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Evaluating the performance of a miniaturized NIR spectrophotometer for predicting intramuscular fat in lamb: A comparison with benchtop and hand-held Vis-NIR spectrophotometers

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ABSTRACT

This study compares a miniaturized spectrophotometer to benchtop and hand-held Vis-NIR instruments in the spectral range of 900–1700 nm for prediction of intramuscular fat (IMF) content of freeze-dried ground lamb meat; and their ability to differentiate fresh lamb meat based on animal age (4 vs 12 months). The performance of the miniaturized spectrophotometer was not affected by sample temperature equilibration time. Partial Least Square regression models for IMF showed R_{cv}^2 = 0.86–0.89 and RMSECV = 0.36–0.40 values for all instruments. Day-to-day instrumental variation adversely affected performance of the miniaturized spectrophotometer (R_p^2 = 0.27, RMSEP = 1.28). This negative effect was overcome by representing day-to-day variation in the model. The benchtop spectrophotometer and miniaturized spectrophotometer differentiated lamb meat by animal age. The miniaturized spectrophotometer has potential to be a fast, ultra-compact and cost-effective device for predicting IMF in freeze-dried ground lamb meat and for age classification of fresh lamb meat.

1. Introduction

Flavor and texture are significant contributors to consumer acceptability of lamb meat (Craigie et al., 2017; Oltra et al., 2015). Intramuscular fat (IMF) is an important quality indicator in lamb due to its influence on properties such as flavor, juiciness, aroma and tenderness. IMF is directly affected by animal background as well as feeding, which can result in variation in levels of IMF across meat products. Thus the interest to detect IMF at meat processing plant to enable classification of meat cuts based on the level of IMF associates to consumer preference (Pannier et al., 2018).

Spectroscopy in the visible and near infrared (Vis-NIR) region has been widely applied to beef, lamb and pork with the aim for developing a non-invasive and rapid tool for measuring various properties related to product quality (Dixit et al., 2017; Holman, Alvarenga, van de Ven, & Hopkins, 2015; Porep, Kammerer, & Carle, 2015; Prieto, Roehe, Lavín, Batten, & Andrés, 2009). Hitherto Vis-NIR spectroscopy systems were expensive, less-portable and accessible by laboratories only. More recently, there has been a growing number of miniaturized, user-friendly, and cost-effective spectrometers introduced into the market. These instruments appeal to a wide range of demographics including scholars and businesses; thus, motivating performance evaluations of these spectrometers for assessment of food. A number of studies have been conducted in order to evaluate the efficacy of these miniaturized spectrophotometers for detecting food adulteration, classifying food products as well as quantifying various compositional and quality attributes (Harpreet, Rainer, & Andrew, 2017; Kovacs, Bazar, Darvish, Nieuwenhuijs, & Hoffmann, 2017; Marques, de Freitas, Pimentel, & Pasquini, 2015). A miniaturized spectrophotometer covering the spectral range between 900 and 1700 nm (Tellspec Inc., Denmark, www.tellspec.com) has been tested for meat with respect to adulteration detection, identification and compositional quantification. The device was used for differentiating freeze-dried pork and rabbit meat. Additionally, the fat content of pork and rabbit meat were also determined with good accuracy, yielding a R_c^2 *of* 0.93 and 0.98, respectively (Bazar, Kovacs, Pinter, Darvish, & Hoffman, 2016). In another study, the device was used to develop multivariate models for differentiating beef cuts and prediction of aging time based on NIR spectra, where 85.37% of sirloin and tenderloin samples were classified correctly in independent validation tests. Calibration models on meat aging were obtained with an accuracy of 1 or 1.5 days for sirloin or tenderloin, respectively, after omitting initial and final days (Bazar, Kovacs, &

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Table 1
Specifications and settings of spectrophotometers used in the study.

Spectrophotometer	Spectral range	Spectral resolution	Spectral sampling (nm)	Sub-scans	Probe/lamp	Manufacturer
Labspec5000	350-2500 nm	3 nm @700 nm 10 nm @ 1400/ 2100 nm,	1	40	• Contact probe • Light source: Halogen light source, 2900 K, 12–18 VDC, 6.5 W • Viewed area 20 mm∅	ASD Inc., Boulder, CO, USA
Labspec4	350-2500 nm	3 nm @700 nm 6 nm @ 1400/ 2100 nm	1	40	• Contact probe • Light source: Halogen light source, 2900 K, 12–18 VDC, 6.5 W. • Viewed area 20 mm∅	ASD Inc., Boulder, CO, USA
Trek	350-2500 nm	3 nm @ 700 nm 9.8 nm @ 1400 nm 8.1 nm @ 2100 nm	1	50	• Contact device • Viewed area 10 mm∅	ASD Inc., Boulder, CO, USA
NIRScan Nano	900–1700 nm	10 nm	0.96–2.6	15	• Contact device • Light source: two lens-end broadband tungsten filament lamps, 1.4 W • Viewed area 2.5 mm∅	Texas instruments Inc., Texas, USA

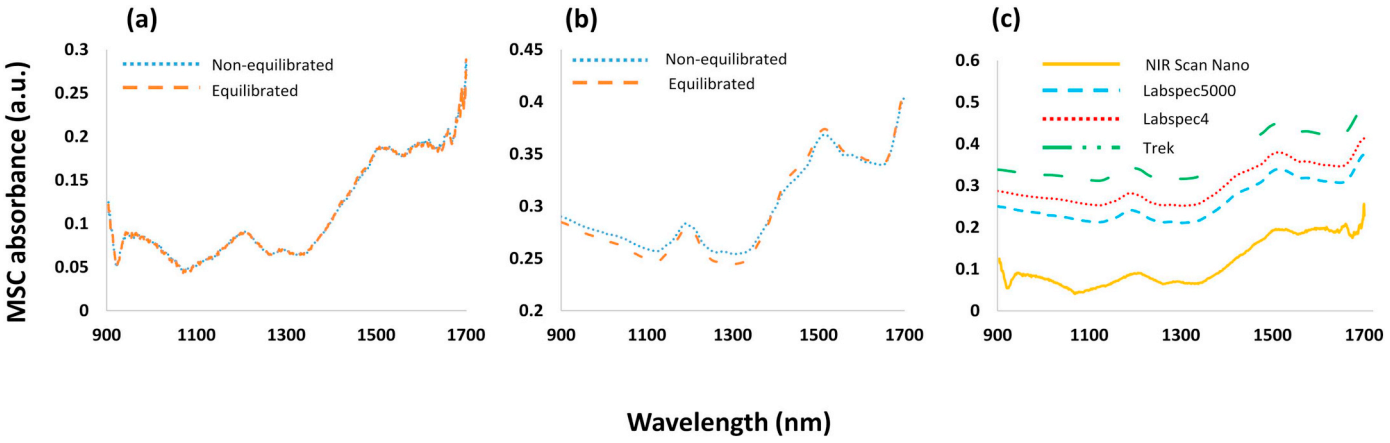

Fig. 1. Spectra; (a) non-equilibrated and equilibrated sample with NIRscan Nano, (b) non-equilibrated and equilibrated sample with Labspec5000 and (c) Comparing spectra from all spectrophotometers for freeze dried ground lamb meat samples (equilibrated).

