Quantitation of Lamb Content in Mixtures with Raw Minced Beef Using Visible, Near and Mid-Infrared Spectroscopy

J. McElhinney, G. Downey and C. O'Donnell

ABSTRACT

Samples of beef (32), lamb (33) and 5%, 10% and 20% (w/w) lamb-in-beef mixtures (33 each) were minced and reflectance scanned in the visible, near and mid-infrared spectral regions. Partial least squares (PLS) regression models were developed to predict percentage lamb content using each spectral region alone and combinations of all three. The most accurate models combined mid-IR (800-2000 cm $^{-1}$) and near IR (1100-2498 nm) spectral data following 2nd derivatization; standard errors of prediction of 0.91% (0–20% range in lamb content) and 4.1% (0–100% range in lamb) were obtained. This technique may be useful for screening such meat mixtures.

Key Words: meat, lamb, beef, spectroscopy, chemometrics

INTRODUCTION

MEAT AUTHENTICITY IS OF CONSIDERABLE CONCERN TO CONSUMers and retailers (Hargin, 1996; Lumley, 1996). Partial substitution of a high value meat with one of lower value or quality is adulteration and may pose religious or potential health problems. While speciation is apparent when meat is examined in large pieces, after mincing it becomes difficult to establish species without sophisticated analytical procedures. Techniques for this purpose have included capillary gas chromatography of lipid fractions (Saeed et al., 1986), electrophoretic separation of proteins (Barai et al., 1992; McCormick et al., 1992; Skrökki and Horni, 1994) , immunological procedures (Pickering et al., 1995) and DNA-based methods (Ebbehoj and Thomsen, 1991a, b). None of these approaches is rapid and they all require sophisticated laboratory procedures.

Visible and infrared spectroscopic procedures are rapid and nondestructive. Near-infrared (NIR) spectroscopy has found considerable application in the food and agricultural industries (Williams and Norris, 1987; Downey, 1996) and the utility of mid-infrared (MIR) spectroscopy in these sectors has also been reported (Wilson, 1990; Lai et al., 1994; Kemsley et al., 1996). In studies of meat authenticity, work on discriminating between fresh and previously-frozen beef using NIR (Downey and Beauchêne, 1997a,b; Thyholt and Isaksson, 1997) has been described. A feasibility study on the application of MIR to determine the freshness and speciation in pork, chicken and turkey meats has been published (Al-Jowder et al., 1997). A preliminary study into the discrimination of raw chicken, pork and turkey meats using visible, near and mid-infrared spectroscopic techniques has also been reported (Rannou and Downey, 1997).

In addition to confirming the identity of mixed cuts of ground meat, a need exists for the quantification of meats in blended materials. Both near and mid-IR spectral regions have been applied separately to this type of study. NIR has been used in the case of coffee varietal mixtures (Downey and Spengler, 1996) and mid-IR for the quantification of chicken/turkey blends (Al-Jowder et al., 1997). An investigation has been reported on combined spectral regions for determining qualitative (Downey et al., 1997) and quantitative (Reeves, 1996) authenticity. The data in each spectral region are related but not identical and their combination may provide a synergistic advantage to spectroscopic analysis. Our objective was, therefore, to investigate such an approach based on mixtures of lamb and beef.

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MATERIALS & METHODS

Meat samples

Beef (n = 32) and lamb (n = 33) samples were purchased from local retail outlets over a period of 10 weeks in 1997. For beef samples, slices of round steak (*m. semimembranosus* ~150g) were used. Loin chops (*m. longissimus dorsi*) were used for the lamb samples. Excess surface moisture was removed by blotting with paper towels. Skin, bone, fatty tissue, connective tissue and visible bloody tissue were removed from each sample to ensure the highest possible quantity of lean meat. Samples were cut into small cubes (~1 cm^3) to facilitate mincing. Single species samples were minced using a model R301 ultra (Robot Coupe SA, Vincennes, France) for 15s each. The mincing bowl was carefully wiped with a tissue paper between samples of the same species while between different species or admixtures it was washed with warm water and a household detergent. In addition to the 100% beef and 100% lamb samples, a series of mixed species samples was prepared. Mixtures (33 samples of each) of 5% lamb:95% beef, 10% lamb:90% beef and 20% lamb:80% beef (w/w) were produced by weighing samples of cubed beef and lamb, placing both in the blender and mincing for 20s to ensure a homogeneous mixture. Once minced, samples were placed in covered plastic containers and stored at 4°C.

