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Acid Detergent Fiber Analysis in Oilseed *Brassicas* by Near-Infrared Spectroscopy

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The potential of near infrared spectroscopy (NIRS) for determining the acid detergent fiber (ADF) in the seed of oilseed *Brassica* (fam. *Brassicaceae*) was assessed. One hundred and fifty accessions belonging to the species Indian mustard (*Brassica juncea* L. Czern.& Coss.), Ethiopian mustard (*B. carinata* A. Braun) and rapeseed (*B. napus* L.) were scanned by NIRS as intact and ground seed, and their ADF values were regressed against different spectra transformations by modified partial least squares regression. The coefficients of determination in the external validation (r^2) for intact and ground seed were 0.83 and 0.85, respectively. The standard deviation to standard error of prediction ratio and range to standard error of prediction ratio were 2.40 and 10.75 for intact seed and 2.62 and 11.76 for ground seed. No significant differences in the prediction were found for both sample presentations. Effects of the C−H and O−H groups of lipids and water, respectively, as well as protein and chlorophyll, were most important in modeling these equations.

KEYWORDS: Oilseed *Brassica*; acid detergent fiber; near-infrared spectroscopy; intact seed; ground seed

INTRODUCTION

Over the past three decades, *Brassica* oilseed production has increased to become one of the most important world sources of vegetable oil. Improvements in the quality of oil and meal have resulted in the recognition of the oil as an edible product of high nutritional value and the meal as an important source of protein for animal nutrition.

One of several approaches undertaken by *Brassica* plant breeders in the last years has been to improve the quality of the meal by reducing fiber content in seed, as fiber has been demonstrated to be negatively correlated with the oil and protein content in the seed (*1*−*3*), and with meal digestibility (*4*). Acid detergent fiber (ADF) is composed of lignocellulose and silica fractions in plant fiber (but not hemicellulose). It has been reported that its correlation with digestibility for ruminant animals is high and could be considered a good indicator of plant quality (*5*).

The standard methods of analysis traditionally used for determining any of the plant fiber constituents (*6*−*8*), usually involve a high cost and labor input, and the assistance of specialized technicians is needed to perform the analysis. Additionally, the analytical procedure is very time consuming, and a period of 20 h for oil extraction plus 1 h for a complete digestion of the plant material is usually necessary. In addition, hazardous chemicals are used in the process, which implies some health risk. Likewise, these methods involve the destruction of the sample to be analyzed, which could be a handicap in the case of valuable and scarce materials.

Near-infrared spectroscopy (NIRS) is a technique that uses the radiation absorbed by a set of samples in the region from 780 to 2500 nm (near-infrared region) to develop calibration curves that are related to sample properties. After calibration, the regression equation permits accurate analysis of many other samples by prediction of data on the basis of the spectra. The most attractive features of analysis using NIRS are its speed, minimal sample preparation, and its being a non destructive method, making it possible to effect a large number of analysis in a short time. In the last 30 years, NIRS has been widely used as a fast and accurate method for qualitative and quantitative analysis of biological and nonbiological materials in the agriculture, food, textile, petrochemical, and pharmaceutical fields. International Standards Committees have formally accepted methods using NIRS for the analysis of many compounds (*9*), including ADF in forage (*10*). Many authors have reported successful results in predicting ADF by NIRS in different commercial and noncommercial forage species (*11*−*14*), including rapeseed (*15*).

In the last years, our research group has been using NIRS for determining several seed quality parameters of interest in different plant species (*16*−*19*). These studies have been carried out on single species and intact seed samples, due to the need to preserve seed material used in the NIR analysis. The current work has the following objectives: (i) to develop a multispecies calibration equation to explore the potential of NIRS to determine the ADF seed content in the genetically related

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allotetraploid species of *Brassica* Indian mustard (*Brassica juncea* L. Czern.& Coss., genome = AABB), Ethiopian mustard (*B. carinata* A. Braun, genome = BBCC), and rapeseed (*B. napus* L., genome = AACC) and (ii) to discuss the accuracy of the technique in the prediction of the ADF content when it is performed on two different sample presentations, intact seed and seed meal.

MATERIALS AND METHODS

One hundred and fifty seed samples of Indian mustard, Ethiopian mustard, and rapeseed from different geographical origins, part of a *Brassica* germplasm collection belonging to the Department of Agronomy and Plant Breeding of the Institute of Sustainable Agriculture (CSIC, Córdoba, Spain), were selected for conducting this work. The entire process consisted of the following five steps: registration of the intact seed sample spectra in an NIR monocromator; grinding of the samples; registration of the spectra of the ground seed samples in the same conditions as the intact samples; chemical analysis of the sample set to obtain the reference values for ADF, and finally, development of the calibration equations and validation of each of them.

