Proteolytic depilation of lambskins

A thesis presented in partial
fulfilment of the requirements
for the degree of
Doctor of philosophy in Bioprocess Engineering at
Massey University, Palmerston North, New Zealand

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2008

Abstract

The processing of lambskins plays an important role in the New Zealand meat industry. The use of enzyme dewooling offers considerable advantages over the conventional depilation method which generates unpleasant working conditions and poses product quality risks when not properly handled. Prior to this work it was unclear from the literature why the practice of enzymatic depilation had not generally been adopted by industry. The aim of this work was to determine the problems associated with enzymatic depilation and provide a mechanistic understanding of the dewooling and damaging processes of enzyme depilation to provide underpinning knowledge for the design of a successful enzymatic depilation system.

It was found that variability in depilation between different regions of the skin resulted in either over exposure of the skin to the enzyme regent and subsequent damage or underexposure of the skin to the enzyme reagent and incomplete depilation. Two approaches were taken in the work: Firstly an attempt was made for the first time to understand the variability in enzymatic depilation so that the variability observed in enzymatic depilation could potentially be reduced, thereby allowing a complete depilation process with no overexposure. Secondly an investigation was made for the first time to understand the cause of damage to skins during the process of enzymatic depilation so that the enzyme depilation process could potentially be modified to avoid damage.

Experimental work characterising the time course of depilation and damage development was carried out and compared with the variation of physical properties across the skin. Correlations between depilation and physical properties such as thickness, grease content and follicle density were found. Reduction in the variability of these properties would likely improve the evenness of depilation but would not reduce it enough to eliminate damage due to over exposure.

A range of techniques including: immunohistology, 2-dimensional electrophoresis, matrix assisted laser desorption ionisation, and atomic force microscopy were used to probe the structural and biochemical mechanism of enzyme depilation and damage. In this way it was found that the removal of minor collagen components were the likely cause of damage observed. In particular the removal of collagen VI was associated with a disruption of the smooth mesh of fine collagen fibres observed at the surface of the leather.

The key requirement identified for a successful enzyme depilation system was the use of a broad spectrum protease which has no activity against collagen VI. The means to select a protease with these attributes was also developed by adopting a micro depilation assay incorporating immunohistology. This knowledge will enable the future development of non damaging enzyme depilatory reagents that could revolutionise the industry.

Acknowledgments

Once in a while a genius comes along and astonishes the world with their accomplishments. I am not one of those people, but I have had the opportunity to work with some astonishing individuals in the preparation of this thesis. The work could not have been accomplished without the help of the following:

Firstly I need to acknowledge the financial support from the foundation for research science and technology (FRST) under the program LSRX0301 through the New Zealand Leather and Shoe Research Association (LASRA) and Tony Passman in particular without whom the project would not have been made available in the first place.

My supervisors in alphabetical order; Timothy Allsop, John Bronlund, Yusuf Chisti, and Gill Norris. Without their guidance this thesis and the work it is based on would have been concise to the point of inconsequence.

Santanu DebChoudhury – thanks for your help with preparation and running of the electrophoresis, the sections involving those very demanding techniques would not have been possible without you. Thanks also to the other members of the Massey Lab who gave their assistance.

Thanks to all the technical staff at LASRA and Katherine Coolbear-Stern in particular – thanks for providing assistance in processing the leather in the tannery and in measurements of physical tests. Thank you also to George particularly for obtaining some of the more unusual fine chemicals that I used in the work.

Merve Birtles and all those people in the Institute of veterinary and biological sciences histology preparation laboratory – thanks for letting me use your flash equipment and your much needed expertise. The immuno-histology parts of the thesis were only made possible through your assistance

Steven Werner – Thanks for suffering all the pitfalls and fishhooks of an Engineering type PhD before me so that I could have some chance to avoid them myself.

Last but not least I need to thank my family, and personal support from my wife Tracy. For the most part they want to say they know someone called Dr Dick but they mean well all the same.

Success is the ability to go from one failure to another with no loss of enthusiasm

Sir Winston Churchill (1874 – 1965)

If you're not part of the solution you're part of the precipitate

Anon

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Preface: Thesis overview

In order to contribute to the knowledge of enzymatic depilation of ovine pelts the following work is reported:

