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Near-infrared reflectance spectroscopy using a portable instrument to measure the nutritive value of oilseed meals as livestock feed

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Introduction: Measurement of the nutritive value of feedstuffs with near infrared reflectance spectroscopy (NIRS) laboratory benchtop spectrometers is well-established. The aim of this study was to examine the reliability and accuracy of a handheld low-cost Tellspec NIRS spectrometer for measurement of the nutritive value of oilseed meals.

Methods: Samples (n=142) comprising byproduct meals from processing linseed, noug seed, cotton seed, groundnut, rapeseed, soybeans, and sunflower seeds were collected from farms, oil factories, wholesalers, and retail shops in central Ethiopia. Samples were scanned 'as received' (UGr) and also following drying and grinding (Gr), and were scanned once, twice, and ten times in a 2x3 factorial experimental design. Laboratory analyses of total nitrogen (TN), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and in vitro organic matter digestibility (IVOMD) provided reference measurements. Calibration models were developed using a subset of 2/3 of the spectra and validated using the remaining 1/3 of sample spectra.

Results: The sample form and the number of scans, and their interactions, all significantly affected the accuracy of the calibration models ($P < 0.001$). The most accurate calibrations were with Gr samples scanned 10 times, where the coefficient of determination of both calibration and validation sets (R^2_{cal} and R^2_{val}) were ≥ 0.90 for most attributes. The respective standard errors of prediction (SEP) (g/kg DM) for Gr, and 'as received', samples respectively that were scanned ten times were: TN (3.2 and 4.7), IVOMD (11.7 and 20.1), NDF (26.9 and 43.8), ADF (25.3 and 44.6), and ADL (6.8 and 10.7). Also, the SEP for each attribute was reduced ($P < 0.05$) by drying and grinding the sample before

scanning. The ratios of the standard deviation of the calibration samples to the SEP (RPD) g/kg DM were 2.68, 2.30, 3.17, 3.05, and 4.06 for TN, IVOMD, NDF, ADF, and ADL, respectively, for Gr samples scanned ten times. Nevertheless, the SEP of samples scanned 'as received' would often be acceptable for routine analyses in the field and market-place under east African circumstances.

Conclusion: In conclusion, NIRS calibrations for a portable handheld Telspec NIRS spectrometer could be developed to measure important nutritional attributes of oilseed cake samples as feeds.

KEYWORDS

near infrared (NIR) spectroscopy, oilseed, Ethiopia, handheld NIRS instrument, nutritive value

Introduction

Knowledge of the nutritive quality of the animal feeds available is one of the most important considerations in livestock production where feed costs often account for the majority (e.g. 75-80%; Lundberg et al., 2004) of the cost of animal production. Also, as discussed in many recommendations of nutritional requirements of livestock (NRC, 2001) the use of feedstuffs with appropriate chemical compositions is essential to optimize livestock production and minimize feeding costs (Tedeschi et al., 2010). However, information about the composition of feed resources, particularly in the context of developing countries, is often lacking due to logistical constraints, high costs, and scarcity of analytical services. Animal feeding trials and conventional laboratory analysis ("wet chemistry") are the two most common methods used for estimation of feed quality. Feeding trials are a labor-intensive and costly method to routinely evaluate the nutritive value of feed resources routinely (Rukundo et al., 2021). Wet chemistry for determining the chemical composition of feeds is also expensive, time-consuming, laborious, and requires the use of potentially unsafe chemical reagents and high-cost instrumentation (Wittkop et al., 2012). In contrast, near infrared reflectance spectroscopy (NIRS) conducted with high quality laboratory benchtop spectrometers can provide rapid and accurate information of feedstuffs with minimal sample preparation, and measurement of many organic constituents from a single spectral measurement (Smith and Flinn, 1991; Wittkop et al., 2012). Moreover, NIRS is a low cost, rapid, high-precision, and high-throughput technique that can predict the concentrations of organic constituents by combining laboratory and spectral data (Ramirez et al., 2015; Wu et al., 2015). NIRS also has the advantage that samples can often be analyzed in their natural form (Font et al., 2006).

In the past, most NIRS spectrometers were high cost and suitable only for well-equipped and well-controlled environments, requiring samples to be sent to a central lab for analysis. However, in recent years there has been extensive development of robust, handheld NIR spectrometers. These instruments offer major advantages in size, weight, robustness, spectral range, simplicity in use and cost (Cabassi et al., 2015) and have the potential to take

the lab to the sample (with on-farm and market-place applications) (Perez Marin et al., 2009; O'Brien et al., 2012).

Handheld instruments working in reflectance mode would increase options for ease of sub-sampling and for rapid, low-cost analysis (Prado et al., 2011; Dela Roza-Delgado et al., 2014). However, the development and testing of handheld NIRS instruments is needed for specific products and sound chemometric methodology and validation is required (Perez-Marin et al., 2010; Garrido-Varo et al., 2016). Many recent research reports have examined the use of handheld spectrometers for measurements of the compositions of agricultural and food products such as grains (Williams, 2001; Chadalavada et al., 2022), seafoods (Brambilla et al., 2020) agro-fruits (Perez Marin et al., 2009; Pierna et al., 2010), meat (Perez-Marin et al., 2010; Prado et al., 2011) and both forage and concentrate feeds for livestock, and have often reported satisfactory measurements of composition. For example, Prasad et al. (2019) evaluated the performance of the Telspec Enterprise NIRS device and reported often very acceptable coefficients of determination of validation data sets (R^2_{val}) for TN (0.87), NDF (0.89), ADF (0.96), and ADL (0.58) in roughage feeds. Similarly, Modrono et al. (2017) predicted the TN content of compound livestock feeds using the Phazir-1624 and Micro NIRS 1700 portable instruments and obtained acceptable R^2_{val} values of 0.89 and 0.87, respectively.