Chemical Analysis. About 3.50 g of seeds of each of the accessions were ground in a mill (Janke & Kunkel, mod. A10, IKA-Labortechnik) for about 20 s to pass a 0.5 mm screen and then oven dried at 75 °C for 20 h. Dried meal (1 g) was defatted with petroleum ether for 20 h, and residual solvent in the meal was eliminated in an air oven at 70 °C for 1 h. ADF content was determined according to procedures described by Goering and Van Soest (*7*) in a Dosi-Fiber apparatus (SELECTA). Samples were analyzed in duplicate, and both determinations were averaged.

NIRS. Intact seeds of each of the accessions used to conduct this work were placed in the NIRS sample holder (3 cm diameter) until it was $^3/_4$ full (seed weight of 3.50 g, approximately). Seed samples were scanned in an NIR spectrometer (NIR Systems model 6500, Foss-NIR Systems, Inc., Silver Spring, MD) in reflectance mode, equipped with a transport module, acquiring their spectra at 2 nm intervals over a wavelength range from 400 to 2500 nm (VIS + NIR regions). The whole set of spectra (n = 150) were split into two subsets, one for calibration and other for validation. Two-thirds of the spectra (n = 102) formed the calibration subset and were used for performing the different calibration equations. The rest of the spectra formed the validation subset and were used for testing the predictive ability of each of the calibration equations obtained. To ensure that the calibration and validation subsets comprised similar spectral (representation by species) and chemical variability (ranges, means and standard deviations), spectra were ordered by species and by their ADF content for each species and then split as described above (**Tables 2**−**5**).

Using the GLOBAL v. 1.50 program (WINISI II, Infrasoft International, LLC, Port Matilda, PA), different calibration equations for ADF were developed on the calibration set (n = 102). Calibration equations were computed using the raw optical data (log $^1/_R$, where R is reflectance), or first or second derivatives of the log $^1/_R$ data, with several combinations of segment (smoothing) and derivative (gap) sizes. Wavelengths from 400 to 2500 nm every 8 nm were used to perform the different calibration equations. Modified partial least squares (MPLS) was the method employed to correlate spectral information and the ADF content in the samples. This regression method constructs a number of factors as linear combinations of the original spectral data, performing a regression on the factor scores to derive a prediction equation (*20*). The final objective is to reduce the huge number of spectral data points (absorbance at each wavelength, i.e., 1050) and to eliminate the correlation of the absorbance values presented by neighboring wavelengths. Standard normal variate and detrend transformations (SNV-DT) (*21*) were used to correct baseline offset due to scattering effects (differences in particle size among samples). Cross-validation was performed on the calibration set to determine the optimum number of terms to be used in the calibration equation and to identify chemical (T) or spectral (H) outliers. "T" outliers are samples with a relationship between reference value and spectrum that is different from the relationship of other samples in the population, and with large residuals (t values >2.5); the "H" outlier identifies a sample that is spectrally different from other samples in the population and has a standardized H value > 3.0 (Mahalanobis distance). Equations were validated with 5 cross-validation groups, and outliers were eliminated in two outlier passes (*22*).

The external validation was conducted with equations developed in the calibration process. These equations were tested on an external group of samples (n = 48) not included in the calibration set. The prediction ability of the different equations obtained was determined on the basis of the optimum combination of the following statistics: high coefficient of determination (r^2), bias (systematic difference between the two sets) and slope close to 0 and 1, respectively, and high ratios standard deviation (SD) to standard error of performance (SEP) (RPD) and range to SEP (RER) (*23*). The standard error of laboratory (SEL) for the ADF analysis was determined and compared with the SEP for both the whole set and individual species. For its determination, all samples in the training set were analyzed in duplicate at different times and by different analysts, to get an estimate of the true total error of the reference method.

Table 1. ADF Composition of the Entire Set of Samples (% DW) (n = 150)

	n^a	range	mean	SD[b]
B. juncea	68	6.98–16.31	11.28	2.33
B. carinata	41	5.33–14.70	9.84	2.10
B. napus	41	8.46–13.35	11.74	1.02

[a] Number of samples. [b] Standard deviation.

RESULTS

Acid Detergent Fiber Measured by the Reference Method. The ADF composition of each of the species of *Brassica* used in this work is shown in **Table 1**. The total range exhibited by the seed samples varied from 5.33% DW (*B. carinata*) to 16.31% DW (*B. juncea*). Ranges presented by *B. juncea* and *B. carinata* were similar to each other and approximately double those shown by *B. napus*.

