NIR Reflectance Spectroscopic Analysis of the FA Composition in Sesame (*Sesamum indicum* L.) Seeds

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ABSTRACT: The feasibility of using NIR reflectance spectroscopy to estimate the FA composition of sesame seed (*Sesamum indicum* L.) samples from the National Institute of Crop Science of Japan and from Myanmar was examined. Multiple linear-regression analyses of NIR spectral data and chemical data for whole seeds were carried out to develop calibration equations for predicting the proportion of each of the four major FA in sesame seeds from the total FA composition. The SE of prediction (SEP) was 0.616% for palmitic acid, 0.348% for stearic acid, 1.051% for oleic acid, and 0.826% for linoleic acid. This NIR method provides a simple, rapid, and nondestructive means of estimating the FA composition of sesame seeds for breeding selection, regardless of the color of the sesame seed coats. However, the proportions of palmitic and stearic acids could not be reliably measured because their SEP were almost as great as the SD of their concentrations in the set of prediction samples. The relationship between NIR spectral patterns and the FA composition of sesame seeds also was examined. The correlation coefficient calculated for the standardized second-derivative NIR spectral readings at 1708 nm and the percentages of linoleic acid was –0.830. A rough estimate of the proportion of linoleic acid in the total FA composition of sesame seeds could be obtained even with single sesame seeds, except for those with a black coat, based on NIR spectral pattern analysis using the wavelength assignments of linoleic acid.

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KEY WORDS: Analysis, FA composition, linoleic acid, near-infrared, oleic acid, second derivative, sesame seed, *Sesamum indicum* L., spectroscopy.

Sesame (*Sesamum indicum* L.), one of the most important oilseed plants, has gained considerable attention as a useful alternative crop in Japan. Thus, improving the FA composition of sesame through breeding programs is an important consideration (1). However, the conventional method for FA determination is tedious. It includes milling, oil extraction, chemical reaction, and analysis with GC equipment. A simple and rapid method for the determination of FA composition is necessary for screening sesame varieties. Furthermore, nondestructive analysis of seeds is needed because individual seeds are sometimes selected from large populations in the breeding process.

NIR spectroscopy has been recognized as one of the most powerful and useful tools for analysis in the fields of agriculture and food testing. NIR also has been used for the analysis of sesame seed constituents (Sato, T., A.A. Maw, and M. Katsuta, unpublished report) and for the identification of geographic origin (2). FA composition is an important index of fat or oil quality for nutritional classification. Therefore, if NIR spectroscopy could be used to analyze the FA composition, it would become an even more useful technique. In this study, the feasibility of using NIR spectroscopy to estimate the FA composition of sesame seeds was examined. In addition, the relationship between NIR spectral patterns and the FA composition of sesame seeds was examined. NIR spectral patterns reflect the FA composition because absorption bands arising from –C–H stretching motions are observed in the NIR region, especially in the 1600–1800 nm range (3–10), and the relative intensities and positions of these bands may be influenced by the degree of unsaturation and the chain length of the FA.

MATERIALS AND METHODS

Samples. Thirty samples were collected as genetic resources in Myanmar by the Seed Bank Project of the Japan International Cooperation Agency (JICA). Another 52 samples of Japanese varieties and lines were cultivated and harvested at a field of the National Institute of Crop Science (NICS, Tsukuba, Japan) in 1997–2001. They included yellowish brown-, dark brown-, black-, and white-coated seeds. These samples were sent to the National Agricultural Research Center for the Kyushu Okinawa Region (KONARC, Kumamoto, Japan) for analysis. The samples are listed in Table 1.

Chemical measurements. The oil was extracted from about 0.5 g of sample seeds with diethyl ether by the Soxhlet method (Soxtec System HT 1043 extraction unit; Tecator, Höganäs, Sweden) after manual crushing. The extracted oils were then used for GC analysis after transesterification according to the conventional method (11). The gas chromatograph was equipped with an FID detector (GC-17A; Shimadzu Co., Kyoto, Japan). The GC conditions used to determine the FAME were the same as those in a previous report (8). Each sample was analyzed twice, and the average of duplicate measurements was calculated. For each sample, the sum of the major FA components—palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2)—was normalized to 95%.

