Nondestructive Determination of Fatty Acid Composition of Husked Sunflower (*Helianthus annua* L.) Seeds by Near-Infrared Spectroscopy

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ABSTRACT: Determination of the fatty acid composition of sunflower (*Helianthus annua* L.) seeds by near-infrared (NIR) spectroscopy was examined. Sunflower seeds were husked (removed from their hulls by a husking machine or manually with a knife). NIR spectra of these seeds were scanned from 1100 to 2500 nm at 2-nm intervals in a whole-grain cell with a wide-angle moving drawer for machine-husked seeds or in a single-grain cup for a manually husked single-grain seed. The extracted oils from machine-husked seeds also were scanned by sandwiching them between a pair of slide glasses to create a thin layer and by placing them on a syrup cup. For extracted oil, the absorption band around 1720 nm filled out to the shorter wavelength region in the NIR second-derivative spectra as the percentage of the linoleic acid moiety increased, because linoleic acid absorbs in this region. On the other hand, for husked seeds and for a single-grain seed, as the percentage of linoleic acid increased, the trough at 1724 nm where oleic and saturated acids absorb decreased in the second-derivative NIR spectra. Determination of the fatty acid composition of sunflower seeds could be carried out successfully according to the NIR spectral pattern for both extracted oil ($r = -0.989$) and kernel seed ($r = -0.993$). This is important, especially for a manually husked single-grain seed ($r = -0.971$), because it can still be germinated after such nondestructive analysis.
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KEY WORDS: Analysis, fatty acid composition, *Helianthus annua* L., husked seed, near-infrared, oil, single grain, spectroscopy, sunflower seed, whole grain.

Sunflower (*Helianthus annua* L.) is one of the major oil production crops. Sunflower oil is widely used for food products (salad oil, etc.), as well as for nonfood use (paints, etc.) (1). The high levels of unsaturated fatty acids in vegetable oils have become recognized as good nutritional characteristics for health. Alteration of the fatty acid composition is one of the objectives for breeding sunflower seeds, with the goal of improving quality. Analysis of the fatty acid composition of sunflower seed oil is important in breeding programs and also for checking raw materials in the oil milling process. However, the conventional analytical method for determining the fatty acid compositions is time-consuming and tedious—it includes oil extraction, sample preparation for analysis (i.e., esterification), and gas-chromatographic (GC) analysis (2–4). So, a rapid, simple, and nondestructive method for the determination of fatty acid compositions is needed for increased demands in breeding projects and for rapidly checking raw materials at oil milling plants. In both cases, useful varieties and individuals are selected from a great number of samples to be tested.

Near-infrared (NIR) spectroscopy is a powerful analytical tool and has been widely used for the simple and rapid analyses of various agricultural, food, and chemical products (5–8). The NIR method has been used for the analyses of major constituents in cereals and oilseeds (9–14). Further, the NIR method has been developed to provide more detailed information than just major constituents because of its potential capacities. As Sato *et al.* (15–17) and Karen *et al.* (18) have reported, the NIR spectral pattern of oil represents its fatty acid composition, because NIR absorption bands around 1600–1800 and 2100–2200 nm are assigned to the straight carbon chain and *cis* double bonds, which reflect fatty acid moieties in fat molecules.

In this report, determination of the fatty acid composition of sunflower seeds by NIR spectroscopy was examined to develop a simple, rapid, and nondestructive determination procedure.

MATERIALS AND METHODS

Samples. Thirty individual sunflower samples from fifteen varieties, which were cultivated on the farm of our experiment station (located in Nishigoshi, Kumamoto, Japan), were used in this study. Samples were divided into three groups according to their seeding seasons (Table 1).

Seeds were husked by a husking machine (Cyclon Mini; Kett Co., Fukuoka, Japan) or manually with a knife. In the former case, they were usually broken into pieces and sustained some damage. In the latter case, the kernel was not damaged even after hull removal (Fig. 1).