By using the *t*-test, ADF mean content exhibited by *B. carinata* was significantly lower ($p < 0.001$) than mean contents exhibited by *B. juncea* and *B. napus*, which presented ADF contents with no significant differences between them. *B. juncea* exhibited the highest SD (2.33% DW), while *B. napus* presented a value (1.02% DW) half that shown by the other two species. The frequency distribution of the ADF content for each species in the whole set (n = 150) is displayed in **Figure 1**.

Intact Seed NIR Calibration Model for Acid Detergent Fiber. As shown in **Table 2**, the lowest standard error of calibration (SEC) and higher coefficient of determination in the calibration (R^2) were obtained transforming the raw optical data into the second derivative (2, 5, 5, 2; (derivative order, gap, first smooth, second smooth)) prior to calibration. A calibration equation of nine terms using five cross-validation groups was developed for that mathematical treatment. A SD to standard error of cross-validation (SECV) ratio (SD/SECV) of 2.34 and a coefficient of determination in the cross-validation ($1 - VR$) of 0.81 were obtained. The use of raw optical data or first derivative of the spectra gave worse results than those obtained for the second derivative. No outliers were detected in the cross-validation for the 0, 0, 1, 1 equation, while first and second derivative calibration equations exhibited three T outliers (two of them being common to both mathematical treatments), which were eliminated from the calibration set. The resulting r^2, RPD, and RER (**Table 3**) in the external validation for the second derivative were the highest of the three equations. A bias of 0.00 and a slope of 0.88 of the linear regression line plotting

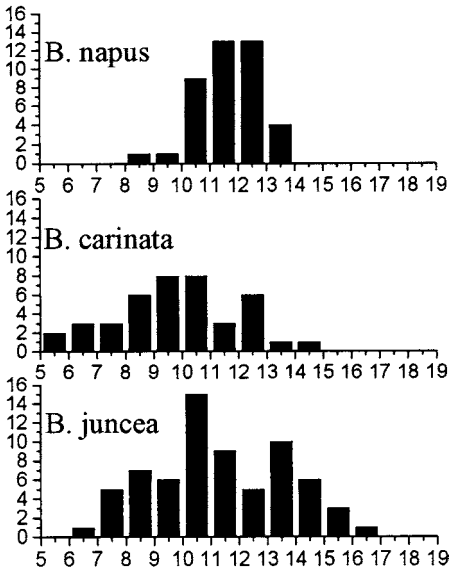

Figure 1. Frequency distribution of the acid detergent fiber content for each of the species forming the sample set. *x*-axis: ADF content (% DW). *y*-axis: number of samples.

Table 2. Calibration and Cross-Validation Statistics for ADF in the Intact Seed Model (% DW) ($n = 102$) for the Different Mathematical Treatments Used

	calibration					cross-validation		
mt[a]	range[b]	mean[c]	SD[d]	SEC[e]	R^{2f}	SD/SECV[g]	1-VR[h]	nt[i]
0,0,1,1	5.33–16.31	11.00	2.18	0.93	0.81	1.81	0.69	10
1,4,4,1				0.80	0.86	2.09	0.76	8
2,5,5,2				0.60	0.92	2.34	0.81	9

[a] Mathematical treatment (derivative, gap, first smooth, second smooth). [b] Range of the reference data. [c] Mean value of the reference data. [d] Standard deviation of the reference data. [e] Standard error of calibration. [f] Coefficient of determination in the calibration. [g] Ratio of the standard deviation of the reference data to standard error of cross-validation. [h] Coefficient of determination in the cross-validation. [i] Number of terms in the calibration model.

Table 3. External Validation Statistics for ADF in the Intact Seed Model (% DW) ($n = 48$) for the Different Mathematical Treatments Used

	external validation								
mt[a]	range[b]	mean[c]	SD[d]	SEP[e]	r^{2f}	bias[g]	slope	RPD[h]	RER[i]
0,0,1,1	6.65–15.47	11.02	1.97	1.06	0.71	–0.01	0.86	1.85	8.32
1,4,4,1				1.01	0.74	0.01	0.82	1.95	8.73
2,5,5,2				0.82	0.83	0.00	0.88	2.40	10.75

[a] Mathematical treatment (derivative, gap, first smooth, second smooth). [b] Range of the reference data. [c] Mean value of the reference data. [d] Standard deviation of the reference data. [e] Standard error of the prediction. [f] Coefficient of determination in the external validation. [g] Difference of means (laboratory minus predicted by NIRS). [h] Ratio of the standard deviation to SEP. [i] Ratio of the range to SEP.

laboratory ADF versus predicted ADF were obtained by this mathematical treatment (**Figure 2**).

