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

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ABSTRACT

A prototype handheld Raman system was used as a rapid non-invasive optical device to measure raw sheep meat to estimate cooked meat tenderness and cooking loss. Raman measurements were conducted on *m. longissimus thoracis et lumborum* samples from two sheep flocks from two different origins which had been aged for five days at 3–4 °C before deep freezing and further analysis. The Raman data of 140 samples were correlated with shear force and cooking loss data using PLS regression. Both sample origins could be discriminated and separate correlation models yielded better correlations than the joint correlation model. For shear force, $R^2 = 0.79$ and $R^2 = 0.86$ were obtained for the two sites. Results for cooking loss were comparable: separate models yielded $R^2 = 0.79$ and $R^2 = 0.83$ for the two sites. The results show the potential usefulness of Raman spectra which can be recorded during meat processing for the prediction of quality traits such as tenderness and cooking loss.

1. Introduction

Flavor, juiciness and tenderness influence the palatability of meat. Among these traits, tenderness is ranked as most important for beef meat (Thompson, 2002), but it has lesser influence in sheep meat (Thompson et al., 2005). The three factors that determine meat tenderness are "background toughness", the toughening phase and the tenderization phase. The toughening and tenderization phases take place during post mortem storage, or aging period (Hopkins & Geesink, 2009). It is now well-established that post mortem proteolysis of myofibrillar and associated proteins is responsible for tenderization, but it is a matter of debate about which proteases are responsible for tenderization and which muscle proteins are degraded (Hopkins & Thompson, 2002; Koohmaraie & Geesink, 2006; Ouali et al., 2006). Nevertheless, as a consequence of this degradation of proteins, the molecular structure of meat changes during aging. Attempts to measure these changes and relate them to instrumental measures of tenderness have been extensive (Damez & Clerjon, 2008). An often used technique is VIS-NIR reflectance spectroscopy. However, many studies have shown that the correlations with shear force measurement were less strong than required for a robust prediction. For example, Rosenvold et al. obtained coefficients of determination of $R^2 = 0.58$ for NIR reflectance spectra with post rigor shear force of bovine *longissimus lumbrorum* muscles (Rosenvold et al., 2009). In another study Yancey, Apple, Meullenet, and Sawyer (2010) reported an $R^2 = 0.64$ to 0.74 for the prediction of Warner–Bratzler shear force of cooked *longissimus thoracis* beef with VIS-NIR spectral data.

Raman spectroscopy is one of the technologies that has come under recent scrutiny (Beattie, Bell, Borggaard, & Moss, 2008; Beattie, Bell, Farmer, Moss, & Patterson, 2004), because it is non-invasive, requires almost no sample preparation and is not influenced by variation in water content. In contrast to NIR and Mid-IR spectroscopy, the Raman effect is not based on the absorption of light, but on inelastical scattering of light which occurs when laser light interacts with molecules and condensed matter. The generally observed process is that the incident light excites molecular vibrations in the material leading to a red-shift of the scattered light which is analyzed. Thus, the Raman spectrum is fundamentally a vibrational spectrum and may be regarded as a "fingerprint" of the scattering material providing qualitative and quantitative information about the molecular composition and structure (Li-Chan, Griffith, & Chalmers, 2010).

In the report by Beattie et al. (2004), Raman spectroscopy was shown to be a useful tool for predicting sensory traits in cooked beef meat that had been aged for 21 days and then frozen. In a later work on raw and cooked pork meat a large amount of the variation ($R^2 = 0.77$) in shear force was explained by Raman spectra (Beattie et al., 2008). In both these studies a bench top instrument with a 785 nm laser was used. Obviously for industrial application or on-line measures handheld devices are required. Such a device has recently been developed based on a laser diode emitting light at 671 nm (Schmidt, Sowoidnich, Maiwald, Sumpf, & Kronfeldt, 2009). Compared to the more convenient 785 nm excitation the shorter laser wavelength allows for faster measurements by a factor of 2

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due to higher scattering intensities according to the λ^{-4} law and due to an increased sensitivity of CCD detectors (Schmidt, Sowoidnich, & Kronfeldt, 2010).

As part of the Cooperative Research Centre (CRC) for Sheep Industry Innovation in Australia, each year 2000 progeny are evaluated for a wide range of meat production and consumer-relevant traits (Hopkins, Jacob, Ball, & Pethick, 2009) which includes tenderness measured objectively as shear force. This paper reports on a study, which uses a subset of these data, to compare whether the measurement of aged meat using a handheld Raman spectroscopic device could be used to predict the shear force of lamb meat. There are no previous reports on the application of Raman to lamb meat for prediction of tenderness.

2. Materials and methods

2.1. Carcasses

Over two days, 140 mixed sex lambs were slaughtered representing both second cross lambs (Terminal sire × Border Leicester × Merino ewes) and first cross lambs (Terminal sire × Border Leicester or Terminal sire × Merino ewes) produced as part of the Information Nucleus for the CRC for Sheep Industry Innovation (Fogarty, Banks, van der Werf, Ball, & Gibson, 2007). The lambs representing 46 different sires were bred (70 per site) at two different research stations (designated A and B). They were slaughtered as two separate groups at 5–6 months of age at the same abattoir. All carcasses were electrically stimulated (800 mA with variable voltage to maintain a constant current, for 25 s at 14 pulses/s, 1 ms pulse width) post-dressing with a mid-voltage unit (Toohey & Hopkins, 2006). The carcasses were weighed and then chilled at a mean temperature of 4–5 °C over a 24 h period.

