Nondestructive Estimation of Fatty Acid Composition in Seeds of *Brassica napus* L. by Near-Infrared Spectroscopy

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ABSTRACT: The feasibility of near-infrared (NIR) spectroscopy for the nondestructive determination of fatty acid composition in rapeseed was examined. NIR spectra were measured on extracted oil, intact rapeseed kernels, and an intact single rapeseed with an InfraAlyzer 500 in a syrup cup or a single-grain cup. NIR spectra were scanned from 1100 to 2500 nm at 2-nm intervals. As the percentage of linoleic acid increased, the spectral values in the region 1696–1724 nm, where linoleic acid has its absorption band, became always stronger downward in second-derivative NIR spectra. As the percentage of erucic acid increased the spectral value at 1728 nm, where erucic acid has its absorption band, became always a little bit stronger downward in the second-derivative NIR spectra. On the basis of their NIR spectral patterns, linoleic acid and erucic acid could be successfully determined in both intact seed kernels and in a single seed of rape without damaging them.
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KEY WORDS: Analysis, *Brassica napus* L., erucic acid, fatty acid composition, intact seeds kernels, intact single seed, near-infrared, nondestructive, rapeseed, spectroscopy.

Rape (*Brassica napus* L.) is one of the major crops used for oil production. Two types of rapeseed are present in commerce: low-erucic acid types, which are used in food applications, and high-erucic acid types, which are used in oleochemicals (1). Erucic acid is also of nutritional interest (2). In oilseed breeding projects, modification of fatty acid patterns is often the main theme (3,4). The analysis of fatty acid compositions of rapeseed is important in breeding programs as well as for checking raw materials in the oil milling process. However, the conventional method for determining fatty acid composition is time-consuming and labor-intensive: it includes grinding, oil extraction, sample pretreatment, and gas chromatographic (GC) analysis. So, a rapid, simple, and nondestructive method to determine the fatty acid composition is necessary for increased demands in breeding projects (3,4) and for rapidly checking raw materials at the oil milling plant. Useful varieties or individual plants are to be selected from a lot of samples to be tested, i.e., the composition of an individual single seed is often important. Sato *et al.* (5–8) and Karen *et al.* (9) reported that a near-infrared (NIR) spectral pattern of oil showed its fatty acid composition. In this report, the nondestructive determination of fatty acid composition in rapeseed by NIR spectroscopy was examined.

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MATERIALS AND METHODS

Samples. Thirty individual rape varieties were cultivated on each of two different farms of the authors' experiment station (located in Nishigoshi, Kumamoto-ken, Japan) for this study. They were sown on October 16, 1996, and were harvested in spring 1997. The varieties were divided into two groups: those with high levels of erucic acid and those with low or no erucic acid. The former included the following named varieties: Michinoku-natane; Tukusi-natane; Isuzu-natane; Tisaya-natane; Aburamasari; Houman-natane; Abukuma-natane; Kogane-natane; Haya-natane; O'omi-natane; Genkai-natane; Tikuzen-natane; and Dairyu'u-natane. The later (low-level erucid acid) varieties comprised the following: Asakano-natane; Kizaki-natane; Touhoku 90; Alto; Aztec; Bounty; Celebra; Delta; Jubel; Karat; Legend; Lergo; Topas; Tornado; Tribute; Vanguard; and Morishi 148. "Natane" is rapeseed in Japanese.

Chemical measurements. Oil was extracted as follows: about 2 g of seed was ground manually in a mortar and dried (105°C for 3 h). Oil was then extracted with diethyl ether by the Soxhlet method (Soxtec System HT 1043 Extraction Unit, Tecator, Sweden). The extracted oils were used for GC analysis after methyl-esterification according to the conventional method (10). The gas chromatograph was equipped with a flame-ionization detector (GC-17A; Shimadzu Co., Kyoto, Japan). The GC conditions for determining the fatty acid methyl esters were as follows: 100 m long × 0.25 mm i.d. WCOT fused-silica capillary column (CP-Sil 88 from Chrompack, Middelburg, The Netherlands); 270°C for injection port, 300°C for detector port, and 190°C for column temperature; H_2, 60 kPa; air, 50 kPa; He, 80 kPa eluting gas pressure. Each sample was analyzed twice.

NIR. An InfraAlyzer 500 [Bran + Luebbe (B+L) GmbH, Norderstedt, Germany] was used to measure the NIR transflectance spectra of the extracted oil in the wavelength range

from 1100 to 2500 nm at 2-nm intervals between glass slides on a syrup cup according to the previous report (8).