Ground Seed NIR Calibration Model for Acid Detergent Fiber. The calibration equation developed with the original spectra (0, 0, 1, 1; (derivative order, gap, first smooth, second smooth)) resulted in the highest SD/SECV and $1 - VR$ values of the three equations performed on the ground seed samples (**Table 4**). Three and two T outliers were identified by cross-validation in the calibration set for the 0, 0, 1, 1 and 1, 4, 4, 1 calibration equations, respectively. No outliers were detected

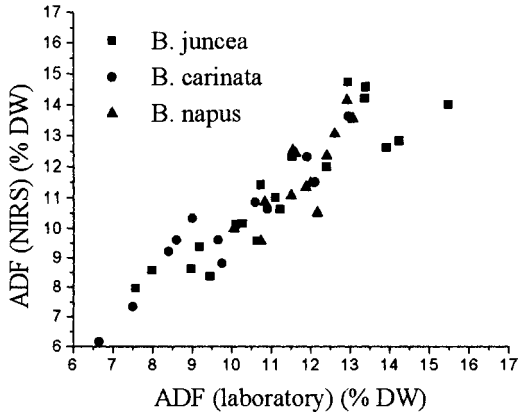

Figure 2. Validation scatter plot for ADF (% DW) in the intact seed model ($n = 48$).

Table 4. Calibration and Cross-Validation Statistics for ADF in the Ground Seed Model (% DW) ($n = 102$) for the Different Mathematical Treatments Used

	calibration					cross-validation		
mt[a]	range[b]	mean[c]	SD[d]	SEC[e]	R^{2f}	SD/SECV[g]	1-VR[h]	nt[i]
0,0,1,1	5.33–16.31	11.00	2.18	0.64	0.91	2.94	0.88	9
1,4,4,1				0.78	0.87	2.62	0.85	4
2,5,5,2				0.77	0.87	2.59	0.85	3

[a] Mathematical treatment (derivative, gap, first smooth, second smooth). [b] Range of the reference data. [c] Mean value of the reference data. [d] Standard deviation of the reference data. [e] Standard error of calibration. [f] Coefficient of determination in the calibration. [g] Ratio of the standard deviation of the reference data to standard error of cross-validation. [h] Coefficient of determination in the cross-validation. [i] Number of terms in the calibration model.

Table 5. External Validation Statistics for ADF in the Ground Seed Model (% DW) ($n = 48$) for the Different Mathematical Treatments Used

	external validation								
mt[a]	range[b]	mean[c]	SD[d]	SEP[e]	r^{2f}	bias[g]	slope	RPD[h]	RER[i]
0,0,1,1	6.65–15.47	11.02	1.97	0.77	0.85	–0.14	0.91	2.55	11.45
1,4,4,1				0.84	0.81	–0.02	0.92	2.34	10.50
2,5,5,2				0.75	0.85	–0.02	0.98	2.62	11.76

[a] Mathematical treatment (derivative, gap, first smooth, second smooth). [b] Range of the reference data. [c] Mean value of the reference data. [d] Standard deviation of the reference data. [e] Standard error of the prediction. [f] Coefficient of determination in the external validation. [g] Difference of means (laboratory minus predicted by NIRS). [h] Ratio of the standard deviation to SEP. [i] Ratio of the range to SEP.

for the second derivative transformation equation in the cross-validation. When performances of the different equations obtained were tested on the independent validation set of samples, the second derivative (2,5,5,2) transformation resulted in the highest RPD and RER and in a bias and slope close to 0.00 and 1.00, respectively (**Table 5**) (**Figure 3**). First derivative transformation yielded the worst combination of the different statistics, presenting the lowest r^2, RPD, and RER. Differences were found in the number of terms used to construct the different equations. While the mathematical model using the raw spectra (0,0,1,1) modeled a nine term-equation, first and second derivative transformations used only four and three terms, respectively. Small differences were found in the biases of the first and second derivative equations, while 0,0,1,1 transformation showed a larger bias than those displayed by the mentioned equations.

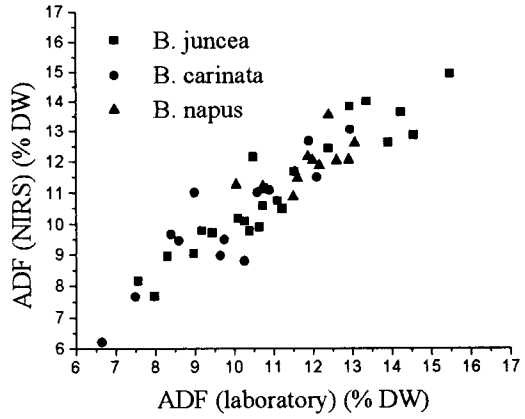

Figure 3. Validation scatter plot for ADF (% DW) in the ground seed model ($n = 48$).

