# final report

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# Development and validation of a probe to measure meat quality for on-line application

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## Abstract

The data analysis and documentation phase of the second experiment is now complete and a paper has been submitted for publication (Approval was given by AMPC/MLA for submission) - Fowler, S.M., Schmidt, H., van de Ven, R., Wynn, P., and Hopkins, D.L. (2014). A new approach to predicting lamb shear force using Raman Spectroscopy and traditional indicators. *Meat Science, (submitted).* A draft of this paper was submitted as part of **Milestone 6.** The draft has been reviewed and a revised draft submitted to the journal. The paper based on Experiment 1 is now published - Fowler, S.M., Schmidt, H., van de Ven, R., Wynn, P., and Hopkins, D.L. (2014). Predicting tenderness of fresh ovine semimembranosus using Raman spectroscopy. *Meat Science,* **97,** 597-601.

Previous milestones have outlined the results of a study conducted by the PhD student to investigate muscle composition using Raman microscopy at the School of Chemistry, Monash University using samples of *semimembranousus* (SM: topside) from the experimentation conducted in April/May 2013. A paper was submitted (Approval was given by AMPC/MLA) to present results of this work at the 60th *International Congress of Meat Science and Technology* to be held, in Punta Del Este, Uruguay in August (see appendix). With the help of researchers at the University of Milan, Italy a system has been developed to interrogate the images generated by the microscope. Preliminary investigation demonstrated that differences in vibrations pertaining to tryptophan, tyrosine, α-helix, C- H deformation and the amide I and III bands can be distinguished between tough (53 – 74N) and tender (26 - 36N) lamb using spectral mapping. This work is being prepared for a journal paper and the significance of the findings will be related back to the work with the Raman Probe. Although not part of the contracted work, this will provide greater insight into the chemical differences between tender and tough topside and thus will complement the current project (Experiment

3).

As mentioned in previous milestones work has also begun on examining Raman spectra against fat traits in muscle like fatty acids (FA) and intramuscular fat (IMF) (Experiment 4). This is the first Raman spectroscopy study to measure FA composition of IMF in intact muscle and there is evidence to suggest that Raman spectroscopy has the potential to predict polyunsaturated fatty acids (PUFA) and PUFA: SFA ratio. A paper was submitted

(Approval was given by AMPC/MLA) to present results of this work at the 60th *International Congress of Meat Science and Technology* to be held, in Punta Del Este, Uruguay in August (see appendix). Further testing and data analysis is underway as the level of the saturated fatty acids (SFA) in the tested samples was lower than expected and so the samples are being re-examined.

As outlined in the last milestone a new phase of experimentation was planned for March/April this year. The following experiments were undertaken with the assistance of Dr Heinar Schmidt (Probe designer and collaborator, University of Bayreuth, Kulmbach, Germany);

**Experiment 5**

Raman spectra were obtained on 80 ovine *semimembranosus* (SM) muscles (20 per day for 4 days) with an in situ measure taken prior to rigor mortis on the carcase, with the subcutaneous fat removed (see photo below). Ten scans/measurements perpendicular to the muscle fibre were taken 20 minutes after slaughter.

The boned out SM was measured again at day 1 post-mortem (PM) and then again at day 5. The objective was to validate the results found in Experiment 1 for the prediction of shear force in 5 day aged SM. To allow for data comparison between aging periods and to validate

the model previously developed with Experiment 1, all spectral data at day 1 and day 5 were obtained using a 671nm laser, with an integration time of 3.75 seconds. To give an indication of how repetitions affect the model, 4 repetitions were stored separately to the initial accumulation to enable a comparison. Saving 4 repetitions separately, but keeping the same integration time will enable a comparison with previous results while still allowing determination of the impact of a longer total accumulation time (3.75 secs vs. 15 secs total accumulation).

As part of this experiment it will be also possible to establish whether Raman measurements obtained prior to rigor mortis are useful in predicting shear force of 5 day aged meat – this hasn't been reported before.