2.2. Measurements

From the loin (product identification number HAM 4910; Anonymous, 1998) the right loin muscle (m. *longissimus thoracis et lumborum*; LL) was removed at 24 h post-mortem and a caudal portion was vacuum packed and held chilled (3–4 °C) until preparation and freezing on day 5. From each of these LL samples, approximately 65 g blocks and a separate 3 cm thick section were cut and frozen (−20 °C) for subsequent shear testing and measurement by Raman spectroscopy respectively. Samples for shear testing were first thawed for 21 h at 3–4 °C in 7 batches of 20 (10 per site per batch randomly allocated) and the pH was measured using a meter with temperature compensation (TPS, WP-80, PTS Pty Ltd) and a polypropylene spear-type gel electrode (Ionode IJ 44), calibrated at chiller temperature. The samples were then weighed (mean 59 g) and cooked for 35 min in plastic bags at 71 °C in a 90 L water bath with a thermoregulator with a 2000 W heating element (Ratek Instruments, Boronia, Victoria, Australia) as previously described (Hopkins, Toohey, Warner, Kerr, & van de Ven, 2010). Once the samples were cooled to room temperature, they were blotted dry using paper towels and re-weighed. Cooking loss percentage was calculated using the difference. From each LL sample, six 1 cm^2 subsamples were cut and these samples were tested using a Lloyd texture analyzer (Model LRX, Lloyd Instruments, Hampshire, UK) with a vee-shaped cutting blade that sheared down through the sample. The crosshead speed of the analyzer was 200 mm/min.

Samples for Raman measurement were transported frozen on dry ice from Australia to Germany and held frozen (−20 °C). The samples were thawed using the same protocol as applied to shear force samples. Prior to measurement, the samples were unpacked and weighed to determine drip-loss caused by processing, transport and storage. Each sample was cut into quarters and the freshly cut surfaces of three of the quarters were measured at five different positions, i.e. 15 Raman spectra per sample were taken. If – occasionally – intramuscular fat was measured this was recognized during the measurement by means of the typical fat tissue Raman patterns (Beattie, Bell, Borggaard, Fearon, & Moss, 2007) and a new position was selected.

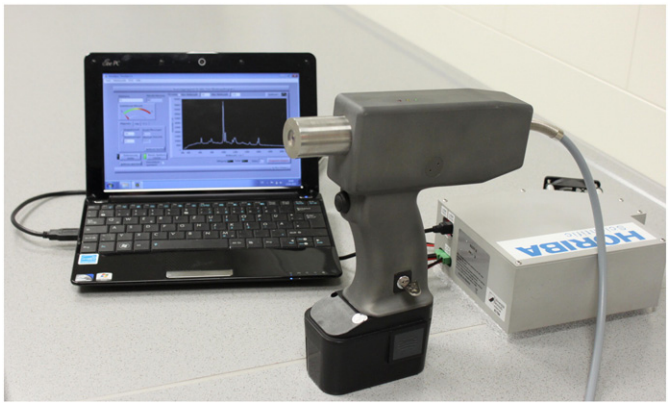

Fig. 1. Portable Raman system for on-line measurements of meat consisting of a hand-held Raman device (center), miniaturized spectrograph (right) and netbook (left).

Raman measurements were conducted with the prototype hand-held Raman device shown in Fig. 1 (Schmidt et al., 2010). Briefly, the 671 nm emission of a microsystem diode laser (Ferdinand-Braun Institute, Berlin, Germany) was focused on the sample by means of an in-house manufactured Raman probe which collected the backscattered radiation and provided the rejection of the elastically scattered light. The wavelength-shifted light was transported with an optical fiber to a custom-made miniature spectrograph covering the 400–2100 cm^{-1} range with 8 cm^{-1} resolution (Horiba Jobin-Yvon, Longjumeau, France) with TE-cooled CCD camera for recording of the Raman spectra. Acquisition and storage of spectra were performed by a net book using commercial 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 due to a higher background intensity level of B samples.

2.3. Statistical analysis

The spectra were normalized to counts per milliwatt and seconds (counts/mWs) by dividing the intensity by laser power and integration time to compare the two different subsets A and B.

Principal component analysis (PCA) and partial least square regression analysis (PLS) were performed with MATLAB 7.9.0 (R2009b) software (The MathWorks Inc., Natick, MA, USA) and the program package PLS Toolbox 5.8.1 (Eigenvector Research, Inc., Wenatchee, WA, USA). As an initial step, a PCA was performed to identify remaining spectra of intramuscular fat in the data set which had not been eliminated during data collection. To this end the spectra were preprocessed with a Savitzky–Golay filter (2nd derivative, 2nd polynomial order, filter width 15). Spectra with scores associated to the fat pattern exceeding a threshold value were excluded from further analysis. The threshold was determined iteratively by lowering the value and subsequently computing a new PCA model with the remaining data until the Raman fat pattern was absent from the first 4 loadings of the PCA model. In this way, 58 of 1058 (5.5%) spectra of flock A and 29 of 1030 (2.8%) spectra of flock B were excluded. For further analysis, the meat spectra were normalized to the baseline intensity at 1517 cm^{-1} as this resulted in better PCA and PLS models. For each sample, the remaining 12–15 different spectra were averaged and preprocessed using Savitzky–Golay smoothing (2nd derivative, 2nd polynomial order) and mean-centering. Six latent variables were used to compute the predictions of the models below. For cross-validation the method “contiguous blocks” using 10 data splits was applied.