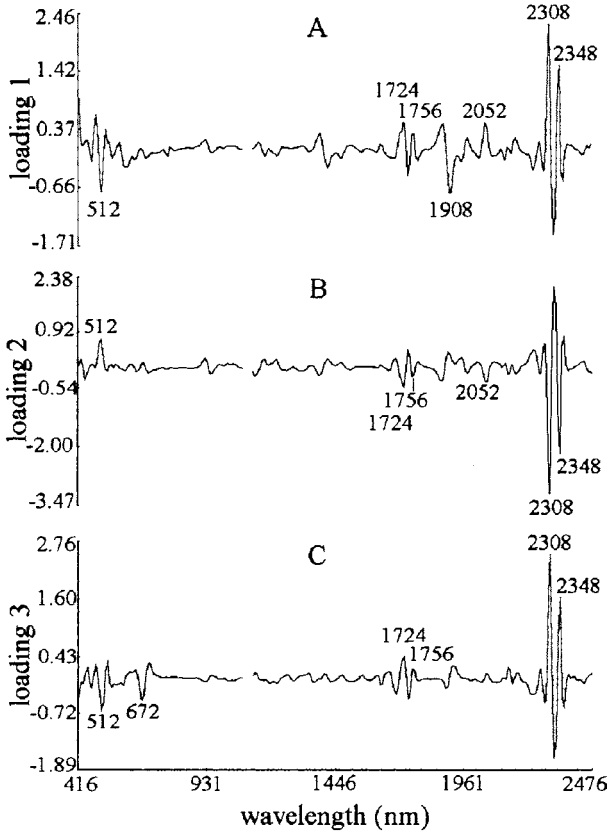

Figure 4. MPLS loading spectra for ADF in the *Brassica* intact seed model for the second derivative (2,5,5,2) transformation. Panels A, B, and C represent loadings for factors 1, 2, and 3, respectively.

The entire set of samples ($n = 150$) was analyzed in duplicate, obtaining a SEL of 0.23%. The SEP/SEL ratios corresponding to the intact and ground seed models for the second derivative transformation were 3.56 and 3.26, respectively.

Linear regression of ADF contents vs NIR predicted values in the intact and ground seed models gave SEP's not significantly different ($p < 0.001$) (*t*-test) between them for the 2,5,5,2 calibration equations.

Modified Partial Least Squares Loadings of the Intact Seed Model. Panels **A**, **B**, and **C** of **Figure 4** represent MPLS loading spectra for factors 1, 2, and 3, respectively. These plots show the regression coefficients of each wavelength to ADF for each factor. Wavelengths represented here as more highly participating in the development of each factor are those of more variation and that are better correlated to ADF in the calibration

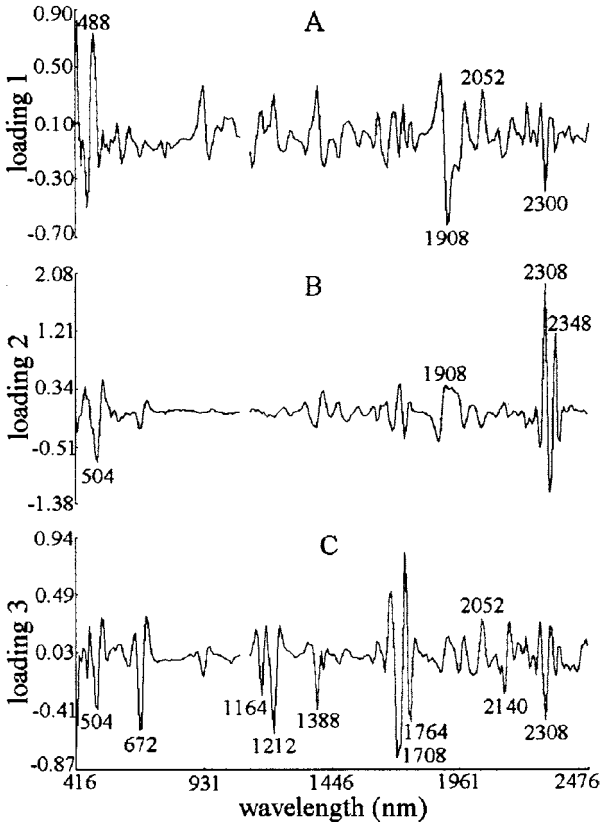

Figure 5. MPLS loading spectra for ADF in *Brassica* ground seed in the second derivative (2,5,5,2) transformation. Panels A, B, and C represent loadings for factors 1, 2, and 3, respectively.

