



The application of modern biotechnology methods and the manufacture of quality leathers

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Great strides in technology are often achieved in a number of small steps. The increased use of biotechnology methods by leading chemical supply companies is impacting the way that leather is produced today. Changes have resulted in improvements to the repeatability of processing, reductions in cost, and a decrease in the environmental footprint along with improvements in leather quality. The overall results of these changes over the past ten years or so, whilst significant, have hardly been noticed by many in the industry.

The advances in biotechnology

The main advances in biotechnology have been in the application of modern methods for the development, selection, standardisation, modification and application of enzymes. Most of this work has happened behind the scenes but has affected the type of enzymes used by tanners, the way these enzymes are formulated, and the way the enzyme formulations are applied in processing. The process areas affected include soaking, unhairing, bating and degreasing.

CHANGES IN ENZYME ORIGIN

Traditionally protease enzymes for the leather industry have been harvested as a by-product of the meat industry from animal pancreases. Fear of pandemic animal diseases—particularly the occurrence of BSE (*Bovine Spongiform Encephalopathy* or *mad cow disease*)—has had a direct impact on the global trade of all animal derived by-products.

But advances in the manufacture of synthetic insulin by various biotechnology companies has been equally important and this development has made the harvesting of the pancreas from slaughtered animals of lesser economic interest. These factors have contributed towards a shift in focus to protease enzymes of micro-organism origin.

Today, enzymes derived from bacteria and/or fungi species have found full acceptance throughout the leather industry. Leading industry products typically have greater uniformity and specificity compared to animal by-product extractions. New enzymes isolated from unique micro-organisms, or organisms that are genetically modified to maximise the desired properties, hold out great promise for improving the versatility and performance of enzymes in leather-making processes.

Bio-engineering techniques can further improve the selectivity and specificity of a particular enzyme. Benefits include enhanced performance at pH and temperature extremes, improved enzyme stability, and increased substrate specificity.

ADVANCES IN BASIC UNDERSTANDING OF ENZYME STRUCTURE AND FUNCTION

Improvements in our understanding of enzyme structure and function, as well as our ability to predict and measure performance of a range of enzymes on different substrates, allows us to better select the enzymes for a particular leather application. Proteases represent the most important group of enzymes for leather processing.

Enzymes function as catalysts and increase reaction rates many-fold (up to a million times) by lowering the energy required to reach the substrate transition state. Almost all enzymes are proteins and rely on their uniquely folded three-dimensional structures for activity.

One critical specification is the identification of the desired pH range. Performance is further moderated by the surrounding conditions which include:

- Temperature.
- Concentration of substrates and reactants.
- Diffusion to the site of activity.
- Other factors including the presence or absence of modifiers such as the calcium ion.

Enzymes are rarely available in pure form. Industrial enzymes are typically produced in batch fermentation vessels where the desired enzymes are expressed from a selected micro-organism species and then harvested for use. Harvesting results in an enzyme 'cocktail' that contains various concentrations of different enzymes with different functionalities. Modern methods that use bio-engineered micro-organisms and special techniques for the isolation, purification and concentration of the desired enzyme have resulted in purer products with a more defined functionality. This is the area where much of the advancement in enzyme technology has been achieved.

To determine fitness for use, a vast range of enzymes are ➤

**Panel 1: Fast Protein Liquid Chromatograph (FPLC)**

typically screened in lab tests. The better candidates progress to further rounds of testing. This involves running many bench scale leather comparisons to identify the best alternatives. Final performance is then evaluated in full-scale tannery trials.

A useful tool when working with enzymes is the protein analyser. Equipment such as the Fast Protein Liquid Chromatograph (*FPLC*) shown in *Panel 1* can be used during screening to separate and even purify enzyme proteins from complex mixtures.

Rapid comparisons and characterisations can be made using this tool, which can separate enzymes based on physical size, charge distribution, and hydrophobicity. The equipment provides an estimate of the molecular weight and even gives an accurate measurement of the quantity of each enzyme present in a sample. This greatly speeds up the work of screening as it allows precise characterisation of enzyme preparations that are being developed for a particular purpose.

