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Determination of forage quality by near-infrared reflectance spectroscopy in soybean

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Abstract: Soybeans have been a favored livestock forage for centuries. However, only a few studies have been conducted to estimate the forage quality of soybean by near-infrared reflectance spectroscopy (NIRS). In this study, 353 forage soybean samples were used to develop near-infrared reflectance (NIR) equations to estimate four forage quality parameters: crude protein (CP), crude fat (CF), neutral detergent fiber (NDF), and acid detergent fiber (ADF). Samples included 181 recombinant inbred lines derived from PI 483463 (*G. soja*) × Hutcheson (*G. max*), 104 cultivated soybeans (*G. max*), and 68 wild soybeans (*G. soja*). Two NIR equations developed for CP and CF (2,5,5,1; multiple scatter correction [MSC]) and for NDF and ADF (1,4,4,1; MSC) were the best prediction equations for estimating these parameters. The coefficients of determination in the external validation set (r^2) were 0.934 for CF, 0.909 for CP, 0.767 for NDF, and 0.748 for ADF. The relative predictive determinant ratios for MSC (2,5,5,1) calibration indicate that the CP (3.25) and CF (3.85) equations were acceptable for quantitative prediction of soybean forage quality, whereas the NDF (2.07) and ADF (1.97) equations were useful for screening purposes. The NIR calibration equations developed in this study will be useful in predicting soybean forage quality for these four quality parameters.

Key words: Near infrared reflectance spectroscopy (NIRS), forage quality, soybean, wild soybean

1. Introduction

Soybean [*Glycine max* (L.) Merr.] is an important annual legume crop for human and animal consumption. In Asia, soybeans, which are rich in edible protein and oil, have been a popular food crop for centuries. However, in other regions (e.g., United States), the early use of soybeans was for forage (Gibson and Benson, 2005). Soybeans are a reliable source of quality forage during the summer, when other forage brassicas or legumes have been harvested or are unavailable (Mihailović et al., 2013). In addition, soybean forage production is advantageous because the production of high-quality forage legumes such as alfalfa (*Medicago sativa* L.) in Eastern Asia has often been challenged by unfavorable environmental conditions, high costs, and carbon footprint issues (Chang et al., 2012). A review by Lima-Orozco et al. (2013) concluded that soybean forage could be successfully used in ruminant diets because it has less impact on the environment through reduced methane emissions with an acceptable energy content and nutrient digestibility. Studies have shown that soybean forage could be a good substitute for imported legume forages because it has high nutritive value at a low cost (Açikgöz et al., 2007; Vargas-Bello-Perez et al., 2008; Chang et al., 2012; Drewnoski and Poore, 2012; Zambom et al., 2012).

The yield and chemical composition of soybean plant biomass depend on genotype, growth stage, management practices, and growing environment (Hintz et al., 1992; Hintz and Albrecht, 1994; Sheaffer et al., 2001; Chang et al., 2012; Asekova et al., 2014). Recent investigations of soybean cultivars specifically developed for forage production concluded that adapted grain cultivars were the most suitable for forage production.

Soybean dry matter yields increase as developmental stages progress from R1 to R7 (Munoz et al., 1983; Hintz et al., 1992). In general, forage quality can be determined by measuring the crude protein (CP), crude fat (CF), acid detergent fiber (ADF), neutral detergent fiber (NDF), total digestible nutrients (TDN), and relative feed value

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(RFV). Heitholt et al. (2004) evaluated the quality of forage soybean cultivars by developmental stage and determined that forage harvested at the full seed stage (R6) had the most optimal yield and quality; CP was 8.7%–17.2%, ADF was 24.1%–33.6%, and NDF was 33.2%–48.9%. Wild soybean (*Glycine soja* Sieb. and Zucc.), the ancestor of the cultivated soybean, is widely available in the Far East and possesses high quality forage with an average mass CP concentration of up to 19.1% (Zhai et al., 2008). The forage yield and quality for soybean lines derived from *G. soja* × *G. max* were evaluated by Lee et al. (2014), and they reported that the interspecific cross between *G. max* and *G. soja* would be an excellent method to develop forage soybean with good yield and quality.