Intact rapeseed kernels. A hole of a single-grain cup was completely filled with intact rapeseed kernels [center hole diameter = 24 mm, (B+L)] and covered with a glass lid to level the sample surface. NIR reflectance spectra were then measured. This was the same type of the single-grain cup used in the previous study (8) The same NIR instrument and wavelength range were used as for the extracted oil.

Single rapeseed kernel. An intact single rapeseed was placed in the hole of an improved single grain cup (Fig. 1). The NIR reflectance spectrum of an individual seed was measured without a glass lid. This sample cup was developed for measuring small seeds, such as rapeseed [center hole diameter = 20 mm (B+L); diameter of a typical rapeseed equals nearly 1 mm].

Standard fatty acid methyl esters. The standard fatty acid methyl esters [oleic acid methyl ester, linoleic acid methyl ester, erucic acid methyl ester, or *cis*-11-eicosenoic acid methyl ester (Sigma Chemical Co., St. Louis, MO] were placed on a syrup cup, covered with a glass lid, and scanned to check their absorption bands. These were also used as authentic samples to check retention time in GC analysis.

Mathematical treatment of NIR spectral data for standardization. The second-derivative mathematical treatment and the standardization of NIR spectral data were carried out according to the previous article (8). The authors used different derivative conditions from the default conditions (8).

RESULTS AND DISCUSSION

NIR spectra of rapeseed. Figure 2 is the raw and the second-derivative NIR spectra of intact rapeseed kernels and an intact single rapeseed. The absorption maximum at 1960 nm is due to water; maxima around 1700–1800 and 2300–2400 nm are due to oil. The absorbing region around 2180 nm may be due to protein. Intact seed kernels and an intact single seed have distinct spectra. In Figure 2A, the NIR spectrum of a single rapeseed was nearly identical to that of a bulk sample of whole kernels. This might be expected because rapeseed contains a high oil content (11,12), almost 40%. The second-derivative math treatment made the absorption characteristics by oil molecules even more evident. However, when the previous single-grain cup was used for an intact single seed, the NIR spectrum was weak, as shown in Figure 2. By the way, absorption bands in the NIR spectrum of extracted rapeseed oil were only due to oil. A single-grain cup itself has an artificial effect around 1400 nm, but it does not affect the oil absorption bands. The NIR spectra of oil were similar to those of fatty acid methyl esters. Thus, NIR spectra provide information about the fatty acid composition. Velasco *et al.* (13,14), Reinhardt *et al.* (15), and Daun *et al.* (16,17) also tried to estimate erucic acid content in mustard, rapeseed, and canola oil by the NIR method. However, they used multilinear regression analysis or partial least square analysis, which is, a statistical method. In the following, we tried to measure the erucic acid ratio of rapeseed from the spectral pattern, i.e., based on the assignments of the absorption bands.

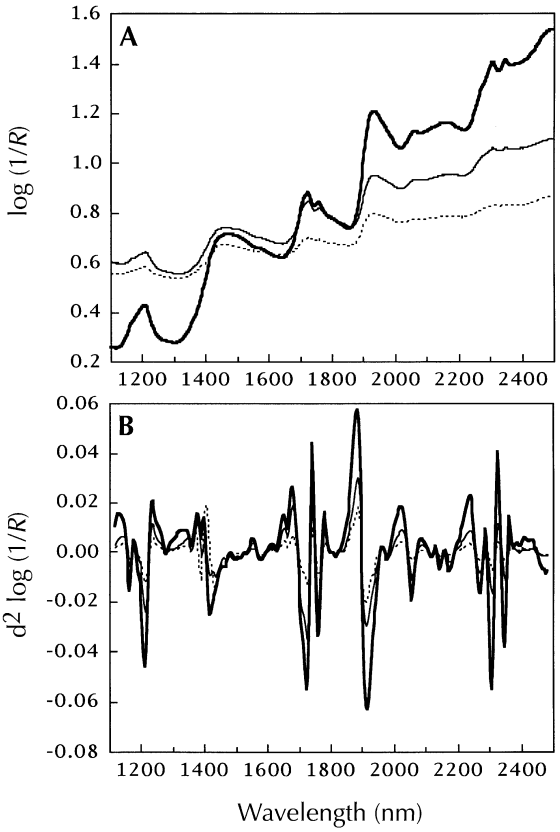

FIG. 2. Original (or nonstandardized) raw (A) and second-derivative (B) near-infrared spectra of rapeseed. (—) Intact seeds; (—) intact single seed (improved single-seed cup); (----) intact single seed (previous type of cup; Ref. 8). *R,* reflectance.

