Assessing the meat quality of venison short-loin from farmed red deer using visible-near infrared spectroscopy

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Abstract

Venison from red deer (*Cervus elaphus*) is marketed as a premium quality, healthy red meat. Ideally, meat-quality attributes need to be measured prior to sale. Near infrared spectroscopy (NIRS) has potential for the measurement of sensory, nutritional and technological parameters in beef, but its use on venison has not yet been considered. The aim was to evaluate NIRS for predicting Warner-Bratzler shear-force parameters, meat colour, ultimate pH, sarcomere length and water holding capacity of venison short-loins. Samples were halved, vacuum packaged, aged for either 3 or 42 d at 1±1°C and frozen, then defrosted for meat-quality assessment and acquisition of spectral data. In total, 154 samples (3 and 42 d aged) were divided into calibration (75%) and validation datasets (25%). Using partial-least squares regression, calibration equations were tested on the validation dataset. Prediction accuracies ranged from R^2 =8.3%, (SE_{pred} =2.42) for meat lightness to R^2 =66.6%, (SE_{pred} =0.10) for ultimate pH, suggesting that NIRS may be useful for predicting some venison quality parameters.

Keywords: Farmed venison; *Cervus elaphus*; Meat-quality; Near-infrared spectroscopy

Introduction

Venison produced from farmed red deer (*Cervus elaphus*) is marketed as a premium red meat, having high iron content, low fat content and superior tenderness (Drew & Seman 1987; Purchas et al. 2010). Due to the variation in venison quality, a non-destructive test to predict desirable attributes would facilitate the supply of meat that caters to market specifications.

Conventional analyses of sensory, technological and nutritional meat qualities are slow, costly and destructive, so are unsuited to routine use by processors. Near-infrared spectroscopy (NIRS) utilizes the visible (~400-700 nm) and near-infrared (~800-2500 nm) regions of the electromagnetic spectrum for determining the chemical composition of a sample. In reflectance (absorbance) mode, spectra are collected from the sample surface, minimising pre-scanning sample preparation. NIRS spectra collection is rapid (1-2 seconds), non-destructive and may allow simultaneous prediction of multiple meat-quality parameters.

NIRS has been assessed for its ability to predict meat-quality parameters and the chemical composition of beef, lamb and pork (Weeranantanaphan et al. 2011). Use of NIRS to evaluate venison meat-quality has not yet been explored. Therefore, the current experiment evaluated the ability of NIRS to predict venison meat-quality characteristics in meat aged for three and 42 days.

Materials and methods

Venison meat samples

At 24 hours post mortem, the short-loin (*M. longissimus lumborum*) was collected from the left side of 78 carcasses of farm-raised red deer. Deer were between 12 and 14 months of age and were processed under commercial conditions in two batches. One batch consisted of 18 red hinds and 19 red stags and the second consisted of 19 wapiti-red crossbred hinds and 20 wapiti-red crossbred stags. Short-loins were halved transversely, the two halves were weighed, vacuum-packaged and the anterior and posterior halves were allocated alternatively to a three-day (3 d) or a 42-day (42 d) aging period prior to freezing at –30°C for a minimum of one week before meat-quality assessment. Purge and thaw weight loss was expressed as a percentage of weight at packing.

Acquisition of NIRS spectra

After aging, freezing, and defrosting, a ~10 mm slice was recovered from the anterior section of each sample (3 and 42 d, n =154) and re-frozen for subsequent NIRS analysis. For NIRS scanning, the samples were defrosted for eight hours at ambient temperature, "butterflied" to create two slices that were 5 mm thick and expose a large surface for NIRS scanning. The samples were bloomed (exposed to air) for at least two minutes before scanning (Prieto et al. 2009). Four scans (350-1830 nm, 1 nm intervals) were collected per sample using a QualitySpec BT spectrometer (ASD, Colorado), rotating the sample 90° between scans. The median absorbance at each

wavelength was calculated from the four scans. Absorbances at the ends of the operating range with excessive noise were removed to form a working range of 560-1600 nm.

Venison meat quality reference measures

Shear-force parameters were measured on a 25 mm thick steak from each short-loin sample. After 90 minutes cooking in a 70°C water bath (Purchas & Aungsupakorn 1993), samples were stored overnight at 1±1°C. Two shears on each of five 13 x 13 mm cross-sectioned cores were measured using a Warner-Bratzler shear-force (WBSF) device (crosshead speed of 230 $mm.min^{-1}$; G-R Electric Manufacturing. Company, Manhattan, Kansas) with a square blade to give force values for initial yield, peak shear, and an index of the work done (Purchas & Aungsupakorn 1993). The average of 10 shears was calculated.