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TABLE 1
List of Samples

Samples from Myanmar, varieties and lines
Pale-21×Hnan Ni No.8; Yetarshay; Ashri×Yoe, Sein(5-1-0); Korea×Ban Ni(13-0-2); MMT-995-501; Ban Net×Hnan Ni(6-0-10-1); Magwe 7/9; Water-logged resistant Kachin; Hnan war lay; Ban Net×Hnan Ni(6-0-3-1); Ashri×Sin-3; Japan Net×Hnan Ni; Hnan Ni; Ban Net×Sin-4(9-0-1-3); Ban Net×Hna Ni(9-7-0); Ashri–118; Ashri×Sin-3 No.237; Sin Yadana-3, A_1; Khway Lay Ni; Me Thi La; Yoe Sein, 9A; Magwe 7/9×China-8(1-9-1-1); Poke Pyay×Hnan Ni; Shwe tasoke; Sin-2×Putt Le-1; Thee Kone(Local variety); Yathae Kyaw; Sin yadana-4

Samples from the National Institute of Crop Science, varieties and lines
Masekin; Iwateguro; Kanto-1; Kanto-2; Kanto-11; 733; 730; TOYAMA016; KOREA58; KOREA54; KUUR6070; KUUR6081; KUUR6082; KUUR6022; Gautemara; TOYAMA062321; TOYAMA146; TOYAMA280; TOYAMA308; TOYAMA40221; TOYAMA3201; TOYAMA942; 3304.2; 9310; Waisei; 114; KOREA39; KOREA45; TOYAMA925; COL/AKITA/TNAES/89004.5/; COL/FUKUSHIMA/TNAES/9001; Azuma; Chichibuzairai; CKL/NAGANO/1988/88026; COL/OKAYAMA/TAKAHASHI/09200; Velvet; COL/MIE/NIAR/1998/029; TOYAMA959; 37803; COL/NAGASAKI/NIAR/1994/11; COL/OKINAWA/NIAR/1991/060

NIR spectroscopy. (i) Intact sesame seed kernels. Intact sesame seed kernels were fully packed in the hole of a single-grain cup [center hole diameter = 24 mm; Bran + Luebbe (B+L) GmbH, Norderstedt, Germany]. They were covered with a glass lid so that the level of the sample surface would be smooth. An InfraAlyzer 500 (B+L) was used to measure their NIR reflectance spectra in the wavelength range from 1100 to 2500 nm at 2-nm intervals. The same NIR instrument and wavelength range were used in the following experiments. Each sample was measured three times at different positions, and the average spectrum was used for analysis. The samples were divided into two sets: a calibration set composed of 55 samples and a prediction set composed of 27 samples, as described in Table 2. Multiple linear-regression analyses of the NIR spectral data and the chemical data were carried out using IDAS software (B+L). When the first- and second-derivative NIR spectra were calculated, the default parameters were used. For the first derivative, the space between output points was 4 nm, the size of the moving average was 8 nm, the size of the derivative segments was 8 nm, and the gap between the derivative segments was 14 nm. For the second derivative, the space between output points was 4 nm, the size of the moving average was 4 nm, the size of the derivative segments was 20 nm, and the gap between the derivative segments was 20 nm.

(ii) Single, intact sesame seed. A single, intact sesame seed was placed in the hole of an improved single-grain cup (INF-B333-01, center hole diameter = 20 mm; B+L), as reported previously (8). The NIR reflectance spectrum of the individual seed was measured without a glass lid. The NIR spectra of the single sesame seed in the modified single-grain cup were clearer because the modified cup had been improved to allow for a better collection of the reflection light from a small sample. This sample cup was developed for measuring a very small seed. Five seeds were measured, and the average spectrum was used for analysis.

(iii) Extracted oil. The NIR transflectance spectra of the extracted oil were measured using glass slides and a syrup cup, as reported previously (8). Because the samples from Myanmar were very small, the NIR analysis of extracted oil was performed on only the samples from NICS.

