Determination of the Fatty Acid Composition of the Oil in Intact-Seed Mustard by Near-Infrared Reflectance Spectroscopy

L. Velasco*, J.M. Fernández-Martínez, and A. De Haro

Instituto de Agricultura Sostenible (C.S.I.C.), ES-14080 Córdoba, Spain

ABSTRACT: Near-infrared reflectance spectroscopy (NIRS) was used to estimate the fatty acid composition of the oil in intact-seed samples of Ethiopian mustard (*Brassica carinata* Braun) within a mutation breeding program that produced seeds with variable fatty acid compositions. Five populations, from 1992 to 1996 crops, were included in this study; and NIRS calibration equations for major fatty acids (palmitic, stearic, oleic, linoleic, linolenic, eicosenoic, and erucic) were developed within each single population. Furthermore, global calibration equations, including samples from the five populations, were developed. After external validation, the NIRS technique permitted us to obtain a reliable and accurate nondestructive estimation of the fatty acid composition of the oil, especially for the major acids—oleic, linoleic, linolenic, and erucic. For these, the r^2 in external validation was higher than 0.95 by using both single-and multipopulation equations, and higher than 0.85 for the remaining fatty acids. Moreover, the multipopulation equations provided an accurate estimation of samples from a population not represented in the calibration data set, with values of coefficient of determination in validation (r^2) from 0.80 (palmitic and eicosenoic acids) to 0.97 (erucic acid). The ability of NIRS to discriminate among different fatty acid profiles was mainly due to changes within six spectral regions, 1140–1240, 1350–1400, 1650–1800, 1880–1920, 2140–2200, and 2240–2380 nm, all of them associated with fatty acid absorbers. Thus, NIRS can be used to estimate the fatty acid composition of Ethiopian mustard seeds with a high degree of accuracy, provided that calibration equations be developed from calibration sets that include large variability for the fatty acid composition of the oil.
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KEY WORDS: *Brassica carinata* Braun, Ethiopian mustard, fatty acid composition, global calibrations, intact-seed samples, near-infrared reflectance spectroscopy, NIRS, spectral analysis.

Near-infrared reflectance spectroscopy (NIRS) is a multitrait technique that fulfills most of the requirements for rapid, reliable, and cost-effective screening for several seed quality traits in intact-seed samples of *Brassica* oilseed species, i.e., rapeseed, turnip rape, and mustards. In these species, it has been demonstrated that NIRS technique permits estimation with high degrees of accuracy and reliability, in the nondestructive analysis of intact-seed samples, of the oil and protein contents (1), chlorophyll content (2), glucosinolate content (3), seed color (4,5), and seed weight (6).

Several studies have been carried out to test the potential of NIRS to estimate the fatty acid composition of the seed oil in the analysis of intact seeds of *Brassica* (7–12), although there is no general agreement on the real possibilities of this technique. The results obtained in these studies are shown in Table 1. Most of them concluded that, although the NIRS technique could discriminate among different fatty acids, the error was too high to consider this technique as acceptable for routine use. The only exception was the estimation of erucic acid content, for which Koester and Paul (7) and Velasco *et al.* (12) developed calibration equations with high coefficients of determination in validation (r^2) and low standard errors of performance (SEP; Table 1).

Because NIRS is being used worldwide as a rapid and nondestructive screening technique for oil, protein, and glucosinolate content in *Brassica* intact-seed samples, the availability of calibration equations to estimate simultaneously the fatty acid composition of the seed oil with high degrees of accuracy and reliability would increase considerably the usefulness of the NIRS technique. Furthermore, this technique could facilitate a considerable savings in time and money when large numbers of samples have to be screened (e.g., mutagenesis programs, surveys, etc.).

The main objective of this work was to test the potential of NIRS to estimate the fatty acid composition of the oil in the analysis of intact-seed samples of Ethiopian mustard. Furthermore, the performance of global calibration equations, which included seed samples from five years, was studied.

MATERIALS AND METHODS

Samples. The samples used in this study consisted of intact seeds from Ethiopian mustard plants, grown in Córdoba, southern Spain, from 1992 to 1996. All plants were part of a mutation breeding program that was focused on widening the

*To whom correspondence should be addressed at Department of Plant Breeding, Institut für Pflanzenbau und Pflanzenzüchtung, Universität Göttingen, von-Siebold-Str. 8, D-37075 Göttingen, Germany.

E-mail: lvelasc@gwdg.de.

TABLE 1
Published Statistics for Near-Infrared Reflectance Spectroscopy Calibration and Prediction of Individual Fatty Acids in the Analysis of Intact-Seed Samples of *Brassica* Species[a]

		Calibration			Prediction			
Fatty acid	Range[b]	r^2	SEC	Mean	r^2	SEP	Mean	Reference
Palmitic	3.1–4.3	NP	NP	NP	0.72	0.14	3.8	10
	4.1–10.3	0.83	0.55	6.18	0.58	0.91	NP	11
Stearic	1.4–2.3	NP	NP	NP	0.73	0.09	1.9	10
Oleic	39.8–73.2	0.86	2.49	58.2	0.80	2.05	59.7	8,9
	55.6–65.6	NP	NP	NP	0.28	1.96	61.5	10
	4.6–20.9	0.90	1.34	10.4	0.55	2.84	NP	11
Linoleic	NP	0.78	NP	NP	0.17	NP	NP	9
	17.5–22.6	NP	NP	NP	0.29	1.14	19.5	10
	8.5–29.7	0.94	0.99	21.0	0.67	2.46	NP	11
Linolenic	NP	0.85	NP	NP	0.10	NP	NP	9
	6.9–12.9	NP	NP	NP	0.73	0.86	9.2	10
	4.9–21.5	0.98	0.47	15.0	0.71	1.75	NP	11
Eicosenoic	2.8–13.7	0.90	0.93	6.84	0.77	1.41	NP	11
Erucic	NP	NP	NP	NP	0.94	4.80	NP	7
	35.0–58.6	0.85	2.59	46.0	0.73	3.75	35.3	8,9
	0.1–6.7	NP	NP	NP	0.00	0.66	0.6	10
	14.7–42.7	0.97	1.43	30.6	0.83	3.20	NP	11
	6.6–52.2	0.99	1.56	31.0	0.96	2.87	31.4	12