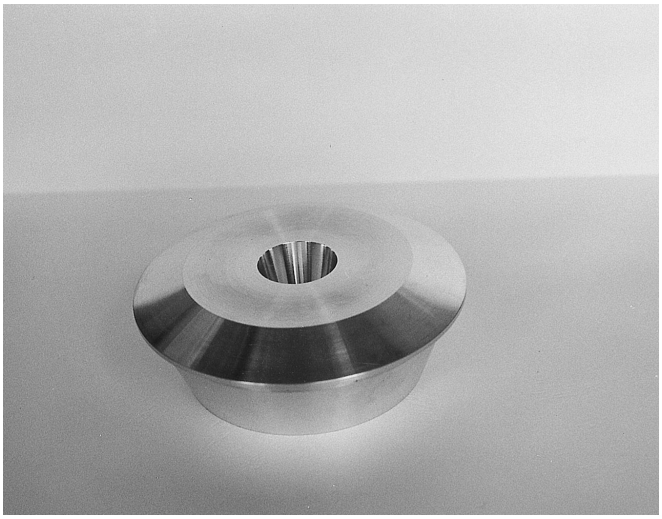

FIG. 1. An improved single-grain cup.

Figure 3 shows the 1650–1750 nm wavelength region of standardized second-derivative NIR spectra of standard methyl esters and of an intact single-grain rapeseed. In Figure

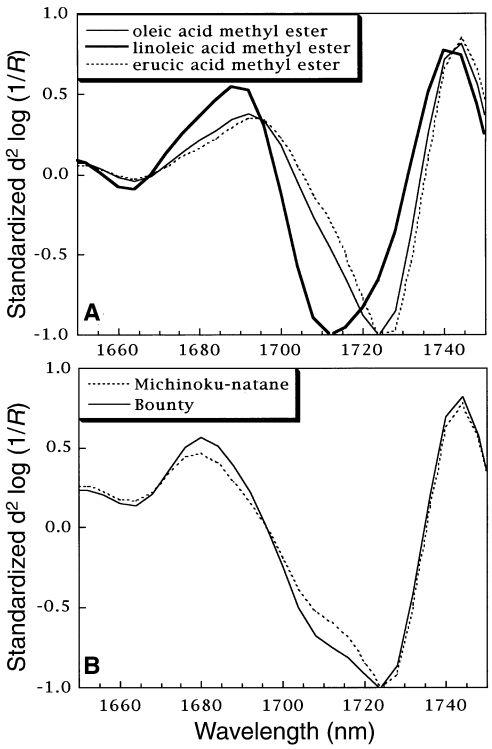

FIG. 3. The 1650–1750 nm wavelength region of standardized second-derivative near-infrared spectra of standard fatty acid methyl esters (A) and an intact single rapeseed (B). Variety names appear in B.

3, the absorption at 1600 nm was set to zero, and the absorption at the minimum around 1724 nm was normalized to −1 according to the calculation method previously reported (7,8). In the following, we used these converted second-derivative NIR spectral values for the analysis. These spectral patterns correspond to the levels of fatty acid moieties. Figure 3A shows NIR spectra of the standard fatty acid methyl esters, and the slope of the straight line connecting the two points at 1724 and 1728 nm was steeper for oleic acid methyl ester than for erucic acid methyl ester. Further, for linoleic acid methyl ester, the absorption band shifted to a shorter wavelength region in the second-derivative NIR spectrum.

Table 1 summarizes the fatty acid compositions of the two groups. Table 1 shows almost the same trends as previous articles (4,18). Two examples were selected for presentation in Figure 3B: Michinoku-natane and Bounty. The former is representative for varieties with high levels of erucic acid, and the latter represents a variety without erucic acid. The main fatty acid compositions of (Michinoku-natane vs. Bounty) samples were as follows: linoleic acid, 13.83 vs. 21.70%; oleic acid, 16.85 vs. 60.64%; erucic acid, 40.98 vs. 0.00%. In Figure 3B, the slope of the straight line between 1724 and 1728 nm was steeper for Bounty than for Michinoku-natane because the percentage of erucic acid decreased. Further, because the percentage of linoleic acid increased, the absorption around 1710 nm became stronger downward in the second-derivative NIR spectrum for Bounty than for Michinoku-natane.

cis-11-eicosenoic acid methyl ester (C20:1) had an intermediate pattern between C18:1 and C22:1. However, the contribution at 1728 nm by this moiety might be weak because its absorption strength and ratio were much smaller than that of C22:1. Further, C20:1 was mainly found in the varieties with high levels of erucic acid, so it strengthened the effects of C22:1 only slightly.