Hoffmann, 2017).

The current study was aimed at investigating a miniaturized spectrophotometer as a compact, mobile, user-friendly, and cost-effective tool for predicting IMF in freeze dried ground lamb, in comparison to other benchtop and hand-held instruments. This approach will enable to assess the ability of the miniaturized spectrophotometer to detect fat signal and its accuracy in doing so. The study consists of two parts: a) Evaluation of the impact of sample state (room-temperature-equilibrated; named as equilibrated and non-equilibrated) on model development and b) Evaluation of the performance of a miniaturized spectrophotometer to predict IMF in freeze dried ground lamb meat samples with other NIR instruments. Additionally, the study also aims at evaluating the performance of miniaturized and benchtop spectrophotometers for differentiating lamb meat based on animal age by utilizing spectral features.

2. Materials and methods

2.1. Sample preparation

2.1.1. Sample preparation: freeze dried ground lamb samples

Lamb meat, devoid of any subcutaneous fat, was minced and frozen at −18 °C followed by freeze drying for 72 h. A total of 609 samples, which consisted of previously prepared freeze-dried samples and recent samples were analyzed. From those, 109 samples were used for the development of models and other 500 samples were used as an independent validation data set. All samples were kept frozen at −18 °C prior to spectral analysis.

2.1.2. Sample preparation: fresh lamb samples

A total of 60 lambs were processed in a commercial processing plant. The lambs were a composite of different breeds and genders; and were in 2 age groups i.e. 4 and 12 months old. At 24 h post mortem, the left *Musculus Longissimus thoracis* (MLT) was removed from the carcass for spectral analysis. Half (n = 30) of the lambs were 4 month old wethers of a composite breed slaughtered at weaning that had been suckling and grazing on their mother's diet (chicory and red clover mix). The remaining 30 animals were wethers (n = 15) and cryptorchid (n = 15) merino lambs that had been grazing a mixed pasture (ryegrass and white clover mix) and slaughtered at 12 months old. Mean carcass weight and fat depth were 18.6 ± 0.16 kg, 19.5 ± 0.23 kg and 19.0 ± 0.23 kg, and 8.9 ± 0.44 mm, 4.5 ± 0.62 mm and 4.1 ± 062 mm for weaned lambs, Merino cryptorchid and Merino wether lambs, respectively. Decisions regarding the animals that were sent for slaughter were made by the farmer based on criteria to obtain a target carcass weight of 17–21 kg. Thirty carcasses from weaned lambs, fifteen from Merino cryptorchid lambs and fifteen from Merino wether lambs were randomly selected from the slaughter chain from a larger group of lambs.

2.1.3. IMF analysis

The AOAC approved method (AOAC, 2000) involving gas chromatography (GC) was used for determining IMF content of all freeze-dried lamb meat samples (n = 609). The IMF content in the freeze dried powder was converted to IMF% in wet meat sample using a conversion factor as described in (Craigie et al., 2017). Briefly, a conversion factor (weight of dried sample − weight of bag and tag) / (weight of wet

Table 2
Fitness of equilibrated model for fat prediction fitted with non- equilibrated and equilibrated ground meat lamb samples data.

Instrument	Calibration						Non-equilibrated			Equilibrated		
	n	LV	RMSEC	R_c^2	RMSECV	R_{cv}^2	n	RMSEP	R_p^2	n	RMSEP	R_p^2
LabSpec5000	66	5	0.34	0.92	0.40	0.89	43	1.12	0.75	43	1.49	0.83
NIRscan Nano	61	5	0.40	0.89	0.47	0.85	42	0.39	0.83	42	0.38	0.82

Note:
n: number of samples.
LV: Latent Variables.
RMSEC: Root Mean Squared Error of Calibration.
RMSECV: Root Mean Squared Error of Cross-Validation.
R_{cv}^2: Coefficient of determination in cross-validation.
RMSEP: Root Mean Squared Error of Prediction.
R_p^2: Coefficient of determination in prediction.