**Experiment 6**

An experiment was undertaken to establish whether Raman measurements obtained from frozen/thawed or fresh intact muscle alter the prediction of shear force values of 5 day aged lamb SM. This relates to the original work conducted on the probe prior to this project which measured frozen/thawed lamb (Schmidt, H., Scheier, R. and Hopkins, D.L. (2013). Preliminary investigation on the relationship of Raman spectra of sheep meat with shear force and cooking loss. *Meat Science* **93,** 79-84) and some evidence that this treatment might improve the prediction of shear force. From 30 lamb carcases per day the SM was collected at 1 day post mortem for 3 consecutive days (n= 90). Each SM was scanned with the Raman probe at 1 day PM, 5 days PM after ageing in a chiller, then frozen and subsequently frozen and thawed and re-scanned, before shear force testing.

## Project objectives

1. Establish whether measurement of sheep meat at 1 day post-mortem using a Raman hand held probe is useful for predicting shear force after an ageing period of 5 days.

2. Establish whether the probe can be used to predict shear force of 5 day aged meat.

3. Establish what biochemical and biophysical changes the probe is detecting in relation to tenderisation and to explore the potential of the probe to provide measures of other traits such as fatty acids and intramuscular fat.

4. Provide the framework for a PhD program.

## Success in achieving milestone

The milestone has been met, with a full journal paper submitted to Meat Science, reviewed and revised – it is anticipated that the paper will be accepted. Also 2 new conference papers have been submitted and experiments 5 and 6 have been conducted.

## Overall progress of the project

There were plans for two further experiments to be conducted in March/April this year (Experiments 7 and 8), but what appears to be an electronic issue arose with the probe and it had to be sent back to Germany, this could be the result of extended use under very cold chiller conditions, but this is yet to be confirmed. Currently sufficient experimentation has been completed to draw conclusions about the capability of the probe for use commercially, but the team will consider undertaking the further experiments (7 and 8) subject to timing and probe availability as it is believed these would give a fuller assessment of the probes usefulness.

Despite this issue the project is progressing well and the PhD student is on track to complete the PhD. The next milestone will provide a full report on the use of the probe for predicting FA and IMF and provide preliminarily results for experiments 5 and 6. The PhD student will also be presenting results at the 60th *International Congress of Meat Science and Technology* to be held in Uruguay in August and the Joint ISNH/ISRP International Conference in conjunction with the Australian Society of Animal Production conference in Canberra in September**.** The project is still on track to enable solid conclusions to be reached about the applicability of the probe for prediction of tenderness and is being expanded as required.

The following papers have been published from the project to date;

1. Fowler, S.M., Schmidt, H., van de Ven, R., Wynn, P., and Hopkins, D.L. (2014). Predicting tenderness of fresh ovine semimembranosus using Raman spectroscopy. *Meat Science,* **97,** 597-601.
2. Fowler, S., Schmidt, H., van de Ven, R., Wynn, P. and Hopkins, D. (2013). Predicting tenderness of fresh intact ovine *longissimus thoracis lumborum* using Raman Spectroscopy. *Proc. 59th International Congress of Meat Science and Technology.* S7B-5, pp 1-4, Izmir, Turkey.
3. Fowler, S., Schmidt, H., van de Ven, R., Wynn, P. and Hopkins, D. (2013). Predicting tenderness of fresh intact ovine *semimembranosus* using Raman Spectroscopy. *Proc. 59th International Congress of Meat Science and Technology.* O-19, pp 1-4, Izmir, Turkey.
4. Schmidt, H., Fowler, S., Scheier, R., van de Ven, R., Wynn, P, and Hopkins, D.L. (2013). Correlation of Raman spectra of sheep meat with shear force – can we measure or predict toughness with an optical measurement? *Proc. BIT's 2nd World Congress of Food Science and Technology*, p 88, Hangzhou, China.
5. Fowler, S.M, Wood, B., Wynn, P. and Hopkins, D.L. (2013). Characterising tenderness of intact ovine *semimembranosus* using Raman Microscopy. *Proc CRC Annual Postgraduate Conference*, Coffs Harbour, NSW.

