PREDICTING TENDERNESS OF FRESH INTACT OVINE *Longissimus thoracis lumborum* USING RAMAN SPECTROSCOPY

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Abstract – Fresh intact lamb muscle was measured using a 671nm hand held Raman probe with a view to prediction of shear force at 1 and 5 days post mortem. Raman measurements were conducted on pairs of subsamples, aged at 1°C for 1 and 5 days respectively, from 80 samples of *longissimus thoracis lumborum* (loin) from different carcases. Each subsample was also measured for shear force (SF), cooking loss, sarcomere length and pHu. Shear force values were regressed on the Raman data using partial least squares (PLS) regression and on sarcomere length, cooking loss and pHu using ordinary least squares regression. For SF at 1 day, the root mean square error of prediction (RMSEP) was 13.16N and the correlation between predicted and observed values (R^2_{cv}) was equal to 0.09. Corresponding values for 5 day aged SF were R^2_{cv} = 0.02 and an RMSEP of 9.90N. This is the first Raman spectroscopy study to measure fresh lamb. Although prediction of SF is poor, the error of prediction is comparable with the traditional indicators. A combination approach of Raman spectra and traditional indicators gave a small reduction in the error of prediction. Further work is required to capture the potential benefits of Raman spectroscopy.

Key Words – Shear force, Meat quality assessment, Sheep

I. INTRODUCTION

Tenderness is an important factor in the consumer acceptability of meat. Meat tenderness is determined by the contribution of connective tissue, myofibrillar structure, the links between them and the changes in structure with ageing [1, 2]. Due to the importance of tenderness, many attempts have been made to measure these changes [3]. Of the technologies that have been used, Raman spectroscopy has been highlighted as having potential as it is rapid, non-destructive, non-invasive and is insensitive to varying water contents [4]. Recent research has shown it has potential in predicting sensory traits of beef [5]; the effect of aging and cooking on pork [6]; and the relationship between spectra, shear force and cooking loss in lamb [7]. However, the application of these studies to the use of Raman spectroscopy for online meat assessment and prediction are limited as samples have been cooked, homogenized or frozen for measurement and bench top Raman devices have been used in most studies. In this study, the potential of a Raman hand held spectroscopic device to predict tenderness of fresh intact lamb is reported for the first time.

II. MATERIALS AND METHODS

Samples of *m. longissimus thoracis lumborum* (LL) were taken from 80 lamb carcases sampled over four days (20 per day). One LL was removed from each carcase and was split into a caudal and medial section and randomized to two ageing periods (1 and 5 days) and held at 1°C. Each subsample was measured immediately following its assigned ageing period.

Raman measurements were taken using a hand held Raman probe [8]. Spectra were recorded with 70 mW of laser power and an integration time of 3 seconds. Ten Raman scans were completed on intact muscle perpendicular to the muscle fibres, with the silverskin removed. LL samples allocated to 5 days post slaughter ageing period were scanned for Raman measurements on day 1, vacuum packed and held at 1°C for 5 days. After ageing, the vacuum packs were opened and samples were allowed to 'bloom' for 2 hours before a freshly cut surface was re-scanned. The 10 Raman spectra per sample were averaged and normalized by dividing by its l_2-norm (square root of sum of squared intensities).

For shear tests, a section was weighed after scanning (mean 64 g). The samples were cooked for 35 min in plastic bags at 71°C in a water bath as previously described [9]. Samples were weighed once cooked to determine cooking loss (CL). Sarcomere length (SL) was measured using the laser diffraction method [10] and pHu was determined as previously described [11] on 1 day aged samples.

Prediction models for shear force using Raman spectra and alternative indicators (CL, SL and pHu) were fitted using Principal components analysis (PCA) and partial least square (PLS) regression analysis performed using R computer software [12]. For PLS, the optimal number of latent variables included was determined on the model having minimum root mean square error of prediction (RMSEP) based on 20 replications of 8-k fold cross validation. Simple linear regression was used for prediction based on cooking loss, sarcomere length and/or pHu.

III. RESULTS AND DISCUSSION

Summary results for shear force, cooking loss, sarcomere length and pHu measurements are given in Table 1.

Table 1. Mean, standard deviation (SD) and range for shear force (SF; N), cooking loss (CL; %), sarcomere length (SL; µm) and pHu.

Trait	Ageing (days)	Mean	SD	Range (min, max)
SF	1	60	13.7	34.8 - 87.1
SF	5	30.0	9.9	20.9 - 64.6
CL	1	17.3	2.7	0.3 - 25.9
CL	5	19.1	4.3	0.2 - 28.8
SL	N/A	1.66	2.96	1.36 - 1.86
pHu	N/A	5.96	0.16	5.54 - 6.29

In Table 2, the RMSEP values for prediction of shear force at 1 and 5 days using different models are summarised.

Table 2. RMSEP for models using traditional indicators and Raman spectra to predict shear force values (N) of 1 and 5 day aged lamb loin.