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TABLE 1
Fatty Acid Compositions (wt%) of Extracted Sunflower Oil as Methyl Esters

	Vital	870348	Sun Wheat101	910152	Sun Wheat101	DE 2841
Group 1[a]						
C16:0	5.60	5.72	5.54	5.01	5.50	5.45
C18:0	5.29	4.49	5.63	4.22	4.66	3.85
C18:1	21.01	19.28	18.09	19.56	18.43	18.93
C18:2	66.03	68.41	68.46	69.19	69.46	69.90
	DO 827	Kitakou No.2	Kitakou No.1	ST3450	ST3450	910270
C16:0	5.13	5.50	5.68	5.50	5.63	5.20
C18:0	4.50	4.53	4.31	3.73	3.94	3.76
C18:1	18.48	17.87	17.51	17.11	16.93	16.70
C18:2	69.93	69.94	70.49	71.34	71.45	72.31
	Vidoc	Peredovik	D 151	Hokkai S1	D 181	Peredovik[d]
C16:0	5.32	5.03	6.08	5.25	5.89	5.33
C18:0	4.06	3.29	3.54	2.94	3.43	3.10
C18:1	15.31	15.47	13.55	14.27	12.84	12.56
C18:2	73.52	74.33	74.86	75.79	75.90	76.97
Group 2[b]	Hokkai S1	Peredovik	Peredovik[d]	ST3450	Sun Wheat101	ST3450
C16:0	5.82	6.65	5.93	6.40	7.35	7.12
C18:0	4.45	4.39	4.90	3.32	6.30	3.68
C18:1	27.58	26.38	26.48	25.60	22.23	24.40
C18:2	58.65	59.55	60.63	61.49	62.20	62.36
Group 3	Sun Wheat101	Sun Wheat101	Peredovik[d]	Peredovik	ST3450	ST3450
C16:0	3.30	4.94	5.55	6.03	6.60	5.85
C18:0	4.95	5.00	3.41	3.27	2.17	2.09
C18:1	56.14	55.07	56.45	54.13	53.63	52.82
C18:2	30.63	32.21	32.63	34.99	35.85	37.15

[a]Seeded on August 12, 1993. [b]Seeded on July 12, 1994. [c]Seeded on June 3, 1996. [d]Adopted as reprentatives.

The oil was also extracted by shaking a 5-g sample of seeds, which were husked by a husking machine, with 20 mL diethyl ether for one hour. The organic solvent layer was separated, and the solvent was evaporated.

Chemical measurements. The extracted oils were analyzed by GC after methyl esterification according to the conventional method (2–4). The gas chromatograph was equipped with a flame-ionization detector (GC-14A; Shimadzu Co., Kyoto, Japan). The GC conditions for determining the fatty acid methyl esters were: 50 m long × 0.25 mm i.d. WCOT fused-silica capillary column (CP-Sil 88; Chrompack, Middelburg, The Netherlands); 230°C for injection- and detector-port temperature; column temperature programmed from 132 to 230°C at 10°C/min, holding for 17 min at 230°C; H_2, 0.58 kg/cm^2; air, 0.55 kg/cm^2; He, 1.6 kg/cm^2. Each sample was analyzed twice.

NIR spectroscopy: extracted oil. The extracted oils were scanned by the NIR procedure. Figure 2 shows the sample presentation method. On one of the glass slides, post-it tape (Cover-up Tape 658, Scotch; 3M, St. Paul MN) was pasted on both edges, extracted oil was applied in the space between, and another slide glass was pressed on top to sandwich the oil layer to make a thin layer between the slides. The assembly was placed on a syrup cup [Bran + Luebbe (B + L) GmbH, Norderstedt, Germany]. There is no need to clean up the cup after each measurement in this procedure, because the slides are disposable and oil does not stain the cup. The sample presentation can be set up easily and reduces the time needed for measurements. An InfraAlyzer 500 (B + L) was used to measure the NIR transflectance spectra in the wavelength range from 1100 to 2500 nm at 2- nm intervals. In the following experiments, the same NIR instrument and the same wavelength range were used.