## Next Phase

Over the next 6 months the work program will cover the following;

1. Further testing of samples from Experiment 4 for FA and IMF and analysis against Raman Spectra.
2. Measurement of samples taken for Experiments 5 and 6 and analysis against Raman Spectra.
3. Preparation of the paper on Raman microscopy results.

## Appendices

## Appendix 1.

Papers submitted and accepted for presentation at the 60th International Congress of Meat Science and Technology, Punta Del Este, Uruguay

# IMAGING OF INTACT OVINE *M. SEMIMEMBRANOSUS* BY CONFOCAL RAMAN MICROSCOPY

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**Abstract – Chemical imaging of intact lamb using Raman microscopy is a useful technique which can provide insight about the differences in chemical composition and spatial orientation of the myofibril in its native state. This paper explores the potential for Raman microscopy to further understand the variation in tenderness of lamb semimembranosus (n = 26). Preliminary investigation demonstrated that differences in vibrations pertaining to tryptophan, tyrosine, α-helix, C- H deformation and the amide I and III bands can be distinguished between tough (53 – 74N) and tender (26 - 36N) lamb using spectral mapping. Furthermore, a new method of chemical image analysis is proposed to facilitate the comparison between multiple chemical images of complex samples and to determine the heterogeneity of the chemical vibrations within a single chemical image.**

**Keywords- Raman microscopy, shear force, sheep muscle**

## I. INTRODUCTION

It is well established that tenderness is a critical factor in determining meat eating quality and consumer acceptance of meat products. As tenderness is determined by the interactions between myofibrils and the connective tissue matrix as well as the extent of myofibrillar degradation during ageing, much research has focused on the ability of technologies to objectively measure tenderness [1]. Of these technologies, Raman spectroscopy has been highlighted as having potential, as it is rapid, non-destructive and not sensitive to varying water content [2]. Meat science research has not over looked these advantages and recently studies have applied Raman spectroscopy to investigate the effects of ageing and cooking on pork loin [3], predict sensory quality of beef silverside [4], shear force of frozen and thawed lamb loin [5] and fresh intact lamb semimembranosus [6]. However, as meat is complex, changes in Raman spectra that reflect the variation in tenderness have not yet been fully characterised. Confocal Raman microscopy is an ideal tool for elucidating the composition and structure of cells in their native state [7]. This paper proposes a new approach for using Raman confocal microscopy and chemical imaging to determine the chemical and spatial differences in Raman spectra of ovine *m. semimembranosus* (SM) and the potential for spectral mapping and chemical imaging to further understand variations in tenderness of ovine topsides.

## II. MATERIALS AND METHODS

At 1 day post slaughter, a SM was removed from each of 80 carcases over 4 consecutive days (20 per day) from the same abattoir. Carcases were randomly selected and were from different consignments, thus were of different backgrounds, ages and gender to represent animals typically processed by the abattoir to obtain a spread of shear force values. A section of 1-2g also was removed at 1 day post mortem, fixed in gluteraldehyde

and paraformaldehyde as previously described [8]. After further sections were removed to determine pHu [9] and sarcomere length [10], SMs were vacuum packed then aged for 4 days at -1ºC.

At 5 days post mortem, sections were excised (mean 65g) for shear force and cooking loss measurement [11]. Sections were also removed to determine collagen content [12]. Samples were ranked using the shear force values at 5 days post mortem, and of these 13 of the most tender (26.3 - 36.0N) and 13 of the toughest (53.2 - 74.3N) were cut and set, as previously described [8] onto gold slides. Raman microscopic measurements were conducted on these set samples using a WiTec Raman microscope with 80mW of laser power and a 3.45 sec integration time. Spectral wavenumber values and intensities were mapped over a 40µm area (20µm x 20 µm) of the myofibril using WiTec project software and a 50x microscopic image of the scan area was taken [13]. Band assignments for individual spectra were completed using Opus software [14].

