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Predicting tenderness of fresh ovine *semimembranosus* using Raman spectroscopy

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ABSTRACT

A hand held Raman probe was used to predict shear force (SF) of fresh lamb *m. semimembranosus* (topside). Eighty muscles were measured at 1 day PM and after a further 4 days ageing (5 days PM). At 1 day PM sarcomere length (SL) and particle size (PS) were measured and at 5 days PM, SF, PS, cooking loss (CL) and pH were also measured. SF values were regressed against Raman spectra using partial least squares regression and against traditional predictors (e.g. SL) using linear regression. The best prediction of SF used spectra at 1 day PM which gave a root mean square error of prediction (RMSEP) of 11.5 N (Null = 13.2) and the squared correlation between observed and cross validated predicted values (R^2_{cv}) was 0.27. Prediction of SF based on the traditional predictors had smaller R^2 values than using Raman spectra justifying further study on Raman spectroscopy.

1. Introduction

Tenderness, juiciness and flavour are all factors which influence the eating quality of meat. Of these, tenderness is critical as a tough steak is unacceptable (Wood et al., 2008), although tenderness of lamb may have a lesser importance than other sensory attributes (Thompson et al., 2005). It has been established that the tenderness of meat is determined by the interaction between the connective tissue that creates 'background toughness', the myofibrillar structure (Damez & Clerjon, 2008) and the changes to these structures post mortem (Hopkins & Geesink, 2009). Consequently, considerable research has focused on the ability of technologies to objectively measure tenderness. A review of such technologies has highlighted Raman spectroscopy as having potential to be used for online measurement of meat quality traits (Damez & Clerjon, 2008).

Based on the in-elastic scattering of light which can provide information about molecular composition and structure, Raman spectroscopy has potential for use in muscle food systems as it is rapid, non-destructive, non-invasive, not sensitive to varying water content and is not based on the absorption of light (Li-Chan, 1996). Previous research has not overlooked these advantages and several studies have demonstrated that Raman is a useful tool in predicting sensory traits in cooked beef (Beattie, Bell, Farmer, Moss, & Patterson, 2004) and in explaining a large variation ($R^2 = 0.77$) in shear force of pork (Beattie, Bell, Borggaard, & Moss, 2008). However, these studies used a bench top instrument that is not suitable for industrial application. Alternatively, Schmidt, Scheier, and Hopkins (2013) used a hand held Raman device suitable for online application to predict shear force of lamb meat with good predictability ($R^2 = 0.79$ and 0.86 for two sample groups). Despite using the hand held Raman device, the industrial application of this study is limited as samples in the study were frozen and then thawed prior to measurement. Further to this, none of these studies cited have reported on the measurement of Raman spectroscopy, shear force and other predictors of tenderness on the same piece of meat.

This study reports for the first time, the potential of a Raman hand held device to predict the shear force of fresh intact lamb *m. semimembranosus* (topside) and a comparison of Raman spectroscopy with the traditional predictors of shear force.

2. Materials and methods

2.1. Samples

Topside (product identification number HAM 5077; Anonymous, 2005) samples were removed from 80 lamb carcases over 4 consecutive days (20 samples per day) from the same abattoir. Topsides were randomly selected from different consignments and thus were of unknown

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backgrounds, age and gender, to represent the various animals typically processed by the abattoir in order to achieve a spread in shear force levels. The cap muscle (*m. gracilis*) and *m. adductor* were removed to leave the *m. semimembranosus* (SM) which was the muscle of measurement.

2.2. Raman spectroscopy

Raman spectroscopic measurements were conducted at ambient room temperature on day 1 post mortem on a fresh cut surface of the intact SM with the epimysium removed (Fig. 1). Twelve positions were scanned using a Raman hand held device (Schmidt, Sowoidnich, & Kronfeldt, 2010) perpendicular to the muscle fibre, over the face where the *m. adductor* had been removed. Spectra were recorded using 70 mW of laser power and an integration time of 3.75 s with no repetitions. After scanning and removal of sections for measurement of traditional indicators, SM samples were vacuum packed and held at 1 °C for 4 days. At 5 days post mortem, SM samples were removed from the vacuum packs and allowed to 'bloom' for 2 h before a freshly cut surface was rescanned.

2.3. Traditional predictors of tenderness

At 1 day post mortem, sections were removed for sarcomere length analysis using the laser diffraction method (Bouton, Harris, Shorthose, & Baxter, 1973) and particle size analysis (PSA) (Karumendu, van de Ven, Kerr, Lanza, & Hopkins, 2009).