Temperature was not controlled during mincing but the bowl was allowed to cool between samples and especially after washing. Spectra were collected on the day of comminution; ground meats were stored at 4°C throughout this period and removed only for spectral collection.

Spectroscopic measurements

Combined visible and near infrared spectra were collected in reflectance mode using a model 6500 instrument (NIRSystems Inc., Silver Spring, MD) over the wavelength range 400–2500 nm at 2 nm intervals. Spectrophotometer control and spectral file management were performed using NIRS3 software (version 3.10; ISI International, Port Matilda, PA). Minced meat samples were placed in polyethylene bags; filled sample bags were set into a high fat/moisture cell (~10 cm long) which was mounted in a sample transport module. During the scanning operation, this cell was moved at constant velocity past the outlet slit of the spectrophotometer; 25 separate scans were collected during this movement and averaged after which the mean spectrum of a reference ceramic tile (16 scans) was recorded and subtracted from the mean sample spectrum.

Mid-infrared spectra were recorded on an Infinity Series Fourier-transform spectrophotometer (ATI Mattson, WI) using an attenuated total reflectance (ATR) sample accessory. Instrument control and file manipulation were by WinFirst software. Minced meat samples were carefully layered onto the ATR crystal so as to ensure even and intimate contact. Spectra were recorded over the range 640–4000 cm^{-1} (15,625–2500 nm) with a resolution of 8cm^{-1}; 64 interferograms were co-added before Fourier transformation. Only data from the "fingerprint" region (800–2000 cm^{-1}; 12,500–5000 nm) were used; this restriction accelerated data processing and used spectral regions in which the most applicably distinguishing molecular absorptions occurred.

Multivariate data compression and derivatization

NIR binary spectra were imported into The Unscrambler software (vs. 6.1;CAMO A/S, Trondheim, Norway) via a supplied conversion program; mid-IR spectra were exported from WinFirst as JCAMP.DX files (Rutledge and McIntyre, 1992) and ported directly into Unscrambler using a supplied macro routine. Quantitation was attempted by partial least squares regression (PLSR) using raw spectral data and data pre-treated by the Norris derivation and both 1st and 2nd derivative treatments. Partial least square regression is used to reduce multicollinearity and dataset size encountered in datasets such as NIR spectra. Spectral matrices are decomposed into a reduced number of orthogonal (i.e. uncorrelated) components or loadings; these loadings describe the main variation in both spectral and associated compositional data matrices (Martens and Naes, 1989; Naes and Isaksson, 1992). Thus, while in the case of raw NIR spectra, each sample is represented by a value at each of 1050 wavelengths (variables), in the PLS-treated data, spectra are condensed to be represented by single values on around 20 or fewer newly created axes. These values may then be input into a regression procedure with considerably less likelihood of overfitting (less chance of modeling spurious variations or noise in the dataset). Prior to statistical analysis of merged spectral data sets, variables were normalized to unit variance by dividing each absorbance value by the standard deviation of absorbance at the appropriate wavelength. A potential danger with PLS regression is that of over-fitting the model. This would have the effect of producing an optimistic model on the set of data used for calibration but the model would not perform well on other datasets of similar material. With limited sample numbers, the technique of full cross-validation was utilized to minimize the likelihood of overfitting. Thus, a single sample was removed from the sample set and a calibration developed on the remainder; this calibration was then evaluated on the removed sample and a prediction error calculated. The deleted sample was then reintroduced, a second removed and the calibration and prediction process repeated. This process was continued until every sample had been used for both calibration development and evaluation.

The individual prediction errors were then combined into a standard error of cross-validation while the correlations between actual and predicted lamb content were estimated from the explained variance divided by the total variance for each model. In derivatization, the actual absorbance value at a given wavelength was replaced by a value which represented the rate of change of absorbance around that wavelength (derivatization). This technique resolves overlapping peaks and removes linear baseline effects (Hruschka, 1987). Standard deviations and standard errors of prediction were then determined on the measured/predicted spectra.