The Telspec Enterprise handheld NIRS spectrometer offers many potential benefits, but apart from the study of Prasad et al. (2019) cited above there is a lack of information to understand its limitations and the accuracy and reliability of chemometric models in the light of its narrower spectral range than most benchtop NIRS spectrometers. Additionally, in the context of East Africa, no information is available on the efficacy of this Telspec NIRS spectrometer to measure the chemical composition of high-protein feed samples such as oilseed meals. The objective of the present study was to develop and test the use of the Telspec Enterprise handheld NIRS spectrometer to measure some important nutritional attributes of the wide range of byproduct cakes and meals from processing oilseeds that are produced in Ethiopia. This included examining the effects of drying and grinding of the samples and the number of measurements of the

spectra (i.e. scans) of each sample on the development and reliability of calibration models.

Materials and methods

Study areas

The study was conducted in the Holeta, Adaberga, Sululta, Bishoftu and Adama districts of the Oromia Regional State of Ethiopia (Figure 1). The study areas were selected based on the potential availability of different feed markets and farms. All the study areas are characterized by a cool, sub-tropical climate with bimodal rainfall of 1200 – 1700 mm per year. The study areas are a mixed crop-livestock farming systems with the cultivation of a range of cereal and legume crops and livestock species including dairy and beef cattle, small ruminants and backyard poultry. Livestock are fed on home-grown feed resources and agro-industrial byproducts including oil seed meals.

Sample collection, experimental design and treatments

The oilseed cake (n = 142) samples were collected from selected farms, oil factories, wholesalers, and retail shops, and comprised meals from linseed (also known as flaxseed, n=53), noug seed (n=22), cotton seed cake (n=56), groundnut (n= 7), soybean

(n=2), sunflower (n=1), and rape seed (n=1). The total number of samples were determined according to Williams (2001), who recommended a minimum of 20 to 30 samples for initial calibration with larger numbers of samples improving the robustness of NIRS prediction equations.

The experiment involved collecting spectra data from intact ‘as received’ (Uground, UGr) and dried and ground samples (Gr), with several scan frequencies (one, two and ten scans per sample; 1s, 2s and 10s) and in triplicate. Thus, there were six treatments comprising UGr-1s, UGr-2s, UGr-10s, Gr-1s, Gr-2s and Gr-10s, and these were analysed as a 2x3 factorial completely randomized design with three replicates.

Sample preparation and collection of spectral data

The samples were transported to the animal nutrition laboratory in the International Livestock Research Institute (ILRI), Addis Ababa. The NIRS spectra of each oilseed cake sample was measured in both ‘intact’ (UGr, i.e. ‘as received’) and Gr samples. The UGr samples were scanned before the samples were dried (forced air oven for 48 hours at 60°C), ground (1 mm screen, Wiley mill), and then scanned again. Both intact and Gr samples were packaged into transparent plastic bags (20 cm width and 30 cm length with 20 µm thickness) for scanning, and subsamples were stored for subsequent reference analyses (Figure 2). The spectra of each Ugr and Gr sample was measured

