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
Date of Birth	22/03/1992
Organization	NZ Leather and Shoe Research Association (LASRA)
Address	PO Box 8049
City/State	Palmerston North
Country	New Zealand
Email Address	catherine.maidment@lasra.co.nz
Phone Number	Work: 06 355 9028 Mobile: 021 205 1028
Education (list)	Massey University: Bachelor of Science (2010-2012)
	Massey University: Masters of Science (2015-present)
If student, graduating year	2019
If employed, starting date	19.11.2012

Advisor Information

Name	Dr. Rafea Naffa
Organization	NZ Leather and Shoe Research Association (LASRA)
Email Address	rafea.naffa@lasra.co.nz

By submitting this application, I commit to develop the project as outlined in the attached Research Project Plan and to complete a written report by February 28, 2019 with the following items:

1) Introduction
2) Materials and Methods
3) Results and Discussion
4) Conclusion
5) Suggestion for Future Work 6) References

Leave one empty page

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.

Leave one empty page

3) *Curriculum Vitae* of the Applicant

1a. Personal details				
Full name	Title Miss	First name Catherine	Second name(s) Ann	Family name Maidment
Present position	Research Technician			
Organisation/Employer	NZ Leather and Shoe Research Association (LASRA)			
Contact Address	LASRA			
	PO BOX 8094			
	Palmerston North		Post code	4410
Work telephone	06 355 9028	Mobile	021 205 1028	
Email	catherine.maidment@lasra.co.nz			

1b. Academic qualifications

2013 B.Sc., Animal Science and Biochemistry, Massey University, Palmerston North.

1c. Professional positions held

2012-present, Research Technician, NZ LASRA.

1d. Present research/professional specialty

My research at LASRA has mainly involved investigating the proteomic profiles of different species skin using 1D SDS PAGE and LC-MS and investigating the amino acid and cross link profiles of skin and hide through HPLC and LC-MS. Through doing my masters I have been investigating the molecular building blocks of hide to determine whether they impact the quality of leather, specifically in causing looseness using techniques such as LC-MS, confocal scanning laser microscopy, biochemical assays and HPLC.

1e. Total years research experience,	6 years
1f. Professional distinctions and memberships (including honours, prizes, scholarships, boards or governance roles, etc)	

1. National Certificate in Leather Manufacture Technology (Introductory) obtained 2014
2. National Certificate in Leather Manufacture Technology (Advanced) obtained 2015
3. Runner up in the ‘Massey 3 minute thesis (3MT) Masterate Competition’ 2017

1g. Total number of peer reviewed publications and patents	Journal articles	Books, book chapters, books edited	Conference proceedings	Patents
	3	0	1	0

2a. Research publications and dissemination
Publications, conference proceedings and posters
1. Maidment, C. (2017). Investigating the molecular building blocks of loose and tight cattle hide. Presented at the 68th Annual LASRA Conference, Wellington. 2. Rafea Naffa, **Catherine Maidment,** Geoff Holmes and Gillian Norris "Insights into the molecular compositions of the skins and hides used in leather manufacture', Journal of the American Leather Chemists Association (JALCA), (Accepted, Jan 2019). 3. Rafea Naffa, Seiichiro Watanabe, Wenkai Zhang, **Catherine Maidment**, Preet Singh, Paul Chamber, Maria T. Matyska, and Joseph J. Pesek "Rapid Analysis of Pyridinoline and Deoxypyridinoline in Biological Samples by Ion-Pairing Free Liquid Chromatography-Mass Spectrometry and Silica Hydride Column", Journal of Chromatography B, (Submitted & being reviewed, 2018). 4. Rafea Naffa, **Catherine Maidment**, Meekyung Ahn, Bridget Ingham, and Gillian Norris "Structural insights into skin collagen reveals several factors that influence its architecture", Acta Biomaterialia, (Submitted, 2018).
2b. Previous research work
1. Project: 0801-Ovine leather for footwear leather manufacture (2012-2018). 2. Project: 1301- High performance New Zealand hide and deerskin leathers. (2014-2018).

Leave one empty page

4) *Curriculum Vitae* of the Advisor

1a. Personal details				
Full name	Title Dr.	First name Rafea	Second name(s) Mustafa	Family name Naffa
Present position	Scientist			
Organisation/Employer	NZ Leather and Shoe Research Association (LASRA)			
Contact Address	LASRA			
	PO BOX 8094			
	Palmerston North	Post code	4410	
Work telephone	06 3559028	Mobile	0278326118	
Email	rafea.naffa@lasra.co.nz			

1b. Academic qualifications

2017, Ph.D., Biochemistry, Massey University, New Zealand.
2005, M.Sc., Chemistry, Hashemite University, Jordan.
2001 B.Sc., Chemistry, Hashemite University, Jordan.

1c. Professional positions held

2017-present, Scientist, NZ LASRA.
2004-2014: Lecturer, Department of Chemistry, University of Sharjah, United Arab Emirates.

1d. Present research/professional specialty

My PhD focused on the development of analytical technique to analyse the collagen natural crosslinks using LCMS, GC-MS and HPLC. My research is also focused on understanding the collagen chemistry and its structure using small angle X-ray scattering (SAXS), Transmission Electron Microscope (TEM), Confocal Laser Scattering Microscope (CLSM).

1e. Total years research experience,	16 years

1f. Professional distinctions and memberships (including honours, prizes, scholarships, boards or governance roles, etc)

1. New Zealand Institute of Chemistry (NZIC) 2017.
2. Australasian Proteomics Society (APS) 2015.
3. New Zealand Society for Biochemistry and Molecular Biology (NZSBMB) 2014.
4. American Chemical Society (ACS) 2012.
5. Chemical Safety and Security Officer (CSSO) trained by Sandia National Laboratories 2011.