The conventional methods used to measure the forage quality components CP, CF, ADF, and NDF have been wet chemistry methods. CP content can be determined by N combustion and the micro-Kjeldahl technique, whereas NDF and ADF are determined by refluxing with the required solutions and amylase modification of the sequential analysis procedure (Hintz et al., 1992; Açıkgöz et al., 2013). However, these methods are labor intensive, expensive, and inefficient.

Near-infrared reflectance spectroscopy (NIRS) is an indirect and efficient method of measuring the chemical composition of feedstuffs based on the unique near infrared absorption properties of the major chemical components of a sample. Near-infrared reflectance (NIR) analysis has been used for over 30 years to differentiate the quality of various forage crops. The NIR method has advantages such as rapid determination, minimal preparation of samples, nonconsumptive analyses, multiplicity of sample preparation in one operation, no consumption of reagents, and ultimately low marginal costs of analyses (Lee et al., 2011).

The nutrient compositions of forage crops have been investigated using NIRS for Leucaena species (Wheeler et al., 1996), maize (*Zea mays*) (Volkers et al., 2003; Montes et al., 2009), rye silage (Moe and Carr, 1985), pigeon pea (*Cajanus cajan*) (Berardo et al., 1997), alfalfa (*Medicago sativa*), white clover (*Trifolium repens* L.) (Cozzolino and Moron, 2004), roasted whole cultivated soybeans (Tremblay et al., 1996), and legume–grass mixtures (Locher et al., 2005). However, no study has determined soybean forage quality using NIRS for forage cultivar improvement and feed industry programs.

The objective of this study was to develop calibration equations for NIRS determination of the soybean forage quality parameters CP, CF, NDF, and ADF.

2. Materials and methods

2.1. Soybean materials

To establish NIR equations for soybean forage quality, 353 forage soybean samples were used for this study. The samples were used from 181 recombinant inbred lines (RILs) derived from PI483463 (*G. soja*) × Hutcheson (*G. max*) (Ha et al., 2013), 104 cultivated soybeans (*G. max*) randomly selected from a soybean germplasm collection at Kyungpook National University, and 68 wild soybeans (*G. soja*) that were collected from Korea. The soybean populations were planted on 4 July 2012 at the Gyeongsangbuk-do Agricultural Research Service farm in Daegu, Republic of Korea. The RILs and wild soybean genotypes were planted in hill plots in rows, with 100 cm between hills and 100 cm between rows. For the cultivated soybeans, each soybean was planted in a hill plot with 50 cm between hills and 50 cm between rows. Weeds were controlled manually.

A single soybean plant from each plot was collected at the R6 growth stage (full seed stage) to test the forage quality traits as reported (Munoz et al., 1983; Hintz et al., 1992). To obtain an exact chemical concentration of each forage sample, the moisture content for each sample was determined from 1 g of forage powder by using a Sartorius moisture analyzer MA35 (Sartorius AG, Göttingen, Germany). Each sample was heated by infrared radiation from a tubular metal heating element on aluminum panels within 30 s. Samples including leaves, stems, and soybean pods were dried in a forced-air oven (YS-120203N, Vision Scientific Co., LTD, Korea) at 60 °C until they reached a constant weight. The samples were subsequently ground in a Cyclotec mill (Tecator, DK-3400 Hillerød, Denmark) and passed through a 1-mm sieve. The CP, CF, NDF, and ADF values were calculated on a dry weight basis.