## III. RESULTS AND DISCUSSION

Chemical images of intact ovine SM generated using the total integrated intensity of Raman bands between 500- 2100cm$^{-1}$ (Fig. 1), which represent chemical bonds found in meat [3] gives a good overview of the morphological differences between the tough (Fig 1; A) and tender (Fig 1; B) samples. Based on these example chemical images, spatial changes of chemical bond intensities between tender and tough samples can be elicited using Raman microscopy. Spectral changes observed in the tender sample (Fig 1; B) suggests that the ultra-structural degradation of myofibrillar proteins which results in myofibrillar detachment and leads to changes to the topography of the sample. However, the same is not observed in the tough sample (Fig 1; A). Changes to the spatial intensities of the tough sample may be attributed to the connective tissue matrix which does not degrade during the ageing period and is responsible for background toughness [15].

Extracting underlying spectra (Fig 2), suggests that the orientation of collagen could be responsible for these spectral changes of the tough sample, as the angle of polarisation affects the ratio of intensity between the amide I and III bands at approximately 1300 and 1650cm$^{-1}$ [16]. Furthermore, a shift in the C-H deformation vibration of the tough sample denotes a change in the number of C-H bonds which are stabilised by C-N bonds rather than C-O bonds [17]. This may reduce the ability of proteolysis to cleave these bonds, resulting in less myofibrillar degradation.

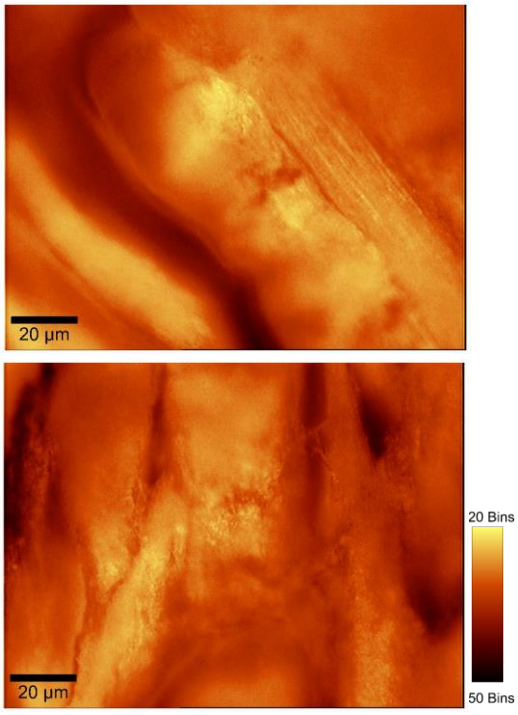


Figure 1 An example of a chemical image of tough (74N; A) and tender (26 N; B) ovine *semimembranosus* showing the morphological differences.

Shifts in amino acid side chain vibrations at approx. 750cm$^{-1}$ as well as 820 and 853cm$^{-1}$ that characterise the vibrations of tryptophan and tyrosine respectively [18], suggests that the ability of these chemical bonds to destabilise during ageing is also reduced in the tough sample.

While this information provides some indication of the spatial and chemical changes between the two examples presented in this paper, it is difficult to ascertain whether the proposed spatial changes and tentative band assignments will be valid across the chemical images for the entire data set.

A limitation in generating single chemical maps that there is no direct way to compare between chemical images of different samples of lamb SM. As intact lamb muscle is complex, with many amino acid vibrations from a variety of proteins in concentrations and orientations, which are unique to each sample causing some spectral features to overlap it is difficult to determine changes in Raman spectra compared to a set reference spectra. However, this information on the changes of spectra between samples holds the information needed to determine what chemical characteristics are linked to variation in tenderness.

