2019 IULTCS/ERRETRE/TFL Young Leather Scientist Grant

Identification: YLSG2019_Catherine Maidment

COMPLETE APPLICATION FORM (click application area)

☑Basic Research ☐ Machinery/Equipment ☐ Environmental/Sustainability

1) Applicant Information

Name	Catherine Ann Maidment
Organization	NZ Leather and Shoe Research Association (LASRA)
Address	PO Box 8049
City/State	Palmerston North
Country	New Zealand

Advisor Information

Name	Dr. Rafea Naffa
Organization	NZ Leather and Shoe Research Association (LASRA)

2) Research Project Plan outline – Maximum 3 pages

Title: Investigating the proteomic profiles of cattle hide resulting in loose and tight leather throughout early processing stages

Introduction:

Skin and hide are the raw material used in producing leather. They consist of many different macromolecules with the most abundant being proteins. These proteins affect the physical appearance and mechanical properties of the leather [1, 2]. During the early stages of leather processing many of the non-collagenous proteins are removed, these processes which include removing the hair, removal of non-collagenous material and opening up of the collagen fibres influence the final properties of the leather including its quality [1, 2]. Although these processes have been investigated a comprehensive analysis of the different proteins found in hide and how they change throughout the early leather processing stages has not been done. A recent method to determine the protein profile in hide or skin is nano liquid chromatography-mass spectrometry (nano-LC-MS). It is highly sensitive and specific and enables large-scale analyses of biological systems [3].

Looseness is a defect found in 7 % of New Zealand cattle hides. It causes a wrinkly appearance in the finished leather when subjected to certain forces that subsequently results reduced leather quality [4, 5]. Investigations into the cause of looseness has resulted in several findings. Microscopy studies have shown an excess of space between the collagen fibres in loose leather as well as an enlarged gap between the grain-corium junction [6-8]. It has also been shown that poor processing techniques during early processing can result in loose leather such as over liming or too much proteolytic enzyme [1, 8, 9].

The large-scale study of proteins from the level of composition, structure and activity helps to understand the link between the genomes, proteins and structure [10]. In recent years, the technological developments in mass spectrometry has largely improved the sensitivity, speed and affordability of proteomics [10]. We at LASRA have been using mass spectrometry to study the skin and leather to gain better understanding of the leather processing regimes. Investigating the protein profile of hide during early processing in samples that produce loose and tight leather will give us a better idea of the cause of looseness and may lead to methods to prevent looseness from occurring in the leather industry.

Objectives:

The main objective is to investigate the proteomic profiles of cattle hide throughout the early stages of leather processing. This will enable us to investigate whether there are any differences in cattle hide that produce tight and loose leather.

- Extract protein from different stages of leather processing in loose and tight cattle hide – raw, lime, delime & bate and pickle.
- Identify and quantify proteins in the different stages for loose and tight cattle hide using mass spectrometry.
- Compare proteins in the different stages and between loose and tight.

Methods:

Extract protein using a combination of lysis/NaCl/Urea buffers followed by TCA precipitation. Resuspend the sample and run on SDS-PAGE gels (7.5 % and 12 %) to examine the protein pattern. Cut out bands and prepare for mass spectrometry by doing trypsin digestion. Run the results on Nano-LC-MS and analyse the peaks using the library to identify the proteins particularly collagen I, III and VI as well as other glycoproteins and proteoglycans. Another option would be to prepare for mass spectrometry in-solution rather than the gel method. This will be carried out at each stage of leather processing (raw, lime, delime & bate and pickle) to identify changes in their protein content and amount.

Hypothesis/Expected Results:

It is expected that a vast majority of the non-collagenous proteins are removed during the early processing stages, leaving mainly the collagenous proteins especially type I and III. Previous results looking at the quality of leather throughout processing have identified a greater amount of non-collagenous proteins remaining in poor quality leather thus it is likely that loose leather will have a greater amount of non-collagenous proteins still present in the later processing stages compared to the tight leathers [11, 12].

Research benefit for the local or global leather industry:

Determining the proteomic profile of hides throughout the early processing stages will give us a greater understanding of how these proteins affect the quality of leather, particularly in response to defects such as looseness. This is the first time where proteomics will be used on large-scale in leather to investigate the effect of protein profile on leather properties particularly loose and tight. The introduction of proteomics into leather science at wide level could potentially have a large transformative effect on the entire leather industry by bridging the gap between our understanding of leather structure and its protein content.

Literature:

1. Covington, A.D. and Covington, T., *Tanning Chemistry: The Science of Leather*. 2009: Royal Society of Chemistry.
2. Mann, B.R. and McMillan, M.M., *The Chemistry of the Leather Industry*. NZ Institute of Chemistry, 2017: p. 1-17.
3. Byron, A., Humphries, J.D., and Humphries, M.J., *Defining the extracellular matrix using proteomics.* International Journal of Experimental Pathology, 2013. **94**: p. 75-92.
4. Cooper, S., *Looseness in leather*, in *Annual Leather and Shoe Research Association Conference*. 2009: Wellington. p. 141-150.
5. Holmes, G., *Eliminating looseness in cattle hides proposed research program*, L.A.S.R. Assoication, Editor. 2012.
6. Liu, C.-K., Laton, N.P., Lee, J., and Cooke, P.H., *Microscopic observations of leather looseness and its effects on mechanical properties.* Journal of the American Leather Chemists Association, 2009. **104**: p. 230-236.
7. Wells, H.C., Holmes, G., and Haverkamp, R.G., *Looseness in bovine leather: microstructural characterization* Journal of the Science of Food and Agriculture, 2015.
8. Wood, B., *Looseness - Tanner's Dilemma.* Leather International, 2000. **64**.
9. Rabinovich, D. and Lowenstein, J.H., *Seeking soft leathers with a tight grain.* World Leather, 2001: p. 27-32.
10. Vogel, C. and Marcotte, E.M., *Insights into the regulation of protein abundance from proteomic and transcriptomic analyses.* Nature Reviews Genetics, 2012. **13**(4): p. 227.
11. Choudhury, S.D., Allsop, T., Passman, A., and Norris, G., *Use of a proteomics approach to identify favourable conditions for production of good quality lambskin leather.* Analytical and bioanalytical chemistry, 2006. **384**(3): p. 723-735.
12. Edmonds, R.L., Choudhury, S.D., Haverkamp, R.G., Birtles, M., Allsop, T.F., and Norris, G.E., *Using proteomics, immunohistology, and atomic force microscopy to characterize surface damage to lambskins observed after enzymatic dewooling.* Journal of agricultural and food chemistry, 2008. **56**(17): p. 7934-7941.