PREDICTING TENDERNESS OF FRESH OVINE *Semimembranous* USING RAMAN SPECTROSCOPY

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Abstract – A hand held 671nm wavelength Raman probe was used to predict shear force of raw intact lamb muscle at day 5 post mortem. Samples (n = 80) of *m. semimembranosus* (topside) from different carcases were measured 24h post mortem and after a further 4 days ageing at 1˚C. At 5 days post mortem shear force (SF) and traditional indicators of shear force (cooking loss, sarcomere length and pHu) were measured. SF values were regressed against Raman spectra using partial least squares (PLS) and against traditional indicators using ordinary least squares regression. For SF prediction using spectra taken 24h post mortem, the root mean square error of prediction (RMSEP) was 11.48N and the correlation between observed and predicted values (R^2_{cv}) was equal to 0.27. Corresponding values for spectra measured 5 days post mortem were RMSEP of 12.20N and R^2_{cv} = 0.17. This is the first study to measure fresh lamb topside using Raman Spectroscopy and there is evidence to suggest Raman spectra taken 24h post mortem is a better predictor of shear force than sarcomere length, cooking loss and/or pHu at 5 days post mortem. Further work to validate the models generated in this study is required to establish the potential benefits of Raman spectroscopy.

Key Words – Shear force, Meat quality assessment, Sheep

I. INTRODUCTION

Tenderness is deemed the most important factor in determining consumer acceptance. It is determined by the interactions between myofibrils and connective tissue and the extent of degradation of myofibrils [1]. Due to this importance, considerable research has focused on the ability of technologies to objectively measure tenderness. A review of such technologies has highlighted Raman Spectroscopy as having the potential to be used for online assessment of meat quality traits [1] as it is non-invasive, rapid, non-destructive and is not sensitive to varying water content [2]. Recent research has not overlooked these advantages and several studies have been conducted to determine the ability of Raman Spectroscopy to predict sensory traits of beef silverside [3], assess the effect of ageing on pork [4] and investigate the relationship between cooking loss and shear force in lamb [5]. While informative, these studies are limited in terms of online application by the cooking, freezing or homogenisation of samples and the use of bench top devices. Further to this no studies have been reported on the measurement of unfrozen meat by Raman Spectroscopy and then measurement of shear force and other traits on the same piece of meat.

This study reports, for the first time, the potential of a Raman hand held device to predict the tenderness of fresh intact lamb *m. semimembranosus* (topside).

II. MATERIALS AND METHODS

One *m. semimembranosus* (SM) was collected from each of 80 lamb carcasses over 4 days (20 per day). Raman spectra were measured with a 671nm Raman hand held sensor head [6] with 70mw laser power and a 3s integration time with no repetitions. At 1 day post mortem each SM had the silverskin removed before being scanned 10 times, perpendicular to the muscle fibres. After measurement the SMs were vacuum packed and held at 1˚C for 4 days. At 5 days post mortem the SM were removed from the vacuum pack and allowed to 'bloom' for 2 hours before a freshly cut surface was rescanned. At 5 days post mortem, sections were excised (mean 65g) for shear tests with a Lloyd texture analyser, as previously described [7]. Samples were weighed before and after cooking to determine cooking loss. Ultimate pH (pHu) was measured using a homogenate method [8] and sarcomere length determined by the laser diffraction method [9] on samples at 1 day post-mortem.

The 10 Raman spectra per SM were averaged then standardised by dividing each by its l_2-norm (square root of sum of squared intensities). The models for predicting shear force which included Raman spectra were fitted using partial least squares (PLS) regression analysis, using R computer software [10]. The number of latent variables (LV) was determined using 20 replications of 8-k fold cross validation and selecting the model with the minimum average root mean square error of prediction (RMSEP). Models not using Raman were fitted using ordinary regression least squares regression. Predictions for each observed shear force value, for each SM sample, were obtained using the Leave-One-Out (LOO) cross validation method.

III. RESULTS AND DISCUSSION

In Table 1, summary results for shear force, sarcomere length, pHu and cooking loss measurements are given. At 5 days post mortem, shear force measurements had a large range (Table 1), but none were below 27N, which indicates very tender samples [11].

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

Trait	Mean	SD	Range (min, max)
Shear Force (N)	51.4	13.1	29.2 - 78.4
Cooking Loss (%)	19.2	3.7	0.24- 28.8
Sarcomere Length (µm)	1.70	0.11	1.46 - 1.99
pHu	5.61	0.11	5.52 - 6.23

The root mean square error of prediction (RMSEP) values for prediction of shear force using the traditional indicators traits and Raman spectra are summarized in Table 2.

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

Model Covariates	RMSEP
Cooking Loss (CL)	13.34
Sarcomere Length (SL)	13.23
pHu	13.75
CL, SL and pHu	13.90
Raman Spectra (1 day)	11.48
Raman Spectra (5 day)	12.20
Raman Spectra (1day) + CL, SL and pHu	12.02
Raman Spectra (5 day) + CL, SL and pHu	13.00

Based on the RMSEP criterion, the best model of those considered for prediction of shear force (Table 2) uses the Raman spectra measured at 1 day post mortem (RMSEP = 11.48, LV= 3). The squared correlation (R^2_{cv}) between cross validated predicted and observed shear force values is R^2_{cv} = 0.27 (Fig 1).