One way of overcoming this limitation is to generate chemical images with restricted wavenumbers isolating the specific bands which have previously been identified as being important to the prediction of tenderness and shear force by previous Raman studies on meat. Such band wavenumbers would include the amino acid side chain vibrations of tryptophan (750 $cm^{-1}$), the tyrosine doublet (826 & 853 $cm^{-1}$) and the α- helix (930 $cm^{-1}$) as well as the peptide backbone vibrations of and the amide I and III bands (1245, 1268, 1300 and 1650 $cm^{-1}$) [3-6], illustrated in Fig 2. These chemical images could then be analysed using image analysis software [19] to distinguish the proportion of the spectral maps which have a high and low intensity at these wavenumbers. By breaking down the Raman map through classifying areas with similar intensity range into a specific class (Fig 3), the total proportion of the area with the same intensity can be compared within and across samples. Proportions of the area across the myofibril associated with high and low intensities for the amino acid vibrations of tryptophan (750$cm^{-1}$) and the tyrosine doublet (826 & 853$cm^{-1}$) will modelled against shear force values, particle size analysis (measure of proteolysis) and sarcomere length, as previous studies have linked the intensity of these peaks with the concentration and structure of these amino acids in tough and tender meat [3,5,6]. Collagen concentrations could be related to the ratio of the amide I and III bands as these peptide backbone vibrations are characteristic of hydroxyproline, which is a typical constituent of connective tissue [5].

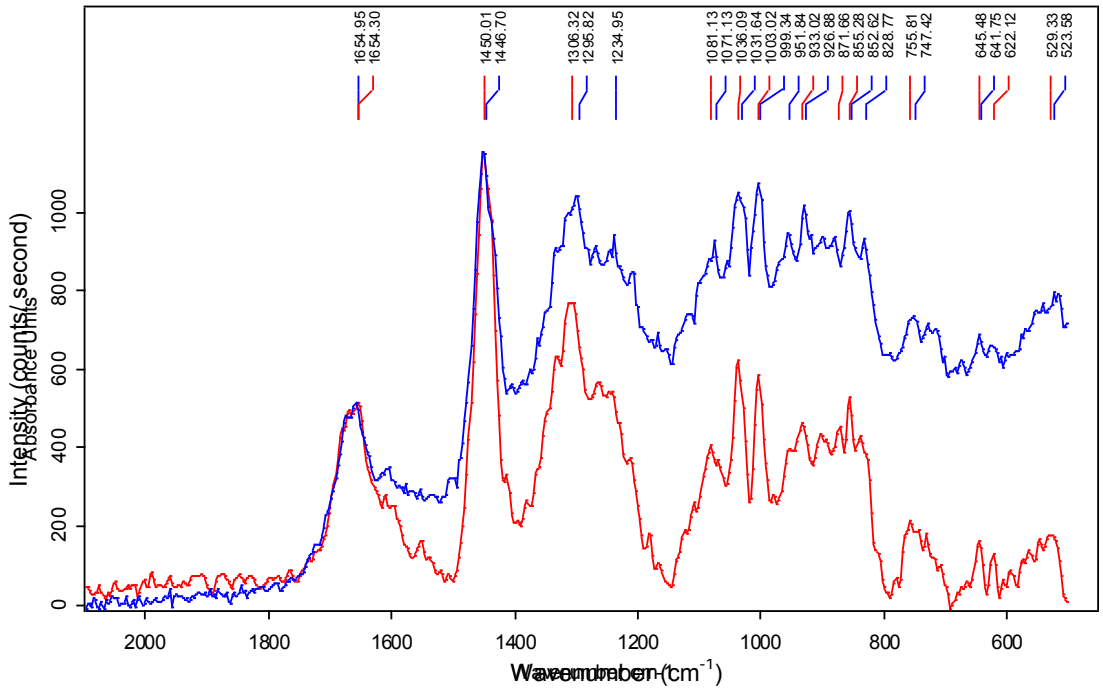


Figure 2. Raman spectra for tender (blue, 26N) and tough (red, 74N) lamb *m. semimembranosus* illustrating changes in key amino side chain vibrations (750, 826, 853$cm^{-1}$), α- helix (930$cm^{-1}$) and amide I (1300$cm^{-1}$) and III bands (1650$cm^{-1}$).

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By conducting an analysis on the intensity ranges at the specific wavenumber targeting these amino acids, and peptide backbone structures, which have been linked to tenderness, the heterogeneity of these specific chemical vibrations across myofibrils at a micrometer level may be ascertained. This information could provide further explanation of the differences between the Raman spectra of tough and tender samples of lamb and why proteolysis is able to degrade some myofibrils and improve tenderness, more than others.

