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Near-infrared reflectance analysis of intramuscular fat in beef

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ABSTRACT

Near-infrared (1100-2500 nm) reflectance spectra were measured on 128 beef ribeye samples using a scanning monochromometer and calibrated against standard laboratory analysis to develop a rapid method for measuring intramuscular fat of beef.

A pilot run was conducted on 38 frozen ribeye samples, ranging in fat content from 94 to 325g/kg dry weight, to evaluate sample preparation methods (frozen slices, thawed slices, coarse mince). After scanning of subsamples, fat content of the sample was then measured using Soxhlet extraction. Standard error of calibration (SEC) was 9g/kg dry weight for spectra collected from minced samples compared to 25g/kg for frozen slices and 15g/kg for thawed slices.

A second set of 90 frozen ribeye samples (fat content range 56-305 g/kg dry weight) was used to test and improve this calibration, using 3 subsamples of minced beef per sample. The ability of the initial calibration equation to predict fat content of these samples was poor. A more robust calibration was then attempted by pooling the data from the two sets. The SEC of this extended set was 16 g/kg dry weight (10% of the sample set mean) and the multiple R^2 of calibration was 0.92. The standard error of prediction for new samples was estimated from internal cross-validation runs as 19 g/kg. Prediction of fat content of an independent validation set of 24 samples had a standard error of 20 g/kg dry weight. Analysis of the calibration results suggested that a major source of error in the calibration was heterogeneity between subsamples, with the standard deviation of prediction of different subsamples from the same ribeye being larger than the standard error of cross validation.

These results indicate that Near-Infrared reflectance spectroscopy may provide a rapid objective method for estimating intramuscular fat of beef.

Keywords: Intramuscular fat; beef; near-infrared reflectance.

INTRODUCTION

Rapid, objective measurement of fat content of meat is necessary for the reproducible assessment of meat quality. Standard analytical methods for measuring fat content involve a solvent extraction step and are usually too time-consuming and costly to be used for routine analysis of large numbers of samples. Near-infrared reflectance (NIR) spectroscopy has been widely used for the rapid analysis of forage quality components, moisture and protein in cereals, and moisture, protein and oil in oilseeds (Osborne and Fearn, 1986). The use of NIR to measure fat content in homogenized beef samples has shown some promise (Kruggel *et al.*, 1981; Lanza, 1983; O'Keefe, 1987). Provided that NIR estimation has sufficient accuracy, this technique would be valuable for both quality grading programmes within meat processing plants and regulatory monitoring of products offered to consumers. The present study was undertaken to evaluate the use of the NIR to estimate fat content in beef using small samples and minimal sample preparation.

MATERIALS AND METHODS

Frozen ribeye muscle (*longissimus dorsi*) samples from the 12th rib of 38 Angus steers (mean carcass weight 368 kg, range 322-424 kg) from a grain finishing trial were used in a pilot run to evaluate the effect of three sample preparation methods on NIR estimation of intramuscular fat. Samples had been stored frozen for 33 months prior to analysis. Ten slices, each approximately 3 mm thick, were cut from each frozen

ribeye sample, using a commercial bacon slicer. A disc of approximately 50 mm diameter was cut from the centre of each slice. Four discs per sample were scanned immediately, while still frozen. These discs were then allowed to thaw and scanned again. The 10 discs per sample were bulked, minced in a kitchen grinder (4 mm opening), mixed, and divided into three subsamples for a final series of scans. After scanning, the subsamples of minced beef were bulked and refrozen pending analysis of chemical fat by Soxhlet extraction using a Tecator Soxtec analyzer.

A second set of 90 frozen ribeye samples was used to test and improve the calibration for minced beef samples. These samples were obtained from the 12th rib of carcasses of Angus steers from a vitamin A supplementation trial (mean carcass weight 322 kg, range 250-393 kg) and had been stored frozen for 6 months. After thawing, a cube of approximately 75 g was cut from the centre of each ribeye, minced using the same grinder as in the first trial and three subsamples of the mince scanned. The subsamples were bulked and refrozen pending chemical fat analysis, as in the first run.