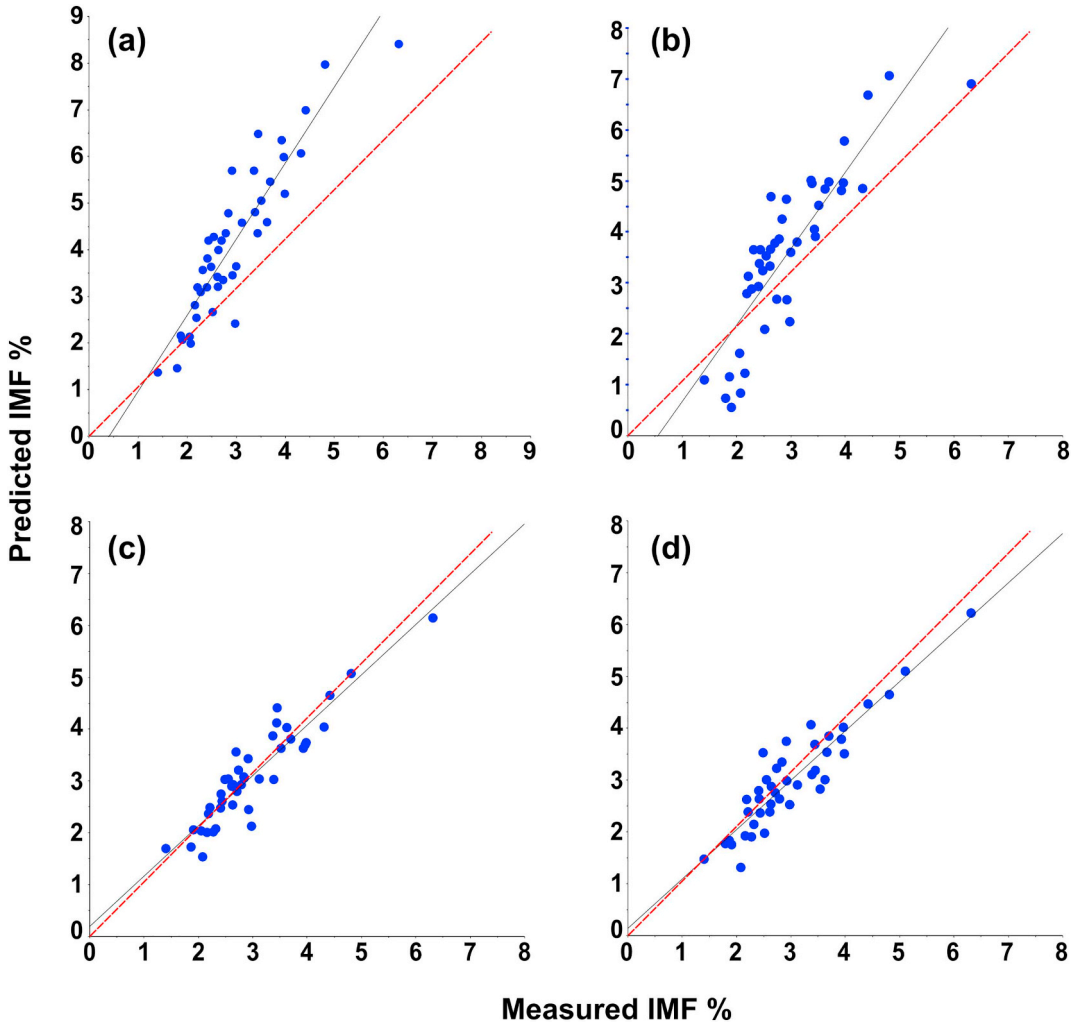

Fig. 2. Prediction plots; (a) Labspec5000 for equilibrated samples, (b) Labspec5000 for non-equilibrated samples (c) NIRscan Nano for equilibrated samples, (d) NIRscan Nano for non-equilibrated samples. The dashed line represents the reference lines which indicates the expected behavior for the regression lines (the solid line).

sample − weight of bag and tag) was applied to the weight of the freeze dried meat to convert from concentration per gram of freeze dried meat to the concentration of the wet meat. The laboratory error for IMF was estimated to be 0.20% (Craigie et al., 2017). The IMF% in wet meat sample were used as reference values to fit the models.

2.2. Spectral analysis

2.2.1. Freeze dried ground lamb samples

Samples were analyzed in two temperature conditions i.e. room-temperature-equilibrated (named as 'equilibrated') and non-equilibrated in order to evaluate the effect of variation in sample temperature between storage at −18 °C and room temperature (20 °C). Non-equilibrated samples were scanned immediately after removal from storage

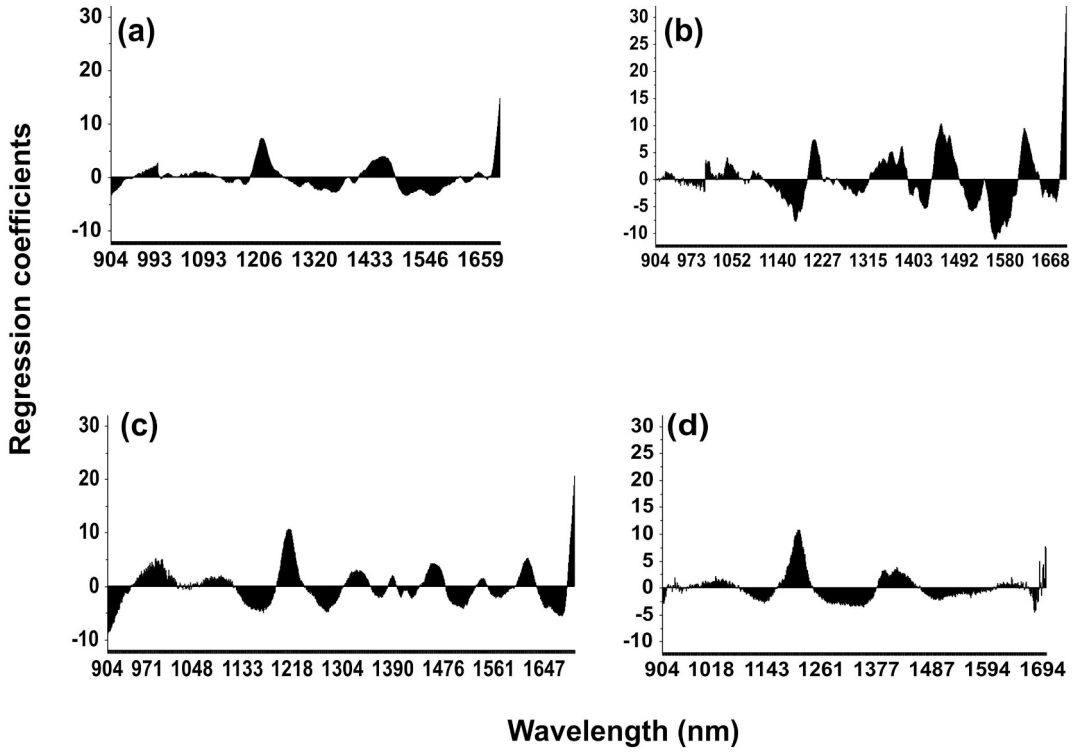

Fig. 3. Regression coefficient plots; (a) Labspec5000, (b) Labspec4, (c) Trek and (d) NIRscan Nano.