At 5 days post mortem, shear force tests were conducted on blocks (mean weight 65 g) cut from the middle of the muscle after Raman scanning. Blocks were cooked at 71 °C for 35 min and analysed using a Lloyd texture analyser with a vee-blade as described by Hopkins, Toohey, Kerr, and van de Ven (2011). The average of 6 repetitions was reported except when the co-efficient of variation exceeded 24% in which case the median of the 6 repetitions was used (Hopkins, Kerr, Kerr, & van de Ven, 2012). Shear force blocks were weighed before and after cooking to determine cooking loss. The pHu was determined using 2.5 g of muscle homogenate in 10 ml of 5 mM iodoacetate/150 mM KCl (pH adjusted to 7.0), as described by Dransfield, Etherington, and Taylor (1992). Another section was taken for particle size analysis (Karumendu et al., 2009) at 5 days post mortem.

Fig. 1. Hand held Raman spectroscopy sensor head measuring a fresh intact lamb SM, with the epimysium removed.

2.4. Data analysis

Background interference was determined using a 'dark' scan, also taken at ambient temperature, which was subtracted from the spectra before they were saved as raw data. Prior to analysis non-meat spectra were identified using Principal Component Analysis (PCA). Scores were calculated with MATLAB 7.9.0 ($R^2$009b) software (The MathWorks Inc., Natick, MA, USA). Any scores outside an established threshold value were checked and removed if intensities corresponding to lamb meat were not present in the spectra. Following this, 12 spectra for each sample were averaged, the wavelength range was restricted to a range of 500–1800 cm^{-1} and then the spectra were pre-processed by dividing each by its l_2-norm (square root of sum of squared intensities). Models for predicting shear force which included Raman spectra were fitted using partial least squares (PLS) regression analysis, using the package *pls* (Martin, Hopkins, Gardner, & Thompson, 2006) under R (R Core Team, 2013). 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). Shear force predictions for each observed shear force value, for each SM sample, were then obtained, based on the selected number of LV, using the Leave-One-Out (LOO) cross validation method. An approach combining Raman spectra and the traditional predictors of shear force was also fitted using PLS regression.

Models for the prediction of shear force based on traditional predictors of shear force, sarcomere length, pHu, cooking loss and/or PSA, omitting Raman spectra, were fitted using linear regression with R computer software (R Core Team, 2013).

3. Results

In Table 1, summary results for the traditional predictors of shear force are given. This data demonstrates that shear force measurements had a large range but none were below 27 N, which indicates none were very tender (Hopkins, Hegarty, Walker, & Pethick, 2006). Comparison of PSA at 1 and 5 days post mortem demonstrates the effects of proteolysis as particle size decreased with ageing.

Prediction errors (as quantified by the root mean square error of prediction (RMSEP)) for models using different combinations of the traditional predictors sarcomere length (SL), cooking loss (CL), pHu and/or PSA, with and without Raman spectra at 1 and 5 days are presented in Table 2. None of the traditional measures were significant predictors of shear force alone or jointly ($P > 0.05$).

Based on the RMSEP criterion, the best model for predicting shear force of SM at 5 days post mortem was based on Raman spectra collected on day 1 (Table 2), using 3 latent variables. The squared correlation between the Leave-One-Out cross validated predicted and observed shear force values was $R^2_{cv} = 0.27$ (Fig. 2.). Changes to Raman spectra due to ageing are complex and are outside the scope of this paper, however the results reported here indicate that the prediction of shear force using Raman spectra taken on day 1 is better ($R^2_{cv} = 0.27$; RMSEP = 11.48) than the prediction using Raman spectra collected 5 days post mortem ($R^2_{cv} = 0.17$; RMSEP = 12.20). Overall, this is a 1.4 N reduction in RMSEP when compared to the Null prediction model

Table 1
Mean, standard deviation, and range of shear force (N), cooking loss (%), sarcomere length (μm), pHu and PSA (μm) of lamb *m. semimembranosus* (n = 80).

Trait	Ageing (days)	Mean	SD	Range (min, max)
Shear force (N)	5	51.4	13.1	29.2–78.4
Cooking loss (%)	5	19.2	3.7	0.24–28.8
Sarcomere length (μm)	1	1.70	0.11	1.46–1.99
pHu	1	5.61	0.11	5.52–6.23
PSA (μm)	1	229	46.8	159–455
PSA (μm)	5	166	40.8	95–322