- Chapter one entails: A definition of the problem at hand, the requirements for work done in this thesis, and describes the approach taken for this work.
- Chapter two contains a detailed description of conventional lime sulfide based depilation and its associated problems; a description of the possible alternatives to lime sulfide based depilation and how they relate to enzyme depilation; a detailed description of the structure of the skin and the bio-chemicals in the skin; and a description of the current understanding of the mechanism of enzymatic depilation.
- Chapter three includes an evaluation of the barriers to a successful enzymatic depilation process and a determination is made of what work was required to understand the enzyme depilation mechanism and so enable the prediction of a successful enzymatic depilation process.
- Chapter four builds on conclusions from chapter three and assesses the issue of variability in depilation across the skin by investigations of heterogeneity in the skin, thickness and diffusion through the skin, and skin composition.
- Chapter five assesses the protein and protease requirements for the production of high quality leather from enzyme depilated material including an assessment of the literature and analysis of protein changes in enzyme depilated skin compared with conventionally depilated skin. Parts of this chapter have been published in (Allsop *et al.*, 2007) and (Edmonds *et al.*, 2008). Immunohistology incorporating cross sections of lambskin exposing all the different layers described in chapter 2 was used extensively in this section
- Chapter six investigates the possibility of controlling the adverse effects noted during enzymatic depilation through the use of salts, and the use of targeted enzyme reagents.
- Chapter seven concludes this thesis, discusses the findings and their implications in enzymatic depilation and suggests possible new directions of research.

Glossary

The following glossary was prepared from a selection of reference sources ((Roether, 1976),(van der Loo *et al.*, 1999), (Harris, 1974))

Table 1: Glossary

word	description
Bate	Reagent applied during bating, usually an enzyme product such as pancreatic trypsin. (n) The process by which interfibrilar proteins are removed and the collagen structure opened up usually carried out after liming at pH 9 and 35°C. (v)
Beamhouse processing	The process of loosening wool or hair from skins and removing non-collagenous proteins from the hide or skin and preserving the skin through a pickling process in such a way that it is ready for tannage. Eg see “conventional processing”
Conventional processing	In the context of this thesis conventional processing is the lime-sulfide based process whereby raw skins are painted with a solution containing hydrated lime, and sodium sulfide, which loosens the wool. The pulled slats are then limed, delimed, bated, and pickled.
Crust	A skin that has been tanned retanned, dyed, and dried but not yet finished.
Delime	The process by which the pH of a slat is brought down from around 12 or 13 down to 8 or 9. This is usually carried out in a processing vessel using a deliming agent such as carbon dioxide or ammonium sulfate.
Depilation	see "Dewool".
Dewool	The process by which skins are treated so as to loosen the wool to an extent that it can be removed easily from the skin.
Drum	A rotating vessel in which skins are processed. (n) Apply mechanical action in a drum through rotation of the vessel. (v)
Enzyme	A protein which acts as a catalyst in chemical reactions especially lysis reactions.
Fell	Describes the skin of an animal as it is removed after slaughter.
Fellmongery	The place at which raw skins are processed to remove wool or hair and other unwanted proteins from a skin as it is converted into a pickle.
Finish	A coating applied to the surface of a finished leather. (n) The process of applying a coating to crust leather in order to generate finished leather. (v)
Finished leather	Leather that is ready for use in a leather object such as a garment or as upholstery for example.
Flesh	The side of the skin that was inside the animal. (n) The process of removing excess fat and muscle from the flesh side of the skin prior to processing. (v)
Float	The liquid in the process vessel in which skins and reagents are drummed. (n) To add liquid to a drum to achieve a desired liquid level. (v)

Grain	The outermost layer of skin remaining after successful processing to crust leather.
Green skin	A skin immediately after its removed from animal at slaughter (also known as raw skin).
Hydro extract	In the context of this work this process involves the use of the LASRA hydro extractor which spins the skins at a constant rate for a constant amount of time and produces a skin with a consistent quantity of associated water.
Liming	The process in which the residual wool and soluble proteins and glycosaminoglycans are removed from the skin after the bulk of loosened wool has been removed.
Opening up	The process by which collagen fibres are separated during processing.
Official sampling position (OSP)	A position on a skin near the back bone and about one third of the way from the butt to the neck. (IUP2, 2000)
Paint	A solution containing depilatory reagents usually applied in a thickened form to the flesh side of a skin. (n) The process of applying a depilatory paint usually to the flesh side of a skin. (v)
Pickle	An intermediate stage in the process of converting raw skins into leather when the skin is preserved in a highly acid state in the presence of salt. (n) The process of salting and acidifying a skin from which the unwanted proteins have be removed. (v)
Potting	The period during which the skins are held after they have been painted with depilatory during which the chemicals penetrate the skin.
Pre-fleshing	See flesh (v).
Protease	An enzyme that catalyses the hydrolysis of proteins.
Pulling	The process by which the wool is removed from the skin after it has been loosened by a depilatory.
Shrinkage	The thermal denaturation of collagen resulting in a change in morphology of the collagen fibres with an associated loss in length.
Slat	The skin after the loosened wool has been removed but before any processing has been carried out.
Slip	Loosened hair or wool.
Wet blue	A skin that has been tanned with chromium but is still wet and has not yet been retanned or dyed.
Wetting back	The process of taking a salted skin or dry pickled pelt and drumming with liquid in order to rehydrate the skin so it can be further processed.