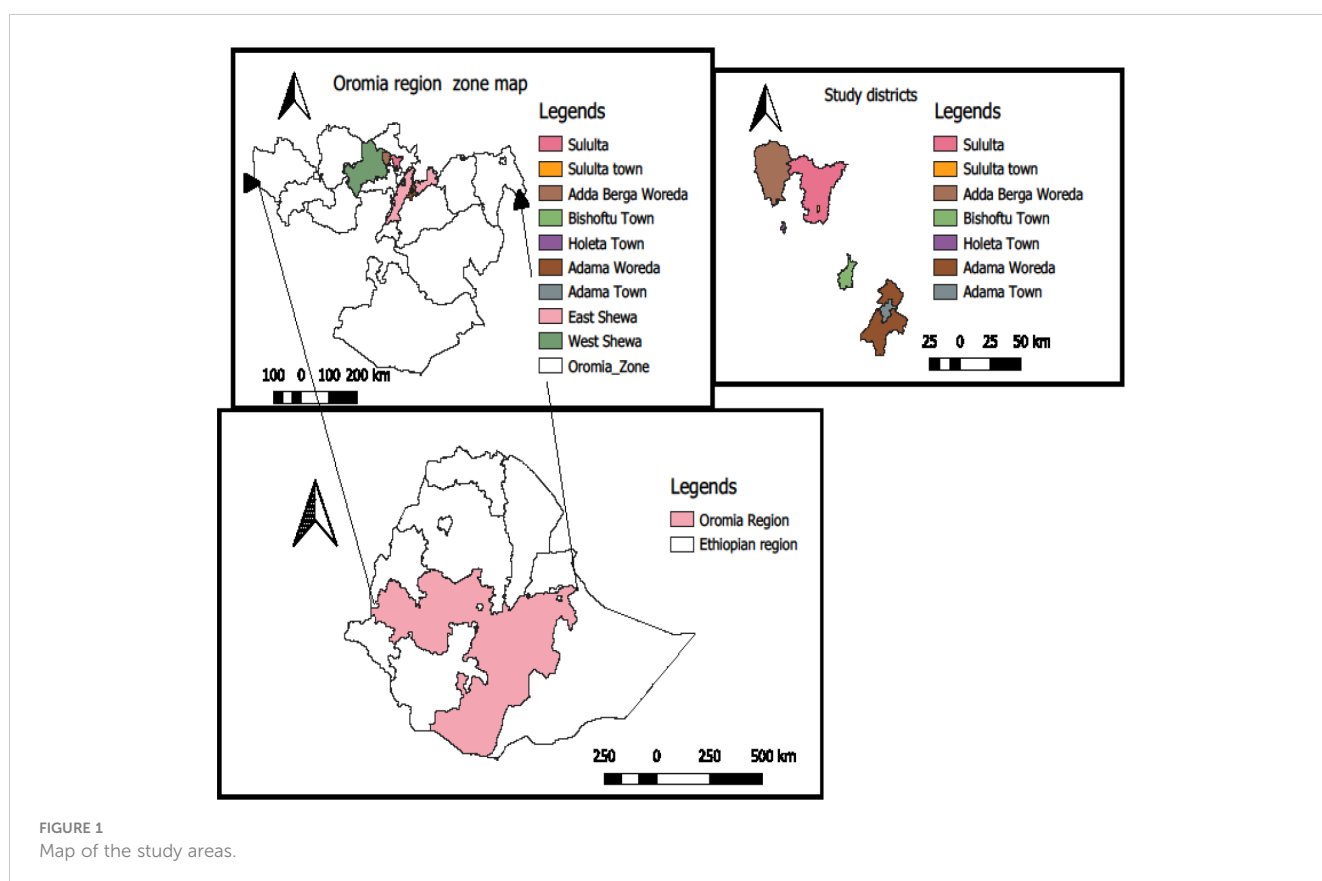




FIGURE 2
Telspec handheld NIR spectrometer (left) and demonstration of how each sample was scanned (right).

with one, two or ten scans (1s, 2s and 10s) in three replicates. Samples were scanned using a Telspec Enterprise model handheld NIR spectrometer (Telspec Enterprise). This NIR spectrometer is a smartphone NIR spectrometer weighing 140 g which measures from 900–1700 nm, with a spectral interval of approx. 3 nm (Croccombe, 2018; Rukundo et al., 2021). Data is collected using a smartphone. The data collection and management software tool designed by Telspec Enterprise (DC&M2) was downloaded from the Google Play-store and installed on a smartphone with a Bluetooth connection to the instrument for spectra collection. The function of the instrument was checked daily using a standard white reference samples supplied by the manufacturer.

Analysis of reference samples

Wet chemistry analyses for each feed attribute in each of the samples was conducted to provide their reference values. Total nitrogen (TN) concentration was determined by the Kjeldahl method with the crude protein (CP) content calculated as $N \times 6.25$ (AOAC, 1995). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) fractions were analyzed according to Van Soest and Robertson (1985). *In vitro* organic matter digestibility (IVOMD) was determined as described by Tilley and Terry (1963).

Spectra data management, calibration and validation

The spectra of the feed samples were exported to the WinISI 3.0 software for chemometric analyses. The spectral data was randomly divided into calibration ($n=95, 2/3$) and validation ($n=47, 1/3$) sets to develop calibration models and then to test their performance (Westad and Marini, 2015; Despal et al., 2020). Calibration equations were developed using modified partial least squares (MPLS) regression after scatter correction using standard normal variate (SNV) and detrend. The mathematical treatment applied was (1, 6, 4, 1), where the first number indicates the order of derivative

(the first derivative of $\log 1/R$), and the gap in nm over which the derivative is calculated, the number of data points used in a first smoothing, and the number of nm over which the second smoothing was applied. The chemometrics were calculated with WinISI version 3.0 software, and the calibration and validation statistics included the standard error of calibration (SEC), $R^2_{cal,SEP}$, R^2_{val} , and the ratio of the SD of the population to the SEP (RPD).

Statistical analysis

The calibration and prediction models were developed by using WINISI 3.0 software. The data obtained from the calibration model and validation SEP were subjected to General Linear Model (GLM) using Statistical Analysis System (SAS, version, 9.0) (SAS, 2002). The Duncan multiple range test was employed for the separation of treatment means after checking for homogeneity of variance.

The model used for calibration and validation statistics mean separation was:

$$Y_{ij} = \mu + F_i + N_j + F_i * N_j + E_{ij}$$

Where, Y_{ij} = Response variable,

μ = overall mean,

F_i = the j^{th} effect of feed form,

N_j = the i^{th} effect of the number of scans,

$F_i * N_j$ = interaction of the i^{th} feed form and the j^{th} the number of scans,

E_{ij} = error.

Results

Composition of oilseed cake samples

The mean, standard deviation (SD) minimum (Min.) and maximum (Max.) values for each feed attribute of the various

TABLE 1 Chemical composition (g/kg DM) of the oilseed cake samples.

	N	Attribute	Min	Max	Mean \pm S.D.
Cotton seed	56	TN	24	51	42 \pm 5
		IVOMD	555	709	653 \pm 30
		NDF	370	653	439 \pm 58
		ADF	314	599	393 \pm 54
		ADL	99	136	112 \pm 8
Linseed	53	TN	42	68	50 \pm 7
		IVOMD	593	716	667 \pm 26
		NDF	187	330	267 \pm 27
		ADF	160	314	229 \pm 31
		ADL	49	120	88 \pm 17
Noug seed	22	TN	42	68	52 \pm 7
		IVOMD	529	659	602 \pm 35
		NDF	266	474	372 \pm 56
		ADF	222	440	306 \pm 50
		ADL	83	156	126 \pm 25
Groundnut	7	TN	61	88	76 \pm 10
		IVOMD	652	815	743 \pm 56
		NDF	178	314	214 \pm 47
		ADF	123	240	163 \pm 40
		ADL	18	97	40 \pm 27
Others	4	TN	42	75	59 \pm 17
		IVOMD	537	786	717 \pm 120
		NDF	202	471	305 \pm 123
		ADF	161	451	241 \pm 141
		ADL	43	147	83 \pm 48
Overall	142	TN	24	88	49 \pm 10
		IVOMD	529	815	657 \pm 47
		NDF	178	653	348 \pm 95
		ADF	123	599	302 \pm 92
		ADL	18	156	100 \pm 27

TN, total nitrogen; IVOMD, In vitro organic matter digestibility; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; others, oilseed meals comprised soybean (n=2), rapeseed (n=1) and sunflower (n=1); S.D., Standard deviation; Min, Minimum; Max, Maximum; n, number of samples.

oilseed cake samples measured by wet chemistry are presented in Table 1. The TN averaged 49 g/kg and ranged from 24 g/kg DM in cotton seed cake to 88 g/kg in a groundnut cake sample. The IVOMD averaged 657 g/kg DM and ranged from 529 g/kg DM in noug seed cake to 815 g/kg DM in a groundnut cake. The NDF, ADF, and ADL contents also had a wide range among the oilseed cake samples. Finally, the mean lignin content of the oil meals was 100 g/kg DM and ranged from 18 g/kg DM in peanut cake to 156 g/kg DM in noug cake.