Prediction errors for new samples were estimated by applying the calibration equations to scans collected from a third set of 24 ribeye samples. These samples were collected over a period of three weeks from cattle supplied by farmers to a commercial meat processor. Carcasses ranged in weight from 289 to 440kg. These samples were stored frozen for 4 to 28 days prior to being minced, scanned (3 replicates/sample) and submitted for chemical fat analysis.

A Pacific Scientific (Perstop Analytical, Silver Spring MD, USA) Model 6250 NIR scanning monochromometer

was used to collect NIR spectra of the beef samples. Standard 'powder' sampling cups, with an effective surface area of 3.5 cm², were used. The wavelength range was 1100 to 2500 nm with a bandpass of 10 nm and an increment of 2 nm between data points. Reflectance (R) data was stored as log(1/R).

Calibration equations were developed using ISI (InfraSoft International, University Park, PA, USA) software. Prior to regressing on chemical fat data, spectral data from replicate scans (4 for frozen and thawed slices, 3 for mince) were averaged. First and second derivatives of log(1/R) were calculated from finite differences. A modified partial least squares regression method with internal cross-validation (Shenk and Westerhaus, 1991b) was used for calibration development. Calibration statistics calculated include the standard error of prediction of the calibration samples (SEC), the proportion of variance explained by the calibration (R²), the standard error of prediction of the cross-validation samples (SECV), and the proportion of variance explained by cross-validation predictions (R²CV). The calculation and interpretation of these statistic are given in Shenk and Westerhaus (1991a).

RESULTS

Chemical fat of the 38 samples used in the initial trial averaged 60 g/kg fresh weight, with a range of 27 to 116 g/kg. On a dry weight basis, the samples averaged 183 g/kg dry weight, ranging from 94 to 325 g/kg.

Evaluation of different first and second derivative transformations of the log(1/R) data gave no clear advantage to any particular data transformation. However, there were consistent differences between sample preparation methods, with the calibrations developed for the minced samples having lower standard errors of calibrations and cross validation. The statistics for calibrations developed from a second derivative transformation, using a moving average interval of 20 nm for smoothing and a gap of 20 nm for calculating second order differences, are presented in Table 1. Similar results were obtained for other transformations of the reflectance data. Based on these results, minced samples were used for further calibration development.

Chemical fat of the second set of 90 samples averaged 45 g/kg fresh weight, with a range of 14 to 90 g/kg. On a dry

TABLE 1. Calibration statistics for NIR estimation of moisture and chemical fat of beef ribeye samples using different sample preparation procedures and a second derivative treatment of spectral data. N=38.

| Variable | Sample preparation | SEC ^a | R ² | SECV | R ² CV |
|---|--------------------|------------------|----------------|------|-------------------|
| Moisture (g/kg FW) | Frozen slices | 7.6 | 0.84 | 10.9 | 0.68 |
| | Thawed slices | 6.4 | 0.89 | 7.8 | 0.83 |
| | Minced | 3.7 | 0.96 | 6.3 | 0.89 |
| Fat Content on fresh weight basis (g/kg FW) | Frozen slices | 9.8 | 0.77 | 13.1 | 0.59 |
| | Thawed Slices | 5.6 | 0.92 | 10.8 | 0.72 |
| | Minced | 3.4 | 0.97 | 6.0 | 0.91 |
| Fat content on dry weight basis (g/kg DW) | Frozen slices | 25.4 | 0.77 | 35.4 | 0.56 |
| | Thawed slices | 14.6 | 0.92 | 29.1 | 0.71 |
| | Minced | 8.5 | 0.97 | 15.6 | 0.92 |

^a Calibration statistics defined in text.

weight basis, the mean was 163 g/kg dry weight, ranging from 56 to 288 g/kg.