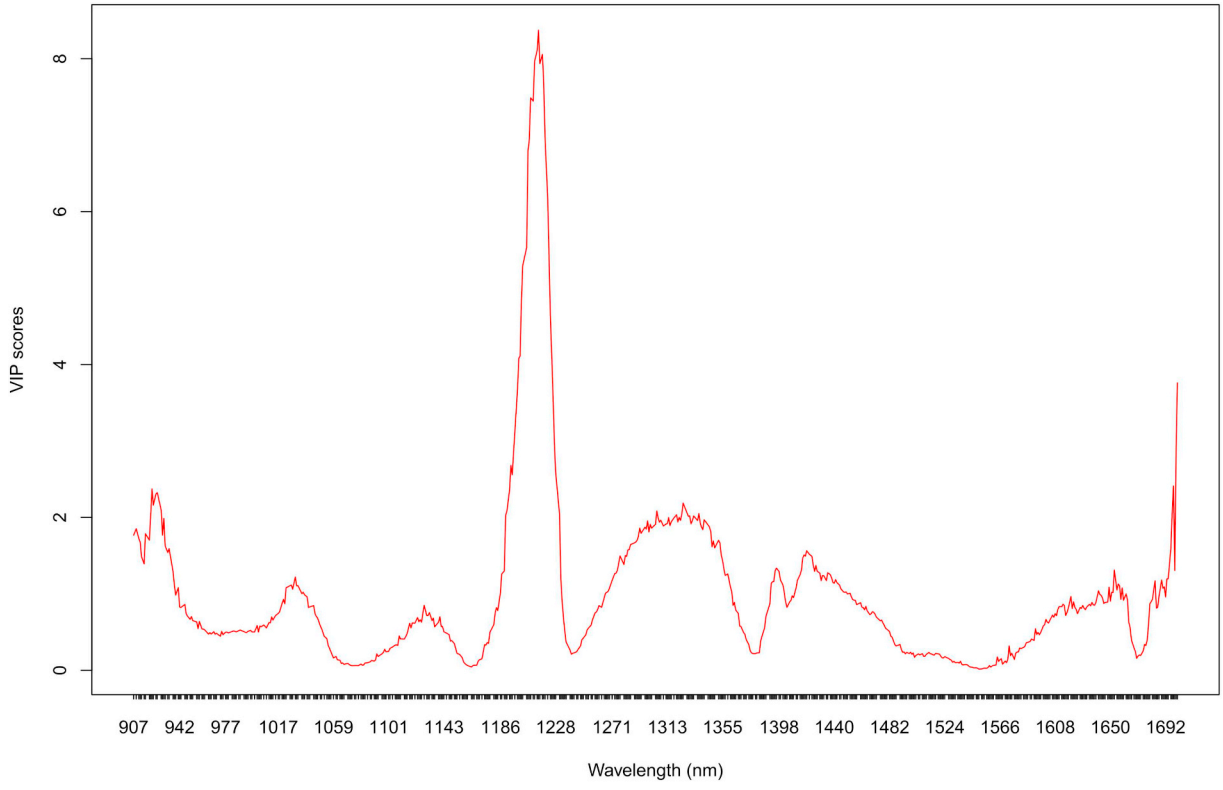

Fig. 4. Variable importance in projection (VIP) scores plot for NIRscan Nano PLS regression model.

(−18 °C) while equilibrated samples were kept at room temperature for 20 min before conducting spectroscopic analysis. Three spectrophotometers i.e. the Labspec5000, Labspec4, Trek and the NIRscan Nano were utilized for the analysis (Table 1). Sample scanning was conducted over a period of 14 days: (a) samples used for calibration were analyzed over the first 4 days and (b) samples used for validation were analyzed over the next 10 days. NIRscan Nano was used with following settings: (a) width: 2.34 nm, (b) digital resolution: 605 nm and (c) method: hadamard (Pham, Agnew, Craigie, & Reis, 2018). Further details about NIRscan Nano can be found elsewhere

Table 3
Comparison of spectrophotometers for prediction of IMF% in temperature equilibrated freeze dried ground lamb samples.

Instrument	Cross-validation						Prediction		
	No. of measurements	LV	RMSEC	R_c^2	RMSECV	R_{cv}^2	No. of measurements	RMSEP	R_p^2
LabSpec5000	152	6	0.35	0.90	0.38	0.88	498	0.41	0.76
LabSpec4	152	9	0.31	0.92	0.36	0.89	499	0.38	0.79
Trek	152	8	0.36	0.89	0.40	0.86	497	0.34	0.83
NIRscan Nano	145	6	0.35	0.89	0.38	0.88	485	1.28	0.27

Note:
LV: Latent Variables.
RMSEC: Root Mean Squared Error of Calibration.
RMSECV: Root Mean Squared Error of Cross-Validation.
R_{cv}^2: Coefficient of determination in cross-validation.
RMSEP: Root Mean Squared Error of Prediction.
R_p^2: Coefficient of determination in prediction.
IMF: Intramuscular fat.

Table 4
F test for model comparison: NIRScan Nano vs other Vis-NIR spectrophotometers.

Instrument	df	SECV	F	F critical
Labspec5000	145	0.40	1.10	1.32
Labspec4	142	0.36	1.11	1.32
Trek	143	0.40	1.10	1.32
NIRScan Nano	138	0.38		

Note:
Degrees of freedom (df) = n-LV-1, n = no of samples/ measurements, LV = Latent Variables.
SECV: Standard Error of Cross-validation.

(Anonymous, 2017). Each freeze-dried lamb powder sample filled a 44 mm × 44 mm × 3.6 mm plastic weighing boat (Thermofisher, USA). The sample surface was flattened out in order to avoid any fluctuations due to surface undulations. Measurements conducted using the LabSpec5000 and the LabSpec4 were performed at a distance of 1 mm between the probe window and the sample. The Trek and NIRscan Nano were operated using contact measurements. All ASD equipment collected 40 scans that were averaged in to one spectrum; while for the NIRscan Nano three spectra were acquired at 3 random locations of the sample surface, in this case each spectrum corresponds to the average of 15 scans on the same location.

2.2.2. Fresh lamb samples

This experiment involved the evaluation of meat samples harvested 24 h post mortem at the meat processing plant. Samples were analyzed using the Labspec5000 and NIRscan Nano instruments. Samples from MLT were cut into 2 cm thick slices and measurements were done on muscle cross-section, without avoiding marbling. Two measurements were acquired per sample at random sample locations using each instrument. A total of 60 samples were analyzed to establish if samples could be distinguished based on animal age. Due to time restriction on sample assessment only Labspec5000 and NIRScan Nano were used for analysis. The Labspec5000 was chosen as it had been tested in similar conditions before (Craigie et al., 2017).