[a]Abbreviations: r^2, coefficient of determination in validation; SEC, standard error of calibration; SEP, standard error of performance; NP, data not published.

[b]Percentage of the total fatty acids (wt%).

existing variability for fatty acid composition in this species (13,14). Seeds collected from the plants grown in 1992 corresponded to the M2 generation, while the seeds collected in 1996 corresponded to the M6 generation within the mutation breeding program.

Analyses by reference method. The intact-seed samples scanned by NIRS were ground with a laboratory mill IKA, model A10 (Janke & Kunkel GmbH & Co. KG, Staufen, Germany) before being analyzed by the reference method. The fatty acid composition of the oil was determined on about 25 mg flour by simultaneous extraction and methylation (15), followed by gas–liquid chromatography (GLC) on a Perkin-Elmer Autosystem gas–liquid chromatograph (Perkin-Elmer Corporation, Norwalk, CT) with a 2-m long column, packed with 3% SP-2310/2% SP-2300 on Chromosorb WAW (Reference 1-1833; Supelco Inc., Bellefonte, PA). A temperature program of 190°C for 10 min, increasing 2°C min^{-1} up to 210°C, and maintained for 7 min was used. The injector and flame-ionization detector were held at 275 and 250°C, respectively. The standard error was calculated by following Windham *et al.* (16), with five different flour samples and 32 replicates.

NIRS scanning. Samples of about 3 g intact seeds were scanned on a monochromator model 6500 (NIR Systems, Inc., Silver Springs, MD) by using a small ring cup (ref. IH-0307, NIR Systems), and reflectance spectra (log 1/*R*) from 400 to 2500 nm were recorded.

Calibration strategy. NIRS was used in this research as a multi-trait screening tool to detect and isolate mutants with altered fatty acid composition. The main objective was to discriminate seed samples according to their fatty acid profile within each generation derived from mutagenesis. With this goal, the samples from each generation were first considered as closed populations, and NIRS calibration equations for individual fatty acids were developed within each population. These equations were used to perform selection for fatty acid composition within each generation of mutagenesis. Second, with a view to develop more robust equations, a global calibration was sequentially expanded each year by combining the samples from the current calibration set with the samples from the previous year's sets.

Calibration and prediction procedures. All procedures were carried out with ISI software, version 3.10 (Infrasoft International, Port Matilda, PA). Original reflectance spectra were corrected, prior to calibration, by applying second-derivative transformation, standard normal variate transformation, and De-trend scatter correction (17). Second derivatives were calculated from the log 1/*R* spectra at gaps of 10 nm and smoothing over segments of length 10 nm. Calibration equations were developed by using the spectral information from 1100 to 2500 nm and modified partial least squares (MPLS) regression. Cross-validation was used to prevent overfitting (18).

Cross-validation statistics were used to estimate the predictive ability of the calibration equations developed during this research. Furthermore, as one of the objectives of this research was to develop robust multipopulation equations to estimate individual fatty acids, the global calibration equations developed in 1995, including samples from 1992 to 1995, were validated (external validation) with a set of samples from the 1996 crop. This set of samples was not included either in the 1996 calibration equations or in the global calibration, and hence was also used to perform an external validation of 1996 single- and multipopulation (1992 to 1996 samples) calibration equations.

Owing to differences in the fatty acid composition among the different populations included in this study, the values of the standard errors of calibration (SEC) and crossvalidation (SECV) were standardized by calculating their ratios to the standard deviation of reference data (RCD and RCVD, respectively). The ratio of the SEP to the standard error of laboratory (SEL) was also calculated.

Spectral analysis. The following procedure was used to identify the main spectral regions associated with NIRS discrimination among different fatty acids. For each individual fatty acid, about 50 samples from the global calibration set that showed low values, 50 samples with middle values, and 50 samples with high values were selected. Three average spectra (low, middle, and high fatty acid content) were then created for each fatty acid. Second-derivative transformation and scatter correction were applied to the new spectra, as described previously, and the standard deviations among average spectra for each fatty acid were studied to identify spectral changes associated with differences in the fatty acid composition of the oil.

RESULTS

Variability for fatty acid composition. Table 2 shows the fatty acid composition of the five single-population calibration sets used in this study. Each set includes intact-seed samples from plants cultivated in the same environment. The variability for fatty acid composition was relatively low in 1992, in which the seed samples were collected from M2 plants, and began to increase in 1993 (M3 plants), and especially in 1994 (M4 plants), where recessive mutants were detected. The largest variability was obtained for oleic, linoleic, and erucic acid, and to a lesser extent for palmitic, stearic, linolenic, and eicosenoic acid.