The ability of the calibrations developed in the pilot run to predict fat content of these 90 samples was very poor. Using a calibration based on second derivative transformations (Table 1), the correlation between laboratory measurements of fat content (g/kg fresh weight) and NIR predictions was moderate (r=0.82). However there was a very serious bias, with over half of the NIR predictions being negative (mean NIR prediction = -0.09 g/kg fresh weight).

The data from the two sets of minced meat samples were pooled to provide a more broadly based calibration set. Calibration statistics, using a second derivative transformation with a 20 nm smooth and a 20 nm gap, are given in Table 2. The standard error of calibration of fat content for each of the three variables was about 10% of the pooled sample set mean.

TABLE 2. Calibration statistics for NIR estimation of moisture and chemical fat of minced beef ribeye samples, using a second derivative transformation of spectral data from both runs. N=128.

| Variable | Mean | SEC ^a | R ² | SECV | R ² CV |
|--|-------|------------------|----------------|------|-------------------|
| Moisture (g/kg FW) | 712.5 | 7.3 | 0.93 | 7.4 | 0.93 |
| Fat content, fresh weight basis (g/kg FW) | 49.6 | 5.0 | 0.93 | 6.7 | 0.88 |
| Fat content, dry weight basis (g/kg DW) | 169.5 | 15.5 | 0.92 | 19.1 | 0.88 |

^a Calibration statistics defined in text.

The calibration equations developed using the average scans over three replicates of each sample were applied to the individual scans of the subsamples to evaluate variability between subsamples. The standard deviation between subsamples for predictions of fat averaged 8.5 g/kg on a fresh weight basis and 26.8 g/kg on a dry weight basis.

Chemical fat of the 24 validation samples not included in the calibration set ranged from 79 to 427 g/kg dry weight. The standard error of NIR prediction of chemical fat of these independent samples was 20.4 g/kg on a dry weight basis (Table 3). This estimate is consistent with prior estimates of prediction error derived from cross-validation runs during calibration development. However, the agreement between laboratory and NIR estimates of moisture and fat content on fresh weight basis was much poorer than expected.

Examination of the relationship between laboratory estimates of chemical and NIR predictions for the calibration and validation samples (Figure 1) show a relatively even spread of errors of prediction over the range of the data.

DISCUSSION

The relatively poor performance of the calibration developed for the frozen or thawed slices compared to the minced samples suggest that heterogeneity of fat distribution in intact samples is major source of sampling error. Any use of NIR to estimate fat content from scans of intact surfaces

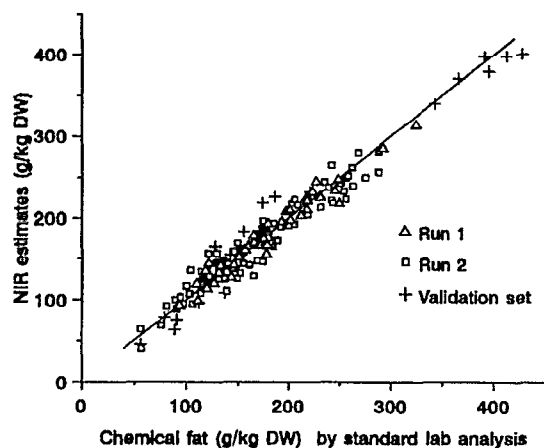
TABLE 3. NIR prediction of moisture and fat of 24 validation samples not included in calibration development.

| Variable | Means | | | R ² (V) ^b |
|---|-------|-----|---------------------|---------------------------------|
| | Lab | NIR | SER(V) ² | |
| Moisture (g/kg FW) | 702 | 710 | 49.7 | 0.34 |
| Fat content, fresh weight basis (g/kg FW) | 66 | 61 | 13.0 | 0.95 |
| Fat content, dry weight basis (g/kg DW) | 207 | 209 | 20.4 | 0.97 |

^a Standard deviation of differences between laboratory and NIR estimates of validation samples.