2.3. Multivariate data analysis

2.3.1. Data analysis: freeze dried ground lamb samples

The raw spectral data was imported into The Unscrambler X statistical software (The Unscrambler, Version 10.2, Camo Software AS, Oslo, Norway). The reflectance spectra (R) collected from the 3 Vis-NIR spectrophotometers were transformed into absorbance i.e. log10 (1/*R*). Three scans obtained for each sample using the NIRscan Nano were averaged into a single absorbance spectrum. Thus, one spectrum per sample per instrument was obtained. The wavelength range of 900–1700 nm was chosen in order to have the same spectral range for all the four NIR instruments. Transformed data were pre-processed by applying multiplicative scattering correction (MSC). Pre-processed data obtained were analyzed and modelled using partial least squares (PLS) regression for predicting IMF. The number of latent variables (LV) for PLS models were chosen using cross-validation strategy in a random mode. Data were analyzed for two studies: (a) evaluating the effect of sample temperature conditions (non-equilibrated and equilibrated) and (b) evaluating the performance of NIRscan Nano for IMF prediction in comparison to the three Vis-NIR spectrophotometers. The Variable Importance in Projection (VIP) scores were also calculated for NIRscan Nano PLS regression model in order to determine the important spectral features (relevant peak related to IMF) captured by the device (Oussama, Elabadi, Platikanov, Kzaiber, & Tauler, 2012; Wold, Johansson, & Cocchi, 1993).

For Study (a) each instrument was used to obtain spectral data from both equilibrated and non-equilibrated samples, but only spectra from the LabSpec5000 and NIRscan Nano were studied. Two sample sets were selected. Set 1: 66 equilibrated samples for Labspec5000 and 61 samples for NIRscan Nano were used to develop the calibration model for IMF prediction. Set 2: 43 samples for Labspec5000 and 42 samples for NIRscan Nano were scanned in both non-equilibrated and equilibrated states, thus obtaining 43 and 42 spectra respectively in both states. These were then used as independent validation sets to assess the performance of models fitted with samples from Set 1 to predict either samples on the non-equilibrated and equilibrated states.

For study (b), the two sample sets in Study (a) were combined and data from the LabSpec4 and Trek added to comprise the calibration set in Study (b). As some of the samples were scanned more than once either non- equilibrated or equilibrated there was in total 152 spectra. Thus, the final numbers of spectra were: 152 measurements for ASD spectrophotometers; and 145 measurements for the NIRscan Nano (some outliers were removed based on abnormal spectral behavior). These spectra were used for developing the calibration models for IMF prediction. These models were then applied in a set of samples reserved for validation, which were a mix of old (previously prepared) and recent freeze-dried samples, for Labspec5000, Labspec4, Trek and NIRscan Nano, respectively. It should be noted that spectra from this independent validation set (500 samples) were collected after the samples used to fit the models were collected. This was to represent normal practice of applying the developed model on new independent samples chronologically separated from calibration dataset. Both calibration and validation data sets had a wide range of fat content. The SECV for NIRScan Nano was compared to those obtained with the other instruments using F test (ASTM International, 2015). Briefly, the ratio between the SECV for NIRScan and SECV for the instrument of interest

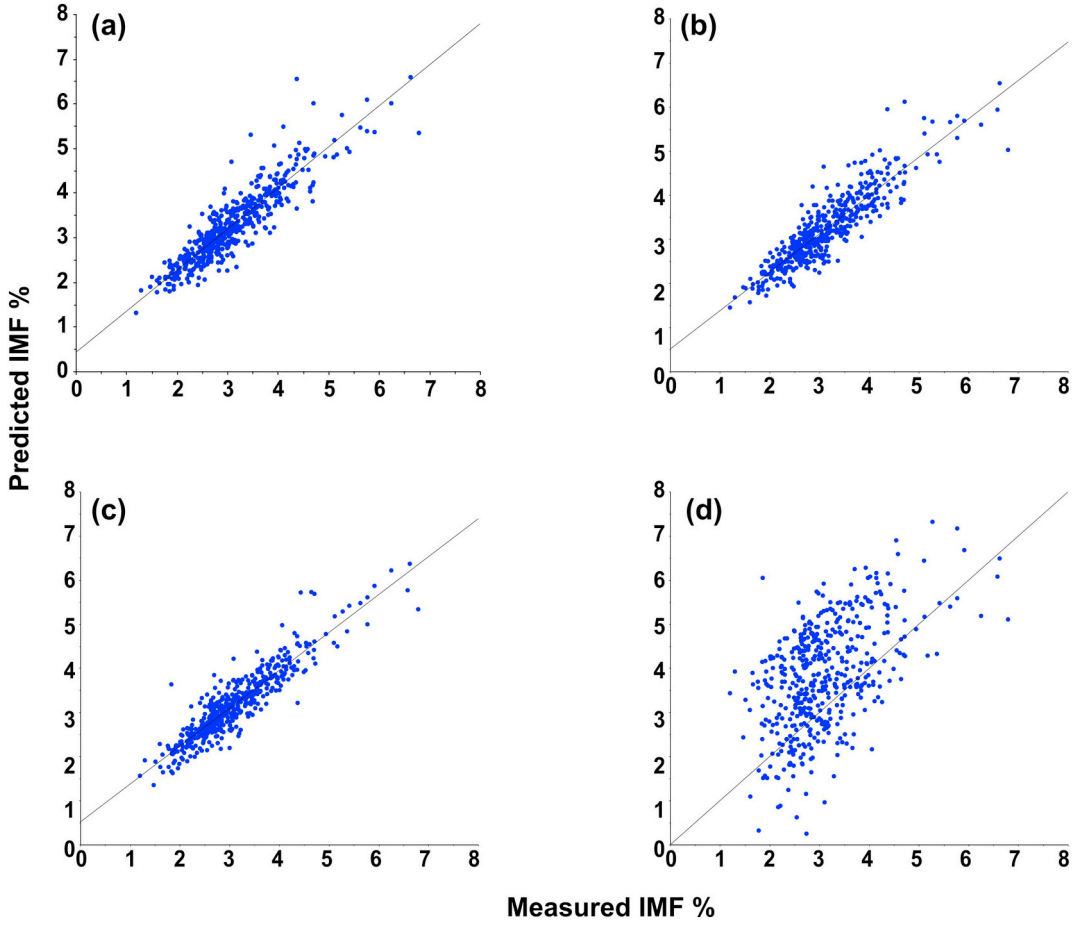

Fig. 5. Prediction plots; (a) Labspec5000, (b) Labspec4, (c) Trek and (d) NIRscan Nano.

was estimated and compared with the F-table for the corresponding number of degrees of freedom (df). The df was estimated as 'n-LV-1', where n is the number of samples, LV is the number of latent variables used in the model to estimate SECV. The ratio was taken as the larger value divided be the smaller value of SECV (ASTM International, 2015).