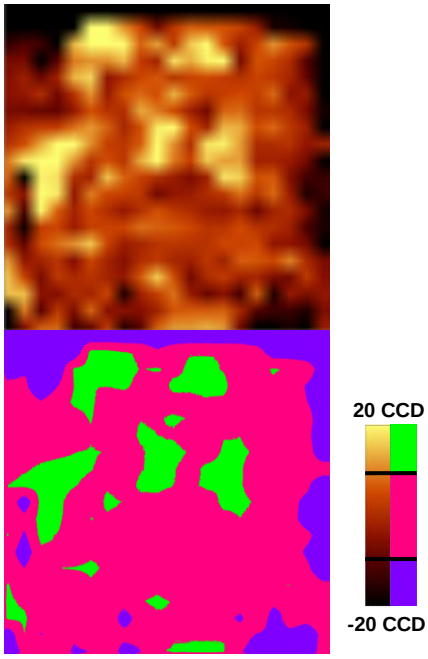


Figure 3. An example of a Raman chemical map for the Tryosine peak (826cm$^{-1}$) illustrating the classification of areas with high (green), moderate (pink) and low (purple) intensity.

## IV. CONCLUSION

Chemical imaging using Raman microscopy is a tool which could be useful in determining what differences in composition and spatial arrangement of amino acids may contribute to the discrimination of tough and tender meat samples. Although current limitations exist in the application of Raman spectroscopy to complex samples of lamb using a combination of confocal Raman microscopy and image analysis, these limitations may be reduced and further insight into the causes of variation of meat quality could be provided.

## ACKNOWLEDGEMENTS

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# PREDICTING FATTY ACID COMPOSITION OF LAMB LOIN USING RAMAN SPECTROSCOPY

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**Abstract – Fresh intact lamb muscle was measured using a Raman hand held device to determine the ability of Raman spectroscopy to predict intramuscular fat (IMF) and fatty acid (FA) composition. Raman measurements were conducted on 80 samples of *longissimus thoracis lumborum* (LL) from different carcases. Measured FA values were regressed on the Raman spectra using partial least squares (PLS) regression. Predicting polyunsaturated fatty acids (PUFA) yielded a squared correlation between predicted and measured values ($R^2_{cv}$) equal to 0.56 and a root mean square error of cross validation (RMSECV) of 16.2mg/ 100g meat. Prediction of PUFA: saturated fatty acids (SFA) gave an $R^2_{cv}$ equalled to 0.15 and a RMSECV of 0.04. This is the first Raman spectroscopy study to measure FA composition of IMF in intact muscle and there is evidence to suggest that Raman spectroscopy has the potential to predict PUFA and PUFA:SFA ratio. Further work is required to validate the models generated in this study and establish the potential benefit of Raman spectroscopy.**

**Keywords- Raman, fatty acids, sheep, IMF**

## I. INTRODUCTION

Fat is an unpopular constituent of meat, despite the contributions of intramuscular fat (IMF) to eating quality and the health benefits of some fatty acids (FA), such as omega-3 FA. As many factors affect the FA composition of meat from ruminants, much research has focused on measuring and predicting IMF and the FA concentrations [1]. Of the technologies that have been used, Raman Spectroscopy has been highlighted as having potential as it is rapid, non-destructive, non- invasive and insensitive to varying water contents and therefore capable of measuring a fresh muscle samples [2]. Recent research has not overlooked these advantages and Raman spectroscopy has been used to predict clarified butter composition [3], classify species of origin for adipose tissues [4] and determine amounts of polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) in porcine subcutaneous fat [5,6]. However, the application of these studies in the context of online assessment of IMF amount and FA composition is limited due to the sampling of subcutaneous fat and measurement with bench top Raman devices. In this study, the potential of a hand held Raman device to predict FA composition and IMF of fresh intact lamb is reported for the first time.