Calibration and accuracy of prediction of total nitrogen and *in-vitro* organic matter digestibility of oilseed meals

Chemometric analysis of the spectra and the b-coefficients after scatter correction, detrend and first derivative math treatment indicated that a number of spectral regions in the measured range (915 - 1647 nm contributed to the modified partial least squares calibration equations (Figure 3). Generally, the most important

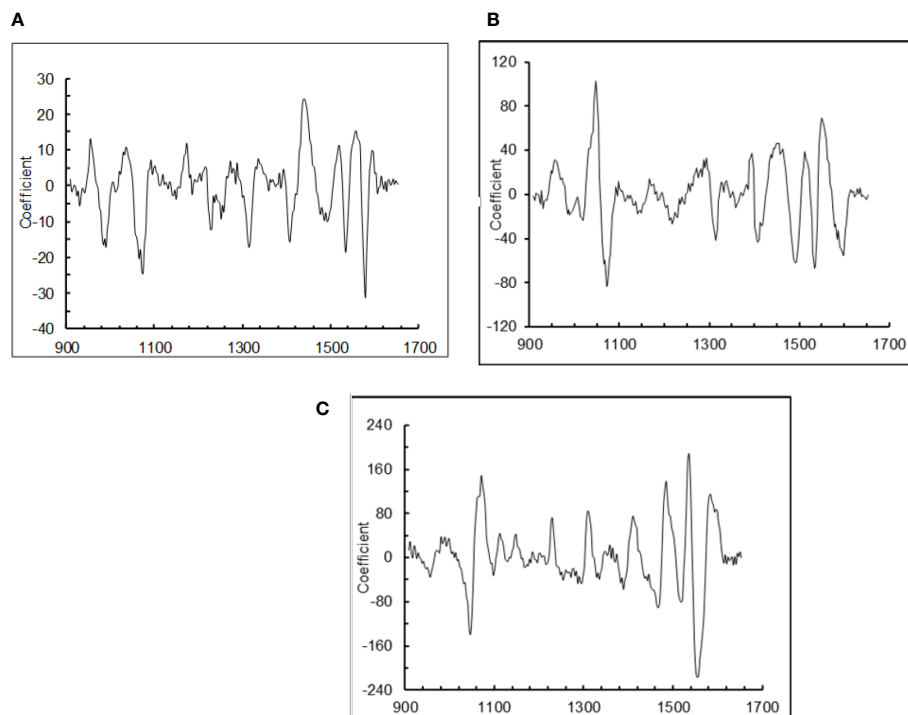


FIGURE 3

B-coefficients for the p1 factors in modified partial least squares (MPLS) calibration models following standard normal variate and detrend and first derivative maths transformation of the spectra of oilseed meals that were dried and ground, and scanned 10 times, for prediction of: (A) total N concentration (TN), (B) *in vitro* organic matter digestibility (IVOMD), and (C) neutral detergent fiber content (NDF) of the oilseed meals. The calibration models used 9, 8 and 8 factors, respectively.

spectral regions contributing to the calibrations were 1000–1100 and 1400–1600 nm, but there were some differences among the feed attributes. The calibration for TN concentration depended primarily on the regions between 940–1080 and 1420–1590 nm. However, calibrations for IVOMD content depended primarily on the regions 1020–1090 and 1430–1560 nm, and for NDF content 1040–1080 and 1480–1590 nm. Hence the 920–1000 nm region was important for TN but not for IVOMD or NDF. The most important spectral regions to predict ADF and ADL tended to be similar to those for NDF.

The calibration and prediction equation statistics for the constituents TN and IVOMD of oilseed meals are shown in Table 2. Both the number of scans (i.e. 1, 2 and 10) and grinding of the samples, and their interactions affected the accuracy of the TN and IVOMD calibrations ($p < 0.001$). However, the number of scans had a much greater effect than the sample form. For TN increasing the number of scans of each sample from 1 to 10 decreased the SEP by 51% (from 6.5 to 3.2 g/kg DM) in Gr samples, but surprisingly had little effect on the SEP in UGr material. However, with measurement of IVOMD there was a different pattern where SEP was reduced by 28% due to increasing the number of scans in both Gr and UGr samples; also the SEP was consistently lower in unground samples. The highest R^2 for calibration and validation for N and IVOMD were obtained for Gr samples scanned ten times (calibration 0.95 and 0.89, validation 0.91 and 0.81, respectively). The relationships between

the reference and predicted values for Gr samples scanned 10x are shown in Figure 4.