Sarcomere length was determined by laser diffraction, and pH_{ult} from 2.0-2.5 g of homogenised meat in 10 mL of distilled water (Purchas et al. 2010). For colour measurements, two measures of L* (lightness), a*(redness) and b* (yellowness) were taken using a Minolta chromameter CR-200, (8 mm measured area diameter, standard illuminant C) (Purchas et al. 2010). Expressed juice (cm^2g^{-1}) for estimating water-holding capacity was evaluated by filter-paper-press method with a 500±10 mg sample pressed on Whatman No1 filter paper for five minutes by a 10 kg weight (Purchas et al. 2010).

Statistical methods

The reference meat-quality data and the NIRS spectra from 3 d and 42 d aged samples were combined into one dataset to generate a model that was applicable across aging times. An analysis of the two aging times separately is available in Craigie (2012). Partial least-squares regression type 1 was used for calibrating and predicting meat-quality traits using median NIRS spectra (560-1600 nm). All analyses were performed using Unscrambler software (version 10.1, Camo Software AS. Oslo). Samples were sorted in ascending order separately for each parameter and every fourth sample was assigned to the prediction dataset, and the intervening three samples allocated to the calibration dataset (Williams 2001). The calibration dataset was used for model development; cross-validation was performed by applying the model back to the spectral data used in calibration to generate predicted values which were compared against the actual values. The prediction dataset is used to test the model on a new data set that was not used in model development with known reference values. Model performance was assessed by the coefficient of determination and standard error for calibration (R^2_{cal}, SE_{cal}), cross-validation (R^2_{cv}, SE_{cv}) and prediction (R^2_{pred}, SE_{pred}). To avoid over-fitting, the optimal number of latent variables used in the regression was determined when the SE_{cv} no longer decreased.

Outliers are either poor spectra or extreme reference values; both disrupt the development of prediction equations. Potential outliers for the meat-quality parameters and spectra were identified and removed as per Westerhaus et al. (2004); through poor calibration (attempt model development), cross-validation (applying the model back to the spectral data used in calibration to generate predicted values that are compared against the actual values) or prediction performance (application of the model to a new data set with known reference values). Confirmation and removal of outliers in reference measures occurred only if there was a known error with the sampling or where measurements were > 3 (standard deviations) from the parameter mean. For spectra, if the spectra of a sample was significantly different ($P < 0.01$) from the mean spectra of the sample population based on the F-test (i.e., if a sample fell outside the Hotelling T^2 ellipse ($\alpha = 0.01$) for any pair of latent variables used in the model), the sample was removed, the method is equivalent to that used by Prieto et al. (2009).

The ratio performance deviation (RPD) statistic is the standard deviation of the dataset used to test the model standard error of prediction as a proportion of the standard error of prediction (Williams 2001). The RPD statistic can be calculated for both the predicted values obtained from internal cross-validation (RPD_{cv}) and the predicted values obtained from testing the model on an independent dataset (RPD_{pred}). The RPD links performance to the variation in the dataset, the predictive performance of models within and between experiments and datasets can be directly compared, hence the RPD statistic is used as the main basis for drawing comparisons.

Results and discussion

The means, standard deviations, and ranges of each venison quality parameter were similar for the calibration and prediction datasets (Table 1).

Prediction of meat quality parameters with NIRS

Table 2 shows the results for calibration, cross-validation and prediction performance along with the number of orthogonal latent variables used in the regression equations.

Multiplicative scatter correction was the only pre-treatment required for pH_{ult}, expressed juice and cooking loss, and the number of latent variables used ranged from 2 for sarcomere length prediction to 10 for pH_{ult} (Table 2). R^2 values fluctuated between calibration, cross-validation and prediction phases. In general R^2, tended to decrease between calibration and prediction phases because models always perform better on the data used to generate them.

In the present experiment, NIRS spectra were collected on short-loin samples that had been aged and frozen prior to scanning, but in the processing plant, scanning would need to be performed on fresh meat. Freezing and thawing alters the NIRS spectra of beef (Downey & Beauchêne 1997) so it is possible that

Table 1 Spectral data for each venison sample and the corresponding meat quality measurements were separated into two datasets: calibration (75% of samples) and prediction (25% of samples), the descriptive statistics for the venison meat-quality parameters for each dataset are presented.