NIR spectroscopy: husked seeds. About 50-g husked seeds was packed in a specially designed sample cell: a whole-grain cell (B + L; Fig. 1A), which was placed on the wide-angle moving drawer (B + L), with the NIR radiation incidents 20 mm apart peripherally from the center, where the reflectance is measured. After each measurement, the cell was rotated 90° on the wide-angle moving drawer and measured again. Each sample was measured four times. The average of four measurements per sample was calculated by InfraAlyzer Data Analysis Software (IDAS; B + L).

NIR spectroscopy: single-grain seed. A single-grain seed was placed in a specially designed sample holder: a single-grain cup (center hole diameter = 24 mm, B + L; Fig. 1B), which was placed on the standard drawer. The NIR spectra of four individual seeds were measured for each sample, and their average was obtained.

Mathematical treatment on NIR spectral data for standardization. Using IDAS, the second-derivative math treatment was carried out on the raw averaged spectra data. The conditions to calculate the second-derivative spectra data were: 4 nm between output points, 4 nm in moving average,

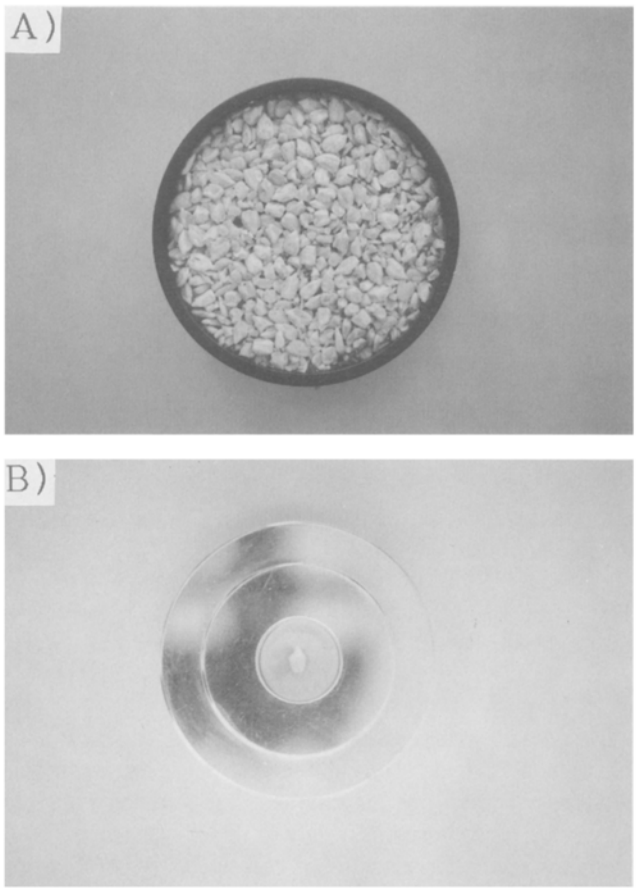

FIG. 1. A, Machine-husked sunflower seeds packed in the whole-grain cell; B, manually-husked single-grain sunflower seed on a single-grain cup.

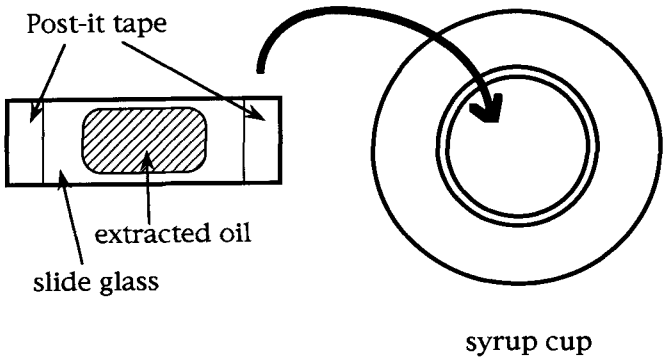

FIG. 2. Sample presentation method of extracted oil for near-infrared measurement.

12 nm per derivative segments, and 12 nm between derivative segments. Thus, the wavelength region of NIR spectra data obtained was from 1120 to 2480 nm at 4-nm intervals.