RESULTS & DISCUSSION

NIR (FIG. 1) AND MID-IR (FIG. 2) SPECTRA FOR THE COLLECTION of meat samples (beef, lamb and lamb-in-beef mixtures) were compared. In the visible-NIR spectra, some apparent differentiation into two bands occurred at wavelengths >1300 nm. The lower band comprised mainly lamb and lamb-in-beef samples, but a few beef samples were also present. Thus, this apparent differentiation may be a matter of physical sample effects (particle size distribution, sample packing) rather than species chemical differences. In the mid-IR spectra, little if any differentiation was visually detectable. Such spectral similarities are common for NIR spectra. Complex multivariate mathematical techniques are required to emphasise any differences which may exist. However, considerable caution must be exercised in the development and evaluation of such predictive models. As explained, cross-validation was implemented and applied to every sample in the calibration and evaluation process to minimize any over-fitting of data.

We made implicit assumptions that (1) differences existed in the chemical composition of beef and lamb and (2) that such differences could be detected by spectroscopic techniques. It is generally accepted that lean meat compositions of beef and lamb are relatively constant, with the major source of variation relating to lipid content (Varnam and Sutherland, 1995). Published analytical data for the two muscle groups of animals produced in Ireland were not available. However, general data for UK animals revealed that lean mutton and beef usually contain between 5 and 10% fat and that removing all visible fat can reduce these values to as low as 2% for beef and 4% for mutton (Varnam and Sutherland, 1995). Of perhaps greater importance is the fact that the composition of fat in the two species is quite different, especially with regard to C14:0, C16:0, C18:0 and C18:1 lipids. Myo-

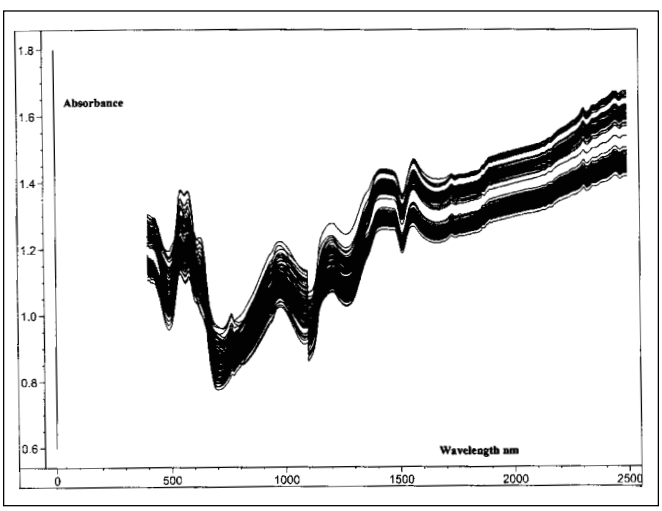

Fig. 1—Visible-NIR spectra of entire meat sample collection.

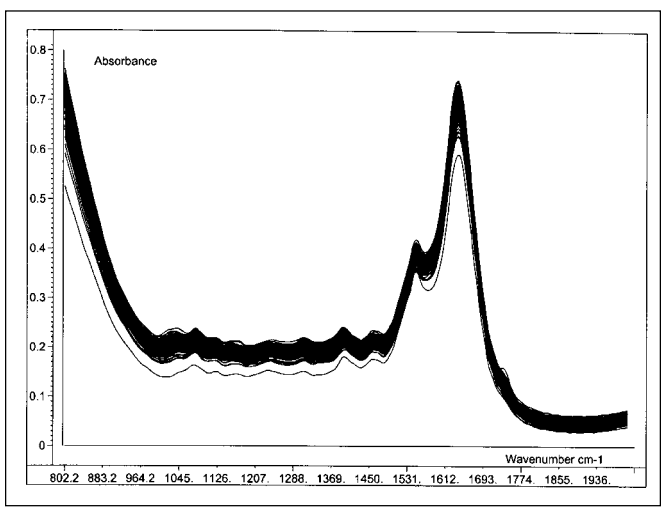

Fig. 2—Mid-infrared (fingerprint region) attenuated total reflectance spectra of entire meat sample collection.

globin is the basic pigment in fresh meat and is found in three forms i.e. reduced myoglobin, oxymyoglobin and metmyoglobin. Meat myoglobin content varies with species and also with age, sex and physical activity (Hedrick *et al.*, 1994; Lawrie, 1998). These chromophores absorb in the visible wavelength and their presence was obvious in the visible-NIR spectra (Fig.1). Protein, fat and water are detected by near infrared spectroscopy at several wavelengths, principally: water (964, 1440 and 1960 nm), protein (908, 1018, 1510, 1980, 2050 and 2180 nm) and fat (928, 1037, 2310 nm) (Osborne and Fearn, 1986). In the mid-infrared, absorbing frequencies for these components have been less-well described although bands at 1650cm^{-1} (water), 1740 cm^{-1} (fat) and protein (1650 and 1550 cm^{-1}) have been reported (Al-Jowder et al., 1997).