2.2. Crude protein and fat analysis

The crude protein content of the forage for each soybean sample was analyzed using the Kjeldahl method AOAC (1990). Approximately 1 g of plant material sample was used to determine the percentage of nitrogen content in the forage. A nitrogen auto-analyzer (VAP50sC, Gerhardt, Germany) was used to measure the nitrogen content. Then the nitrogen percentage was multiplied by 6.25 to determine the protein content.

The crude fat content was determined by the auto-soxhlet method with a Soxtherm apparatus (Gerhardt, Bonn, Germany). Crude fat was determined from about 2 g of dried plant material by extraction in 150 mL of *n*-hexane for 90 min at 180 °C. Samples were allowed to reflux for 4 h.

2.3. NDF and ADF analysis

The ADF and NDF values were determined from 0.5-g plot samples using a Fiber Analyzer (ANKOM^{2000} Fiber Analyzer, Ankom Technology, Macedon, NY, USA) based on the Goering and Van Soest (1970) method and the Van Soest et al. (1991) method. The solvent used for the ADF was an acid detergent solution (FAD20C, ANKOM Tech.). The reagents used for the NDF analysis were neutral

detergent solution (FND20C, ANKOM Tech.), sodium sulfate, and heat stable alpha-amylase.

2.4. Spectra collection and pretreatment

The milled 353 forage samples were scanned on a XDS-NIRS rapid content analyzer (FOSS Analytical, Slangerupgade, Denmark) to compute reflectance spectra and stored as the reciprocal logarithm (log (l/*R*) without replicates over a wavelength range of 400 to 2498 nm at every 2 nm to give a total of 1050 data points. Samples (ca. 5 g) were loaded into a small, round metal cup of 3.75 cm in diameter (US-ISIH-0305) with disposable backs (US-ISIH-0309) to seal the ground samples. Each sample was subsequently scanned 20 times and an average spectrum was collected to process calibration, cross-validation, and external validation. The data from the wet chemistry analyses were entered into the NIRS database to derive a relationship with the absorbance spectra.

2.5. Calibration

The spectra were collected and managed using ISI scan software (Infrasoft International Port Matilda, PA, USA) and calibration models were developed using WinISI software, version 1.50. The calibration was performed using the recommended modified partial least squares (MPLS) as well as partial least squares (PLS) and principal component regression (PCR) in developing compatible calibrations for soybean forage components. Two different outliers, T (reference value – predicted value/SEC) (Shenk and Westerhaus, 1996; Patil et al., 2010) and GH (distance from a sample point to center, Mahalanobis distance) were used for organization of calibration spectra. The number of outlier passes was 4. Outliers with large residuals (T > 2.5, GH > 10) were eliminated and the calibration was performed again. Prior to MPLS regression, a multiple scatter corrections (MSC) algorithm was applied. The MSC was predominantly used to treat scatter on an individual spectrum by performing a linear transformation of each spectrum to best match the mean spectrum of the whole set (Isaksson and Næs, 1988).

Employing a single mathematical treatment for all four (CP, CF, NDF and ADF) constituents for the cross-validation procedure, statistical analysis for fiber contents (NDF and ADF) was not acceptable ($R^2 < 0.7$). To improve the statistical results, two mathematical treatments, 1,4,4,1, from the first derivative (D1) for NDA and ADF and 2,5,5,1, from the second derivative (D2) for CP and CF were used to maximize the calibration in order to more accurately predict these values for forage quality. Each number indicates the order of the derivative with the first number being the first and second derivatives of log l/*R*, the second number being the gap in the data points in which the derivative was calculated, and the third and fourth numbers referring to the number of data points used in the first and second smoothing, respectively.