6. Award for the Best Scientific Content of “2015 Institute of Fundamental Sciences Post-Graduate Student Symposium”, Massey University, New Zealand 2015.
7. Best Teaching Award by College of Sciences, University of Sharjah, United Arab Emirates. 2008 and 2012.
8. First Class Honours in the M.Sc., Hashemite University, Jordan. 2004.
9. Graduate Assistantship, Hashemite University, Jordan. 2001.
10. First Class honor in B.Sc., Hashemite University, Jordan. 2001.
11. University honor list (Three consecutive semesters on the faculty honour list), Hashemite University, Jordan. 1998, 1999 and 2000.

1g. Total number of peer reviewed publications and patents	Journal articles	Books, book chapters, books edited	Conference proceedings	Patents
	8	0	13	0

PART 2

2a. Research publications and dissemination
Peer-reviewed journal articles
1. **Rafea Naffa**, *at al.* “Insights into the molecular compositions of the skins and hides used in leather manufacture’, Journal of the American Leather Chemists Association (JALCA), (Accepted, Jan 2019). 2. **Rafea Naffa**, *at al.* “Rapid Analysis of Pyridinoline and Deoxypyridinoline in Biological Samples by Ion-Pairing Free Liquid Chromatography-Mass Spectrometry and Silica Hydride Column”, Journal of Chromatography B, (Submitted & being reviewed, 2018). 3. **Rafea Naffa**, *at al.* “Structural insights into skin collagen reveals several factors that influence its architecture”, Acta Biomaterialia, (Submitted, 2018). 4. **Rafea Naffa**, *at al.* “Isolation and characterization of collagen type I crosslinks from skin: high resolution NMR reveals diastereomers of hydroxylysinonorleucine and histidinohydroxymerodesmosine crosslinks”, preparative biochemistry and biotechnology paper, (Submitted, 2018). 5. Zhang, Y., Mansel, B. W., **Naffa, R.,** Cheong, S., Yao, Y., Holmes, G., ... & Prabakar, S. (2018). “Revealing molecular level indicators of collagen stability: minimizing chrome usage in leather processing”. ACS Sustainable Chemistry & Engineering, 6(5), 7096-7104. 6. Zhang, Y., Ingham, B., Cheong, S., Ariotti, N., Tilley, R. D., **Naffa, R**., ... & Prabakar, S. (2017). RealTime Synchrotron Small-Angle X-ray Scattering Studies of Collagen Structure during Leather Processing. Industrial & Engineering Chemistry Research, 57(1), 63-69. 7. **Rafea Naffa**, et al. “Liquid chromatography-electrospray ionization mass spectrometry for the simultaneous quantitation of collagen and elastin crosslinks”, Journal of Chromatography A, 1478, 2016, 60-67, http://dx.doi.org/10.1016/j.chroma.2016.11.060. 8. **Rafea Naffa**, *et al.* “Removal of Pesticides Residues from Wastewater Using Activated Carbon Prepared from Olive Mills Wastes (JIFT)”, presented at Sharjah International Conference on Nuclear and Renewable Energy, University of Sharjah, United Arab Emirates, Sharjah, 2011.
2b. Previous research work
1. Project: 0801-Ovine leather for footwear leather manufacture (2014-2018). 2. Project: 1301- High performance New Zealand hide and deerskin leathers. (2014-2018) 3. University of Sharjah and United Arab Emirates University, United Arab Emirates 2013-2014

4. Project: Emirates liver assist device (EmLAD) for liver failure, University of Sharjah, United Arab Emirates, Sharjah (2012-2013).
5. Project: Collection and screening of UAE local plants to be used in wastewater treatment. University of Sharjah, United Arab Emirates, Sharjah (2008-2009).
6. Project: Synthesized of poly alkyl oxides for as Corrosion Inhibitors, sponsored by the College of Graduate Studies & Research".
7. Hashemite University, Jordan/Az-Zarqa (2001-2004).
8. Project: wastewater treatment by utilizing the natural compounds (Zeolite, Bentonite and Charcoal prepared from Olive residues), Hashemite University, Jordan, (2001-2004).

2c. Describe the commercial, social or environmental impact of your previous research work

Leave one empty page

5) Letter of Recommendation from the advisor

Dear Dr. Meyer,

It is my pleasure to recommend Catherine for admission to IULTSC Young Leather Scientist Grant 2019. As here research supervisor for the past few years, I have witnessed the exceptional contributions that Catherine has made to leather industry particularly leather quality and its strength. Catherine has been working in leather industry for six years where she has remarkably contributed to understand fundamentally the changes occurring in skin during leather processing using proteomics. Despite here young age, she has managed to build a strong experience in skin and leather science and has developed several skill sets both in protein analysis and leather processing. This enables her to address many challenges facing the current leather industry and finding solutions using basic science research.

Her idea of using proteomics to investigate the underlying protein composition of loose and tight is novel and to the best of my knowledge, it has not been reported previously in the literature. I believe the identification of proteomic markers for loose and tight skin or leather will help to understand the problem fundamentally. She predicted that the differences observed in the molecular analyses of the different skins will be reflected in their different physical characteristics, allowing her to understand the bases of these differences.

Globally, looseness is a defect found in cow hides and causes a wrinkly appearance in the finished leather reducing its quality. Through her research, she may discover methods, in which hide processes could be improved to produce leather with improved properties. Her research is focused on finding molecular-microstructure relationships between loose and tight cow hides which will provide valuable and accessible information for the industry to refine hide processing protocols to produce leather with improved physical properties.