Development of calibration equations within single populations. Table 3 summarizes the results obtained, in the development of calibration equations for major fatty acids, by using calibration sets from single populations. The calibration equations from both 1992 and 1993 sets showed a high correlation between NIRS-predicted and GLC values, especially for the major fatty acids (oleic, linoleic, linolenic, eicosenoic, and erucic), although the SECV was generally too high. However, a considerable reduction in SECV (indicated by the standardized ratio RCVD) was observed in the calibration equations developed from 1994 onward, when the largest variability for fatty acid composition in the samples was obtained. The only exception was eicosenoic acid, which showed only minor differences in range among populations (Table 2).

Development of global (multipopulation) calibration equations. Table 4 shows the results obtained in the development of multipopulation equation for individual fatty acids from 1993 (including samples from both 1992 and 1993) to 1996, in which samples from five different years (1992 to 1996) were included. As expected (18), the combination of samples from different environments within the same calibration set led to a certain loss of accuracy in comparison with the calibration equations developed from single populations (Table 3). However, the expected robustness of this multipopulation equation, developed in 1996 from a calibration set of 822 seed samples from plants cultivated in five different environments, compensates for the loss of accuracy.

Performance of calibration equations. Although cross-validation provides a useful way to estimate the predictive ability of the calibration equations, an external validation was carried out in 1996 to analyze in more detail the performance of the calibration equations. The validation set included a total of 130 Ethiopian mustard samples, covering all available variability for fatty acid composition in this species. The objective of developing global calibration equations was to obtain a reasonable prediction of the fatty acid composition of samples from plants cultivated in environments different from those included in the calibration set. Therefore, the first step was to use the validation data set, with samples cultivated in 1996, to validate the global equations developed in 1995 from a calibration set that included samples from 1992, 1993, 1994, and 1995 crops. This validation was compared with the results obtained in the validation of the 1996 single- and multipopulation equations. The results are shown in Table 5. For the 1995 equations, the r^2 ranged from values around 0.8 for the prediction of palmitic, stearic, and eicosenoic acids to values as high as 0.97 for the prediction of erucic acid. Although the SEP was too high to consider these calibration equations as adequate to obtain accurate predictions, these results indicated the considerable potential of the NIRS technique to pro-

TABLE 2
Number of Samples and Fatty Acid Composition of the Five Calibration Data Sets Included in this Study

Fatty acid[a]	1992[b]	1993	1994	1995	1996
n	108	150	232	218	114
C16:0	3.1 (2.5–4.5)	3.5 (2.0–5.2)	4.1 (2.5–6.1)	4.2 (1.6–7.5)	3.7 (2.4–5.6)
C18:0	0.8 (0.6–1.1)	0.9 (0.6–1.5)	1.0 (0.5–1.9)	0.9 (0.3–2.0)	1.0 (0.3–2.0)
C18:1	7.7 (4.9–17.4)	10.7 (4.5–22.5)	15.7 (3.1–34.2)	13.9 (4.6–30.4)	20.5 (6.3–41.8)
C18:2	16.5 (12.5–25.0)	19.2 (8.0–31.5)	20.2 (6.8–32.0)	20.1 (7.4–36.2)	19.5 (6.4–33.5)
C18:3	13.7 (6.3–17.8)	13.9 (4.0–20.1)	13.2 (4.0–21.7)	10.4 (1.3–16.7)	10.8 (2.4–19.6)
C20:1	7.7 (4.7–15.5)	7.9 (4.1–16.5)	8.7 (2.8–14.6)	8.6 (1.7–13.6)	8.6 (2.5–12.8)
C22:1	47.1 (24.7–54.4)	40.9 (11.2–53.7)	33.9 (7.6–53.8)	38.8 (6.9–59.4)	33.9 (4.6–56.5)

[a]Fatty acids are expressed as percentage of the total fatty acids.
[b]Each calibration set consisted of seed samples from plants grown in the same environment.
[c]Values given are means, with ranges within parentheses.

TABLE 3
Calibration and Cross-Validation Statistics for Individual Fatty Acids Equations Developed Within Single Populations[a]

Fatty acid	1992	1993	1994	1995	1996
$C_{16:0}$					
r^2	0.71	0.73	0.83	0.90	0.90
SEC/RCD	0.21/0.59	0.32/0.52	0.33/0.41	0.37/0.31	0.26/0.32
SECV/RCVD	0.24/0.60	0.35/0.56	0.35/0.44	0.43/0.36	0.30/0.37
$C_{18:0}$					
r^2	0.59	0.62	0.92	0.90	0.91
SEC/RCD	0.06/0.64	0.12/0.62	0.09/0.29	0.12/0.32	0.14/0.31
SECV/RCVD	0.08/0.79	0.14/0.76	0.12/0.39	0.14/0.38	0.16/0.34
$C_{18:1}$					
r^2	0.94	0.91	0.98	0.97	0.98
SEC/RCD	0.49/0.25	1.25/0.31	1.03/0.13	1.05/0.17	1.51/0.16
SECV/RCVD	1.12/0.59	1.83/0.45	1.52/0.20	1.45/0.23	2.19/0.23
$C_{18:2}$					
r^2	0.94	0.91	0.98	0.98	0.99
SEC/RCD	0.66/0.25	1.14/0.30	1.05/0.16	0.91/0.13	0.74/0.10
SECV/RCVD	1.03/0.39	1.60/0.41	1.43/0.21	1.31/0.18	1.51/0.20
$C_{18:3}$					
r^2	0.95	0.94	0.97	0.98	0.99
SEC/RCD	0.50/0.23	0.74/0.25	0.65/0.17	0.56/0.16	0.45/0.12
SECV/RCVD	0.81/0.37	1.00/0.34	0.88/0.24	0.83/0.24	1.00/0.27
$C_{20:1}$					
r^2	0.96	0.90	0.89	0.92	0.85
SEC/RCD	0.41/0.21	0.85/0.32	0.92/0.33	0.79/0.29	0.90/0.39
SECV/RCVD	0.65/0.34	1.07/0.40	1.14/0.42	0.96/0.35	1.17/0.50
$C_{22:1}$					
r^2	0.97	0.96	0.99	0.98	0.99
SEC/RCD	0.84/0.19	1.67/0.20	1.63/0.11	1.85/0.12	1.46/0.08
SECV/RCVD	2.20/0.49	2.61/0.31	1.95/0.13	2.43/0.16	2.35/0.13