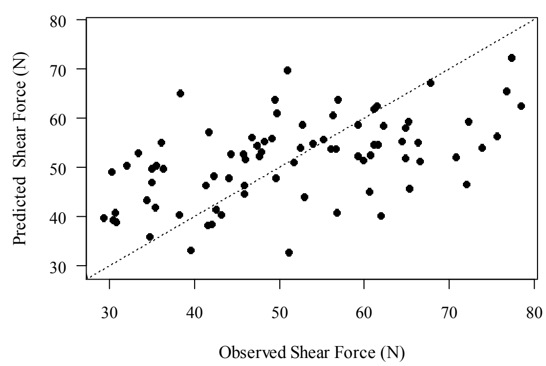

Figure 1. Cross validated prediction of shear force values (N) at 5 days post mortem with Raman spectra collected on day 1 post mortem and analysed with 3 latent vectors.

Although changes to Raman spectra due to ageing are complex and are outside the scope of this paper, results reported here indicate the prediction of shear force using Raman spectra taken on 1 day post mortem is less variable (R^2_{cv} = 0.27; RMSEP= 11.48) than the prediction using Raman spectra collected 5 days post mortem (R^2_{cv} = 0.17; RMSEP = 12.20). As muscle is a closed system, the attributes of muscle which determine the effects of proteolysis and the subsequent amount of myofibrillar degradation during ageing are predetermined by the biophysical and biochemical properties at processing. Whether the ageing process is a result of the interaction of actin and myosin, a change in ionic strength or the action of calcium ions on proteins [12], it is hypothesized that this change or the loss of myofibrillar structure weakens the Raman signal when spectra are taken at 5 days. If changes in ionic strength during ageing are affecting the ability of Raman to predict shear force, collecting spectra as the muscle enters *rigor* may improve predictions because this is the period in which initial changes to electrochemistry occur [12].

None of the traditional indicators were significant predictors of shear force alone or jointly ($P > 0.05$) and combining all three measured with Raman spectra gave no improvement to the predictability of the model. Despite some previous studies linking these indicators to shear force [11, 13], results reported agree with others that suggest these indicators do not explain large amounts of variation in shear force [14]. A wider range of shear force values with some below < 27N would improve this outcome. However, it is also important to acknowledge that aging weakens the relationship established at *rigor* [14], so it is not so surprising that sarcomere length wasn't a significant predictor of shear force in aged meat (Fig 2).

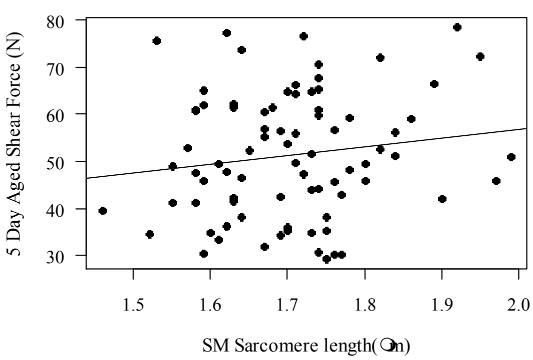

Figure 2.The relationship between shear force values (N) and sarcomere length (μm) for SM samples after 4 days of ageing.

The predictions reported in this paper have lower accuracy compared to coefficients of determination previously reported [3-5]. However, other studies have measured meat that was frozen and thawed, causing myofibrillar disintegration and increased water movement within the muscle [15]. It is hypothesised that increases in shear force prediction accuracy may occur when measuring frozen and thawed meat as Raman is sensitive to changes induced by freezing and thawing [16].

It should also be acknowledged that like other spectroscopic technologies, Raman Spectroscopy is sensitive to variations in experimental design including sampling and sample handling, statistical analysis and equipment parameters [17]. Therefore, it is difficult to compare the results reported against other studies as there is little accordance between experimental design and statistics reported. An example is the difference in integration times used, as previous studies reported times between 2 seconds and 6 minutes [3-5, 18]. Since Raman scattering is relatively weak, spectra of biological samples may be compromised by noise or non-Raman signals and background radiation [17]. Consequently, longer integration times and repetitions or more scans may improve the prediction of shear force by improving the signal to noise ratio. While a 6 minute integration time or 25 scans per sample will not suit an online application, increasing the total accumulation time by increasing integration time or including repetitions may improve the prediction of shear force. Overall, the sensitivity of Raman to experimental parameters emphasises the need for validation of prediction models generated on completely independent samples and as yet no such study has been conducted.

IV. CONCLUSION

Overall it is difficult to determine the ability of Raman spectroscopy to predict shear force values of intact lamb samples, as there is currently no opportunity to evaluate these results reported against other studies which have the same experimental design, equipment and statistical analysis. Therefore, the accuracy and robustness of these predictions need to be validated and the impact of variations in scanning, sampling and chemometric analysis needs to be determined. However, this study suggests that use of Raman may be a better indicator of variation in shear force compared to the traditional indicators, sarcomere length, cooking loss and pHu.

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