set. In the second derivative, peaks pointing downward indicate positive influence of absorbers on the development of the equations, while peaks pointing upward indicate negative correlations. Factor 1 of the intact seed model showed the lowest correlation to ADF, presenting a loading with major positive correlations at 1724, 1756, 2308, and 2348 nm, associated with the absorbance of C−H groups of lipids (**Figure 4 A**) (*24*). This factor was also influenced by water, as indicated by the band at 1908 nm, amide groups in the protein region at 2052 nm (*25*), and a conspicuous peak at 512 nm related to seed color. Factor 2 was the most highly correlated to ADF and had a loading with the greatest absorbances at 2308 and 2348 nm (**Figure 4 B**). Other minor contributions to the development of this factor were wavelengths related to protein (2052 nm), C−H stretch groups of lipids, and seed color (512 nm). The third MPLS loading (**Figure 4 C**) had similar absorption bands to those displayed by the second loading as well as one additional band (672 nm) which has been described as related to chlorophyll (*26*).

Modified Partial Least Squares Loadings of the Ground Seed Model. Factor 1 of this model was highly influenced by water, presenting a prominent absorption band at 1908 nm (**Figure 5 A**). Peaks related to amide groups of protein (2052 nm) and C−H stretch groups of lipids (2300 nm) also participated in the development of this factor. The second MPLS loading was the most highly correlated to ADF, as previously occurred in the intact seed model, and its loading plot had prominent absorption peaks in the spectral region associated to C−H stretch groups of lipid (**Figure 5 B**). The third loading of the ground seed model was much more complex, with positive or negative influences in the entire range of the spectrum (**Figure 5 C**). Thus, in addition to peaks described previously, some wavelengths in the region of the second overtones of C−H

stretch (1164−1388 nm) (*27*) participated in modeling this factor. As previously occurred in the intact seed model, a chlorophyll-related band at 672 nm was used to construct the third factor of the ground seed model.

DISCUSSION

On the basis of the r^2 statistic (*28*), the six equations showed a good precision at the time of predicting the ADF content of the validation set samples. The lowest SEP, and therefore the highest RPD and RER, were obtained with the 2,5,5,2 transformation of the ground seed model (**Table 5**). Although the highest RPD's obtained (2.40 and 2.62) were under the cutoff point of 3, recommended by Williams and Sobering (*23*) for using the equation for screening purposes, the RERs displayed by four equations (**Tables 3** and **5**) were over 10, which is considered by the same authors as indicative of equations with an acceptable accuracy on the basis of the set range. The ratios of SEP/SEL obtained for the second derivative equations of the ground and intact seed models (SEP/SEL = 3.26 and 3.56, respectively) were indicative of high accuracy in the prediction of the ADF content performed on an external set of samples.

Michalski et al. (*15*), in a study of *B. napus* performed in transmittance mode on intact seed samples, reported satisfactory results for ADF prediction (SEC = 1.62% DW, $R^2 = 0.85$) in a range from 15.7 to 25.1% DW, and for neutral detergent fiber (SEC = 1.73% DW, $R^2 = 0.78$) in samples ranging from 22.1 to 31.4 DW. On the basis of the ratio of the range to SEC, results presented by us in this work for the second derivative of the intact seed model are 3 times more accurate than those reported by these authors for *B. napus*. Kays et al. (*29*) reported NIR predictions of total dietary fiber (TDF) on a broad variety of ground cereal products. These authors obtained RPD's of 7.07 and 8.63 for products with high fat content and products with both high fat and high sugar content, and coefficients of determination of 0.98 and 0.99, respectively, which implies a high precision in the TDF prediction for these products. Other authors (*30*) have reported NIR predictions of chemical and physical properties of peas and chickpeas performed on intact and ground seed, obtaining higher accuracy in the prediction for the different chemical parameters when the seeds were previously ground. The same study also concluded that physical properties of the seed were better predicted on intact material rather than ground. The fact that fiber in *Brassica* is mainly located in the coat of the seed allows NIR radiation to easily reach it and be absorbed. Additionally, the seed coat color (*3*) of these species is a characteristic highly correlated with fiber content (the higher proportion of yellow seeds in the sample, the lower content of lignin). Thus, seed color participated in the performance of the prediction equations, as is demonstrated in **Figures 4** and **5**, where wavelengths associated with absorbances at the visible region of the spectrum were used to develop the calibration equation.

An examination of the intact and ground seed model loadings (**Figures 4** and **5**) of the second derivative transformation suggests that C−H groups of lipids were the most important associations for modeling these factors. The loading more highly correlated with ADF was the second in both cases. Water and protein mainly contributed to the development of the first factor of both models. The use of any tool for correcting the scattering effects leads to water having a major preponderance in the first loadings, which explains part of the existing variability but not all. Wavelengths in the visible region also participated in all factors, but mainly in the second and first intact and ground seed equation factors, respectively. Shape and positioning of the bands presented by the different loadings described previously very closely resembled those reported by Kays et al. (*29*) for cereal products, in which effects due to C−H groups of lipids and O−H groups of water were most important in the model. These differences found in band selection between intact and ground seed presentations at the time of calibration are caused by small differences in radiation path lengths reaching the sample, as has been stated by Panford et al. (*31*).