Calibration and accuracy of prediction of neutral detergent fiber, acid detergent fiber, and acid detergent lignin concentration of oilseed meals

The fiber composition of oilseed cake samples as NDF, ADF, and ADL were accurately predicted (Table 3). The statistics of R^2_{cal} , SEC, R^2_{val} , and SEP were significantly affected by the interaction between the numbers of scans and grinding of samples ($P < 0.001$). The highest R^2_{cal} for NDF and ADL were found for Gr (0.98 and 0.93, respectively) and UGr samples scanned ten times (0.97 and 0.93, respectively), whereas the maximum (0.92 and 0.94) R^2_{val} for these parameters were obtained for Gr samples scanned ten times, respectively. Similarly, the best R^2_{cal} and R^2_{val} for ADF were observed for Gr samples scanned ten times (0.96 and 0.90), respectively. For NDF and ADF increasing the number of scans of each sample from 1 to 10 reduced the SEP in Gr samples by 50% (from 53.8 to 26.9 g/kg DM) and 52% (from 53.1 to 25.3 g/kg DM), respectively, but not in UGr samples. However, when ADL was measured, the SEP was lowered by 67% (from 20.4 to 6.8 g/kg DM) due to increasing the number of scans in Gr samples, but there was little effect in UGr samples (23% reduction from 13.8 to 10.7). The

TABLE 2 Effects of scanning and sample form on the accuracy of predicting of total nitrogen (TN) and *in-vitro* organic matter digestibility (IVOMD) content of oilseed meals using a Tellspec handheld NIRS.

		TN					IVOMD				
		R ² cal	SEC	R ² val	SEP	RPD	R ² cal	SEC	R ² val	SEP	RPD
Sample form	Number of scans										
Ground	1	0.87 ^d	3.1 ^c	0.53 ^d	6.5 ^a	1.25 ^c	0.81 ^c	12.4 ^d	0.58 ^c	16.2 ^d	1.47 ^d
	2	0.91 ^b	4.0 ^b	0.61 ^b	4.8 ^b	1.54 ^b	0.85 ^b	10.1 ^f	0.73 ^b	13.1 ^e	1.54 ^c
	10	0.95 ^a	2.2 ^d	0.91 ^a	3.2 ^d	2.68 ^a	0.89 ^a	10.7 ^e	0.81 ^a	11.7 ^f	2.3 ^a
Unground	1	0.82 ^e	4.7 ^a	0.47 ^e	4.2 ^c	1.34 ^c	0.13 ^e	23.3 ^b	0.06 ^f	27.9 ^a	1.49 ^{cd}
	2	0.89 ^c	3.9 ^b	0.55 ^d	4.7 ^b	1.39 ^c	0.14 ^e	24.5 ^a	0.09 ^e	26.7 ^b	1.90 ^b
	10	0.94 ^a	2.4 ^d	0.90 ^a	4.5 ^{bc}	1.39 ^c	0.60 ^d	18.2 ^c	0.28 ^d	20.1 ^c	1.94 ^b
	Mean	0.90	3.4	0.66	4.6	1.60	0.57	16.5	0.43	19.3	1.77
SEM	Sample form (SF)	0.18	0.44	0.38	0.05	0.17	0.33	0.37	0.36	0.38	0.57
	Number of scans (NS)	0.23	0.54	0.47	0.62	0.21	0.40	0.46	0.45	0.47	0.70
	SF*NS	0.19	0.38	0.15	0.49	0.31	0.46	0.07	0.26	0.31	0.99
Sig.	SF	***	***	***	***	***	***	***	***	***	ns
	NS	***	***	***	***	***	***	***	***	***	***
	SF*NS	***	***	***	***	***	***	***	***	***	***

^{a-f} means within columns having different superscript are significantly different at ***($P < 0.001$); ns, non-significance; R²cal, Coefficient of determination of calibration; R²val, Coefficient of determination of validation; SEC, Standard error of calibration (g/kg DM); RPD, the ratio of standard deviation of the calibration samples to the SEP; SEM, Standard Error of Mean; Sig, Significance level; SEP, Standard error of performance (g/kg DM).

relationships between the reference and predicted values for Gr samples scanned 10x are shown in Figure 5.

Discussion

Chemical composition of reference samples

The high variation in the chemical composition of the oilseed meals analyzed is a positive factor for calibration when predicting the composition of different types of feeds (Naes et al., 2002; Arzani et al., 2012). The overall mean of the total nitrogen (TN) content in the current study were in accordance with other studies e.g. the

findings of Adugna (2008), who found TN content of oilseed meals ranging from 46.7 to 58.3 g/kg DM, and Fekede et al. (2015), who reported an average TN content of 45.7 to 57.2 g/kg DM g/kg DM for oilseed meals in the central highlands of Ethiopia. Likewise, the observed mean IVOMD was comparable with those reported by the above authors. However, in the current study, the mean ADF content was higher than reported by Fekede et al. (2015). The observed variations in the chemical composition of oilseed cake samples might be associated with the type of the oilseed cake, the method, and efficiency of extracting oil from the seeds, and environmental conditions for crop growth (Adugna, 2008; Fekede et al., 2015). This large variability in chemical composition of reference samples was considered suitable to develop NIRS calibrations (Dardenne et al., 2000).

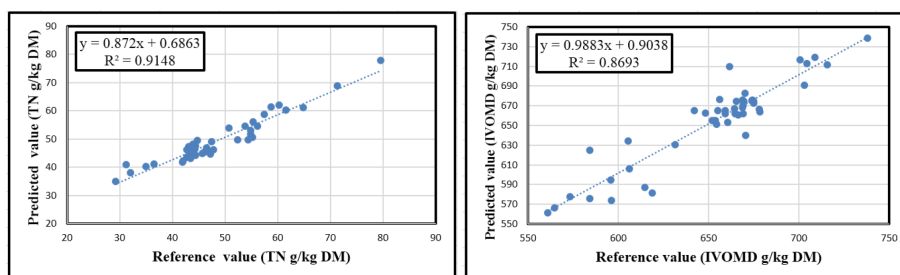


FIGURE 4 The relationships between laboratory determined and NIRS predicted values for TN (right) and IVOMD (left) values of Gr oilseed cake samples scanned ten times.

TABLE 3 Effect of the number of scans, sample form and their interaction on the accuracy of predicting neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) content of oilseed meals using a Telspec handheld NIRS.