[a]r^2, coefficient of determination; SEC, standard error of calibration; RCD, ratio of SEC to standard deviation of reference data; SECV, standard error of cross-validation; RCVD, ratio of SECV to standard deviation of reference data.

TABLE 4
Evolution of Calibration and Cross-Validation Statistics for Individual Fatty Acid Equations During the Development of Global (multipopulation) Calibration Equations[a]

Fatty acid	1992	1992–1993	1992–1994	1992–1995	1992–1996
$C_{16:0}$					
r^2	0.71	0.75	0.86	0.88	0.85
SEC/RCD	0.21/0.54	0.27/0.50	0.29/0.38	0.32/0.35	0.35/0.38
SECV/RCVD	0.24/0.60	0.28/0.51	0.31/0.40	0.35/0.37	0.37/0.41
$C_{18:0}$					
r^2	0.59	0.68	0.80	0.83	0.84
SEC/RCD	0.06/0.64	0.08/0.57	0.11/0.45	0.12/0.41	0.12/0.40
SECV/RCVD	0.08/0.79	0.10/0.67	0.12/0.51	0.13/0.46	0.13/0.43
$C_{18:1}$					
r^2	0.94	0.88	0.96	0.95	0.95
SEC/RCD	0.49/0.25	1.13/0.34	1.42/0.21	1.43/0.22	1.65/0.22
SECV/RCVD	1.12/0.59	1.43/0.44	1.65/0.24	1.56/0.24	1.81/0.24
$C_{18:2}$					
r^2	0.94	0.91	0.95	0.95	0.95
SEC/RCD	0.66/0.25	1.09/0.31	1.21/0.22	1.31/0.22	1.39/0.22
SECV/RCVD	1.03/0.39	1.29/0.37	1.38/0.25	1.45/0.24	1.51/0.24
$C_{18:3}$					
r^2	0.95	0.92	0.93	0.94	0.94
SEC/RCD	0.50/0.23	0.79/0.29	0.84/0.26	0.85/0.24	0.86/0.24
SECV/RCVD	0.81/0.37	0.98/0.36	0.98/0.30	0.95/0.26	0.96/0.26
$C_{20:1}$					
r^2	0.96	0.89	0.77	0.82	0.83
SEC/RCD	0.41/0.21	0.74/0.33	1.20/0.48	1.11/0.43	1.03/0.42
SECV/RCVD	0.65/0.34	0.89/0.40	1.28/0.51	1.21/0.47	1.12/0.45
$C_{22:1}$					
r^2	0.97	0.93	0.98	0.98	0.98
SEC/RCD	0.84/0.19	1.92/0.26	1.79/0.14	2.02/0.15	2.20/0.16
SECV/RCVD	2.20/0.49	2.44/0.33	2.03/0.16	2.19/0.17	2.37/0.17

[a]For abbreviations see Table 3.

TABLE 5
External Validation of 1992–1995 and 1992–1996 Global Calibration Equations and 1996 Single-Population Equations with a Set of Samples from 1996[a]

				1992 to 1995 set		1992 to 1996 set		1996 set	
Fatty acid	Mean	Range	SEL	r^2	SEP/RPL	r^2	SEP/RPL	r^2	SEP/RPL
$C_{16:0}$	3.9	2.4–5.5	0.14	0.80	0.34/2.43	0.85	0.31/2.21	0.86	0.30/2.14
$C_{18:0}$	0.9	0.3–2.0	0.05	0.81	0.17/3.40	0.90	0.12/2.40	0.89	0.13/2.60
$C_{18:1}$	18.6	6.7–41.8	0.72	0.91	2.58/3.58	0.96	1.72/2.39	0.97	1.46/2.03
$C_{18:2}$	20.8	6.5–31.3	0.59	0.93	1.72/2.92	0.95	1.54/2.61	0.97	1.06/1.80
$C_{18:3}$	10.7	2.9–19.3	0.55	0.89	1.29/2.35	0.95	0.91/1.65	0.96	0.77/1.40
$C_{20:1}$	8.4	2.5–13.0	0.26	0.80	0.98/3.77	0.85	0.93/3.58	0.85	0.89/3.42
$C_{22:1}$	34.7	4.6–56.5	1.01	0.97	2.78/2.75	0.98	2.33/2.31	0.99	1.69/1.67

[a]Abbreviations: SEL, standard error of the laboratory (reference method); r^2, coefficient of determination in external validation; SEP, standard error of performance; RPL, ratio of SEP to SEL.

vide a reliable approximation to the fatty acid composition of Ethiopian mustard samples, especially if the fact that validation samples were obtained from a population not represented in the calibration file is taken into account. The goodness of fit between NIRS-predicted and GLC data for the major fatty acids can be observed in Figure 1.

