Preliminary investigation on the relationship of Raman spectra of sheep meat with shear force and cooking loss

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Abstract—**We have tested a prototype handheld 671nm Raman system as a rapid non-invasive and non-destructive optical device to estimate cooked meat tenderness and cooking loss using Raman measurements of raw sheep meat.**

***M. longissimus thoracis et lumborum* samples from two sheep flocks originating from two different sites and two slaughters were aged for five days at 3-4°C before deep freezing and further analysis. Shear force, cooking loss and Raman measurements were performed on 140 thawed samples.**

The Raman data from the samples could be correlated with shear force and cooking loss data using PLS regression analysis. Sample origin could be discriminated and separate correlation yielded better correlation models than the joint models for both sites. For shear force, coefficients of determination of R^2 = 0.79 and R^2 = 0.86 were obtained. Standard errors of calibration (RMSEC's) were 10.9% and 10.4%. Results for cooking loss were similar yielding R^2 = 0.79 and R^2 = 0.83. The standard errors of calibration were better for cooking loss: RMSEC = 3.2% and RMSEC = 2.6%.

These first results of the handheld device demonstrate the potential usefulness of Raman spectra which can be recorded during meat production for the prediction of quality traits such as tenderness and cooking loss.

Keywords— **Raman spectroscopy, sheep meat, shear force.**

I. INTRODUCTION

Tenderness is an essential quality trait for the palatability of meat. It is now well-accepted that degradation of muscle proteins and the incurred changes of the molecular structure is responsible for tenderisation of meat during post mortem ageing. Numerous attempts have been made to measure these changes and relate them to physical measures of tenderness [1]. In this context, Raman spectroscopy is one of the techniques that has gained attention because it is non-invasive, capable of providing structural information of proteins and not influenced by variation in water content.

Using bench top instruments, Beattie et al. showed Raman spectroscopy to be suitable for the prediction of sensory traits in beef meat [2]. Later, they could explain a large amount of the variation (R^2 = 0.77) in shear force of pork meat using Raman spectra [3]. However, for industrial application on-line measures and hand held devices are required. Such a device has recently been developed based on a laser diode emitting light at 671nm [4].

In this study, we report on the first use of this hand held Raman spectroscopic device to predict the shear force of aged lamb meat. This work is part of the CRC for Sheep Industry Innovation in Australia which evaluates lamb meat for a wide range of meat production and consumer-relevant traits [5].

II. MATERIALS AND METHODS

Meat samples were taken from 140 lambs which were bred at two research stations (referred to as A and B) and which were slaughtered at the age of 5-6 months as part of the Information Nucleus for the CRC for Sheep Industry Innovation (Fogarty, Banks, van der Werf, Ball, & Gibson, 2007). The 70 lambs per site represented 46 different sires and included both second cross lambs (Terminal sire x Border Leicester x Merino ewes) and first cross lambs (Terminal sire x Border Leicester or Terminal sire x Merino ewes). All carcasses were electrically stimulated as described in [6], weighed and chilled at 4–5°C for 24 h. Then, the right loin muscle (*m. longiss. thoracis et lumborum*; LL) was removed and a caudal portion was aged vacuum packed for ageing for four days at 3–4°C. On day 5, the samples were prepared into small blocks, vacuum packaged and then frozen at –20°C. For shear tests, samples were thawed for 21h at 3–4°C, weighed (mean 59 g) and the pH was measured. The samples were cooked for 35 min in plastic bags at 71°C in a water bath as previously described [7]. Cooking loss was determined using the weight of the cooked samples which were dried with paper towels. From each sample, six 1 cm^2 subsamples were tested using a Lloyd texture analyzer with a v-shaped cutting blade.

For Raman measurements, samples were transported on dry ice from Australia to Germany and held frozen (-20°C). The samples were thawed as described above, unpacked and Raman spectra were taken at 15 different positions per sample on a fresh section. The measurements were conducted with the prototype 671nm hand-held Raman device described by Schmidt et al. [8] (Figure 1). This Raman probe was coupled via optical fibre to a miniature spectrograph with TE-cooled CCD camera covering the 400-2100 cm^{-1} range (Horiba Jobin-Yvon, Longjumeau, France). Acquisition and storage of spectra were performed by a net book using programs Versa spec® (Horiba) and MS Excel®. Spectra were recorded with 80 mW of laser power and integration times of 5 s for A samples and 4 s for B samples. The spectra were normalized by dividing the intensity by laser power and integration time.