Mathematical treatment of NIR spectral data for standardization. The parameters used to calculate the second-derivative spectra were as follows: The size of the moving average was 4 nm, the size of the derivative segments was 12 nm, and the gap between the derivative segments was 12 nm. Furthermore, to facilitate comparison of spectra, these NIR spectra were standardized by setting the spectral value at 1600 nm as 0.0 and that at 1724 nm as –1.0. The second-derivative mathematical treatment and the standardization of the NIR spectral data were carried out as reported in previous articles (8,10).

RESULTS AND DISCUSSION

Estimation of FA composition by the NIR method. FA were found in the following ranges (in %): 16:0, 7.64–10.66; 18:0, 4.06–6.60; 18:1, 33.78–44.36; and 18:2, 34.21–47.70 (see Table 2). These ranges were nearly the same as those reported by Yermanos *et al.* (12) and by Brar (13). The proportions of linoleic and oleic acids were inversely correlated, with a cor-

TABLE 2
FA Composition for Calibration and Prediction Sets

	Palmitic acid (%)			Stearic acid (%)			Oleic acid (%)			Linoleic acid (%)		
	Mean	Min.–max.[a]	SD	Mean	Min.–max.	SD	Mean	Min.–max.	SD	Mean	Min.–max.	SD
Calibration set												
Myanmar (*n* = 20)	9.47	8.15–10.42	0.68	5.43	4.49–6.60	0.47	40.25	36.93–44.15	1.95	39.85	35.96–43.08	1.89
NICS (*n* = 35)	9.31	8.41–10.52	0.55	4.61	4.06–6.11	0.40	38.34	33.78–44.12	2.28	42.74	38.05–47.70	2.35
Prediction set												
Myanmar (*n* = 10)	9.25	7.64–10.66	0.99	5.65	5.31–6.54	0.47	40.84	38.00–44.36	2.26	39.27	34.21–43.45	2.90
NICS (*n* = 17)	9.07	7.97–10.27	0.65	4.81	4.11–5.87	0.46	38.58	35.26–41.16	1.93	42.55	40.37–46.62	2.00

[a] Min.–max., minimum–maximum; NICS, National Institute of Crop Science.

TABLE 3
Multiple Linear Regression Analysis of the Values Obtained by the NIR Method

	Calibration set			Prediction set		
	Calibration equations	*r*	SEC	SEP	Mean corrected SEP	Bias
Palmitic acid	7.604+40.506×L(1436)–45.916×L(1476)+132.248×L(1912) –140.292×L(1924)+19.780×L(1952)	0.593	0.509	0.616	0.580	0.236
Stearic acid	6.618–67.482×d2L(1358)–37.980×d2L(1410)+139.014×d2L(1686) –814.339×d2L(1810)–85.129×d2L(2166)+96.323×d2L(2322)	0.869	0.305	0.348	0.331	–0.126
Oleic acid	40.927+438.761×d1L(1479)–3391.399×d1L(1719) –2461.745×d1L(1747)+2412.552×d1L(1755)+633.051×d1L(1879) +1061.221×d1L(2003)–469.015×d1L(2279)	0.898	1.105	1.051	1.063	–0.126
Linoleic acid	42.372–1957.603×d1L(1707)+3783.052×d1L(1715) –2951.203×d1L(1847)–2705.246×d1L(2059)+2266.029×d1L(2067)	0.942	0.917	0.826	0.799	0.259

[a] SEC, SE of calibration; SEP, SE of prediction; L(1436), raw spectral data at 1436 nm; d1L(1479), first-derivative spectral data at 1479 nm; d2L(1358), second-derivative spectral data at 1358 nm.

relation coefficient of –0.950; this value was quite similar to that reported by El Tinay *et al.* (14).