The NIR spectral region of 1600–1800 nm was especially notable in this examination, because the variation of this region was more pronounced than in the region of 2100–2200 nm. Further, because the intensity level of the original spectral values were different, the spectra were standardized to facilitate the comparison between the NIR second-derivative spectral patterns was obtained as follows: the level of the starting point at 1600 nm was set at 0.0, and the minimal value at 1720 nm (extracted oil) or at 1708 nm (husked seeds) was corrected to −1.0 according to the previous report (16,17).

RESULTS AND DISCUSSION

Fatty acid composition by GC method. Table 1 shows the fatty acid composition (wt%) of the husked sunflower seeds. The standard deviation was 0.00–0.24% (average of SD: 0.06%). In group 1, sunflowers were cultivated by standard procedures, and main fatty acids in the kernel were linoleic acid ($C_{18:2}$, 66.03–76.97%), oleic acid ($C_{18:1}$, 12.56–21.01%), palmitic acid ($C_{16:0}$, 5.01–6.08%), and stearic acid ($C_{18:0}$, 2.94–5.63%). Linolenic acid ($C_{18:3}$) was less than 0.20%. These results approximate the data from Yamazaki *et al.* (19). Samples from group 2 had the same inclination, with an increase in the percentage of $C_{18:1}$ and a decrease in that of $C_{18:2}$. However, the samples from group 3, which were seeded in an earlier season, had very different results—the percentage of $C_{18:1}$ was much higher than that of $C_{18:2}$. Morishita *et al.* (20) have reported and discussed such situations, i.e., the influence of the seeding season on grain yield, oil content, and fatty acid composition of sunflower cultivated in the warmer areas of Japan.

From Table 1, the following three samples of Peredovik were adopted as representatives based on linoleic acid contents: highest level of linoleic acid (group 1, 77.0%), moderate level of linoleic acid (group 2, 60.6%), and lower level of linoleic acid (group 3, 32.6%).

Extracted oil. Figure 3 shows NIR spectra of extracted oil of sunflower seeds. These NIR spectra have characteristic oil absorption bands, as previously reported (16): maximal values at 1720, 1760, 2144, 2306, and 2348 nm in the raw NIR spectra (Fig. 3A), and minimal bands at 1660, 1720, 1760, 2120, 2144, 2180, 2308, and 2348 nm in the second-derivative spectra (Fig. 3B).

Figure 4 shows the 1600–1800 nm wavelength region of standardized, second-derivative NIR spectra of the extracted oils at the three levels of linoleic acid moiety. As the percentage of linoleic acid increased, the trough at 1720 nm became broader. Because the absorption band of linoleic acid occurs at the lower wavelength region, as reported (16), the observed NIR spectra reflected the fatty acid composition.

The sum of the spectral values from 1696 to 1724 nm was calculated for each sample as an index of the area of this region, and the higher the percentage of linoleic acid, the broader the absorption becomes or the lower this sum becomes. Figure 5 shows good correlation between the percentage of the linoleic acid moiety and the sum of these spectral values. The correlation coefficient for (group 1 + group 2 + group 3) was −0.989, that for (group 1 + group 2) was −0.939, and that for (group 1) was −0.943. As for the extracted oil, NIR spectral patterns were well reflected by the fatty acid