The results obtained in the validation of the NIRS calibration equations of 1996, both from single- and multipopulations, were considerably better than those obtained from 1995 equations (Table 5). For equations developed from multipopulations, the coefficient of determination was higher than 0.95 for oleic, linoleic, linolenic, and erucic acids (see Fig. 2). Furthermore, the SEP obtained in the validation of the single-population equations for these four fatty acids was within two times the standard error of the laboratory (SEL), which is the limit usually considered to accept NIRS equations for accurate routine use (16). These results demonstrate that a highly reliable and accurate nondestructive determination of the fatty acid composition of Ethiopian mustard seeds can be performed by using the NIRS technique, especially for the four major fatty acids (oleic, linoleic, linolenic, and erucic).

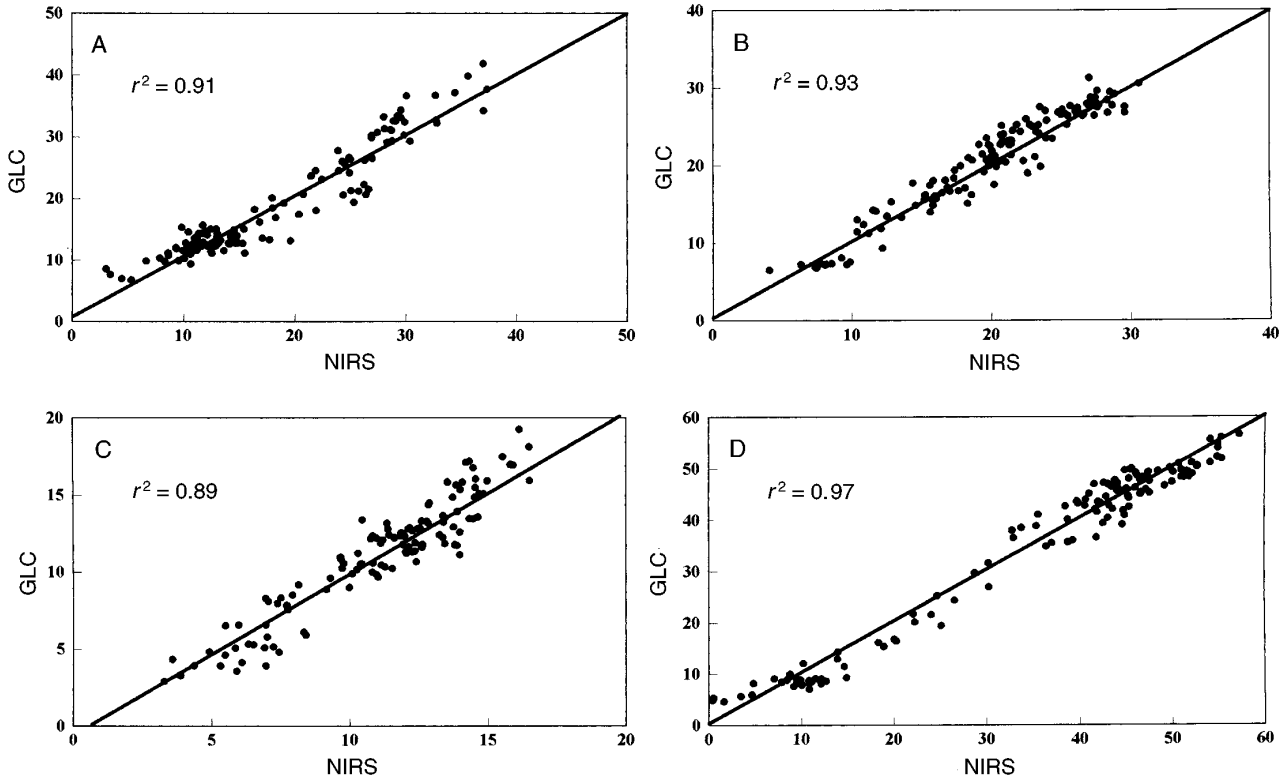

FIG. 1. Prediction plots for oleic (A), linoleic (B), linolenic (C), and erucic acids (D), in external validation of the multipopulation equations of 1995 (including samples from 1992 to 1995) with a set of samples from 1996. The fatty acids are expressed as the percentage of total fatty acids. Abbreviations: GLC, gas–liquid chromatography; NIRS, near-infrared reflectance spectroscopy; r^2, coefficient of determination in validation.