The NIR calibration was performed by multiple linear-regression analysis of the data for 55 samples in the calibration set (Table 2). Table 3 presents the calibration equations, the correlation coefficients, and the SE of calibration (SEC). These calibration equations provided the best predictions. The wavelengths were selected to reflect the chemical structures of the constituents. According to Osborne and Fearn (15), oils have absorption bands around 1700–1750, 2100–2200, and 2300–2400 nm. In particular, the absorption at ~1710 nm provides information about unsaturation. Table 3 also shows the results obtained for the 27 samples in the prediction set. Figure 1 shows correlations of the values estimated by the NIR method with those determined chemically. The correlation coefficient (*r*) between the proportion of palmitic acid in the total FA composition obtained by chemical analysis and values estimated by NIR analysis was 0.699, and the SE of prediction (SEP) was 0.616%. For the proportion of stearic acid, *r* was 0.849, and the SEP was 0.348%; for that of oleic acid, *r* was 0.889, and the SEP was 1.051%; and for linoleic acid, *r* was 0.959, and the SEP was 0.826%. The NIR method worked better for oleic and linoleic acids because the proportion of palmitic acid was small and the SEC for stearic acid was almost as great as the SD of the stearic acid concentrations for the set of prediction samples. These findings indicate that NIR spectroscopy can be successfully applied as a simple, rapid, and nondestructive screening method for the selection of sesame seeds in breeding programs, regardless of the color of the sesame seed coats.

Velasco and Mollers (16) analyzed the FA composition of rapeseeds by NIR spectroscopy. They obtained good results for the concentrations of 18:1 ($r^2 = 0.93$), 18:3 ($r^2 = 0.95$), and 22:1 ($r^2 = 0.94$). On the other hand, the calibration equations for the contents of 16:0, 18:0, and 18:2 were less reliable ($r^2 = 0.67$ to 0.73), and that for the content of 20:1 was the least reliable ($r^2 = 0.31$). Perez-Vich *et al.* (17) determined the FA composition in sunflower by using NIR spectroscopy. Their results showed that the NIR method was reliable and accurate for estimating the major FA components in sunflower oil (validation r^2 ranged from 0.97 to 0.99), meal (r^2 from 0.92 to 0.98), and husked seeds (r^2 from 0.90 to 0.97). Our findings concerning the NIR performance were similar to those in the studies mentioned above.

Relationship between NIR spectral patterns and FA composition. NIR analyses have often been carried out using a statistical method, i.e., multiple linear-regression analysis, as described above. The transfer and management of the calibration equations is a problem when they are applied to different sample populations, i.e., samples harvested in different years or areas, different sample types (liquid, meal, or intact seed), or when a different instrument is used. In the present study, another approach was examined. The NIR spectral patterns reflect the FA composition because they are affected by the

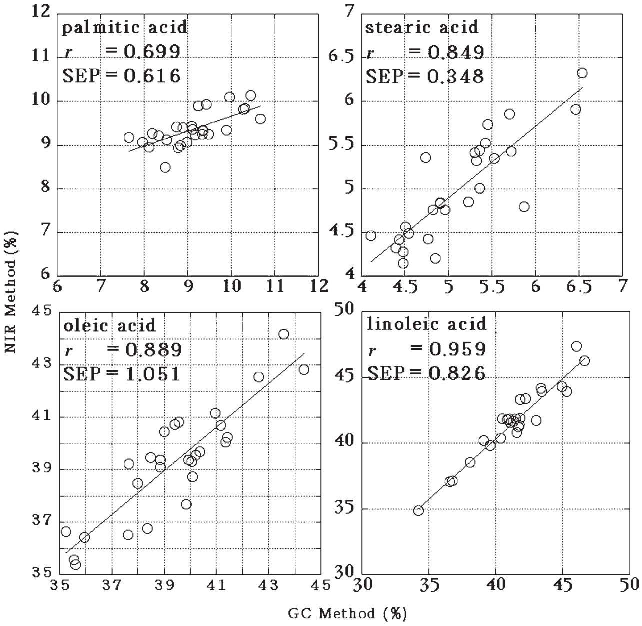

FIG. 1. Correlation between the proportions of FA in the total FA composition as estimated by the NIR method and by the GC method. SEP, SE of prediction.

unsaturation and the carbon chain lengths of the FA, especially in the 1600–1800 nm region (3–10). However, in the original NIR spectra, spectral differences attributable to differences in FA composition could not be detected. In addition, no differences were detected in the second-derivative NIR spectra calculated with the default parameters (18).