Fig. 1 Handheld Raman probe for measurements of meat

Principal components analysis (PCA) and partial least square regression analysis (PLS) were performed with MATLAB software (MathWorks Inc., Natick, MA, USA) and PLS Toolbox (Eigenvector Research, Inc.; Wenatchee, WA, USA). For each sample, the spectra were averaged and preprocessed using 2^{nd} derivative Savitzky-Golay smoothing and mean-centering.

III. RESULTS AND DISCUSSION

The measured shear force and cook loss data of lamb LL samples from both sites are summarized in Table 1. The shear force data had a large variance for a correlation of these traits with the Raman spectra, while variation of cook loss was lower.

Table 1: Mean, standard deviation (SD), minimum and maximum for shear force (N/cm^2), and cooking loss (%)

Trait (Site)	Mean ±SD	Min.	Max.
Shear force (A)	27.5 ± 7.7	15.8	58.5
Shear force (B)	23.1 ± 5.6	14.1	44.1
Cooking loss (A)	25.4 ± 1.6	22.0	29.9
Cooking loss (B)	24.8 ± 1.7	21.0	29.6

PLS regression analysis of the shear force data of both sites A and B yielded a prediction model with a coefficient of determination of $R^2 = 0.72$, a root mean square error of calibration (RMSEC) of 15%, and a root mean square error of validation (RMSECV) of 27.4%. When PLS analysis was performed separately for A and B sites the prediction models for the shear-force values of both data subsets yielded improved coefficients of determination of $R^2 = 0.79$ and $R^2 = 0.86$ for sites A and B, respectively. The result of the analysis is depicted in Figure 2. The RMSEC's were 10.9% (A) and 10.4% (B). The validity of these PLS models is described by the RMSECV's which were 31% for A and 26% for B. These values are quite high and might be due to an unbalanced distribution of the shear-force values: the majority of samples had values around 23 N, only few samples had high shear force values. Thus, predictability for samples with low and medium shear force values was high, but it was reduced for samples with high shear force values.

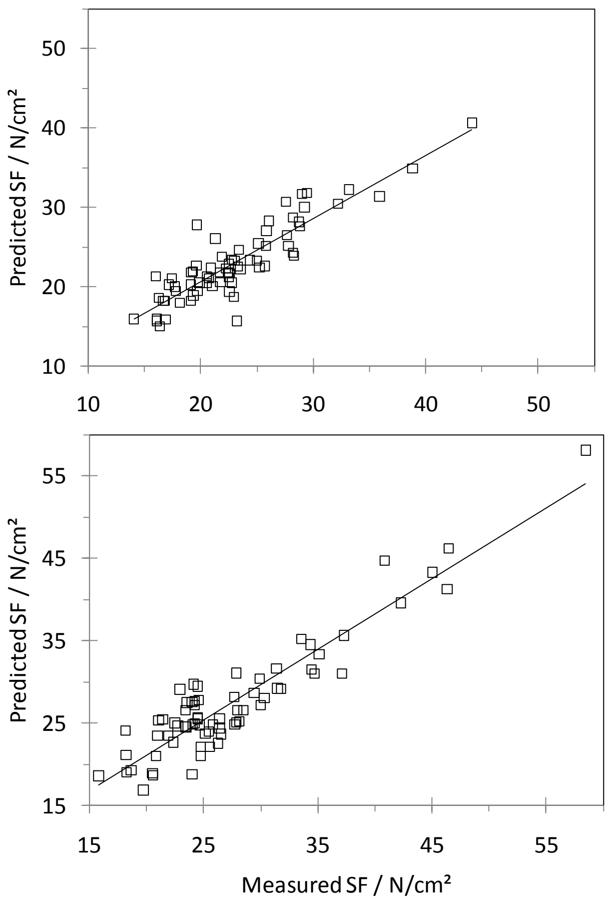

Fig. 2 PLS-Correlation of shear force (SF) and Raman data for site A (top) and B (bottom)

In a second step, the cook loss data was correlated with the Raman spectra. Again, the joint treatment of A and B data sets produced a less accurate model. The coefficient of determination was low ($R^2 = 0.55$), which is also a consequence of the lower variance in the cook loss data. The RMSEC and RMSECV yield acceptable 4.5% and 8.5%, respectively. Separate treatment for sites A and B improved the cook loss correlation. The coefficients for both data sets were $R^2 = 0.79$ for A and $R^2 = 0.83$ for B samples. The standard errors of the calibration models were slightly better for the B site: RMSEC = 3.2% and RMSECV = 8.7% for A samples and RMSEC = 2.6% and RMSECV = 7.6% for B samples.