CHANGES IN HOW ENZYME ACTIVITY IS DETERMINED

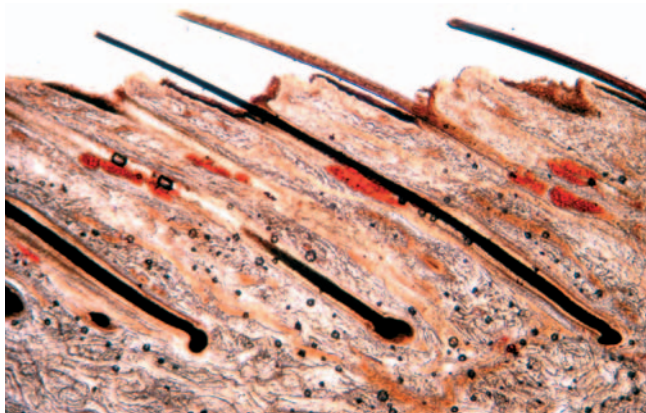
Several methods have been used to determine the activity of proteolytic enzymes. The most widely used method in the leather industry expresses the activity in LVU/g (*Lohlein-Volhard Units*). This technique uses an alkaline casein substrate to determine relative proteolytic activity. The assumption is made that the greater the enzyme activity against the protein casein, the greater the activity will be as a bating enzyme. In general there is a correlation between casein degradation and

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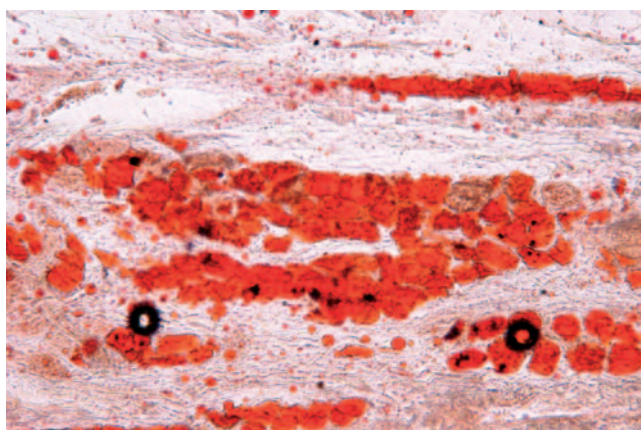
Panel 2: Cross-section of the grain side of a properly soaked North American hide stained with Sudan IV.



pancreatic enzyme activity on leather and so the LVU method is widely regarded as a suitable method for the standardisation of these enzymes.

The LVU does not, however, adequately represent the proper activity of the new generation of enzymes from micro-organism origin. We can observe that some enzymes have a high LVU activity but little or no effect on the hide substrate. Conversely, some enzymes showing low LVU may be very damaging to the hide structure. A corollary is that enzyme preparations of equal

Panel 3: Improved degreasing by improved enzyme hydration [Flesh side of the soaked hide depicted in Panel 2]



The stain of Sudan IV indicates diffuse fat deposits that are characteristic of natural fats emulsification. Partial opening of the fat cells can also be seen.

LVU activity may very well show significantly different results in the tannery. Various researchers have commented on this; nevertheless, LVU/g is still widely used by the industry as an indicator and this remains an area of confusion.

Buckman Laboratories has developed its own analytical ➤

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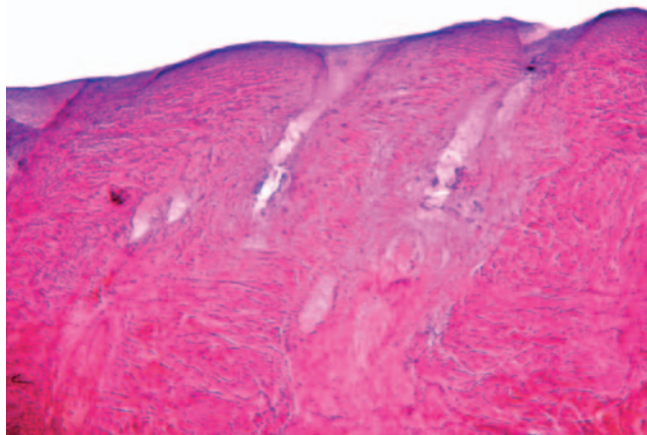
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Panel 4: Cross-section of typical unhaired hide after filtration using haematoxylin stain



method to determine the proteolytic activity of its bio-engineered leather enzymes. This proprietary method uses a synthetic substrate and the measured proteolytic activity is expressed using the Buckman Protease Unit (*BPU*). It has proven more consistent, more predictable, and much quicker than the Lohlein-Volhard analysis. The new method is applicable for the standardisation of production batches and confirmation of enzyme activity in enzyme stock solutions.