	Calibration				Prediction			
Meat-quality parameter	n[b]	Mean	SD[c]	Range	n[b]	Mean	SD[c]	Range
Ultimate pH (pH_{ult})	116	5.60	0.17	5.41-6.31	38	5.59	0.16	5.42-6.2
Purge (%)	116	3.92	1.61	0.00-8.85	38	3.87	1.52	0.76-7.32
Expressed juice (cm^2g^{-1})	114	29.38	3.71	21.51-39.10	37	29.33	3.45	22.48-36.46
Cooking loss (%)	116	28.09	2.03	21.76-32.19	38	28.06	1.95	22.34-31.12
Sarcomere length (µm)	116	1.57	0.10	1.23-2.00	38	1.57	0.08	1.30-1.71
Lightness (L*)	116	36.34	2.37	29.66-41.12	38	36.30	2.29	30.25-39.87
Redness (a*)	116	12.01	1.55	6.87-14.86	38	12.00	1.47	8.59-14.60
Yellowness (b*)	116	3.11	0.80	0.92-4.75	38	3.10	0.78	1.32-4.58
WBSF[a] peak shear-force (kgF)	116	6.43	2.04	3.13-13.07	38	6.35	1.90	3.31-10.61
WBSF initial yield force (IYF) (kgF)	116	5.54	1.84	2.64-11.57	38	5.48	1.74	2.77-9.63
WBSF peak shear-force – IYF (kg)	116	0.88	0.50	0.15-2.58	38	0.87	0.47	0.19-2.10
WBSF work done	116	1.99	0.56	1.00-3.48	38	1.98	0.54	1.02-3.27

[a] WBSF = Warner-Bratzler shear-force.
[b] n = number of samples.
[c] SD = standard deviation of reference meat quality measurements.

spectra from frozen then thawed venison would also differ from spectra of fresh venison.

Prediction of ultimate pH

The pH_{ult} was predicted with an R^2_{pred} =66% (RPD =1.63) and the model correctly identified the three samples in the prediction dataset with pH_{ult} values >5.80, but misclassified one sample as having a pH_{ult} >5.80. The calibration performance (Table 2) was lower than that that reported by Andrés et al. (2008) where R^2_{cal} was 97% (SE_{cv} =0.10) when NIRS assessment was done on *M. longissimus thoracis* (LT) from 30 bulls. Performance (R^2_{cal}) was similar to the R^2_{cal} values of 81% (SE_{cv} =0.18) obtained on 100 beef LT samples reported by Cozzolino & Murray (2002) and the 85% (SE_{cv} =0.20) obtained on LT from 26 Hereford steers (Rosenvold et al. 2009). Prieto et al. (2008) reported an R^2_{cal} of 41% (RPD_{cv} =1.12) for LT pH_{ult} for 53 steers.

Prediction of purge and expressed juice

To develop a model for purge, the minimum (0%) and maximum (8.86%) purge values were excluded. Two further samples were removed due to spectra that were significantly different from the mean spectra of the population (P <0.01). One sample was removed from the prediction dataset based on poor spectra (P <0.01).

Comparison of present and previous NIRS performance for predicting purge is difficult due to methodological variations. Leroy et al. (2004) analysed purge in a plastic bag on LT samples from 88 bulls and reported R^2_{cv} =51% (RPD_{cv} =1.40) for 2-day aged samples and R^2_{cv} =54% (RPD_{cv} =1.46) for 8-day aged samples. Prieto et al. (2008) reported R^2_{cal} =26% (RPD =1.04) for drip loss using the Honikel bag method on LT samples from 53 steers and R^2_{cal} =20% (RPD_{cv} =1.02) on 67 young cattle.

For expressed juice, one sample was removed from the prediction dataset due to having anomalous spectra (P <0.001). Retaining this sample reduced the R^2_{pred} to 23% (SE_{pred} =3.07). Prieto et al. (2008) reported an R^2_{cal} of 48% (RPD_{cv} =1.11) for expressed juice on LT from 53 steers and R^2_{cal} =58% (RPD_{cv} =1.30) for 67 young cattle. Ripoll et al. (2008) obtained a R^2_{pred} of 89.2 (RPD_{pred} =1.76) for expressed juice on LT from 190 bulls using calibration equations developed on 75% of the samples and applied to the remaining 25% of the samples. Rosenvold et al. (2009) reported R^2_{pred} = 67% (SE_{pred} =2.8 cm^2g^{-1}) for expressed juice on LT samples from 40 Hereford steers, spectra and expressed juice reference measures were recorded on intact meat, which is the most similar to the present NIRS protocol. Prieto et al. (2008) and Ripoll et al. (2008) collected spectra from homogenised samples.