Near infrared spectral data

Predictive models for the estimation of lamb content in this sample set were developed. The range of composition in the mixed samples extended only up to 20% and the 100% lamb samples could unduly influence the development of models through leverage effects. Thus, experiments were performed using (a) the entire sample set and (b) the 100% beef and lamb-in-beef mixtures only. Additionally, three wavelength ranges were separately investigated, i.e., 400–2498 nm, 400–750 nm and 1100–2498 nm (Table 1).

Considering the complete sample collection, i.e., samples covering the entire compositional range, the optimum model developed from raw spectral data in the 400–2498 nm range produced a standard error of cross-validation of 10.6 % ($^{w}/_{w}$) lamb using 10 PLS loadings. The effect of each derivative mathematical pre-treatment was to reduce the number of loadings required to produce the optimum model without any major reduction in prediction error. Such a reduction may also increase the robustness of the models. When spectral data covering the visible range only (400–750 nm) were similarly treated, prediction errors in the range 11.0 to 11.6% resulted. The optimum number of loadings utilized was slightly lower than with the complete 400–2498 nm wavelength range. The lowest prediction accuracies were achieved using the wavelength range 1100–2498 nm; these ranged from 12.4% to a minimum of 6.7% after 2nd derivative pre-treatment. The number of loadings required for these models did not exhibit the same decrease as the complete range but the numbers were not excessive. Considering the rate of reduction in residual variance achieved by modelling 2nd derivative data in the range 1100–2498 nm (Fig. 3), use of a specific lower number of loadings would probably not be justified. Results for models developed using a restricted compositional range by exclusion of the 100% lamb samples were also compared (Table 1). In most cases, a larger number of PLS loadings was used by the optimum models than when the unrestricted range was considered. Additionally, correlation coefficients were slightly lower for models generated using the restricted compositional range as we expected. However, as before, the best predictive model was obtained using the 1100–2498 nm wavelength range and a 2nd derivative pre-treatment (Fig. 4). The accuracy of lamb prediction achieved (SEP=1.08%) strongly suggests that this model should at least be useful as a screening method for mixture analysis.

Table 1—Prediction of lamb content using some near infrared wavelength ranges, data pre-treatments and composition ranges[a]

	Complete sample collection			Collection without 100% lamb samples		
	n	*SEP*	*R*	*n*	*SEP*	*R*
			400-2498 nm wavelength range			
Raw data	10	10.6	0.96	20	3.0	0.92
Norris derivative	6	10.3	0.96	20	1.7	0.97
1st derivative	6	9.4	0.97	17	1.4	0.98
2nd derivative	5	9.6	0.97	14	1.8	0.97
			400-750 nm wavelength range			
Raw data	9	11	0.96	17	2.9	0.92
Norris derivative	7	11	0.96	14	3.6	0.88
1st derivative	5	11.4	0.95	18	2.4	0.94
2nd derivative	3	11.6	0.95	13	3.4	0.89
			1100-2498 nm wavelength range			
Raw data	12	12.4	0.94	20	2.8	0.93
Norris derivative	8	11	0.96	17	1.5	0.98
1st derivative	9	8.1	0.98	14	1.1	0.99
2nd derivative	10	6.7	0.98	10	1.1	0.99

[a]*n*=number of PLS loadings; *SEP*=standard error of prediction using cross-validation; *R*=correlation coefficient.

Mid infrared spectral data

The performance of models developed using mid-infrared spectra was compared (Table 2). Norris derivatization was not applied since it requires an integer value for the between-data point gap and this did not apply for mid-IR data. Considering samples covering the entire compositional range, raw mid-IR data produced a model with about the same predictive accuracy as the raw NIR and/or visible wavelength ranges. This was achieved through use of a greater number of PLS loadings (16) than the 10, 9 or 12 for 400–2498 nm, 400–750 nm and 1100–2498 nm ranges, respectively. When mid-IR spectra were derivatized, little or no change in predictive accuracy was noted. However, the number of terms required in optimal models was reduced to 10 (1st derivative) and 11 (2nd derivative), respectively. This was in marked contrast to the visible and NIR wavelengths where the numbers of loadings and/or predictive accuracy achieved subsequent to derivatization were decreased.Restricting the compositional range of the meat samples to 0 to 20%($^{w}/_{w}$) lamb again produced predictive models with high correlation coefficients. They also required higher