Using the mathematical treatments of the NIR spectra described in the Materials and Methods section, a clearer difference was obtained. The solid lines in Figure 2 show the average spectrum in the spectral region where a pattern characteristic of the FA composition was observed. This pattern was especially characteristic for the proportions of oleic acid (18:1) and linoleic acid (18:2). As the percentage of linoleic acid increased, the absorption band around 1708 nm was stronger downward in the second-derivative NIR spectra because the spectral phase was reversed in the second-derivative spectra.

For comparison, the second-derivative NIR spectral value at 1724 nm was set the same as that shown in Figure 2. As the percentage of linoleic acid (18:2) increased, the spectral value around 1708 nm, where linoleic acid (18:2) had an effect because of its broad absorption band and larger deviations, became slightly stronger downward in the second-derivative NIR spectra. This is shown by the thin and thick solid lines in Figure 2A. Figure 2A shows the outcome with multiple seeds, and the pattern was nearly the same with a single seed. In a typical case, when the absorption band near 1660 nm was stronger downward in the second-derivative spectra, the band near 1708 nm was also stronger downward, as shown by the solid line. However, in the case of some samples of black-coated seeds, a different pattern was observed. The dotted line in Figure 2A shows that the absorption band near 1660 nm was shifted and that it was stronger downward in the second-derivative spectra; however, the band near 1708 nm was not stronger downward. On the other hand, all samples of extracted oil, including that from black-coated seeds, showed the same spectral pattern. As the percentage of linoleic acid (18:2) increased, the spectral value at around 1708 nm, where the linoleic acid (18:2) had an effect because of its broad absorption band and larger deviations, became slightly stronger downward in the second-derivative NIR spectra. This is shown by the thin and thick solid lines in Figure 2B. The narrow range of FA compositions accounts for the smaller spectral differences than those noted with other oilseeds (7,8,10). In the case of the intact seeds, the spectral differences were greater owing to the heterogeneity of the samples, whereas in the case of the extracted oil, the samples were more homogeneous.

Based on the above observations, the standardized reading at 1708 nm was selected to predict the linoleic acid content as a proportion of the total FA composition. The correlation between the linoleic acid content and the standardized reading at 1708 nm is shown in Figure 3. The scatter plots show that the stronger downward the band at 1708 nm, the higher the proportion of linoleic acid in the total FA composition. The solid circles in Figure 3 are derived from the spectra represented by the dotted curve in Figure 2A and were excluded as outliers in calculating the correlation coefficients. For the oil samples, there were no outliers (Fig. 3C). The correlation coefficient was –0.830 for a single sesame seed (Fig. 3A), –0.786 for the sesame seeds (Fig. 3B), and –0.925 for the extracted oil (Fig. 3C). The second-derivative NIR reading at 1708 nm provided an index of the proportion of linoleic acid in the total FA composition. Thus, the results showed that a rough estimation of the percentage of linoleic acid (18:2) was possible. On the other hand, the proportion of oleic acid (18:1) in the total FA composition and the readings at 1708 nm showed an inverse correlation: The correlation coefficients were 0.771 for a single sesame seed, 0.732 for the bulk sesame seeds, and 0.850 for the extracted oil.

The method described above can be used to select sesame seed varieties or lines that have only minute differences in their levels of linoleic (or oleic) acid. This method does not require empirical calibration equations; instead, it is based on spectral pattern analysis. There is no need to develop a calibration equation.