## II. MATERIALS AND METHODS

At 24 hrs post mortem, samples of *m. longissimus thoracis lumborum* (LL) were taken from 80 lamb carcases sampled over four days (20 per day). Samples were randomly selected from different consignments and thus were of unknown background, age and gender to represent animals typically processed. One LL was removed from each carcase, the cranial portion measured with a Raman hand held device [7], removed and stored for further analysis.

Spectra were recorded using 70mW of laser power with an integration time of 3.75 seconds. Spectra of IMF were identified and separated from meat spectra using Principal Components Analysis (PCA) and saved separately for analysis. Raman scans of IMF were completed on each intact muscle perpendicular to the muscle fibres, with the

silverskin removed, until 10 scans of IMF were observed. The 10 Raman spectra per sample were averaged, background corrected by fitting to a 7th order polynomial (at wavenumbers 523, 761, 982, 1139, 1383, 1526, 1712, 1859cm$^{-1}$) and normalised by dividing each intensity by the integration time multiplied by the laser power. Spectral wave numbers were restricted to 500 – 1800cm$^{-1}$.

Reference measurements were conducted on freeze dried and ground samples using a soxhlet method [8] to determine total IMF and a one- step extraction and methylation procedure [9] for measurement of FA. Total combined abundance of the main FA categories (PUFA, MUFA and SFA) were determined by the addition of identified FAs for that category.

Prediction models for FA traits were fitted using partial least square (PLS) regression analysis performed using R [10] and MATLAB [11] computer software. For PLS, the optimal number of latent variables included was determined for the model having the minimum root mean square error of cross validation (RMSECV) based on averages over 20 replications of 8-k fold cross validation. RMSECV for a model, including the Null (0 latent vector model), are based on leave one out cross validation.

## III. RESULTS AND DISCUSSION

Summary results for key FA composition measurements are given in Table 1.

Table 1. Mean, standard deviation (SD) and range for polyunsaturated (PUFA), monounsaturated (MUFA), saturated (SFA) fatty acids and PUFA:SFA ratio.

| Fatty Acid (mg/100g meat) | Mean ± SD | Range (min - max) |
|---|---|---|
| IMF | 4.0 ± 1.1 | 2.02 – 7.73 |
| PUFA | 149 ± 24 | 105 - 205 |
| MUFA | 665 ± 165 | 211 – 1032 |
| SFA | 785 ± 157 | 455 – 1271 |
| PUFA:SFA | 0.1 ± 0.04 | 0.1 – 0.2 |

In Table 2, the root mean square error of cross validation (Null and Optimal model) and the squared correlation between cross validated predictions and observed values ($R^2_{cv}$) for prediction models of key FA composition measurements are summarised.

Table 2. RMSECV and $R^2_{cv}$ for models to predict key FA composition traits (mg/100g meat) of intact lamb.

| Fatty Acid Trait (mg/100g) | Null RMSECV | Optimal RMSECV (Latent Variables) | $R^2_{cv}$ |
|---|---|---|---|
| IMF | 1.12 | 1.12 (2) | 0.01 |
| PUFA | 24.58 | 16.23 (9) | 0.56 |
| MUFA | 165.86 | 161.35 (1) | 0.04 |
| SFA | 253.76 | 250.43 (1) | 0.02 |
| PUFA:SFA | 0.038 | 0.035 (2) | 0.15 |

The square correlation between cross validated predicted and observed values ($R^2_{cv}$) indicates that there is potential for Raman spectra to predict PUFA ($R^2_{cv}$ = 0.56). Optimal RMSECV for PUFA equals 16.2 (9LV) which was a 34% reduction in RMSECV compared to the null model. Plots of cross validated predictions versus observed PUFA are given in Fig. 1.