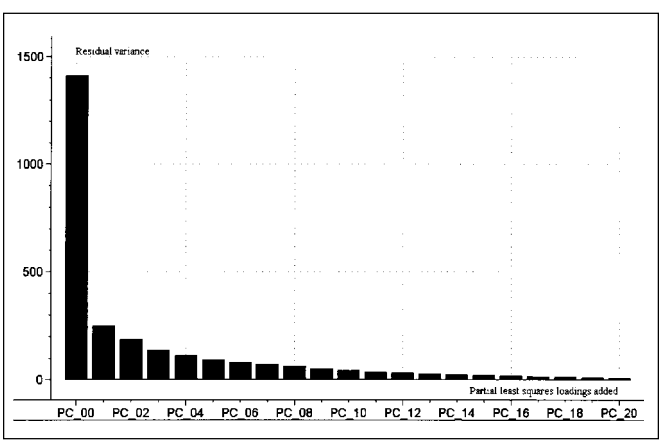

Fig. 3—Residual variance for NIR partial least squares regression model (1100-2498 nm; 0-100% lamb; 2nd derivative data pre-treatment).

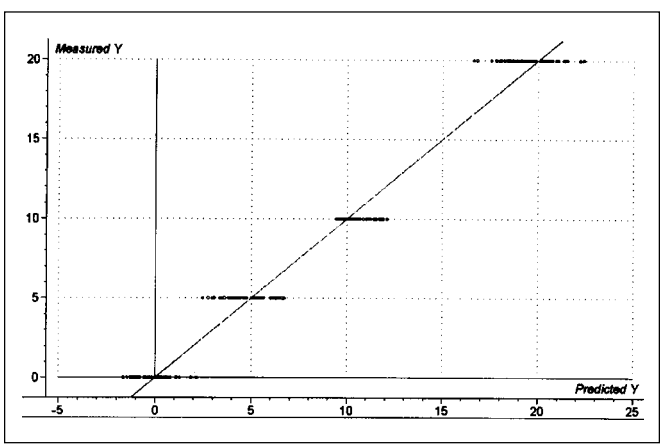

Fig. 4—Prediction of %lamb in beef and beef+lamb mixtures (1100-2498 nm; 0-20% lamb; 2nd derivative data pre-treatment).

Table 2—Prediction of lamb content using mid-IR fingerprint range spectral data as related to data pre-treatments and composition ranges[a]

	Complete sample collection			Collection without 100% lamb samples		
	n	SEP	R	n	SEP	R
Raw data	16	10.6	0.96	10	4.3	0.82
1st derivative	10	11.1	0.96	16	2.0	0.96
2nd derivative	11	9.8	0.97	12	1.9	0.97

[a] *n*=number of PLS loadings; *SEP*=standard error of prediction using cross-validation; *R*=correlation coefficient.

Table 3—Prediction of lamb content using combined NIR and midIR fingerprint range spectral data as related to data pre-treatments and composition ranges[a]

	Complete sample collection			Collection without 100% lamb samples		
	n	SEP	R	n	SEP	R
Entire NIR + FTIR (fingerprint) ranges						
Raw data	10	10.2	0.96	9	4.4	0.81
1st derivative	6	7.4	0.98	10	1.06	0.99
2nd derivative	7	4.1	0.99	7	1.05	0.99
VIS (400-750 nm) + FTIR (fingerprint) ranges						
Raw data	8	11.9	0.95	11	4.2	0.83
1st derivative	4	10	0.96	5	3.2	0.90
2nd derivative	5	7.9	0.98	4	3.3	0.90
NIR (1100-2498 nm) + FTIR (fingerprint) ranges						
Raw data	9	13.4	0.93	8	4.7	0.78
1st derivative	6	8.5	0.97	10	1.1	0.99
2nd derivative	7	5.5	0.99	8	0.91	0.99

[a] *n*=number of PLS loadings; *SEP*=standard error of prediction using cross-validation, *R*=correlation coefficient.

numbers of loadings than was the case for the 0–100% compositional range (Table 2; Fig. 5). Examination of the residual variance data for these models revealed a smooth reduction up to very high numbers of loadings and no clear indication appeared for the manual selection of any specific model with fewer loadings.