We conclude that NIRS can evaluate ADF content in *Brassica* oilseeds with sufficient accuracy for use as a screening tool in plant breeding programs. The inclusion of different oilseed *Brassica* species in the calibration model increments the robustness of the equation, because inter-specific variability is increased. Equations obtained over intact and ground seed material gave similar accuracy in the prediction on an external validation set of samples, and therefore, ADF analysis can be performed on intact seed without loss of accuracy. The development of a global equation for ADF in oilseed *Brassica* species would require the addition of new cultivars collected across multiple years and environments, which could increase the spectral and chemical variability presented by the accessions used in this work.

ABBREVIATIONS USED

ADF, acid detergent fiber; MPLS, modified partial least squares; NIRS, near infra-red spectroscopy; RER, ratio of the range to standard error of prediction; RPD, ratio of the standard deviation to standard error of prediction; SEC, standard error of calibration; SECV, standard error of cross validation; SEL, standard error of laboratory; SEP, standard error of prediction; SNV-DT, standard normal variate-detrending; TDF, total dietary fiber; VIS, visible; R^2, coefficient of determination in the calibration; r^2, coefficient of determination in the external validation; 1 − VR, coefficient of determination in the cross validation

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LITERATURE CITED

(1) Stringam, G. D.; McGregor, D. I.; Pawlowski, S. H. Chemical and morphological characteristics associated with seed coat color in rapeseed. In *Proceedings of the 4th International Rapeseed Congress*; Organizing Committee of the 4th International Rapeseed Congress: Giessen, Germany, 1974; pp 99−108.

(2) Simbaya, J.; Slominski, B. A.; Bogdan A.; Rakow G.; Campbell, L. D.; Downey, R. K.; Bell, J. M. Quality characteristics of yellow-seeded *Brassica* seed meals: protein, carbohydrates, and dietary fiber components. *J. Agric. Food Chem.* **1995**, *43*, 2062−2066.

(3) Slominski, B. A. Developments in the breeding of low fibre rapeseed/canola. *J. Anim. Feed Sci.* **1997**, *6*, 303−317.

(4) Bjergegaard, C.; Eggum, B. O.; Jensen, S. K.; Sorensen, H. Dietary fibres in oilseed rape: physiological and antinutritional effects in rats of isolated IDF and SDF added to a standard diet. *J. Anim. Physiol. Anim. Nutrition.* **1991**, *66*, 69−79.

(5) Van Soest, P. J.; Robertson, J. B. In *Analysis of forages and fibrous foods.* Cornell University Publication: Ithaca, New York, 1985; p 165.

(6) Entwistle, P. Van; Hunter, W. L. Report on crude fiber. *J. Assoc. Offic. Agr. Chemists.* **1949**, *32*, 651−652.

(7) Goering, H. K.; Van Soest, P. J. Forage fiber analysis; apparatus, reagents, procedures, and some applications. USDA-ARS Agric. Handbook No. 379. U. S. Gov. Print. Office: Washington, DC, 1970.

(8) AOAC. Animal Feed (Crude fiber method 962.09). In *Official methods of analysis*, 15th edition; Helrich, K., Eds.; Association of Official Analytical Chemists, Inc.: Arlington, VA, 1990; Vol. I, pp 80−81.

(9) Batten, G. D. Plant analysis using near-infrared reflectance spectroscopy: the potential and the limitations. *Aust. J. Exp. Agric*. **1998**, *38*, 697−706.

(10) Barton, F. E. II; Windham, W. R. Determination of acid detergent fiber and crude protein in forages by near-infrared spectroscopy: Collaborative study. *J. Assoc. Off. Anal. Chem.* **1988**, *71*, 1162−1167.

(11) Marten, G. C.; Halgerson, J. L.; Cherney, J. H. Quality prediction of small grain forages by near-infrared reflectance spectroscopy. *Crop Sci.* **1983**, *23*, 94−96.

(12) Marten, G. C.; Brink, G. E.; Buxton, D. R.; Halgerson, J. L.; Hornstein, J. S. Near-infrared reflectance spectroscopy analysis of forage quality in four legume species. *Crop Sci.* **1984**, *24*, 1179−1182.

(13) Blosser, T. H.; Reeves III, J. B.; Bond, J. Factors affecting analysis of the chemical composition of tall fescue with near-infrared reflectance spectroscopy. *J. Dairy Sci.* **1988**, *71*, 398−408.

