength kg/cm} = \frac{\text{Load in kg to burst the sample}}{\text{Leather thickness in cm.}}$$

SHRINKAGE TEMPERATUER :
The temperature at which the leather just starts shrinking when heated in a liquid medium is its shrinkage temperature or hydrothermal stability in that liquid. This test gives an idea of the extent of tanning action.

The media is generallt water. If the shrinkage temperature of the leather is above 100°C. a mixture of glycerin and water in the ratio of 3: 1 iodine used.

LIABILITY TO WRINKLE (PHYSICAL TEST METHOD PM 44)

SOURCE: SATRA 44:1994

SCOPE:

This method is intended to determine how much a material can be shortened or compressed in a edge wise direction with out folding or forming pleats. The method is mainly applicable to footwear upper materials but can be used with any type of flexible sheet material.

PRINCIPLE:

A rectangular test specimen in held at two positions along its length so that there is a known amount of excess material forming a hump. The specimen is then transversely compressed between two flat surface, at least one of which is transparent and the degree of wrinkling is determined.
Here Dmax is calculated from the observation when unexpected result comes out .

Liability to wrinkle= Dmax-25 mm.

RESULTS OF THE TEST AND ITS DISCUSSION:

TABLE-5

TENSILE STRENGTH

SL. NO.	Name of the sample	Parallel (kg/sq cm)	Vertical (kg/ sq cm)
01	EN-1	171.4	191.6
02	NON EN-1	250	254

TABLE-06

SL. NO.	Name of the sample	Parallel(kg/sq cm)	Vertical (kg/sq cm)
01	EN-1	71.4	76.6
02	NON EN-1	50	54

TABLE-07

SL. NO.	Name of the sample	Grain crack strength(kg/ cm)	Grain burst strength(kg/ cm)
01	EN-1	381.4	400
02	NON EN-1	165	315

TABLE-08

Shrinkage Temperature

SL. NO.	Name of the sample	Shrinkage Temperature(°C)
01	EN-1	106°C
02	NON EN-1	Above106°C

TABLE-09

Liability to wrinkle

SL. NO.	Name of the sample	Parallel(mm)	Vertical (mm)
01	EN-1	9	8
02	NON EN-1	6	7

Sample Exhibition

SL. NO.	Name of the sample	Attachments)
01	EN-1	
02	NON EN-1	

Conclution

Through this work and investigation, I have attempted to highlight some of the possibilities that are now available to tanners for exploiting enzymes more effectively in the beam house.

Although current enzymes technology still primarily dependent upon proteolitic enzymes, better characterization and more sensitive methods of control can provide the basis for using these products more selectively during soaking and bating for using these products more specific type of product.

There is now also the opportunity of using selected proteases curing chemical unhairing/liming to improve beam house processing, based on a clearer understanding of the opening up process and the role of proteoglycan.commercial trial and production runs have now confirmed the viability of enzyme assisted chemical unhairing under practical conditions and range of key options are available to the tanners, depending of the priorities of production:

- shortening of liming time
- increased opening –up of the fibre structure and the production of softer leather.
- Improved pulping and the production of cleaner grains.
- Reduction of sulfide requirement.
- Increased area yields.

REFERENCE

1.R.Puvanakrishnan & Susil C. Dhar "Enzzyme technology in beam house practice " CLRI 1988.

2.Dutta, S.S., "AN introduction to The Practice Of Leather Manufacture" 3rd edition.

3. Sarker, K.T "Theory and Practice of Leather Manufacture" Revised edition –1995, India.

4. Sharphouse, J.J. " Leather technicians hand book" .

5."Pocket Book for The Leather Technologist" –BASF, 2nd edition.

6.Dutta, S.S."An Introduction to the principle of physical testing of leather"
7.Gerhard john, "possible defects in leather production".
8."Technical literature", Eanshaw, U K.
9. Technical literature,Hadgson,U.K.
10. Technical literature, Alpa Italy.
11.Jean J. Tancous, "Skin HIDE AND Leather Defects".
12.JALCA Nov. 1994, Page 265-270.
14.JALCA Vol. 83 1988, Page-314.
15. JALCA Vol. 81 1986 Page- 85-100.
16. JALCA Vol.XCIV, No-,Nov 1999.
17 JALCA Vol. XCV,No-2,Feb-March-2000.
18. JALCA Vol. XC111, No, October-1998.
19.Prof. Dr.Heidemann, "Fundamentals of Leather Manufacture".
20. JSLTCVol.80 May-June1996
21. JSLTCVol. 83, Jan-Feb1999.
22. . JSLTCVol. 69, December 1985. Page164.
23. JSLTCVol.80, Mar-April1996.Page-52 .
24. JSLTCVol. 69, December 1985. Page160.
25. . JSLTCVol.82, Sep- Oct, 1998.
26. . JSLTCVol. 84, Sep-Oct, 2000.
27.James R. Mckeemckee,"Biochemistry,An Introduction".
28.AS Saini,"Textbook of Biochemistry". 2nd edition.
29.Geoffrey L.Zubay," Biochemistr"4th edition.
30.World leather May-1998 Page 54-60.