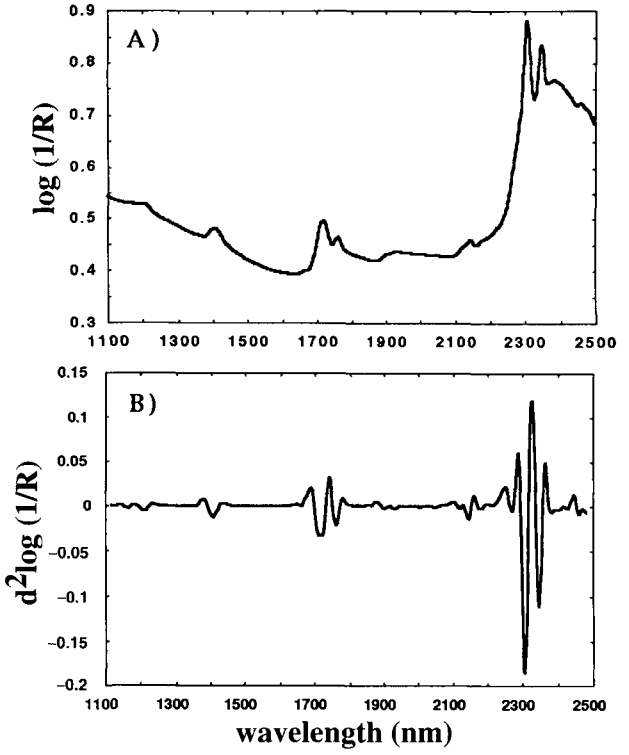

FIG. 3. Original raw (A) and second-derivative (B) near-infrared spectra of extracted sunflower oil.

composition. Good correlation was obtained even if the population concerned was divided into smaller group.

Kernel seeds without hulls. Figure 6 shows NIR spectra of sunflower seeds before and after removing hulls—intact seeds and husked seeds. As for intact seed, the absorptions were weak, owing to oil. After removing hulls, the NIR spectra were especially characteristic for absorption bands of the oil component. This should be expected because sunflower seeds contain high oil content (1,20), more than 40%. These NIR spectra had characteristic oil absorption bands—maximal values at 1722, 1760, 2308, and 2348 nm in the raw NIR spectra (Fig. 6A), and minimal bands at 1664, 1708, 1720, 1760, 2120, 2144, 2176, 2308, and 2348 nm in the second-derivative spectra (Fig. 6B). These wavelengths were similar to those of oils. However, the absorption of oil at 1720 nm was slightly separated into two parts for kernel seeds—the minima were at 1708 and 1720 nm, with the maximal or inclination point at 1716 nm. Thus, the second-derivative math treatment made the absorption characteristics of fat molecules more evident. Because the spectra of intact hulls were poor in absorption characteristics, the second-derivative NIR spectra of kernels were mainly used in the following analysis.

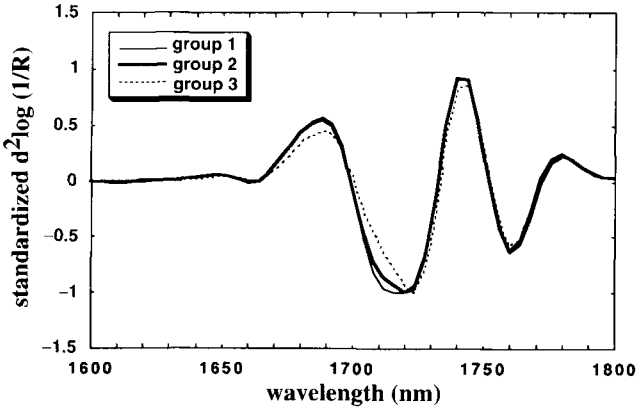

FIG. 4. The 1600–1800 nm wavelength region of standardized, second-derivative near-infrared spectra of extracted sunflower oils. The percentage of linoleic acid moiety is 77.0% for group 1, 60.6% for group 2, and 32.6% for group 3.

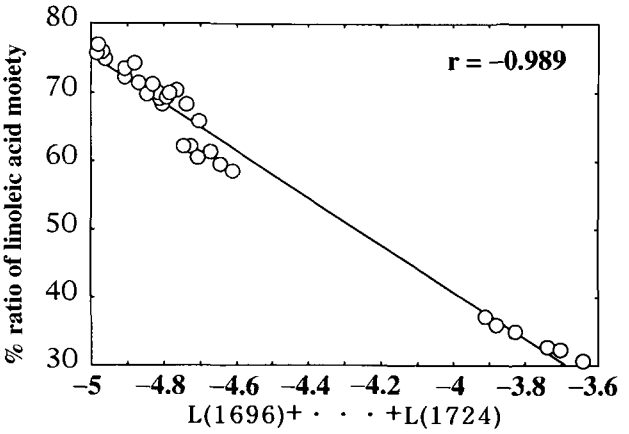

FIG. 5. Correlation between the percentage of linoleic acid and the sum of the spectral values from 1696 to 1724 nm for extracted oil.