(14) García-Ciudad, A.; García-Criado, B.; Pérez-Corona, M. E.; Vázquez De Aldana, B. R.; Ruano-Ramos, M. A. Application of near-infrared reflectance spectroscopy to chemical analysis of heterogeneous and botanically complex grassland samples. *J. Sci. Food Agric.* **1993**, *63*, 419−426.

(15) Michalski, K.; Ochodzki, P.; Cicha, B. Determination of fibre, sulphur amino acids, and lysine in oilseed rape by NIT. In *Making Light Work: Advances in Near Infrared Spectroscopy*, 1st edition; Murray, I., Cowe, I. A., Eds.; VCH Verlagsgesellschaft: Weinheim, Germany, and VCH Publishers: NY, 1992; pp 333−335.

(16) De Haro, A.; López-Medina, J.; Cabrera, A.; Martín, A. Determination of tannin in the seeds of *Vicia faba* by NIR. In *Recent Advances of Research in Antinutritional Factors in Legume Seeds*, Huisman, Ed.; Liener. Pudoc.: Wageningen, The Netherlands, 1989; pp 96−103

(17) Velasco, L.; Fernández-Martínez, J. M.; De Haro, A. Screening Ethiopian mustard for erucic acid by near-infrared reflectance spectroscopy. *Crop Sci.* **1996**, *36*, 1068−1071.

(18) Font, R.; Del Río, M.; Fernández-Martínez, J. M.; De Haro, A. Determining quality components in Indian mustard by NIRS. *Cruciferae Newsletter* **1998**, *20*, 67−68.

(19) Font, R.; Del Río, M.; Fernández-Martínez, J. M.; De Haro, A. Using near-infrared spectroscopy (NIRS) for the determination of bulk density in Indian mustard seed. *Cruciferae Newsletter* **1999**, *21*, 75−76.

(20) Shenk J. S.; Westerhaus, M. O. Population structuring of near-infrared spectra and modified partial least squares regression. *Crop Sci.* **1991**, *31*, 1548−1555.

(21) Barnes, R. J.; Dhanoa, M. S.; Lister, S. J. Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Appl. Spectrosc.* **1989**, *43*, 772−777.

(22) Shenk J. S.; Westerhaus, M. O. Population definition, sample selection, and calibration procedures for near-infrared reflectance spectroscopy. *Crop Sci.* **1991**, *31*, 469−474.

(23) Williams P. C.; Sobering D. C. How do we do it: a brief summary of the methods we use in developing near-infrared calibrations. In *Near Infrared Spectroscopy: The Future Waves.* Davies, A. M. C., Williams, P. C., Eds; Nir Publications: Chichester, U.K., 1996; pp 185−188.

(24) Murray, I.; Williams, P. C. Chemical principles of near-infrared technology. In *Near-Infrared Technology in the Agricultural and Food Industries*, 1st edition; Williams, P. C., Norris, K. H., Eds.; American Association of Cereal Chemists: St. Paul, MN, 1987; pp 17−34.

(25) Krikorian, S. E.; Mahpour, M. The identification and origin of N−H overtone and combination bands in the near-infrared spectra of simple primary and secondary amides. *Spectrochimica Acta*. **1973**, *29A*, 1233−1246.

(26) Tkachuk, R.; Kuzina, F. D. Chlorophyll analysis of whole rapeseed kernels by near-infrared reflectance. *Can. J. Plant Sci.* **1982**, *62*, 875−884.

(27) Osborne, B. G.; Fearn, T.; Hindle, P. H. Theory of near-infrared spectrophotometry. In *Practical NIR spectroscopy with applications in food and beverage analysis*, 2nd edition; Browning, D., Ed.; Longman Scientific and Technical: Harlow, England, 1993; pp 13−35.

(28) Shenk, J. S.; Westerhaus, M. O. Calibration the ISI way. In *Near-infrared spectroscopy: The future waves*. Davies, A. M. C., Williams, P. C., Eds.; NIR publications: Chichester, UK, 1996; pp 198-202.

(29) Kays, S. E.; Windham, W. R.; Barton II, F. E. Prediction of total dietary fiber by near-infrared reflectance spectroscopy in high-fat-and high-sugar-containing cereal products. *J. Agric. Food Chem.* **1998**, *46*, 854−861.

(30) Flinn, P. C.; Black, R. G.; Iyer, L.; Brouwer, J. B.; Meares, C. Estimating the food processing characteristics of pulses by near-infrared spectroscopy, using ground or whole samples. *J. NIRS* **1998**, *6*, 213−220.

(31) Panford, A.; Williams, P. C.; Man, J. M. Analysis of oilseeds for protein, oil, fiber and moisture by near-infrared reflectance spectroscopy. *JAOCS*. **1988**, *65*, 1627−1634.

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