Sample form	Number of scans	NDF					ADF					ADL				
		R ² cal	SEC	R ² val	SEP	RPD	R ² cal	SEC	R ² val	SEP	RPD	R ² cal	SEC	R ² val	SEP	RPD
	1	0.86 ^d	35.3 ^b	0.60 ^c	53.8 ^b	1.36 ^e	0.88 ^d	32.1 ^a	0.50 ^f	53.1 ^a	1.27 ^f	0.82 ^c	11.3 ^a	0.53 ^f	20.4 ^a	1.31 ^d
Ground	2	0.95 ^{bc}	24.2 ^d	0.57 ^f	57.4 ^a	1.33 ^f	0.89 ^{cd}	30.2 ^b	0.59 ^e	50.4 ^b	1.43 ^e	0.83 ^c	11.2 ^a	0.58 ^e	20.8 ^a	1.36 ^d
	10	0.98 ^a	16.8 ^e	0.92 ^a	26.9 ^f	3.17 ^a	0.96 ^a	17.3 ^f	0.90 ^a	25.3 ^f	3.05 ^a	0.93 ^a	6.6 ^e	0.94 ^a	6.8 ^e	4.06 ^a
Unground	1	0.84 ^d	25.3 ^c	0.73 ^d	37.5 ^e	1.91 ^d	0.90 ^c	26.7 ^d	0.77 ^d	31.2 ^e	2.07 ^c	0.87 ^b	10.4 ^b	0.67 ^d	13.8 ^b	1.67 ^c
	2	0.93 ^c	36.2 ^a	0.76 ^c	39.4 ^d	1.97 ^b	0.90 ^c	28.7 ^c	0.80 ^c	32.5 ^d	2.20 ^b	0.89 ^b	9.2 ^c	0.77 ^c	12.1 ^c	2.03 ^b
	10	0.97 ^{ab}	16.8 ^e	0.85 ^b	43.8 ^c	1.94 ^c	0.92 ^b	26.2 ^e	0.87 ^b	44.6 ^c	1.74 ^d	0.93 ^a	7.6 ^d	0.84 ^b	10.7 ^d	1.74 ^c
Mean		0.92	25.8	0.74	43.1	1.95	0.91	26.9	0.74	39.5	1.96	0.88	9.4	0.72	14.1	2.03
	Sample form (SF)	0.33	0.27	0.29	0.31	0.23	0.22	0.24	0.24	0.29	0.26	0.24	0.49	0.35	0.52	0.12
SEM	No of scans (NS)	0.41	0.33	0.36	0.38	0.28	0.27	0.32	0.31	0.36	0.31	0.34	0.65	0.43	0.63	0.14
	SF*NS	0.46	0.25	0.15	0.36	0.4	0.24	0.18	0.34	0.65	0.45	0.38	0.54	0.95	0.19	0.2
	Sample form (SF)	**	***	***	***	***	ns	***	***	***	***	***	***	***	***	***
Sig.	No of scans (NS)	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
	SF*NS	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***

^{a-f}means within columns having different superscript are significantly different at **($P < 0.01$); ***($P < 0.001$); ns, non-significance; R²cal, Coefficient of determination of calibration; R²val, Coefficient of determination of validation; SEC, Standard error of calibration (g/kg DM); RPD, the ratio of standard deviation of the calibration samples to the SEP; SEM, Standard Error of Mean; Sig, Significance level; SEP, Standard error of performance (g/kg DM).

Calibration and validation statistics

There is extensive evidence in the literature that NIR spectroscopy is suitable for evaluating the TN content of various types of animal feeds (Andueza et al., 2011; Chen et al., 2015; Rushing et al., 2016). The observed R² values for prediction of TN in the present study were consistent with findings from Rukundo et al. (2021), who reported R²cal (0.96) and R²val (0.90) on mixed feeds using Foss and ASD Quality NIRS instruments, respectively. However, the R²cal value was higher than the reports of Aufrere et al. (1996); Modrono et al. (2017), and Wiedemair et al. (2019) who reported using Foss 6500 (0.92), Phazir (0.91), and Phazir (0.57) spectrophotometers for compound feeds, respectively, and lower than the study of Perez-Marin et al. (2004) who found 0.98 and 0.97 on ground and unground compound feeds, respectively, using Foss 6500 instrument. In the present study, the standard error of calibration (SEC) and standard error of prediction (SEP) for TN were lower than in previous studies reported by Aufrere et al. (1996); Swart et al. (2012), and Modrono et al. (2017). Also the results of the current study showed that as the number of scans increased from one to ten, the SEC decreased by 29% (from 3.1 to 2.2 g/kg DM) and 49% (from 4.7 to 2.4 g/kg DM) on D&Gr and UGr samples, respectively. Likewise, the SEP decreases by 51% (from 6.5 at one scan to 3.2 g/kg DM at ten scans) on Gr samples.

Apart from the TN content, the quality trait most frequently measured by NIR spectroscopy in forage crops is digestibility. The