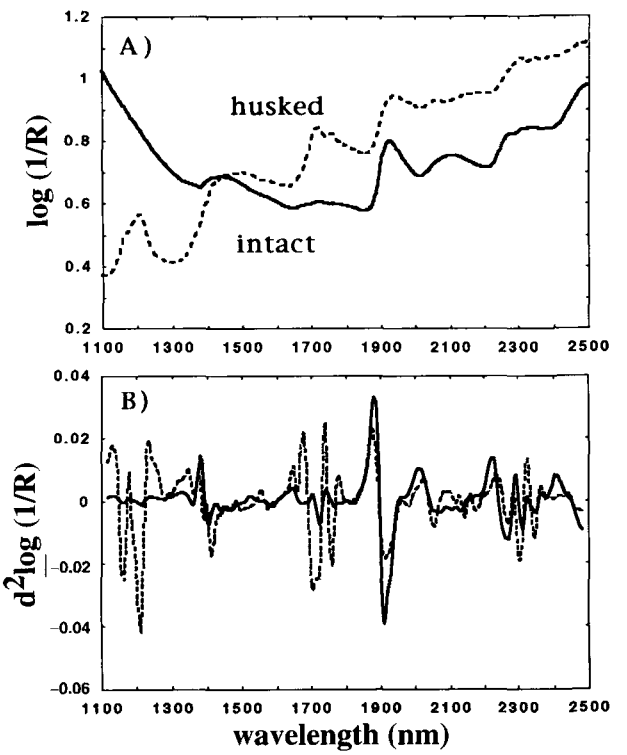

FIG. 6. Original (or nonstandardized) raw (A) and second-derivative (B) near-infrared spectra of sunflower seeds: solid line for intact seed, and dotted line for husked seed.

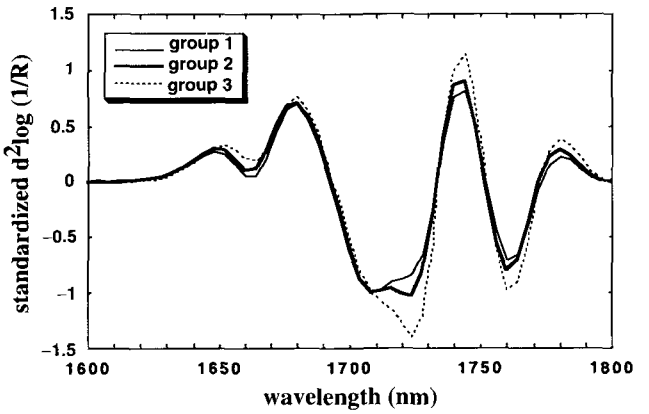

FIG. 7. The 1600–1800 nm wavelength region of standardized, second-derivative near-infrared spectra of machine-husked sunflower seeds. The percentage of linoleic acid is 77.0% for group 1, 60.6% for group 2, and 32.6% for group 3.

Figure 7 shows the 1600–1800 nm wavelength region of standardized, second-derivative NIR spectra. These spectra correspond to the three levels of linoleic acid. In Figure 7, the absorption at 1708 nm (linoleic acid moiety) was normalized to the same value, −1, and this means that the strength of this absorption due to linoleic acid was fixed. On the other hand, the absorption at 1724 nm was due to the oleic and saturated acid moieties. In Figure 7, the slope of the straight line connecting two minimal points at 1716 and 1724 nm changed from a positive value, through zero, to a minus value, as the percentage of linoleic acid decreased. As the percentage of linoleic acid decreased, the trough at 1724 nm became deeper in the second-derivative NIR spectrum.

The difference between L(1716) and L(1724) was calculated as an index for the relative concentrations of the fatty acids. Figure 8 shows good correlation between [L(1716) − L(1724)] and the percentage of linoleic acid moiety. The correlation coefficient for (group 1 + group 2 + group 3) was −0.993, that for (group 1 + group 2) was −0.945, and that for (group 1) was −0.897. As for husked whole-grain seeds, NIR spectra also reflected the fatty acid composition well.