2.6. Cross-validation

The Score and Global programs in WinISI were used in developing compatible calibrations and cross-validations for the soybean forage components. The Score algorithm ranks spectra according to H statistics (Mahalanobis distance) from the average spectrum of the file using principle component scores to identify outliers; spectra with H > 3.0 and samples with H < 0.6 from their nearest neighboring samples were eliminated. The Global program provided several regression techniques (MPLS, PLS, PCR) in the ISI package (Shenk and Westerhaus, 1996). The performances of the different equations obtained in the calibration were determined from cross-validation as an internal method. Four cross-validation groups were used to select the optimal number of PLS terms and to avoid overfitting the equations by selecting 16 PLS terms in each model suggested from the software for the more than 300 samples. The standard error of calibration (SEC), the coefficient of determination (R^2), the standard error of cross validation (SECV), and one minus the ratio of unexplained variance to total variance (1 – VR) were calculated to evaluate the predictive ability of the models (Windham et al., 1989). Relative predictive determination for cross-validation (RPDc) was calculated (SD of reference data/SECV) to test the accuracy of the calibration models developed (Font et al., 2003; Patil et al., 2010). The equations for each soybean forage parameter were screened based on minimizing the SEC and SECV, and maximizing the 1 – VR. The R^2 and RPDc values were used as criteria for evaluation of the optimal performance of the calibration equations (Patil et al., 2010).

2.7. External validation

From the calibration sample set (n = 353), 100 samples were randomly selected using the Monitor program in WinISI to check the NIR calibration equations independently. It is preferable that samples used in the validation should be obtained from different sources. Hence, an independent test set representing a complete range of proximate (crude protein, crude fat) and fiber (NDF/ADF) compositions were used for validation of each model. The procedure defines two control limits to determine if a meaningful bias is occurring and if a meaningful increase in unexplained error is occurring. The output includes bias limits and values for global (GH) and neighborhood (NH) spectral distances. As a part of the validation, the coefficient of determination in validation (r^2), the standard error of performance (SEP), the standard error of prediction corrected for bias [SEP(C)], the bias (mean difference between NIR predicted and reference concentration), the relative predictive determinant [RPDv = SD of the external validation set data/SEP(C)] to determine the accuracy of prediction (Williams and Norris, 2001), and the range to error ratio (RER) [Range/SEP(C)] were used as additional techniques to evaluate the predictive ability of the models.

3. Results

The descriptive statistics for the calibration and validation sample sets, the mean, range, and standard deviation are given in Table 1. In the sample set, there was a wide range in variation for CP, CF, NDF, and ADF concentrations. The range and mean values in the calibration sets for CP, CF, NDF, and ADF were, respectively, 10.9%–25.7% with an average of 19.6%, 0.7%–10.5% with an average of 4.4%, 37.4%–66.6% with an average of 50.7%, and 22.6%–38.1% with an average of 29.4%. The validation sample sets also had similar range and mean values for CP, CF, NDF, and ADF with the actual calibration sets (Table 1). The mean values of CP, CF, NDF, and ADF for the validation sample sets were 19.5%, 3.9%, 48.9%, and 28.8%, respectively.

3.1. Cross-validation

The coefficient of determination in calibration (R^2), SECV, SEC, and the subtracted value of the ratio of unexplained variance to total variance from unity (1 – VR) were obtained by the MPLS regression method and are shown in Tables 2 and 3.

In developing the NIRS equation for CP and CF, the second derivative transformation equation (2,5,5,1; MSC) was best for assessing forage quality components as compared to the other mathematical treatments tested. Likewise, the first derivative transformation equation (1,4,4,1; MSC) of the raw (log l/*R*) data was best for NDF and ADF (Tables 2 and 3).

The R^2 values from the calibration statistics for CP and CF were 0.922 and 0.942, respectively (Table 2), while the R^2 for NDF and ADF were 0.848 and 0.789, respectively (Table 3). The 1 – VR values developed were 0.911 for CP, 0.916 for CF, 0.818 for NDF, and 0.749 for ADF.

The RPDc was 3.34 for CP, 3.45 for CF, 2.34 for NDF, and 1.97 for ADF (Tables 2 and 3) for all calibration equations. The calibration equations for CP and CF had RPDc values higher than 3.0 while the RPDc values were between 1.5 and 2.4 for the NDF and ADF equations.