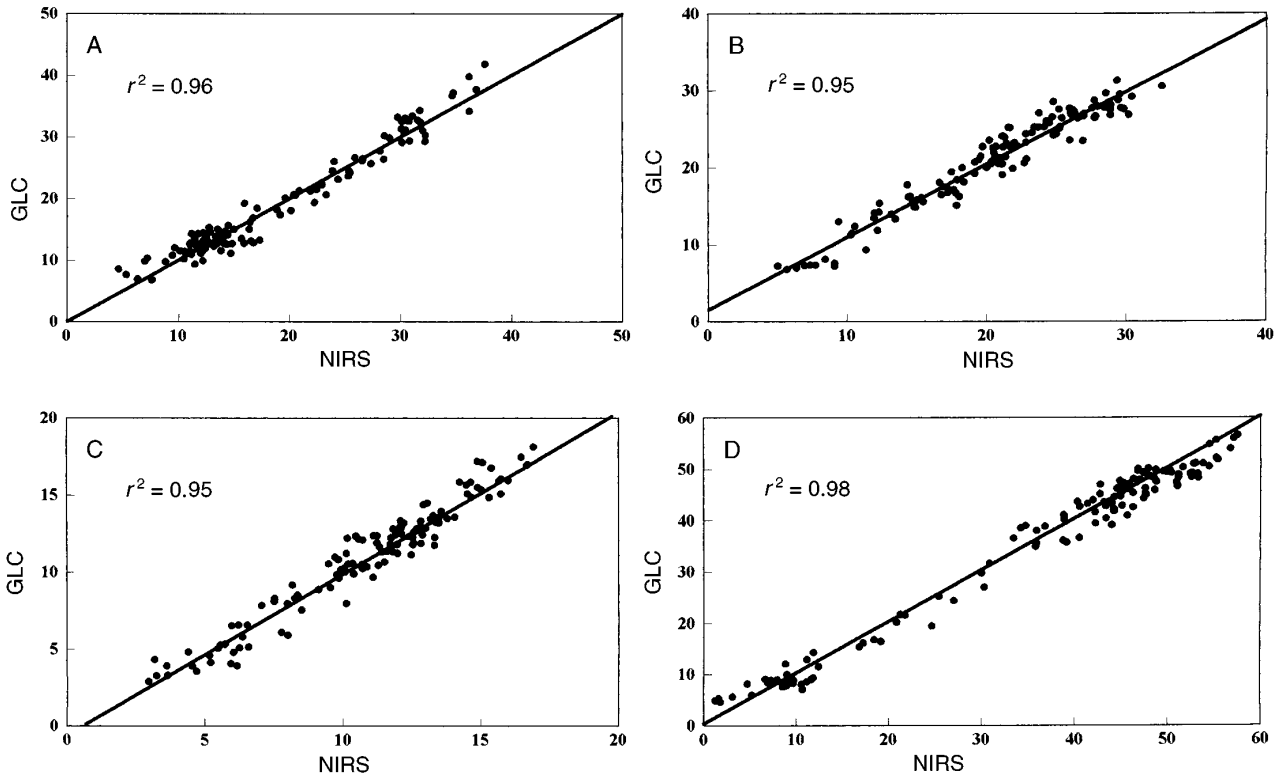

FIG. 2. Prediction plot for oleic (A), linoleic (B), linolenic (C), and erucic acids (D), in external validation of the multipopulation equations of 1996 (including samples from 1992 to 1996) with a set of samples from 1996. The fatty acids are expressed as the percentage of total fatty acids. For abbreviations see Figure 1.

Spectral analysis. Differences in the fatty acid composition of the samples were associated with spectral differences, mainly located at six different spectral regions (Fig. 3). These spectral regions include several absorption bands associated with fatty acids (19,20), which are shown in Table 6. The spectral pattern within these regions was different for each fatty acid, which represents the basis of NIRS discrimination among different fatty acids.

DISCUSSION

The results obtained in this work represent a considerable improvement in the estimation of the fatty acid composition of *Brassica* intact-seed samples by NIRS in comparison with previous studies (Table 1). Up to now, it had only been possible to estimate accurately by NIRS the erucic acid content of *Brassica* intact-seed samples, but not the other fatty acids. The results of this study suggest that poor results in previous studies were mainly due to the lack of samples with a large variability in fatty acid composition. Thus, calibration equations improved considerably when the range for fatty acid values within the calibration data set was increased.

The calibration equations developed in this study were exclusively based on Ethiopian mustard samples, and therefore their application is restricted to this species, which is currently of little economic importance and only cultivated in a limited region of Africa. However, this species has considerable agronomic potential for other semiarid regions (21,22), although its spread to a higher-scale cultivation is mainly limited by inadequate seed oil and meal quality. Intensive breeding efforts to develop high-quality Ethiopian mustard are currently in progress (14,23), and the NIRS equations reported in this work have played an important role in the improvement of the fatty acid composition of the seed oil in this species (13,14). Furthermore, the results obtained in this study may be applicable to other *Brassica* oilseed species (rapeseed, turnip rape, and Indian mustard), owing to their similar seed characteristics.

We conclude that the NIRS technique has high potential to

TABLE 6
Principal Spectral Regions Associated with Differences in the Fatty Acid Composition of the Samples, and Absorption Bands Associated with Fatty Acids Absorbers

Spectral region (nm)	Bond vibration	Structures
1140–1240	C-H second overtone	CH_2, CH_3
1350–1400	First overtone of C-H combinations	CH_2, CH_3
1650–1800	C-H first overtone	CH_2, CH_3
1880–1920	C=O second overtone	$-CO_2H$
2140–2200	=C-H + C=C combinations	HC=CH
2240–2380	C-H combinations	CH_2, CH_3

