Investigation of the Confinement Odour Problem in Exported Lamb using NMR-based Metabolomics

A thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Chemistry at Massey University, Manawatū, New Zealand.

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2015
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Dedicated to the Memory of Moira Lynette Fay Olivecrona
Abstract

Recent changes to the supply chain practices of meat exporters has increased the potential for consumers to be exposed to the phenomenon of confinement odour, the smell produced by vacuum or modified atmosphere packaged meat which has been chilled and stored for extended periods. This harmless odour, which does not indicate meat spoilage, can lead to the rejection of the product by consumers. This is a problem for NZ lamb meat producers as they form the largest group of exporters of lamb meat in the world, and their largest market is the UK and other EU countries.

The processes behind confinement odour development are poorly understood. In this thesis, NMR spectra were acquired of meat, and drip extracts of meat from two different processing plants stored under different temperatures for 11-13 weeks to simulate conditions of exported meat during overseas shipment, transport to warehouse and retail display. The spectra were analysed by multivariate data analysis to find metabolic differences between meat which produces confinement odour and meat which produces either spoilage odour or no odour. Optimisation of extraction of metabolites from meat and drip samples was also carried out.

The best sample preparation method for meat and drip included homogenisation by bead beating (meat samples only), protein precipitation using an acetonitrile, methanol and acetone solvent mixture, and removal of solvent by vacuum centrifugation.

Multivariate data analysis demonstrated the ability to discriminate drip samples with confinement odour from spoiled samples and the former showed increased lactate concentration with low levels of leucine indicating the presence of Lactic Acid bacteria. The spoiled samples had increased butyrate levels which is indicative of the presence of Clostridium spp. Both bacterial populations were in a late stage of growth. This is consistent with confinement odour as an early indicator of spoilage. This result indicates the potential for drip to be utilised more widely for the analysis of meat metabolites.

Additionally, samples could be discriminated by processing plant of origin using multivariate data analysis. Increased levels of pyruvate and decreased levels of glucose in samples from Plant 2 indicated their bacterial populations had progressed to a later stage of growth than the bacterial populations in samples from Plant 1.
Acknowledgements

First I would like to thank my supervisor Dr Patrick Edwards who encouraged me to pursue this project and introduced me to the field of metabolomics. His wealth of knowledge of NMR spectroscopy and valuable time was freely shared and hugely appreciated. I would also like to thank my supervisor Dr Linda Samuelsson, who provided endless encouragement, proofreading of my thesis and expert knowledge of so many of the techniques and methods used in this research. Many thanks to Dr Mariza Reis and my supervisor Dr Marlon Reis for providing the framework and samples for this work, and their guidance throughout the entirety of my research. Thanks to the C. Alma Baker Trust for the provision of a scholarship, and AgResearch for providing a stipend without which I would not have been able to complete these studies. Particular thanks go to Assoc. Prof. Kathryn Stowell for her assistance and guidance in helping me obtain a suspension to my studies during a particularly distressing time. I would like to thank my mum Moira Olivecrona for her constant support and encouragement; she was my greatest rock and my strongest advocate. Sadly she passed away before this could be completed but she never doubted for a moment that it would be. Thanks to my dad Stenfinn and my sister Courtney who contributed with their love, support and in so many other innumerable ways. Without them this would never have happened. Finally I would like to thank Jim for sharing every happy, sad and frustrating moment and supporting me every step of the way.
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Glossary of Abbreviations

NMR Experiments
HSQC .......................................................... Heteronuclear Single Quantum Coherence
NOESY .......................................................... Nuclear Overhauser Effect Spectroscopy
TOCSY .......................................................... Total Correlation Spectroscopy
CPMG .......................................................... Carr Purcell Meiboom Gill

Statistical Analysis
PCA .......................................................... Principal Components Analysis
PLS-DA .................................................. Partial Least Squares Projection to Latent Structures-Discriminant Analysis
OPLS-DA ................................................ Orthogonal Partial Least Squares-Discriminant Analysis
UV .......................................................... Unit Variance (Scaling)
VIP .......................................................... Variable Importance in Projection

Meat Science
MAP .......................................................... Modified Atmosphere Packaging
LAB .......................................................... Lactic Acid Bacteria
GN .......................................................... Gram Negative
GP .......................................................... Gram Positive