3.2. External validation of calibration models

The external validation results of the calibration models developed with the MPLS regression method for CP, CF, NDF, and ADF soybean forage quality traits are shown in Tables 4 and 5. The variation in the samples was well depicted by the NIRS predictions. Based on the bias, standard error of prediction corrected for bias SEP(C), and r^2 statistics, the NIR calibration equation models accurately predicted CP, CF, NDF, and ADF constituents of the validation set (Tables 4 and 5). The validation r^2 values for the CP and CF (Table 4) as well as for the NDF

Table 1. Descriptive statistics of soybean parameters used in calibration and validation sets for predicting forage quality with near-infrared reflectance spectroscopy (NIRS)[a].

Groups	CP (%)		CF (%)		NDF (%)		ADF (%)	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Calibration (n = 353)	10.9–25.7	19.6 ± 2.2	0.7–10.5	4.4 ± 1.7	37.4–66.6	50.7 ± 4.6	22.6–38.1	29.4 ± 2.8
Validation (n = 100)	14.4–24.8	19.5 ± 2.2	1.1–10.5	3.9 ± 2.1	37.4–58.8	48.9 ± 4.1	22.6–37.6	28.8 ± 2.8

[a] = Reported values are means of a replicate ± standard deviation. CP, CF, NDF, and ADF are crude protein, crude fat, neutral detergent fiber, and acid detergent fiber, respectively.

Table 2. Equation development statistics using modified partial least squares (MPLS) and multiple scatter correction MSC (D2) (2,5,5,1) to predict forage quality by using near-infrared reflectance spectroscopy (NIRS)[a].

Groups	N	Mean	SD	Calibration		Cross-validation		RPDc
				SEC	R^2	1 – VR	SECV	
Crude protein	336	19.62	2.17	0.608	0.922	0.911	0.650	3.34
Crude fat	319	4.41	1.61	0.387	0.942	0.916	0.467	3.45

[a] = Reported values were calculated from the number of samples (N) collected from outlier filtration used to perform the calibration model. SEC = standard error of calibration, R^2 = the coefficient of determination in calibration, 1 – VR = one minus the ratio of the unexplained variance to the total variance, SECV = the standard error of cross-validation, RPDc and SD/SECV = the ratio of SD (standard deviation of reference data) to SECV in the calibration set, and D2 = the second derivative.

Table 3. Equation development statistics using modified partial least squares (MPLS) and multiple scatter correction (MSC; D1) (1,4,4,1) to predict forage quality by using near-infrared reflectance spectroscopy (NIRS)[a].

Groups	N	Mean	SD	SEL	Calibration		Cross-validation		RPDc
					SEC	R^2	1 – VR	SECV	
NDF	332	50.64	4.3	0.027	1.677	0.848	0.818	1.836	2.34
ADF	336	29.19	2.6	0.002	1.192	0.789	0.749	1.320	1.97

[a] = Reported values were calculated from the number of samples (N) collected from outlier filtration used to perform the calibration model. SEC = standard error of calibration, R^2 = the coefficient of determination in calibration, 1 – VR = one minus the ratio of the unexplained variance to the total variance, SECV = the standard error of cross-validation, RPDc and SD/SECV = the ratio of SD (standard deviation of reference data) to SECV in the calibration set, D1 = the first derivative, SEL = the standard error of the laboratory calculated by the standard deviation between the two laboratory measurements.

Table 4. Monitoring statistics of the eternal validation set (modified partial least squares [MPLS] and multiple scatter correction [MSC] '2,5,5,1') to predict forage quality by using near-infrared reflectance spectroscopy (NIRS)[a].