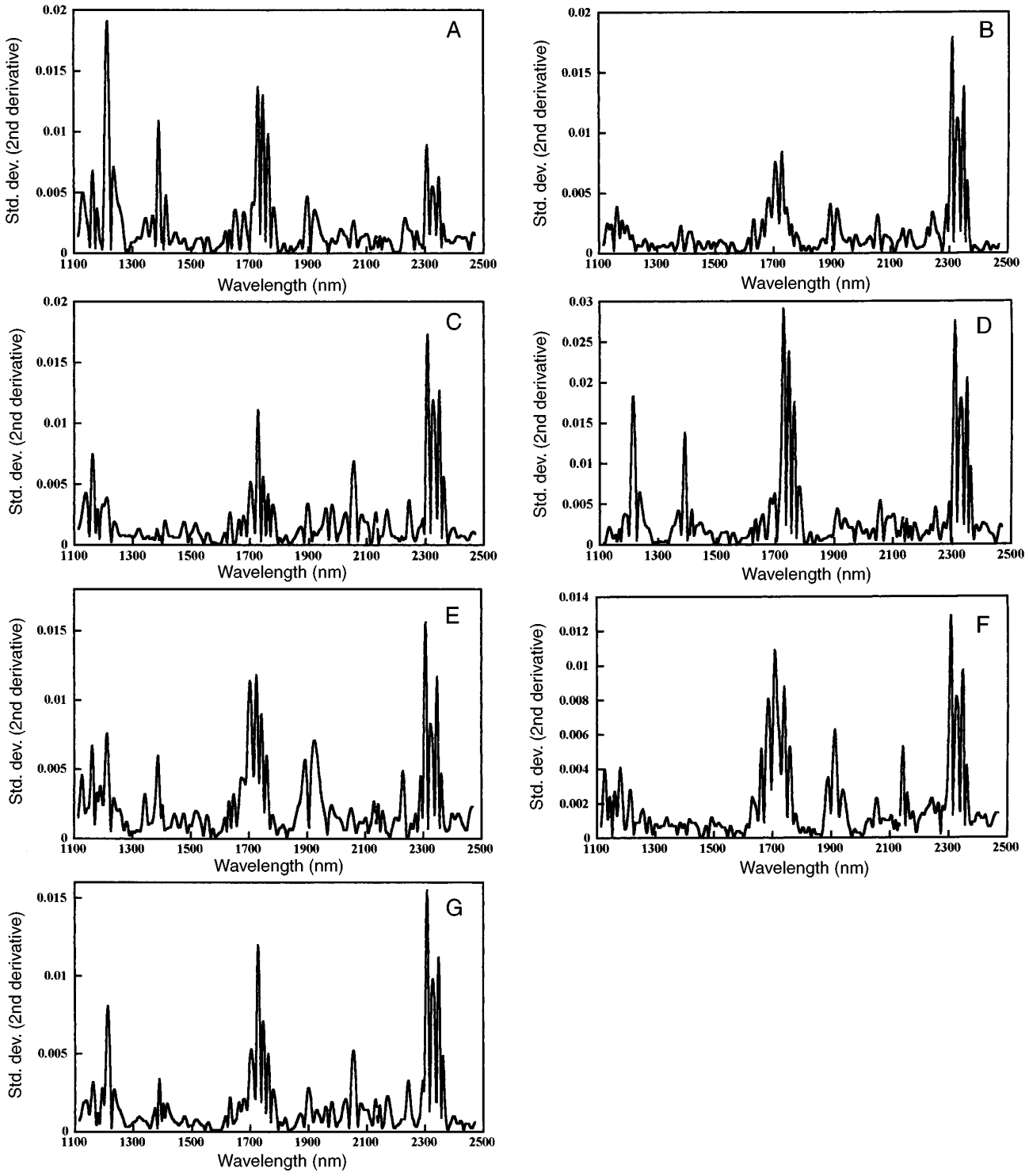

FIG. 3. Standard deviations (Std. dev.) among second-derivative average spectra (low, middle, high fatty acid content). A, $C_{16:0}$; B, $C_{18:0}$; C, $C_{18:1}$; D, $C_{18:2}$; E, $C_{18:3}$; F, $C_{20:1}$; G, $C_{22:1}$.

estimate in a nondestructive way and with a high degree of accuracy the fatty acid composition of the oil in intact-seed Ethiopian mustard samples. These results have special significance because NIRS is a multitrait technique, i.e., fatty acid composition may be determined simultaneously with other traits, such as oil, protein, and glucosinolate content. Therefore, a simple, rapid, and reliable overall characterization of seed quality traits in this species may be obtained at a low cost, which may have a high impact in applications in which large numbers of samples have to be analyzed, such as breed-

ing programs, surveys, and quality control in the rapeseed and mustard processing industry.

Finally, two aspects have to be taken into account for the successful development of accurate calibration equations to estimate the fatty acid composition of *Brassica* seed samples by NIRS: a calibration set with large variability for fatty acid composition, and good repeatability in the analyses by the reference method.