CHANGES IN ENZYME DELIVERY — LIQUID STABILISATION

It is important to first differentiate between enzyme activity and enzyme concentration:

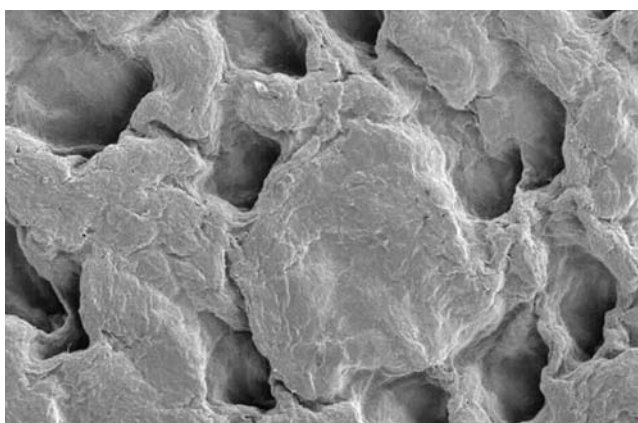
- Enzyme activity may be measured as units of change in a specified substrate over a period of time.
- Enzyme concentration may be defined as the quantity of enzyme protein present in the sample.

If we double the concentration of enzyme present in solution, is the activity doubled? The simple answer is 'no'. There is no linear relation between concentration and activity. As stated above, enzymes are catalysts and the rate at which they act upon a substrate is a complex function involving many different factors. This means that an enzyme described as 'concentrated' is not necessarily the same thing as an enzyme characterised as having 'high activity'. This is especially relevant when we consider how an enzyme works when dispersed in a large commercial tanning vessel.

Traditional enzyme products are fragile and are typically sold as very dilute powder formulations. To make the powder, 'wet' enzyme extracts are dried into a large volume of inert substrate. Drying removes moisture and this slows down normal degradation. The inert carrier material also increases product volume and thereby assists in the distribution of the powder when it is added to the tanning vessel. This technology has remained unchanged for almost 100 years, but has certain drawbacks as:

- Powders will absorb moisture in humid conditions.
- Degradation of the enzyme will ensue.
- There will be a reduction in activity.
- There may be poor distribution in a processing drum resulting in inconsistent action.
- They are subject to dusting and so can pose a health risk.

Panel 5: Electron microscopy picture of the wet blue after wringing (Magnification. x100)



The production of enzymes in granulated or 'pill' form can offset some of these 'dry' enzyme problems, but this adds significantly to the cost.

Patented technology has been developed that stabilises 'wet' enzymes in a liquid medium. This stability is confirmed by subjecting the enzyme formulation to multiple 'freeze-thaw' cycles and by exposing it to a temperature of 50°C for up to four weeks. Enzyme activity must remain close to 100% under these extremely harsh test conditions before being given the seal of approval as a stable liquid formulation.

This ensures a sufficiently long shelf life and means that stabilised liquid enzymes can withstand the harsh storage and transport conditions found in much of the leather industry. Benefits of liquid enzymes are:

- easy handling,
- rapid distribution in a large drum,
- no dusting hazard.

Accurate measurement of concentrated liquid enzymes is important but, once a protocol for measurement is in place, the ease of using these concentrated products is apparent. These advances in technology turn fragile enzymes into robust industrial chemicals that are safe to use, reliable and consistent. They allow the tanner to use enzymes with confidence.

A case study at Samyang Tonsang Leather Company: The application of bio-engineered liquid enzymes

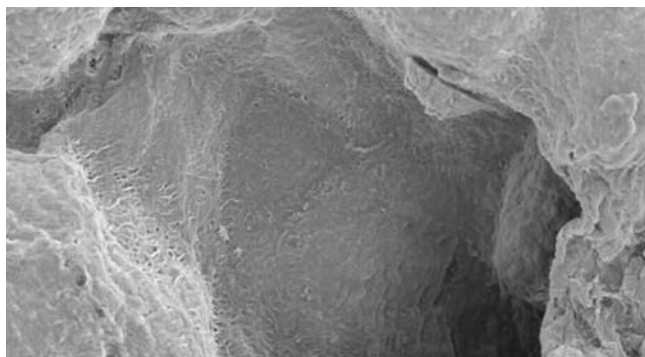
Samyang Tonsang Leather Company, Korea, is a leading manufacturer of full grain leathers for shoe uppers, leather goods and automotive upholstery. In 2003 the company faced the environmental challenge of reducing nitrogen content from liquid wastewater to meet local regulations. The tannery mostly processes North American fleshed salted hides in 10-ton beamhouse drums, and a new hair-saving process was needed that could produce the same quality as obtained with a traditional hair-burn method.

The new approach, which included two liquid enzymes, proved to be an efficient way to reduce the nitrogen load in the effluent. The result was an effluent that met nitrogen requirements for the wastewater and produced leather of equal or better quality compared with the traditional process.