Groups	N	Mean	Range	SD	SEP	r^2	SEP(C)	bias	slope	RPDv	RER
Crude protein	93	19.34	14.4–24.8	2.11	0.912	0.909	0.649	0.644	1.067	3.25	16.02
Crude fat	93	3.70	1.1–10.5	1.91	0.537	0.934	0.497	–0.208	1.040	3.85	18.91

[a] = Reported values were calculated from the number of samples (N) in the external validation set. SEP = standard error of performance, r^2 = the coefficient of determination in the external validation, SEP(C) = the corrected standard error of prediction, bias = the average difference between the reference and NIRS values, slope = the steepness of a straight line curve, RPD_v and SD/SEP(C) = the ratio of SD (standard deviation of the reference data in the validation set) to SEP(C) in the validation set, and RER = the range-to-error ratio (RER) [Range/SEP(C)].

Table 5. Monitoring statistics of the external validation set (modified partial least squares [MPLS] and multiple scatter correction [MSC] '1,4,4,1') to predict forage quality by using near-infrared reflectance spectroscopy (NIRS)[a].

Groups	N	Mean	Range	SD	SEP	r^2	SEP(C)	bias	slope	RPDv	RER
NDF	98	49.00	37.4–58.8	4.00	2.053	0.767	1.934	–0.715	0.978	2.07	11.07
ADF	96	28.83	22.6–37.6	2.80	1.557	0.748	1.419	–0.658	1.085	1.97	10.57

[a] = Reported values were calculated from the number of samples (N) in the external validation set. SEP = standard error of performance, r^2 = the coefficient of determination in the external validation, SEP(C) = the corrected standard error of prediction, bias = the average difference between the reference and NIRS values, slope = the steepness of a straight line curve, RPD_v and SD/SEP(C) = the ratio of SD (standard deviation of the reference data in the validation set) to SEP(C) in the validation set, and RER = the range-to-error ratio (RER) [Range/SEP(C)].

and ADF (Table 5) equations ranged from 0.748 to 0.934. High r^2 values were observed for CF (0.934, $P < 0.05$) and CP (0.909, $P < 0.05$), and moderate values were observed for NDF (0.767, $P < 0.05$) and ADF (0.748, $P < 0.05$). The standard error of prediction and r^2 were also in good agreement with the related parameters SECV and 1 – VR of the cross-validation statistics.

4. Discussion

NIR has been widely used for the determination of seed or plant components in soybean (Lee et al., 2011). However, studies describing the application of NIR to predict forage quality in soybean are limited. Prediction of forage quality parameters for several forage crops has been investigated using NIR to accurately predict the chemical composition.

In this study, we developed NIR equations for CP, CF, NDF, and ADF to determine forage quality and to use for screening purposes in soybean breeding programs. Generally, the coefficients of determination ($r^2 > 0.9$) for CP and CF were higher than those for the NDF and ADF constituents ($r^2 < 0.9$), which was in agreement with previous studies (Shenk and Westerhaus, 1996; Hoffman et al., 1998; Starks and Brown, 2010).

A lower prediction accuracy using NIRS for NDF and ADF in comparison to CP was reported by Hoffman et al. (1998). Results for 121 samples of legume and grass silages were collected from commercial forage testing laboratories, and the results also showed that NIRS performed much better in predicting CP and CF than in predicting NDF and ADF. Shenk and Westerhaus (1996) reported an r^2 value of 0.83 from an NIR equation for estimating ADF, although RPD was under the cutoff point of 3 (2.40), which was also the cutoff point recommended by Williams and Sobering (1996) for screening purposes. It is normal that the standard errors obtained by validation were slightly higher and the r^2 values slightly lower than those of the cross-validation. MPLS loading plots for ADF were also published by Font et al. (2003), although the results were not comparable because the authors developed the plots following the second derivative (2,5,5,2) transformation of the spectra. Calibration results for ADF were best calculated using 2,5,5,2 math treatments for intact seed and ground seed samples in rapeseed species of *Brassica* (Font et al., 2003). They reported an RPDc (SD/SECV) of 2.34 and an RPDv of 2.40 for ADF in intact seeds and an RPDc of 2.59 and an RPDv of 2.62 in the ground seed model. These results were comparable with the results of this study. We obtained an RPDc and an RPDv of 2.02 and 2.57, respectively, for ADF when the same model for CP and CF (2,5,5,1) was calibrated and validated for ADF (data not shown) on a ground soybean plant basis. Dimov et al. (2011) accurately predicted NDF and ADF using the MPLS (1,4,4,1) model, but used a scatter correction of "SNV and Detrend" on samples of black-seeded winter oilseed rapes (*Brassica napus* L.). SD/SECV (RPDc) and RPDv values were 1.95 and 1.92 and 1.86 and 2.22, and RER values were 8.76 and 10.03, respectively.