Model Covariates	Shear Force (1 Day)	Shear Force (5 Day)
Cooking Loss (CL)	13.45	9.74
Sarcomere Length (SL)	12.94	10.07
pHu	13.98	10.12
CL, SL and pHu	12.82	10.03
Raman Spectra (1 day)	13.16	9.90
Raman Spectra (5 day)	N/A	9.92
Raman Spectra (1day) + CL, SL and pHu	12.46	9.78
Raman Spectra (5 day) + CL, SL and pHu	N/A	9.76

The best prediction using Raman, based on the square correlation between cross validated predictions and observed values (R^2_{cv}) was the prediction of shear force 1 day post mortem using Raman spectra measured on day 1. This gave a RMSEP of 13.16, using 2 latent vectors (Fig.1), with R^2_{cv} = 0.09.

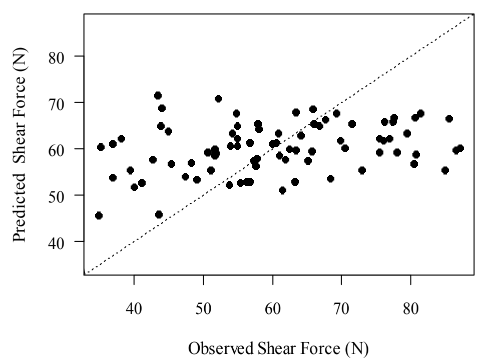

Figure 1. Prediction of shear force values at 1 day post mortem using Raman spectra analysed with 2 latent vectors.

The models for predicting shear force of 5 day aged LL with 1 or 5 day spectra, based on 2 and 1 latent vectors respectively, gave an RMSEP of approximately 9.9 with R^2_{cv} = 0.02, at best.

Of the traditional indicators, sarcomere length was a slightly better predictor of shear force (RMSEP= 12.94) than cooking loss (RMSEP= 13.45) or pHu

(RMSEP= 13.98) the relationship between shear force and sarcomere length is weak (R^2 = 0.14; Fig 2) at 1 day and by day 5 there was no relationship. This highlights the significance of fibre contraction during *rigor mortis* in determining initial tenderness, while proteolysis and the subsequent degradation of the myofibrillar structure diminishes some of the influence of sarcomere length for aged meat [13].

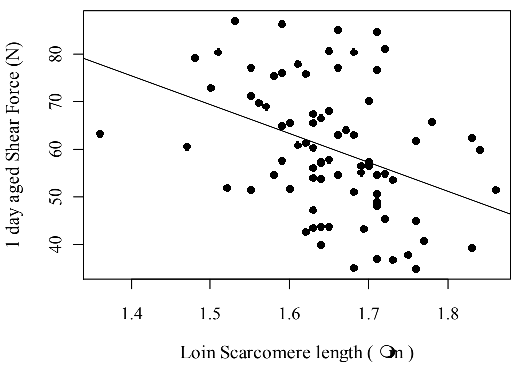

Figure 2. The relationship between shear force values (N) at 1 day post mortem and sarcomere length (µm).

Cooking loss had a weak relationship with shear force values at both 1 and 5 days post slaughter (R^2 = 0.07 and R^2 = 0.06 respectively). Less variation in the shear force values at day 5 (Table 1) contributed to the lower error at 5 days (RMSEP = 9.74) and although this is the lowest prediction error of any of the models, it is not significantly different to the prediction error of Raman spectra predictions at 5 days.

It has been established in literature that pHu also contributes to shear force [13, 14] however, data in this study shows no relationship between pHu and either 1 or 5 day shear force ($P > 0.05$). Although there was significant variation in shear force data (Table 1), there were no measurements in this study that would be classified as being very tender (below 27N) [7]. Combined with few samples that had a pH above 5.8, it is not surprising that pH wasn't significant predictor effect in our study.

Co-efficient of determination levels in this study are low in comparison to the previous Raman study conducted on lamb which had a co-efficient of R^2 = 0.72 [7]. It should be stressed that the one previous report with this probe was for lamb that was frozen and thawed prior to measurement. As Raman spectroscopy is sensitive to the changes that occur during freezing and thawing [15, 16], it is hypothesised that movement of cellular water and changes in ionic charges as the muscle cell structures change during freezing and thawing [17] improves the ability of Raman to predict shear force.

Previous studies using Raman spectroscopy to measure porcine *m. longissimus* and beef silverside also produced relatively high R^2_{cv} values of 0.77 and 0.75 for predicting shear force respectively [5, 6]. As the Raman signal is weak in comparison to fluorescence of biological samples, it is sensitive to spectral acquisition parameters needed to improve signal to noise ratios and the concentrations that can be quantitatively measured [18]. Acquiring several repetitions in the same position via longer accumulation times during scanning can overcome this [18]. Consequently it is expected that a 3 minute accumulation time [5] would improve prediction models in comparison to results reported here where only a 3 second accumulation was used. While 3 minutes is too long for online application, it is proposed that increasing the accumulation time by 10-12 seconds will improve the prediction.

IV. CONCLUSION

The measurement of 80 LL lamb samples with a hand held Raman probe showed a poor ability to predict shear force based on the Raman spectra. Prediction of shear force 1 day post mortem had a slightly better accuracy in comparison to predicting shear force based on spectra measured on day 5. In comparison traditional meat science measures sarcomere length and pHu were also poor predictors of shear force. However measurement of LL on-line in lamb carcases using Raman Spectroscopy using these experimental parameters for prediction of shear force does not appear to provide the improvement in accuracy that industry would require. This conclusion though is restricted to the LL and these experimental parameters and excludes other muscles and also the measurement of other traits, such as intramuscular fat and other experimental parameters such as longer accumulation times, which are yet to be studied.

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