Two additional analyses were performed to evaluate the performance of NIRscan Nano. The first involved applying the fitted model on individual spectra from the NIRscan Nano to evaluate the effect of sample heterogeneity on prediction variability. The second evaluation involved fitting a model for all spectra (n = 630; 145 + 485) to test whether the variation between different days of scanning could be modelled. In this case, repeated double cross validation (RDCV) was used to fit PLS regression models to assess the relationship between IMF and the spectral attributes (Filzmoser, Liebmann, & Varmuza, 2009; Szymańska, Saccenti, Smilde, & Westerhuis, 2012; Westerhuis, van Velzen, Hoefsloot, & Smilde, 2010). In RDCV, the whole procedure was repeated ten times and the average of ten predictions for each sample is used as final prediction. For each sample there were three predictions.

2.3.2. Data analysis: fresh lamb samples

The reflectance spectra (R) collected from the Labspec5000 were transformed into absorbance i.e. log10 (1/R) in order to have the data similar to the NIRscan Nano. Transformed data were pre-processed by applying MSC and subjected to principal component analysis (PCA) for differentiating samples based on the animal age.

3. Results and discussion

3.1. IMF results

IMF content for all the original fresh meat samples (n = 609) were obtained in the range of 1.2–6.79% on wet basis. The average and standard deviation of the IMF% values were 3.10 and ± 0.85% on wet basis, respectively. IMF% in wet meat sample was used to fit the models.

3.2. Visual spectral analysis

Fig. 1a illustrates the pre-processed spectra obtained for freeze dried ground lamb meat samples in both non-equilibrated and equilibrated state using the NIRscan Nano while Fig. 1b illustrates the similar spectra using Labspec5000. It can be observed that the condition of the freeze-dried meat samples did not significantly affect the spectral information from NIRscan Nano, however resulted in small variation in the spectra from Labspec5000. Thus, the time required to equilibrate samples could be eliminated for NIRscan Nano. Fig. 1c illustrates the pre-processed spectra obtained by averaging the individual spectrum of each equilibrated sample (without outliers) using all the four spectrophotometers in the wavelength range of 900–1700 nm. The NIRscan Nano generated similar spectral features in comparison to other spectrophotometers in the region 1160–1238 nm and 1380–1490 nm. The absorption band at 1215 nm related to the second overtone of C—H stretching vibration is associated with intramuscular fat (Dixit et al., 2016a; Dixit et al., 2016b; ElMasry, Sun, & Allen, 2013). Although a low signal-to-noise ratio (SNR) was observed for the spectra obtained from the NIRscan Nano which could be related to the smaller scanned

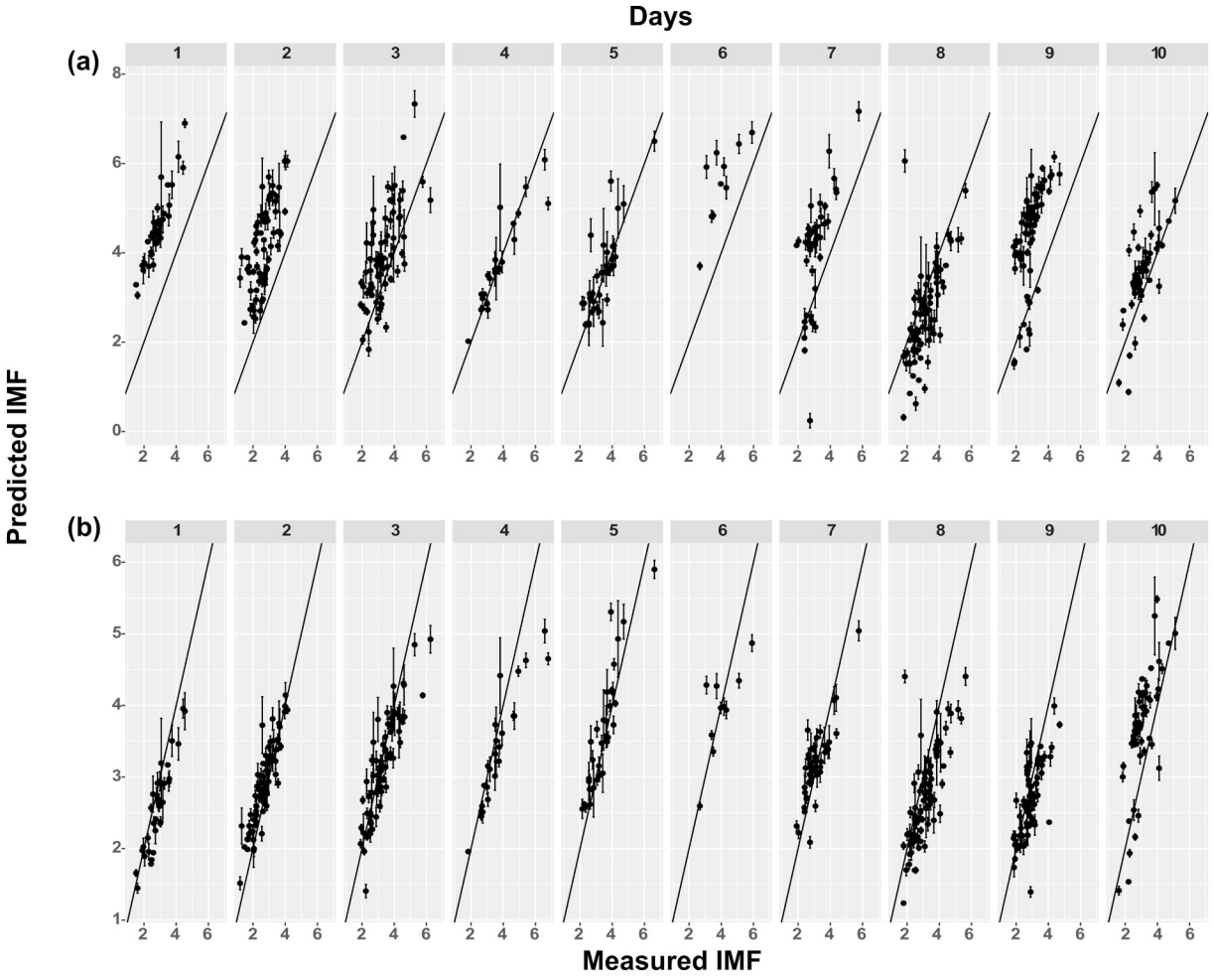

Fig. 6. Prediction plots: (a) Samples analyzed at different days using the original calibration model and (b) Samples analyzed at different days validated with corresponding calibration model. Error bars denote standard errors.

area in the sample. However, the spectra showed relevant spectral features, especially the IMF related peak at 1215 nm.