Relationship between NIR spectral values and fatty acid compositional ratio. The sum of the spectral values from 1696 to 1724 nm was calculated for each sample as an index for the area of this region to show that: the higher the percentage of linoleic acid is, the broader the absorption becomes. Figure 4 shows good correlations between the percentage of linoleic acid and the sum of these spectral values. The correlation coefficient for extracted oil was –0.926, for intact rapeseed kernels –0.866, and for an intact single rapeseed –0.858. Especially for the extracted oil, NIR spectral patterns were well reflected by the fatty acid composition.

The difference between L(1728) and L(1724) was calculated as an index for the relative concentrations of oleic and erucic acids. L (xxxx) means calculated spectral value at xxxhm. Figures 5 and 6 show good relationships between [L(1728) − L(1724)] and the percentages of oleic acid and erucic acid, respectively. For intact seed kernels or a single seed (Fig. 6), the line $y = 0.12$ divided the samples into two groups: varieties with high levels of erucic acid, and those with low or no erucic acid. For extracted oil, separation of the groups was much clearer. As reference data, fatty acid compositions on whole-grain seeds were also used for the NIR spectra of single grain seeds. Certainly, even in a homogeneous variety, there is a considerable range in fatty acid compositions for single seeds.

The correlation for the extracted oils was best. In turn, the correlation for a batch of intact seed kernels was better than that for the intact single seed. The oil extraction for GC analysis in this study was carried out on a number of whole seeds, not on each single seed. So, the fatty acid variation was some-

TABLE 1
Summary of Fatty Acid Compositions of Rapeseed Containing Differing Amounts of Erucic Acid[a]

Erucic acid	C16:0	C18:0	C18:1	C18:2	C20:0	C20:1	C18:3	C22:1
High level	3.47 ± 0.40	1.44 ± 0.23	19.20 ± 2.10	14.02 ± 0.90	0.94 ± 0.16	9.27 ± 1.08	8.57 ± 0.57	38.59 ± 1.50
(n = 26)	(2.62 ~ 4.32)	(1.06 ~ 1.96)	(14.85 ~ 22.21)	(12.61 ~ 15.93)	(0.74 ~ 1.33)	(7.06 ~ 11.13)	(7.50 ~ 9.72)	(35.54 ~ 42.20)
Low or none	4.27 ± 0.50	2.02 ± 0.51	60.79 ± 3.19	18.98 ± 2.00	0.62 ± 0.18	1.92 ± 1.53	8.50 ± 0.82	1.01 ± 1.69
(n = 34)	(3.09 ~ 5.56)	(1.27 ~ 3.59)	(51.45 ~ 65.93)	(14.53 ~ 22.33)	(0.21 ~ 1.04)	(0.74 ~ 7.85)	(6.62 ~ 10.06)	(0.00 ~ 6.48)

[a]Mean ± SD (minimum ~ maximum). Based on extracted oil (wt%).

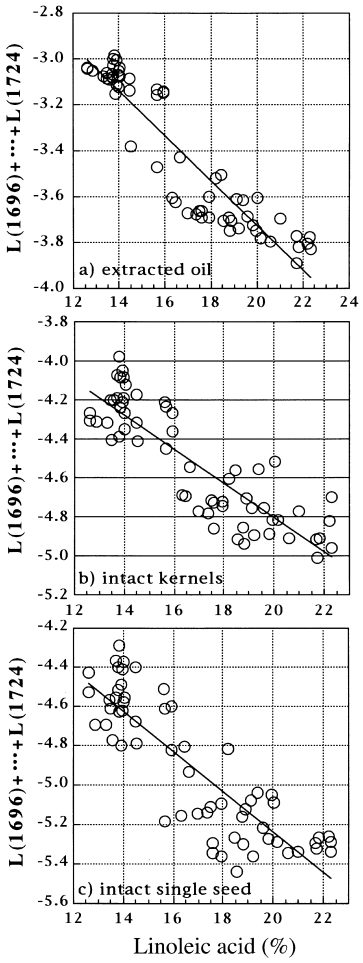

FIG. 4. Correlations between the percentages of linoleic acid in rapeseed and the sum of their converted second-derivative near-infrared spectral values from 1696 to 1724 nm.