It was interesting to identify spectral features which could be used for discriminating tender and tough meat. However, due to the increased noise in 2nd derivative spectra, the loadings of the PLS analysis did not allow us for an identification of faint signals. Therefore, we have sorted the Raman spectra into two groups, designated as "normal" and "tough" meat using a threshold of 27 N/cm^2, because lamb meat with shear force values around or below 27 N/cm^2 has been shown to be preferable for consumer acceptance [9]. Figure 4 depicts the averaged Raman spectra of all lamb samples from both sites with shear force below 27 N/cm^2 (black curve, normal) and with a shear force larger than 27 N/cm^2 (red curve, tough). The spectra are typical of muscle tissue, notably the amide I and III bands at 1650 cm^{-1} and 1250 – 1300 cm^{-1}, C-C skeleton vibrations at 935 cm^{-1} of the protein backbone as well as signals of the aromatic amino acids such as phenylalanine at 1000 cm^{-1} [10].

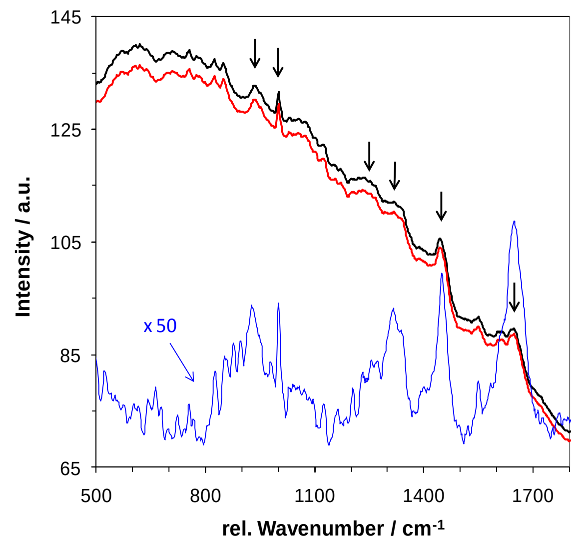

Fig. 3 Averaged Raman spectra of lamb meat (LL) samples from both sites according to their shear force values < 27 N/cm^2 (normal, black) and > 27 N/cm^2 (tough, red) and the difference spectrum after baseline correction (blue)

The Raman spectra of the two shear force groups can be distinguished spectrally by broad band differences in the baseline of between 500 cm^{-1} and 1200 cm^{-1} (Figure 3). This is suggesting a slightly lower fluorescence and scattering background of the tough samples. In the difference spectrum between normal and tough meat, weak Raman signals were observed which could be ascribed to α-helical proteins which appear to be stronger in the tough spectra, e.g.

amide I band at 1646cm^{-1}, Amide III band at 1304cm^{-1} and 1270cm^{-1} as well as from the C-C stretch skeletal mode at 930cm^{-1}. The same was observed for peaks of tryptophan and of cystine bridges [11].

IV. CONCLUSIONS

The Raman data of (LL) lamb samples from two different sites could be correlated with shear force and cook loss data using PLS regression analysis.

The separate correlation of Raman data subsets A and B yielded better correlation models than the joint correlation models for the full data set. A comparison of the Raman spectra of lamb meat with shear force values over 27 N/cm^2 (tough meat) with those with shear force values below 27 N/cm^2 (normal or tender meat) exhibited slightly stronger signals for proteins in α-helical conformation, for tryptophan and for cystine groups in the spectra of the tough meat.

The results show that Raman spectroscopy has the potential for predicting quality traits such as tenderness or cooking loss of aged meat.

For future work, it would be interesting to investigate the prediction of the tenderness of aged meat when the spectra are collected on meat on the day of slaughter or before boning.

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