Williams and Sobering (1996) indicated that RPD values should be at least 3 and RER values should be more than 10 for reliable quantification. Although RER should be more than 10, it is often more than 20. Williams and Norris (2001) also stated that an RPD value of more than 2.4 is desirable for good calibration equations, whereas equations with an RPD of less than 1.5 are unusable. Galvez-Sola et al. (2010) suggested guidelines for setting performance calibrations for samples over environments as follows: excellent calibrations should have $r^2 > 0.95$ and an RPD of >4, successful calibrations should have $r^2 = 0.9–0.95$ and an RPD of 3–4, moderately successful calibrations should have $r^2 = 0.8–0.9$ and an RPD of 2.25–3, and moderately useful calibrations should have $r^2 = 0.7–0.8$ and an RPD of 1.75–2.25. Some calibrations with $r^2 > 0.7$ may be useful for screening purposes.

Using these criteria in this study, successful calibration equations were obtained for the CP and CF model, and moderately useful calibration equations were found for the NDF and ADF models (Tables 2 and 3). The statistical indicators r^2, RPDv, and RER for NDF were 0.767, 2.07, and 11.07, respectively, and for ADF were 0.748, 1.97, and 10.57, respectively. In addition, there was excellent agreement between the NIR and laboratory analyses. The intercepts (bias) and slopes were close to 0 and 1, respectively (Tables 4 and 5) for the MPLS regression. Roggo et al. (2004) utilized RPD and RER statistics in screening of sugar beet (*Beta vulgaris*) quality and found that RPD values of 1–3 and RER values more than 10 indicate the usefulness of NIR for screening. This accuracy can be considered as acceptable for certain applications.

The low r^2 and RPDv for NDF and ADF may be due to their negative correlation with the oil and protein content as well as their NIR absorption characteristics of hemicellulose and cellulose fractions (Buxton and Mertens, 1991; Hoffman et al., 1998; Dimov et al., 2011). Nevertheless, RER values of more than 10 indicate that the calibrations should be useful in future analysis of fiber fraction contents in forage-type soybean genotypes.

The usefulness of NIR in predicting desirable feed contents in milled samples facilitates rapid estimation of samples, which is in stark contrast to the intensive and time-consuming analytical chemistry techniques. The predictive ability of NIR equation models expressed by lower SEC and SECV with relatively high R^2 and 1 – VR values was obtained for CP, CF, NDF, and ADF in this study. Our results showed that NIR could constitute a feasible technique to quantify several essential soybean forage quality parameters such as CP and CF. This research is the first step towards using the NIR equation on the chemical constituents of whole forage type soybeans and developing additional chemometric models. The NIR calibration equations developed in this study are reliable and will be useful in predicting the contents of crude protein and crude fat; however, further studies are needed to improve the performance of the calibrations for both NDF and ADF in forage soybeans. The NIRS predictions for NDF and ADF composition were characterized by high bias or systematic error, which has been previously reported for oilseed rapes. Even though a more accurate selection or prediction is required, a combination of NIRS prescreening followed by laboratory wet analysis on selected samples would help to reduce time and costs.

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