Prediction of sarcomere length

After removal of one sample from the calibration dataset (sarcomere length was >4 SD from the mean), a prediction equation was developed that performed better in the prediction than the calibration phase (R^2_{cal} =17.4 vs. R^2_{pred} =36.7, Table 2). Removing scatter effects reduced performance (data not shown) which suggests that variation in sarcomere length may be responsible for some of the scatter effects in the absorbance NIRS spectra. The absorbance spectra differed between samples with long (>2.0 µm) and short (<1.6 µm) sarcomeres in the spectral region below about 1150 nm, shorter sarcomeres having a higher absorption than long sarcomeres in LT samples from 12 young bulls (Rødbotten et al. 2001). They also

Table 2 The calibration dataset (75% of the samples) was used to develop models to predict venison meat-quality parameters from the NIRS spectral data of each sample. Models were subsequently tested on the prediction dataset (25% of the samples) to test their performance when applied to new spectral data. The performance of NIRS calibration equations is presented both on the spectral data used to calibrate them (cross-validation) and the spectral data reserved for testing (prediction).

Meat-quality parameter		Calibration			Cross-validation			Prediction			
	LV[b]	n[c]	R^2_{cal}[d]	SE_{cal}[e]	R^2_{cv}[f]	SE_{cv}[g]	RPD_{cv}[h]	n[i]	R^2_{pred}[j]	SE_{pred}[k]	RPD_{pred}[l]
Ultimate pH (pH_{ult})[a]	10	116	78.8	0.08	68.0	0.10	1.75	38	66.4	0.10	1.63
Purge (%)	2	112	19.4	1.35	15.0	1.41	1.15	37	29.6	1.30	1.18
Expressed juice (cm^2g^{-1})[a]	7	116	35.0	2.98	13.5	3.48	1.07	36	34.5	2.67	1.23
Cooking loss (%)[a]	3	116	21.5	1.79	16.2	1.88	1.08	38	na[m]	na[m]	na[m]
Sarcomere length (µm)	2	115	17.4	0.08	11.4	0.08	1.15	38	36.7	0.06	1.28
Lightness (L*)	4	116	40.4	1.82	33.2	1.95	1.21	38	8.3	2.42	0.94
Redness (a*)	4	115	42.8	1.15	35.1	1.24	1.25	38	62.3	0.92	1.60
Yellowness (b*)	3	115	53.4	0.54	50.0	0.57	1.40	38	40.4	0.59	1.31
WBSF[n] peak shear-force (kgF)	3	114	54.5	1.31	51.6	1.37	1.49	34	27.0	1.55	1.20
WBSF[n] initial yield force (IYF) (kgF)	3	110	39.0	1.6	35.0	1.42	1.29	37	54.4	1.16	1.50
WBSF[n] peak shear-force – IYF (kg)	3	116	45.4	0.37	40.6	0.39	1.29	38	50.8	0.32	1.47
WBSF[n] work done	4	112	48.7	0.39	44.4	0.48	1.15	38	50.0	0.40	1.35

[a] Indicates meat-quality parameters where multiplicative scatter correction (MSC) has been applied to the spectra prior to analysis.
[b] LV = Number of orthogonal latent variables used in the regression.
[c] n = Number of samples included in calibration and cross-validation phases.
[d] R^2_{cal} = Coefficient of determination obtained in the calibration phase.
[e] SE_{cal} = Standard error of calibration.
[f] R^2_{cv} = Coefficient of determination obtained in the cross-validation phase.
[g] SE_{cv} = Standard error of cross-validation.
[h] RPD_{cv} = Ratio of performance deviation is the standard deviation of the Y variable in the calibration dataset (after removal of outliers) divided by the SE_{cv}.
[i] n = Number of samples used for the prediction phase.
[j] R^2_{pred} = Coefficient of determination obtained in the prediction phase.
[k] SE_{pred} = Standard error of prediction.
[l] RPD_{pred} = Ratio of performance deviation (standard deviation of the prediction dataset divided by the SE_{pred}.
[m] na = Not available, the prediction phase failed for cooking loss so no results were obtained.
[n] WBSF = Warner-Bratzler shear-force

reported a significant correlation between WBSF peak shear-force and sarcomere length ($r = –0.67$, $P < 0.001$) and postulated that different absorption patterns (associated with shortened sarcomeres) may underpin WBSF peak shear-force prediction but, they did not predict sarcomere length directly. In the current analysis, the correlation between WBSF peak shear-force and sarcomere length for 3 d and 42 d aged samples was –0.35, ($P = 0.002$) and –0.48 ($P < 0.001$) respectively.