Velasco and colleagues (19,20) successfully analyzed the protein content and the FA composition of single, intact rapeseeds by the NIR method. In previous experiments conducted by our team, the NIR method was also successful in analyzing the FA composition of a single seed and minute amounts

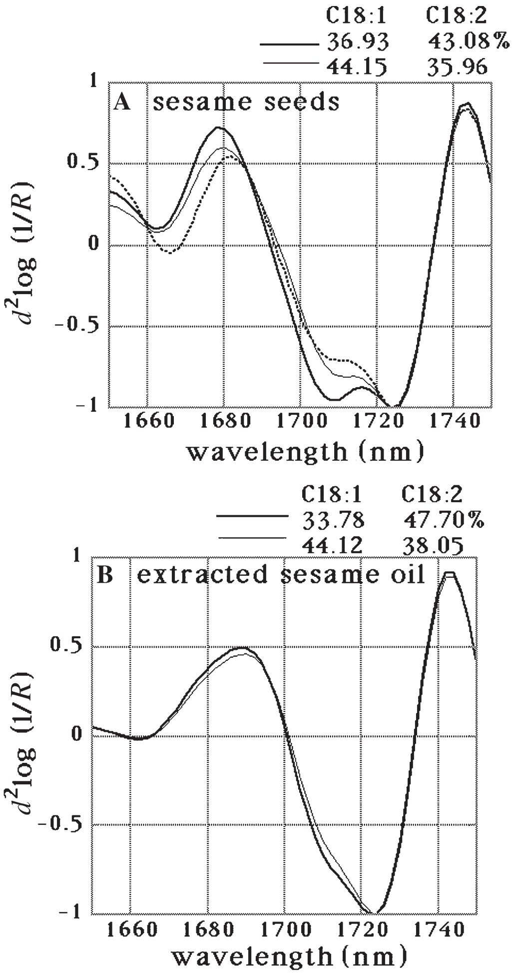

FIG. 2. Standardized second-derivative NIR spectra in the 1680–1740 nm region using modified parameters. The dotted line indicates the spectrum of black-coated sesame, which appeared differently.

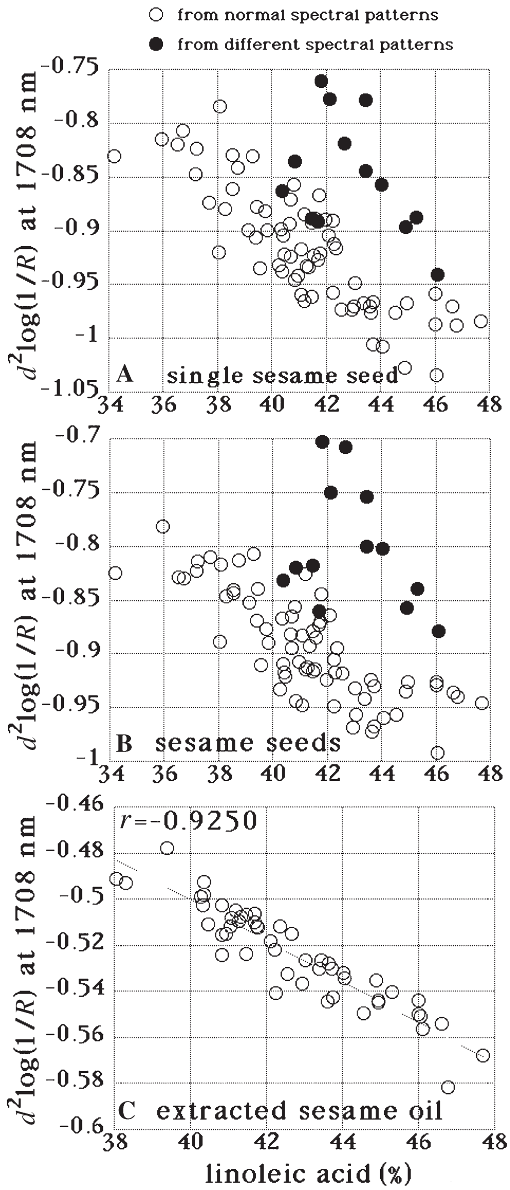

FIG. 3. Correlation between the proportion of linoleic acid in the total FA composition and the standardized NIR spectral readings at 1708 nm for (A) a single sesame seed, (B) multiple sesame seeds, and (C) extracted sesame oil.

of seed of, for example, sunflower (7), rapeseed (8), and soy (10). The present report also includes the results from experiments with a single sesame seed.

This nondestructive method can be adopted to screen varieties, not only for the required FA composition but also for useful properties linked with their FA composition.

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