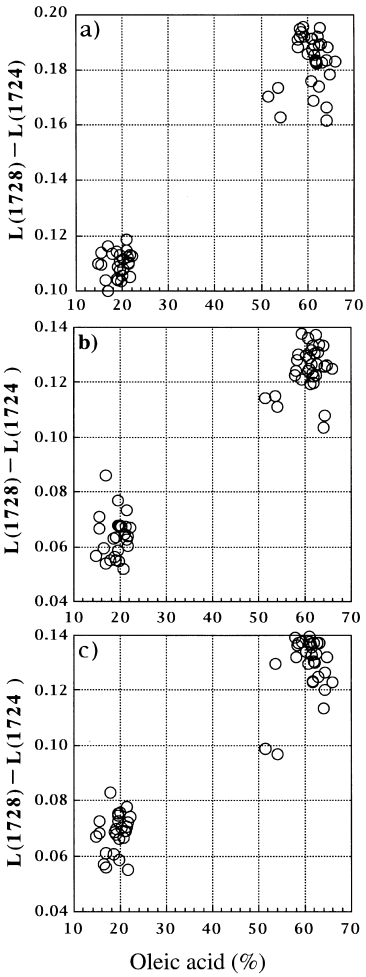

FIG. 5. Correlation between the percentages of oleic acid in rapeseed and the difference between their converted second-derivative near-infrared spectral values at 1728 and at 1724 nm.

what averaged. On the other hand, with a single seed, the variation of the individuality of a single seed still remains. This explains the poorer correlation.

Downey (19) developed the half-cutting method for the analysis used in breeding. It includes cutting a seed into halves, extracting oil from one half, and analyzing fatty acid composition by GC. Our method might be an improvement. The capability of NIR for the nondestructive analysis of the fatty acid composition of rapeseed has been shown. There is no need to husk the hulls of rapeseed. The level of erucic acid in the fatty acid composition can be detected nondestructively by NIR. Further, precision can be improved, if average spectra are obtained by scanning the same seed kernels or a single seed several times. Especially for an intact single seed, nondestructive analysis can be carried out successfully even when the seed is very small, and subsequent germination can be ensured. There are other types of rapeseed: *Brassica rapa*, which is rarely cultivated in Japan, contains high content of erucic acid, and L(1728) might be stronger downward in the second-derivative spectra. Then, the positions in the scatter graphs (Fig. 5 and 6) may be changed as well.

Reinhardt *et al.* (15) reported that the main band for erucic acid in rapeseed at 1750 nm. This difference might be the result of statistical analysis. Our techniques did not depend on statistical methods but on the assignment of the absorption band. Because the spectral pattern used was due to the assignments of fatty acid moieties, these results can be adopted for determining the fatty acid composition of oilseeds generally, and one can choose the method depending on the sample type or the purpose. Also, this method does not depend on the populations used in the study. The index is based on assignment of the absorption band. So, it may be adopted for other instrumental results as well as for other populations and other oilseeds. This method can be used for the selection not only of varieties with or without erucic acid but also of varieties with useful properties linked with erucic acid genes: the detection of erucic acid may thus be used as an index or a marker in breeding projects.

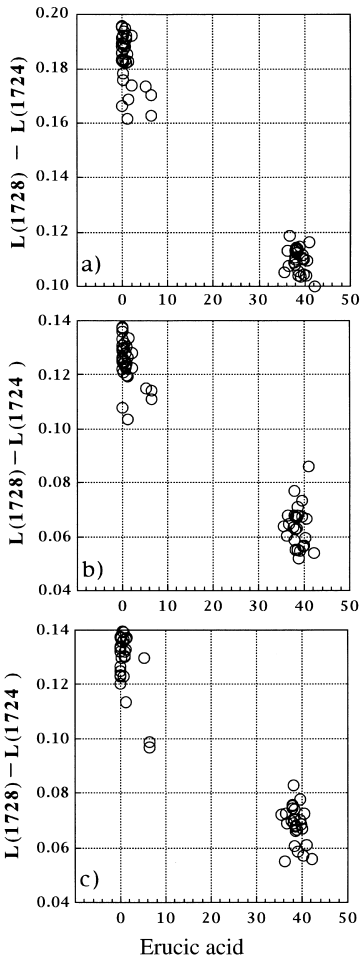

FIG. 6. Correlation between the percentages of erucic acid in rapeseed and the difference between their converted second-derivative near-infrared spectral values at 1728 and at 1724 nm.

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