There are few reports where sarcomere length has been predicted by NIRS. Andrés et al. (2008) reported an R^2_{cal} of 16% (SE_{cal} =0.08 µm) and a R^2_{cv} of 2% (RPD_{cv} = 0.84) for LT sarcomere length on a sample of 30 young bulls, while Shackelford et al. (2012) reported that the mean sarcomere length was significantly shorter in LT classified "not tender" compared to LT classified "tender", although they did not predict or classify samples based on the sarcomere length. Shackelford et al. (2012) postulated that the biochemical basis for classifying LT into tenderness classes was sarcomere length and post-mortem proteolysis as indicators of the extent of desmin degradation.

Prediction of venison colour

No spectral pre-treatments were necessary for prediction of colour traits. This is consistent with Prieto et al. (2009), where scatter effects were informative for the prediction of colour in beef LT. Good model performance during calibration and cross validation (RPD_{cv} =1.21) for venison lightness prediction did not translate into good prediction performance (RPD_{pred} =0.94). In contrast, NIRS was better at predicting venison redness (RPD_{pred} =1.60) and yellowness (RPD_{pred} =1.31) parameters and performance was broadly similar to that observed in other experiments. Andrés et al. (2008) reported an R^2_{cv} =75% (RPD_{cv} =2.07) for L*, R^2_{cv} =29% (RPD_{cv} =0.90) for redness and R^2_{cv} =46% (RPD_{cv} =1.37) for

yellowness for 30 bull LT samples after 60 minutes bloom time. Prieto et al. (2009) obtained R^2_{cv} =83% (RPD_{cv} =2.47) for lightness, R^2_{cv} =76% (RPD_{cv} =2.02) for redness and R^2_{cv} =69% (RPD_{cv} =2.48) for yellowness for beef LT samples after 45 minutes bloom time.

Prediction of venison shear-force parameters

When developing a prediction for shear-force parameters two samples were removed, one due to anomalous spectra (P <0.005) and the maximum (13.07 kgF) value. The strong calibration and cross-validation performance for WBSF peak shear-force did not translate into strong prediction performance. This suggests the model is not particularly robust, and that cross-validation is not always indicative of future prediction performance. In contrast, the prediction for initial yield force was much stronger than for cross-validation (Table 2). Six outliers from the calibration dataset and two samples from the prediction dataset were removed during prediction model development and testing for WBSF initial yield force, retaining outliers reduced the R^2_{cv} to 25% (SE_{cv} =1.60) and R^2_{pred} to 26.0% (SE_{pred} =1.52). In the present dataset, WBSF peak shear-force and initial yield force were correlated (r = 0.97, P <0.001) which is consistent with the correlation between the two parameters reported by Peachey et al. (2002) for beef (r =0.98, P <0.001). It is not clear why the prediction performance was stronger for WBSF initial yield force than for peak shear-force, but the model for initial yield force may be more robust. The RPD_{pred} is greater than the RPD_{cv} for WBSF initial yield force and for work done, which indicates that prediction performance was stronger in the prediction phase than the cross-validation phase for both parameters (Table 2).

Four outliers were removed from the calibration phase for WBSF work done throughout the shear. Work done was highly correlated (r =0.95, P <0.001) to WBSF peak shear-force, the samples that have a high WBSF peak shear-force also have a high work done index value, in this instance, the model for predicting work done from the spectral data was more robust than that for WBSF peak shear-force.

Conclusion

NIRS prediction equations were developed to predict technological meat-quality parameters of venison short-loin across two aging times. Although there were limitations in the experimental design, the results demonstrate that NIRS can predict venison meat-quality parameters with varying levels of accuracy. Ultimate pH, redness and some shear-force traits were well predicted whilst cooking loss, purge and lightness were not well predicted. NIRS may be a useful tool for venison processors with meat-quality specifications to meet, and in deer breeding programmes to aid selection for desirable traits. NIRS scanning would need to be undertaken at boning, and the feasibility of this needs further investigation. With further development, NIRS could be a useful tool for the online assessment of venison meat-quality. To address this, future work to calibrate NIRS for venison meat-quality assessment should be undertaken under commercial conditions, on a number of different muscles and include a validation step on independent data.

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