3.3. Multivariate data analysis

3.3.1. Freeze dried ground lamb samples

PLS regression was performed on pre-processed spectral data for all the spectrophotometers in order to develop predictive models for IMF. Data were analyzed for two studies: (a) evaluating the effect of sample temperature conditions (non-equilibrated and equilibrated) (b) evaluating the performance of the NIRscan Nano for IMF prediction in comparison to 3 other Vis-NIR spectrophotometers.

3.3.1.1. Study (a): evaluating the effect of sample temperature conditions (non-equilibrated and equilibrated). Table 2 shows the performance summary of PLS regression models for IMF prediction obtained using data from the Labspec5000 and the NIRscan Nano for non-equilibrated and equilibrated samples. It presents the root mean squared error and coefficient of determination for calibration (RMSEC;R_c^2); cross validation (RMSECV;R_{cv}^2); and for prediction (RMSEP;R_P^2); along with the number of latent variables (LVs) used. Two validation sets were used, one with non-equilibrated samples and the other with equilibrated samples. The PLS regression model for NIRscan Nano showed good fit, indicated by a high R_{cv}^2 (0.85) and a low RMSECV (0.47); moreover, the model showed good fit in comparison to Labspec5000 which yielded slightly higher R_{cv}^2 (0.89) and lower RMSECV (0.40) (Table 2). The PLS regression model for NIRscan Nano showed good prediction ability for both non-equilibrated and equilibrated samples as indicated by R_p^2 as 0.83 and 0.82, respectively. The PLS regression model for the Labspec5000 also showed good predictions, achieving R_p^2 of 0.75 for non-equilibrated and 0.83 for equilibrated samples. Furthermore, the RMSEP values for NIRscan Nano and Labspec5000 instruments were in the ranges of 0.38–0.39 and 1.12–1.49% in the validation dataset, respectively. The RMSEP values for NIRscan Nano were similar as reported in previous studies by Pullanagari, Yule, and Agnew (2015), Craigie et al. (2017) and Andersen, Wold, Gjerlaug-Enger, & Veiseth-Kent, 2018. However, the reason behind higher values of RMSEP for Labspec5000 is not clear. Fig. 2 presents the prediction plots for non-equilibrated and equilibrated samples using both spectrophotometers. Both non-equilibrated and equilibrated samples showed similar distribution along the regression lines using either spectrophotometer. In the prediction plot for equilibrated samples (Fig. 2a) using the Labspec5000, data points were more clustered along the regression line, resulting in the high R_p^2. Fig. 2a also shows that the regression line is over the reference line (dashed line) showing an overestimation of predicted values. Whereas prediction plot for both non-equilibrated and equilibrated samples using NIRscan Nano showed very similar regression plots as justified by R_p^2 (Fig. 2c and d). Overall, it can be concluded that sample equilibration is not a crucial step for NIR scanning with respect to freeze dried lamb meat samples.

3.3.1.2. Study (b): comparing the performance of miniaturized spectrophotometer for IMF prediction to three Vis-NIR

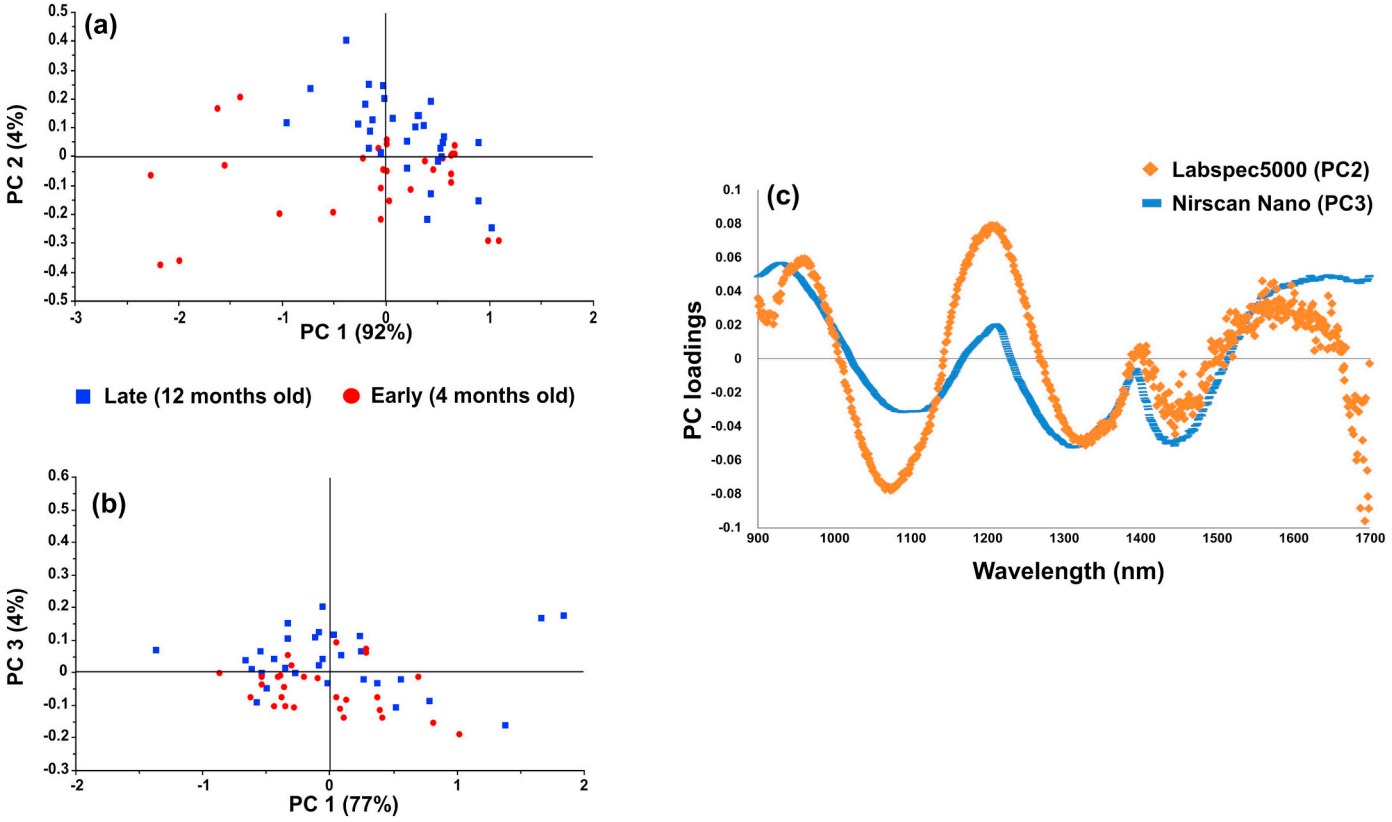

Fig. 7. PCA plots distinguishing early (4 months old) and late (12 months old) lambs; (a) PCA score plot: Labspec5000, (b) PCA score plot: NIRscan Nano and (c) PC Loadings plot (PC2: Labspec 5000, PC3: NIRscan Nano).