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REFERENCES

1. Tkachuk, R., Oil and Protein Analysis of Whole Rapeseed Kernels by Near Infrared Reflectance Spectroscopy, *J. Am. Oil Chem. Soc. 58*:819–822 (1981).
2. Tkachuk, R., and F.D. Kuzina, Chlorophyll Analysis of Whole Rapeseed Kernels by Near Infrared Reflectance, *Can. J. Plant Sci. 62*:875–884 (1982).
3. Renard, M., C. Bernard, M. Deschamps, V. Furtoss, M. Lila, and A. Quinsac, Glucosinolate Analysis in Whole Rapeseed by Near Infrared Reflectance Spectroscopy, in *Glucosinolates in Rapeseeds: Analytical Aspects*, edited by J.-P. Wathelet, Martinus Nijhoff Publishers, Dordrecht, The Netherlands, 1987, pp. 173–176.
4. Van Deynze, A.E., and K.P. Pauls, Seed Colour Assessment in *Brassica napus* Using a Near Infrared Reflectance Spectrometer Adapted for Visible Light Measurements, *Euphytica 76*:45–51 (1994).
5. Velasco, L., J.M. Fernández-Martínez, and A. De Haro, An Efficient Method for Screening Seed Colour in Ethiopian Mustard Using Visible Reflectance Spectroscopy and Multivariate Analysis, *Ibid. 90*:359–363 (1996).
6. Velasco, L., J.M. Fernández-Martínez, and A. De Haro, Use of Near Infrared Reflectance Spectroscopy to Screen Ethiopian Mustard for Seed Weight, *Agron. J. 89*:150–153 (1997).
7. Koester, S., and C. Paul, Application of Near Infrared Reflectance Spectroscopy (NIRS) in Breeding Rapeseed for Improved Quality, in *Science for Plant Breeding* (Book of Poster Abstracts, Vol. II, XII Eucarpia Congress), edited by European Association for Research on Plant Breeding (EUCARPIA), Paul Parey Publishers, Berlin and Hamburg, 1989, poster 31-4.
8. Reinhardt, T.-C., and G. Röbbelen, Quantitative Analysis of Fatty Acids in Intact Rapeseed by Near Infrared Reflectance Spectroscopy, in *Proceedings of the 8th International Rapeseed Conference*, Saskatoon, Canada. 9–11 July 1991, edited by Groupe Consultatif International de Recherche sur le Colza (GCIRC), Saskatoon, pp. 1380–1384.
9. Reinhardt, T.-C., C. Paul, and G. Röbbelen, Quantitative Analysis of Fatty Acids in Intact Rapeseed by NIRS, in *Making Light Work. Advances in Near Infrared Spectroscopy*, edited by I. Murray and I. Cowe, VCH, London, 1992, pp. 1380–1384.
10. Daun, J.K., K.M. Clear, and P. Williams, Comparison of Three Whole Seed Near-Infrared Analyzers for Measuring Quality Components of Canola Seed, *J. Am. Oil Chem. Soc. 71*:1063–1068 (1994).
11. Velasco, L., J.M. Fernández-Martínez, M. Del Rio, and A. De Haro, The Applicability of NIRS for Estimating Multiple Seed Quality Components in Ethiopian Mustard, in *Proceedings of the 9th International Rapeseed Congress*, Cambridge, United Kingdom 4–7 July 1995, edited by GCIRC, Henry Ling Limited, Dorchester, pp. 867–869.
12. Velasco, L., J.M. Fernández-Martínez, and A. De Haro, Screening Ethiopian Mustard for Erucic Acid by Near Infrared Reflectance Spectroscopy, *Crop Sci. 36*:1068–1071 (1996).
13. Velasco, L., J.M. Fernández-Martínez, and A. De Haro, Isolation of Induced Mutants in Ethiopian Mustard (*Brassica carinata* Braun) with Low Levels of Erucic Acid, *Plant Breed. 114*:454–456 (1995).
14. Velasco, L., J.M. Fernández-Martínez, and A. De Haro, Induced Variability for C18 Unsaturated Fatty Acids in Ethiopian Mustard, *Can. J. Plant Sci. 77*:91–95 (1997).
15. Garcés, R., and M. Mancha, One-Step Lipid Extraction and Fatty Acid Methyl Esters Preparation from Fresh Plant Tissues, *Anal. Biochem. 211*:139–143 (1993).
16. Windham, W.R., D.R. Mertens, and F.E. Barton, II., Protocol for NIRS Calibration: Sample Selection and Equation Development and Validation, in *Near Infrared Reflectance Spectroscopy (NIRS): Analysis of Forage Quality*, edited by G.C. Marten, J.S. Shenk, and F.E. Barton, II, Agricultured Handbook No. 643, USDA-ARS, U.S. Government Printing Office, Washington, DC, 1989, pp. 96–103.
17. Barnes, R.J., M.S. Dhanoa, and S.J. Lister, Standard Normal Variate Transformation and De-Trending of Near-Infrared Diffuse Reflectance Spectra, *Appl. Spectrosc. 43*:772–777 (1989).
18. Shenk, J., and M.O. Westerhaus, Analysis of Agriculture and Food Products by Near Infrared Reflectance Spectroscopy, Infrasoft International, Port Matilda, 1993, 116 pp.
19. Murray, I., Forage Analysis by Near Infrared Spectroscopy, in *Sward Measurement Handbook,* 2nd edn., edited by A. Davies, R.D. Baker, S.A. Grant, and A.S. Laidlaw, The British Grassland Society, Reading, United Kingdom, 1993, pp. 285–312.
20. Osborne, B.G., T. Fearn, and P.H. Hindle, *Practical NIR Spectroscopy with Applications in Food and Beverage Analysis*, Longman Scientifical & Technical, Harlow, 1993, pp. 13–35.
21. Fereres, E., J.M. Fernández-Martínez, I. Minguez, and J. Domínguez, Productivity of *Brassica juncea* and *B. carinata* in Relation to Rapeseed, in *Proceedings of the 6th International Rapeseed Conference*, Paris, 17–19 May 1983, edited by GCIRC, Paris, pp. 293–298.
22. Knowles, P.F., T.E. Kearney, and D.B. Cohen, Species of Rapeseed and Mustard as Oil Crops in California, in *New Sources of Fats and Oils*, edited by E.H. Pryde, L.H. Princen, and K.D. Mukherjee, American Oil Chemists' Society, Champaign, 1981, pp. 255–268.
23. Getinet, A., G. Rakow, J.P. Raney, and R.K. Downey, The Inheritance of Erucic Acid Content in Ethiopian Mustard, *Can. J. Plant Sci. 77*:33–41 (1997).

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