A soaking enzyme was employed to react with the endodermis fibres, thus improving hide hydration and preparing it for ➤



Panel 6: Electron photomicrograph of the wet blue with detail of the hair follicle (Magnification. x400)



Panel 7: Full grain crust observed by light microscope



unhairing. The enzyme helps to improve soaking and produces cleaner hides with improved uniformity in temper. A photomicrograph of the soaked hide treated with a soaking enzyme is shown in *Panel 2*.

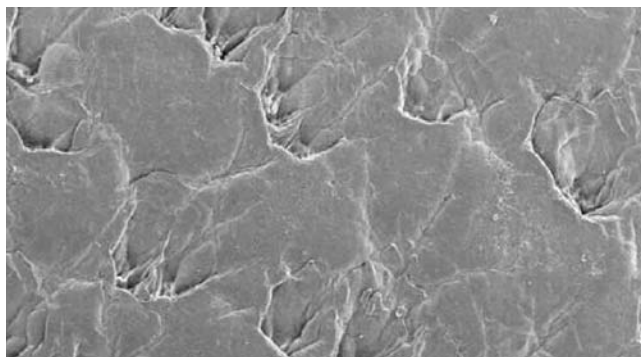
The typical soak lasts between 15 and 20 hours depending on the hide weight and degree of hydration of the raw material. A dosage of 0.05% of a concentrated liquid soaking enzyme based on salted hide weight (*500g of soaking enzyme/tone of salted hides*) was sufficient to improve the soaking operation significantly. The soaking enzyme improves hydration and acts on the endodermis thereby promoting better penetration of degreasers to the fatty structures and improving fat emulsification, distribution and degreasing. The improved degreasing is illustrated in *Panel 3*.

The unhairing process starts with a hair conditioning (*immunisation*) step in which the hair structure is temporarily preserved against sulfide degradation by using an alkali. The next step in hair removal proceeds with a 0.2% dosage of unhairing enzyme alone. This is then complemented by the addition of a hydrosulfide and sulfide combination.

The unhairing enzyme assists the unhairing process by promoting the disruption of the epidermis to allow better and more uniform penetration of the unhairing chemicals. After the addition of hydrosulfide and sulfide a typical 90-minute filtering step begins.

Filtration is carried out by recirculation of the liquid from the drum through a continuous dewatering device that separates the liquid and hair. In one 10-ton load of salted hides between 600kg and 900kg of hair along with 50-60% of moisture is removed.

Panel 8: Electron photomicrograph of the crust, showing the grain quality (Magnification. x50)



Typical grain appearance after unhairing is depicted in *Panel 4*.

After hair filtration, the liming proceeds for an additional 12-16 hours with typical stop/run cycles. Hides are fleshed in a throughfeed fleshing machine, sorted, trimmed and lime split.

The limed hides sorted after fleshing have cleaner grain, with less pronounced neck wrinkles and draw as observed with the same type of hides in the traditional hair-burn process. The hides are delimed, bated and chrome tanned. *Panel 5* shows the grain structure of the wet blue, and the cleanness of the hair follicle of this process is depicted in *Panel 6*.

The enzymes used in this process are bio-engineered from a micro-organism origin and are stabilised using patented liquid stabilisation technology^[Legend 1]. This ensures constant activity over the shelf life of the product, which is up to one year. Enzyme standardisation and confirmation of activity are made using the BPU analysis for consistent performance.

The resulting leathers obtained with this hair-save beamhouse were very comparable with the hair-burn process with respect to grain quality, veins, draw, grain tightness and area yield. Finished automotive upholstery leathers processed from the wet blue show equal performance with regard to leather attributes and physical properties.

Photomicrographs of crust shoe upper leather made from the wet blue are shown following retanning, fatliquoring and drying to demonstrate the cleanness of the grain surface. *Panel 7* shows full grain leather observed using an optical microscope, whilst the grain quality shown in *Panel 8* was observed using a scanning electron microscope.

Conclusions

Most tanners are rightly conservative when it comes to adopting new technologies or making significant changes in the extremely important beamhouse area. Enzymes have been around in the leather industry for a long time, but recent technological advances have resulted in products that can be used with increased confidence and safety and will yield greater uniformity and better quality leather. 

Acknowledgements

The authors would like to thank Samyang Tonsang Leather Company for allowing their process to be described and for the hide/leather cuttings that made the photomicrographs possible.

Legend

1] Buckman Laboratories markets its stabilised liquid enzymes under the trade name Buzyme.