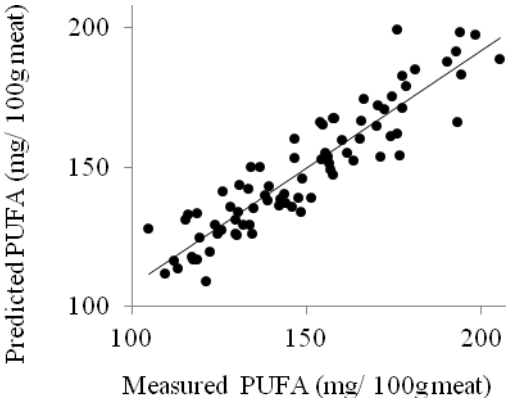


Figure 1. Prediction of polyunsaturated fatty acids (PUFA; mg/ 100g meat)

As Raman spectra are a reflection of the energy exchange between the excitation laser and the electrons involved in chemical bonds [12], the increase in C double bonds present in the fatty acid chains of PUFA increases the Raman signal at 1270cm$^{-1}$ (=C-H deformation), 1650cm$^{-1}$ (HC=CH stretch) and 1660cm$^{-1}$ (HC= CH trans stretch) [4]. Overall, the higher prediction accuracy for the PUFA concentration indicates that the increase in these intensity peaks within the Raman spectra enables the discrimination of samples which have higher amounts of PUFA.

Even though the cross validated squared correlation between predicted and observed

values of SFA is poor ($R^2_{cv}$ = 0.02, 1 LV), Raman spectra still explain some of the variation of the PUFA:SFA ratio ($R^2_{cv}$ = 0.15) despite having poor predictive power (optimal RMSECV= 0.37, 1 LV). The ability of Raman spectra to explain some variation in the PUFA:SFA ratio may be evidence of changing ratios between 1059cm$^{-1}$ (C-C stretch) and 1126cm$^{-1}$ (C-C in phase stretch), as well as increasing intensities of the shoulders of peaks at 1078cm$^{-1}$ (C-C aliphatic stretch) and 1265cm$^{-1}$ (=C-H deformation) [5], but this is yet to be validated.

Previous Raman studies of singular SFAs [13,14], suggest that the vibrations of SFAs are split over multiple spectral regions, depending on the polymorphic form. Therefore, the use of intensity ratios may provide much clearer information regarding the SFA composition. Since the FA composition of IMF from an intact muscle is complex, there is likely to be overlap of spectral regions pertaining to individual FA characteristics. It is hypothesised that this results in a better prediction for PUFA: SFA ratio in comparison to SFA alone, as the spectra can discriminate on both the PUFA peak increases and the changes to the spectral intensity ratios of the SFA, without losing the split vibrations of the SFA within the strong vibrations of the ester bonds of the other adjoining FAs.

The predictions for the main FA categories found by this study were lower than the correlations ($R$ = 0.91 – 0.96) previously reported for prediction of FA composition of porcine subcutaneous adipose tissue [6]. However, large differences exist in the chemical properties and FA of lamb muscle and pork subcutaneous adipose tissue, which results in differences between spectral parameters, particularly increased intensity peak shoulders that correspond to *trans* isomers in lamb [4]. It is hypothesised that these differences contributed to the overlap effect reducing the prediction of FA composition in this study.

While the prediction of IMF values was low ($R^2_{cv}$ = 0.01, RMSEP = 1.12 (2 LV)) this is the first study to report on the potential of Raman spectroscopy to predict IMF amount and composition, as previous Raman spectroscopic studies on animal fats have focused on porcine subcutaneous adipose tissue [6] and classifying species of origin based on subcutaneous adipose tissue composition. Therefore the results found in this study need to be validated to determine the merit of this approach over a larger number of samples and whether the prediction can be improved by altering Raman spectroscopic parameters such as integration time.

## IV. CONCLUSION

Overall it is difficult to determine the ability of Raman spectroscopy to predict FA composition of IMF within intact lamb loin, as there is currently no opportunity to compare the results found in this study against other studies sampling the same species and intramuscular fat. Therefore, the accuracy and robustness need to be validated and the impact of complex FA composition on Raman spectra needs to be determined. However, this study suggests that there is potential for Raman spectroscopy to predict the PUFA and PUFA: SFA composition of IMF, but not IMF itself.

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