^b Squared correlation between laboratory and NIR estimates of validation samples.

FIGURE 1. Relationship between NIR predictions and standard laboratory analysis of fat for calibration and validation samples.



will require the scanning of larger surface area than used in this study. This may be achieved by either increasing the number of replicate scans, or using a different method of sample presentation, either a larger sample cell or a remote reflectance module that could be located at several positions on the intact sample. Both of these methods of sample presentation are available with more recent NIR scanners.

Mincing of the beef samples appears to have reduced sampling error somewhat. However, the very large deviations between predictions made from subsamples of the same ribeye sample suggest that sampling error is still a major problem with this procedure. Using meat samples that had been emulsified to a smooth paste, Lanza (1983) developed calibration equations with standard errors of prediction for fat of 2.7 g/kg fresh weight (cf. SECV of 6.7g/kg in the present study). Although this level of accuracy may be achieved by increased replication of scans on coarsely minced sample, it may be more efficient to spend the time required for scanning (packing and scanning the small sample cells used in this study takes about 2 minutes per scan) on more thorough sample homogenization.

The inaccuracy of the calibration developed in the first run for predicting the fat content of the 90 samples used in the second run suggest that the two sample sets represented very different populations. The most obvious difference is in sample age: the first set of samples having been frozen for 33 months prior to analysis, the second having been stored for 6 months. Changes in chemical constituents - e.g. oxidation of fatty acids into shorter chain compounds - could have resulted in the first calibration giving disproportionate weight to wavelengths representing fat breakdown products. Further work is needed to define critical factors in storage and handling of meat samples prior to analysis by NIR.

The accuracy of the final calibration equations developed in this preliminary investigation in predicting fat content (on a dry weight basis) of 24 independent samples is encouraging. Particularly encouraging is the observation that the calibration was able to provide good estimates of the fat content of six validation samples which exceeded the range of the calibration set (Figure 1). However, the poor agreement of laboratory estimates with NIR estimates of moisture and fat content (on a fresh weight basis) in this validation set (Table 3) indicates that further testing is needed to develop calibrations with acceptable accuracy over the range of carcase variation that is likely in commercial beef grading environments.

ACKNOWLEDGEMENTS

We gratefully acknowledge Richmond Ltd, Hastings for supplying the ribeye samples used in this project; Thai Phung, AgResearch Grasslands, Palmerston North, for assistance in developing the sample preparation and scanning protocol; and Peter Isherwood, Veterinary and Animal Sciences Group, Lincoln University for performing the laboratory analyses of fat and moisture. This project was funded by FRST.

REFERENCES

- Kruggel, W.G.; Field, R.A.; Riley, M.L.; Radloff, H.D.; Horton, K.M. 1981. Near-Infrared reflectance determination of fat, protein, and moisture in fresh meat. *J. Assoc. Off. Anal. Chem.* 64: 692-696.
- Lanza, E. 1983. Determination of moisture fat and calories in raw pork and beef by near infrared spectroscopy. *Journal of Food Science* 48: 471-474.
- O'Keefe, M. 1987. Proximate analysis of beef samples by near-infrared reflectance spectroscopy. Proceedings of the 33rd International Congress on Meat Science and Technology, Vol 2. Helsinki, 2-7 August 1987. pp 368-369.
- Osborne, B.G.; Fearn, T. 1986. Near Infrared Spectroscopy in Food Analysis. Longman, Essex, UK.
- Shenk, J.S.; Westerhaus, M.O. 1991a. Population definition, sample selection and calibration procedures for near infrared reflectance spectroscopy. *Crop Science* 31: 469-474.
- Shenk, J.S.; Westerhaus, M.O. 1991b. Population structuring of near infrared spectra and modified partial least squares regression. *Crop Science* 31: 1548-1555.