IVOMD calibration model had a greater R²cal (0.89) than those found previously by Rukundo et al. (2021) on mixed feeds and forage with a Foss XDS (0.82), Foss 6500 (0.80), and Telspec (0.83) NIRS instruments and by Xiccato et al. (2003) who found (0.84) using a Foss 6500 with compound feeds. However, the R²cal and R²val values for the IVOMD model were lower than the values reported by Prasad et al. (2019) on roughage feeds using a Phazir (0.96) and Telspec (0.91). This could be related to the narrow range of values found in the samples analysed for this parameter, in contrast to that of TN, NDF, ADF and ADL. The SEC value for IVOMD in the present study was in line with the report of Prasad et al. (2019) (26.3 g/kg DM) using a Telspec handheld NIRS instrument on roughage feeds but lower than the report of Dereje et al. (2010) on natural pasture hay (41.2 g/kg DM) using a Foss 5000, and higher than the findings of Andueza et al. (2001) on alfalfa hay (20.5 g/kg DM) using a Foss 6500, and Prasad et al. (2019) on roughage feeds (20.8 g/kg DM) using a Phazir instrument. Similarly, the SEP for IVOMD prediction was lower than the study carried out by Rukundo et al. (2021) using Telspec (34.5), Foss XDS (40.0), Foss 6500 (41.7), and ASD Quality (40.8) instruments. The present study indicated that when the number of scans increased from one to ten, the SEC decreased 14% (from 12.4 to 10.7 g/kg DM) and 22% (from 23.3 to 18.2 g/kg DM) on ground and unground samples, respectively. The SEP IVOMD prediction decreased as well by 29% (from 27.9 to 20.1) on UGr samples, and by 28% (from 16.2 to 17 g/kg DM) on Gr samples

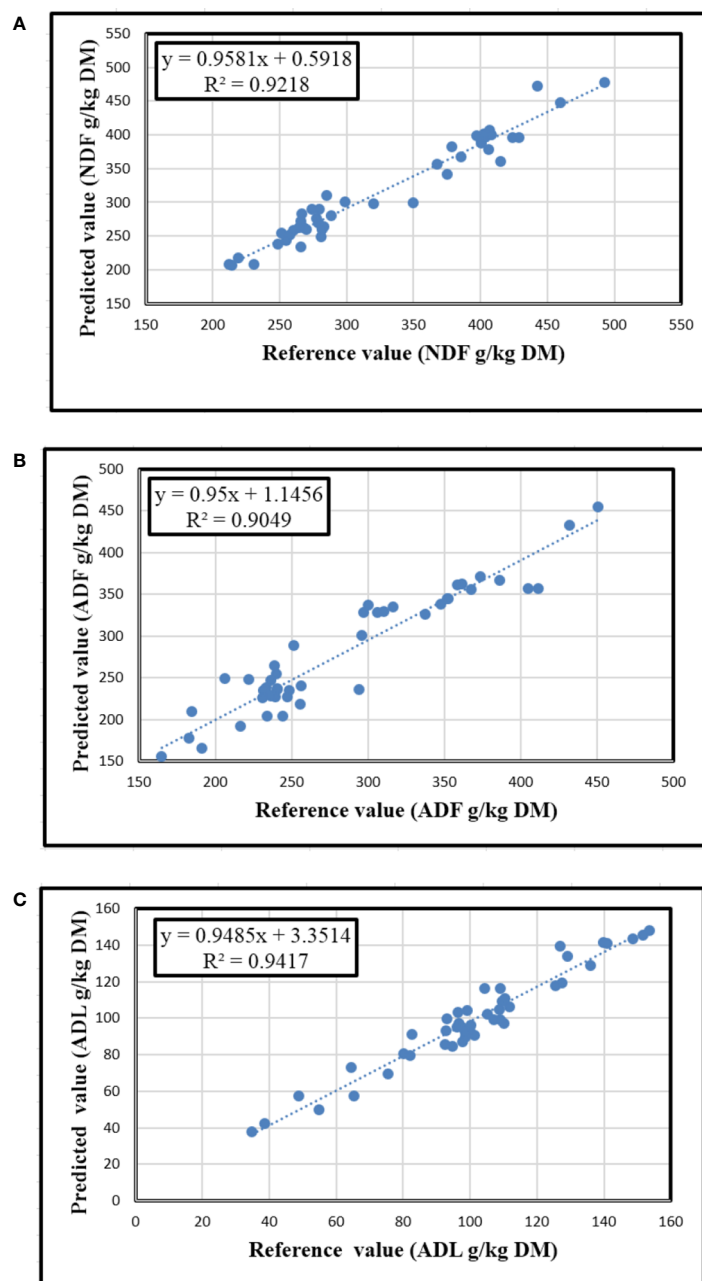


FIGURE 5

The relationships between laboratory determined and NIRS predicted values for NDF (A), ADF (B), and ADL (C) contents of Gr oilseed cake samples scanned ten times.

when the scanning frequency increased from one scan to ten scans.

Many studies showed that NDF and ADF concentrations could be well predicted by NIRS in forage (Chen et al., 2015; Rushing et al., 2016). In this study, the performance of the models for NDF and ADF were successful and the precision of the models was consistent with previous studies (Prasad et al., 2019; Mazabel et al., 2020). The R^2_{cal} value for the NDF equation in the current study was higher than those from the study reported by Aufreire et al. (1996); Modrono et al. (2017), and Swart et al. (2012) in compound feed and total mixed ration using Foss 6500 (0.92), Phazir (0.91),

and FT-NIR (0.94), respectively. This finding shows that 98% and 97% of the observed variation in NDF concentration in both UGr and Gr oilseed cake samples, respectively, was explained by NIRS scans when samples were scanned ten times. Similarly, the accuracy of the Telspec handheld NIRS to predict the NDF content of ground oilseed cake samples scanned in locally available plastic bags was excellent and the observed R^2_{val} (92%) fitted the regression line, or 92% of the variation observed on dependent variables was explained by independent variables. The SEC for the NDF equation was in accordance with the study conducted by Prasad et al. (2019). Likewise, the R^2_{val} value for the NDF prediction was in agreement

with the report of Mazabel et al. (2020) and Andueza et al. (2001) on Brachiaria grass (0.92) and alfalfa hay (0.92), respectively. Similarly to other parameters, the SEC and SEP for NDF decreases by 52 and 50% as the scanning frequency increase from one to ten, respectively. Although the precision of the NDF (0.92) and ADF (0.90) models was also lower when compared to ADL (0.94), they were still sufficient to differentiate between high and low NDF and ADF content in oilseed meals.