spectrophotometers. Fig. 3 shows the PLS regression coefficients plots for the Labspec5000, Labspec4, Trek and NIRscan Nano. Regression coefficients plot of the NIRscan Nano showed similar features to all ASD spectrophotometers especially Labspec5000 (Fig. 3). These observations were expected since NIRscan Nano and the LabSpec5000 models utilized the same number of latent variables i.e. 6, while the LabSpec4 and the Trek models utilized 9 and 8 LVs, respectively. The PLS regression models for these two sets might differ slightly on the information captured in the model. The NIRscan Nano and the LabSpec5000 models were dominated by the fat absorption region around 1215 nm. Also, Fig. 4 shows the VIP scores plot for NIRscan Nano PLS regression model where the highest score is observed at 1215 nm and thus confirms the ability of the instrument to capture the peak relevant to IMF.

Table 3 shows the performance summary of 4 PLSR models for predicting IMF in equilibrated freeze-dried ground lamb meat samples obtained using data from 3 Vis-NIR spectrophotometers and the NIRscan Nano. All the four calibration models showed good fit as indicated by high R_{cv}^2 in the range of 0.86–0.89 and low RMSEC as well as RMSECV in the range of 0.31–0.40. The goodness of fit for the models decreased in the following order: LabSpec4, NIRscan Nano/LabSpec5000 and Trek. A standard F-test was conducted using SECV values of PLSR models in order to compare the performance of NIRscan Nano to the three Vis-NIR spectrophotometers (ASTM International, 2015). Table 4 shows that the F values obtained for all 3 comparisons were less than their corresponding F-critical values at a 95% confidence level which verifies that the SECV for NIRscan Nano was not significantly greater than the SECV values of other spectrophotometers. Thus, based on the available data there is no evidence that the performance of NIRscan Nano is inferior to the other devices.

Good prediction ability was observed for the three Vis-NIR spectrophotometers as indicated by high R_p^2 in the range of 0.76–0.83 and low RMSEP in the range of 0.34–0.41. The instrument of interest i.e. NIRscan Nano showed poor predictions as indicated by low R_p^2 and RMSEP values (Table 3). The RMSEP value of NIRscan Nano (1.28) was just over three times larger than the RMSEP of the LabSpec5000 (0.41). This suggests that there is variation in spectral data from new (validation) samples compared to the calibration data set. Fig. 5 shows the prediction plots for IMF using all spectrophotometers. It can be observed from Fig. 5a, b and c that the data points were more clustered along the regression line, which is reflected in the high R_p^2 for these instruments.

However, Fig. 5d shows that the data points in the predictions plot for NIRscan Nano deviate from the expected trend, indicated by the reference line in the plot, and the samples seem to be divided in two groups. Fig. 6a shows the scatter plot corresponding to Fig. 5d but split according to the day of scanning and with prediction for individual spectrum. It shows that variation among individual spectrum on the same sample (size of the error bars) is relatively small compared to the range of IMF investigated. It suggests that there is a small effect of sample heterogeneity contributing to poor prediction performance. Fig. 6a also shows that predictions follow the expected trend but there is a significant bias on particular days. The bias can be observed in the prediction plot for samples analyzed on day 1 and day 2. Similarly, bias can be observed for some samples analyzed on day 6, 7 and 9. The presence of this bias explains the drop off in performance of NIRscan Nano when comparing the R_{cv}^2value (0.88) to the R_p^2 (0.27). This type of bias was not observed in other instruments (Fig. 5a-c) suggesting that variation showed in Fig. 6a is not associated to day-to-day variation in the samples. Furthermore, the same procedure for sample processing and operator were involved in the sample scanning. Thus, it can be suggested that the bias observed in Fig. 6a is due to instrumental variation. However, it is not clear what is the exact source of this variation. The spectra corresponding to these ten days (Fig. 6a) were used in the repeated double cross validation. Fig. 6b shows the prediction plots for the new calibration models where the bias was eliminated in all the

plots. These results show that the effects of day-to-day variation can be accommodated, and that this type of variation should be taken in to consideration when developing calibration for NIRscan Nano.

3.3.2. Fresh lamb samples

Fig. 7a and b shows the PCA score plots generated from the spectral data obtained for fresh lamb meat samples using the Labspec5000 and the NIRscan Nano, respectively. It can be observed that samples were separated among themselves based on the age of lambs. PC2 from the Labspec5000 and PC3 from the NIRscan Nano were the main contributors to the separation of lamb samples based on their age. Fig. 7c illustrates the loadings plot for PC 2 and PC 3 from the Labspec5000 and NIRscan Nano, respectively. Both loadings show similar features which includes an absorption peak at 1215 related to fat content. The loading also includes a peak comprised of two very weak bands related to fat at 1392 and 1414 nm which are dominated by water content of the samples (peak at 1140 nm). A second water peak could also be observed at 970 nm. Peaks related to water content had the greatest contribution to the loading values because water is the main compositional component of fresh meat samples (ElMasry et al., 2013).

4. Conclusion

It was observed that variation in the time for temperature equilibration of the samples did not affect the prediction performance of models for IMF% in the freeze dried powder.

The PLS regression calibration models obtained for IMF in freeze dried samples using the LabSpec5000, the LabSpec4 and the Trek showed good fit. However poor prediction in the validation set of NIRscan Nano was observed, and it was found that bias was associated with the data for particular days. The observed bias is thought to be largely related to instrumental variations. It was observed that representing the day-to-day variation in the calibration model drastically improved the prediction ability of the NIRScan Nano, i.e. the design of the calibration model should take in consideration instrumental variation involving samples measured over several days.

Both the Labspec5000 and the NIRscan Nano could be used to differentiate fresh lamb meat according to the animal age by exploiting the compositional difference between early and late-season lambs. NIRscan Nano instrument has the potential to be a fast, ultra-compact and cost-effective NIR spectrophotometer for predicting IMF in freeze dried ground lamb meat. Also, the NIRscan Nano could be a potential alternative to other benchtop and hand-held spectrophotometers for rapid and real-time classification of fresh lamb meat.

Declaration of Competing Interest

None.

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