Combined near and mid-infrared spectral data

Results from predictive models using combined and normalized spectra and sub-sets of them were compared (Table 3).With samples covering the entire range, the most accurate predictive model using raw spectral data was that based on the complete spectral ranges. In this case, a 10 loading model produced a standard error of prediction of 10.2% lamb, very similar to the optimum model using 400–2498 nm data. The use of sub-sets of visible-NIR spectra generally produced optimal models using fewer loadings but the associated predictive errors were >10.2%. In each of the three spectral combinations (Table 3), the most accurate model was produced using a 2nd derivative data pretreatment. Greatest accuracy was achieved using both entire ranges (standard error of prediction of 4.1% lamb in a 7 component model). In the compositional range 0–20% lamb, the use of derivative spectra also improved predictive accuracy. The visible-fingerprint combination proved least applicable while the other two were of comparable accuracy. There appeared to be a marginal gain by using the NIR-fingerprint combination after calculation of a second derivative. In this case, an 8 component model predicted lamb content with a standard error of 0.9% (Fig. 6).

CONCLUSIONS

COMPARISON OF THE PERFORMANCE OF SEPARATE AND COMBINED wavelength ranges indicated that for the 0-100% lamb range, the most accurate predictive models were generated by the NIR-fingerprint combination. Adding the fingerprint mid-IR data in all cases improved the predictive accuracy over that of NIR or visible data alone. The most accurate model predicted lamb content with a standard error of prediction of 4.1% ($^{w}/_{w}$). With the narrower compositional range, the combined spectral ranges proved more accurate than either alone. The model of choice for maximum predictive accuracy was the 2nd derivative of combined NIR and fingerprint spectral data. The associated standard error of prediction was 0.91% lamb. Results suggest that this analytical approach may have the required accuracy and ease-of-use for potential application by manufacturers and regulatory authorities.

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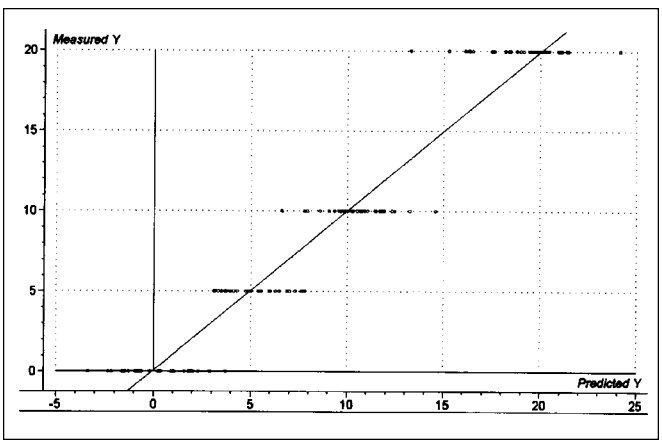

Fig. 5—Prediction of %lamb in beef and beef+lamb mixtures (mid-IR fingerprint region; 0-20% lamb; 2nd derivative data pre-treatment; PLS regression model with 12 loadings).

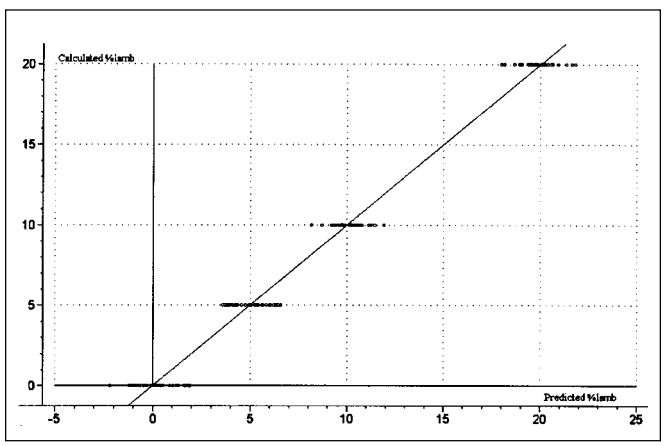

Fig. 6—Prediction of %lamb in beef and beef+lamb mixtures (combined NIR-mid-IR fingerprint region; 0-20% lamb; 2nd derivative data pre-treatment; PLS regression model with 12 loadings).

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