The findings in the present study revealed that a ‘robust’ calibration was developed for the prediction of ADF content in oilseed cake samples, with higher R^2_{cal} (0.96) and R^2_{val} (0.90) and lower SEC (32.1 g/kg DM) and SEP (53.1 g/kg DM) values when compared with the results reported by other authors. The precision of the calibration model pertaining to the R^2_{cal} value obtained in this study was consistent with Aufrere et al. (1996) on compound feed using Foss 6500 (0.95), Swart et al. (2012) on the total mixed ration using an Infralyzer 500 (0.95), and Prasad et al. (2019) on roughage feeds using Telspec (0.96), but higher than Xiccato et al. (2003) on compound feed using an InfraAnalyzer 500 (0.84) instrument. Lyons and Stuth (1992) stated that an R^2 value of above 0.90 is considered good for agricultural products. Similarly, the R^2_{val} value obtained in this study was in accord with the report of Acosta et al. (2020) on grass (0.87) using a Nano NIRS instrument. It was higher than the study of Rukundo et al. (2021) who reported (0.63) R^2_{val} for mixed feed and forage using a Telspec, and lower than Prasad et al. (2019), who obtained 0.97 and 0.96, respectively, using Phazir and Telspec instruments on roughage feeds. The SEC and SEP for ADF reduced by 46 and 52%, respectively, when the scanning frequency increases from one to ten on Gr samples.

Among the fiber components, lignin is the one most commonly measured by NIR spectroscopy. Lignin calibrations have been reported mostly for grasses and legume species commonly used to produce hay and pasture in subtropical regions (Roberts et al., 2004). The R^2_{cal} (0.93) and R^2_{val} (0.94) for ADL was higher than the study reported by Goi et al. (2020) on ground ($R^2_{cal} = 0.76$) and intact ($R^2_{cal} = 0.78$) kibbles using a SCiO instrument, and Despal et al. (2020) on roughage feeds ($R^2_{val} = 0.77$) by FT-NIR, and Rukundo et al. (2021) on mixed feeds ($R^2_{val} = 0.62$). The R^2 of validation for ADL in the current study might be an indication of the high performance of the Telspec instrument to predict the ADL content of unknown samples, and 94% of the observed data fitted the model. The SEC for ADL was comparable with the study conducted by Despal et al. (2020) on roughage feeds (10.9 g/kg DM) by using a FT-NIR instrument and higher than the study of Goi et al. (2020) who reported 0.30 and 0.29 on ground and intact kibble samples, respectively, using a SCiO NIR spectrometer device. The current result indicates that Telspec has the potential to measure the ADL concentration of both Gr and UGr oilseed cake samples. The observed SEP value for ADL was higher than Despal et al. (2020) findings for roughage feeds (10.7 g/kg DM) using FT-NIR, but lower than Rukundo et al. (2021) results for mixed feeds (13.58) using the Telspec instrument.

For most of the variables the ratio of standard deviation to the SEP (RPD) g/kg DM found was higher than 3 for NDF (RPD = 3.17), ADF (RPD = 3.05), and ADL (4.06), while RPD were less than 3 for TN (RPD = 2.68) and IVOMD (RPD = 2.30). The RPD values represent the ability of the NIRS model to predict the nutrient content of animal feeds (Williams and Sobering, 1993). An RPD value of more than 2 was

categorized as a relevant prediction of NIRS (Baillères et al., 2002). In this study, the developed oilseed meals prediction equation was relevant in predicting the oilseed cake samples composition because the RPD value found for TN, IVOMD, NDF, ADF, and ADL were higher than 2 for Gr samples scanned ten times. However, Lobos et al. (2013) gave a higher category (RPD > 2.5) as a valid measurement ability of a NIRS model to predict the constituent of a particular feed. In this case, the IVOMD database produced needs to be improved. The low RPD found in the prediction of IVOMD might be related to the interaction between laboratory measurements and the collected spectra of a particular feed. According to Williams (2004) there are five categories of prediction accuracy based on RPD values, i.e., 1) the RPD < 1.5 indicated an unusable; 2) the 1.5 < RPD < 2.0 categorized as the ability of the prediction to distinguish between high and low values; 3) the 2.0 < RPD < 2.5 produced an “approximate” quantitative prediction; 4) the 2.5 < RPD < 3.0 reflected a “good” quantitative prediction; and 5) the RPD > 3.0 indicated an “excellent” quantitative prediction.

The discrepancy in R^2_{cal} , R^2_{val} , SEC, SEP, and RPD for TN, IVOMD, NDF, ADF, and ADL might be associated with instrumental differences, feed samples, feed forms, number of scans, scanning material, environmental conditions of crop growth, and the interaction between feed forms and number of scans. Generally, the current results indicate that increasing the number of scans leads to higher R-square values and lower standard errors of calibration (SEC) and standard errors of prediction (SEP). By scanning multiple times the various traits measured could be predicted with reasonable precision, implying that the composition predicted by NIRS agreed closely with that of chemical analysis for the studied quality components.

Conclusion

The results of this study indicate that there is strong potential to use Telspec handheld NIRS devices to predict the chemical composition of UGr and Gr oilseed cake samples. The accuracy of the prediction was improved by increasing the number of scans per sample and drying and grinding prior to scanning. Therefore it would be important to consider using mobile grinders that can be used *in situ* prior to spectra collection with a Telspec NIRS device. Further work to refine the calibration equations taking into account environmental and sample handling factors that affect the spectra data would be important.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

MW did the experimental design work, conducted the experiment, conducted NIRS scans, did the laboratory analysis, analyzed the data, wrote, and revised the manuscript. YA and KP

supported laboratory analysis, data analysis, and revised the manuscript. AD and MB supported in project conception. FM, MBD, MD, CJ, RD and AD provided support in developing and revising the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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