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.

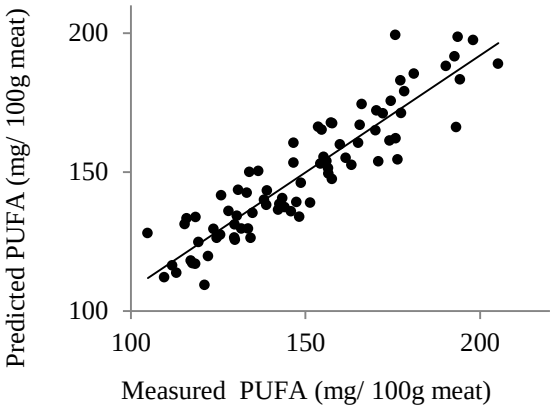

Figure 1. Prediction of polyunsaturated fatty acids (PUFA; mg/ 100g meat)

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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