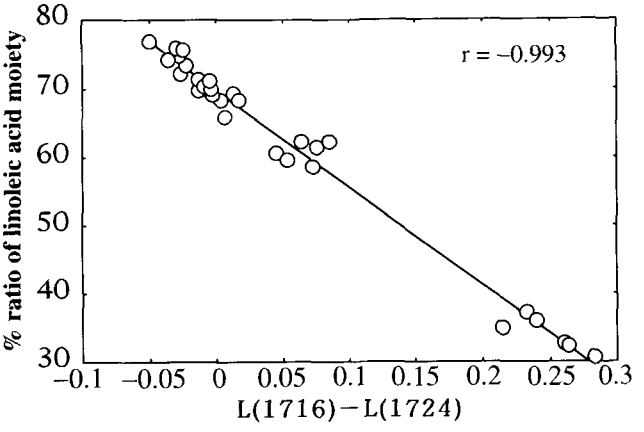

FIG. 8. Correlation between the percentage of linoleic acid and the difference between the spectral values at 1716 and at 1724 nm [L(1716) − L(1724)], for machine-husked sunflower seeds.

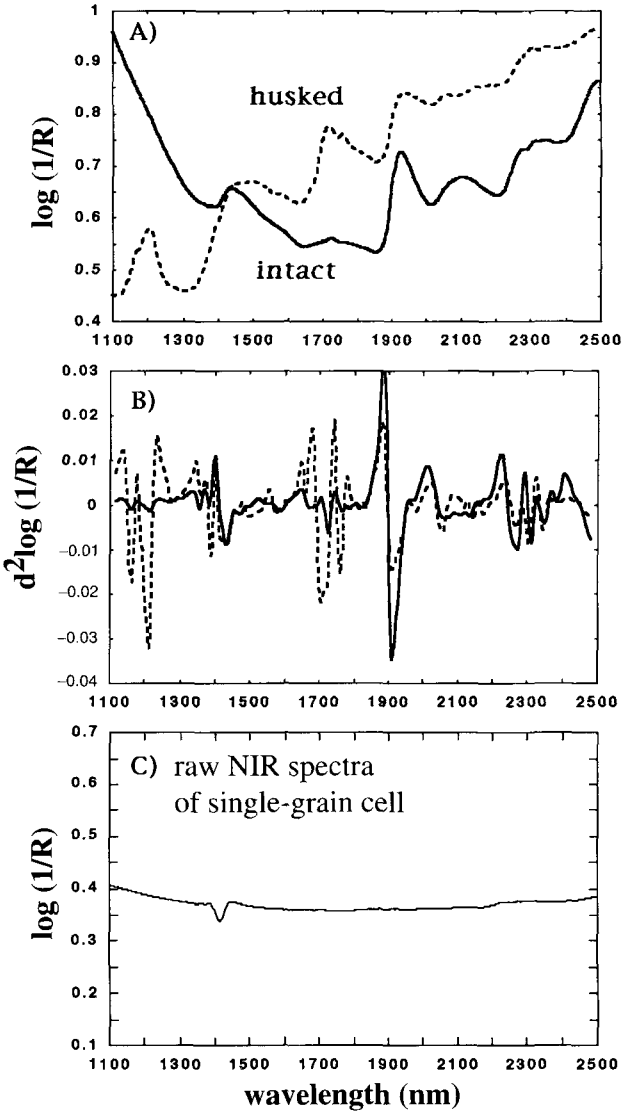

FIG. 9. Original (or nonstandardized) raw (A) and second-derivative (B) near-infrafed spectra of manually-husked, single-grain sunflower seeds: solid line for intact seed, and dotted line for husked seed. C, Original raw spectra of single-grain cell only (blank).

Single-grain seed without hulls. Figure 9 shows NIR spectra of sunflower seeds before and after removing hulls—intact seed and husked seed. After removing the hull, the NIR spectrum had especially characteristic absorption bands for the oil component. These NIR spectra have characteristic oil absorption bands: maximal values at 1722, 1760, 2308, and 2348 nm in the raw NIR spectra (Fig. 9A), and minimal bands at 1664, 1708, 1720, 1760, 2120, 2144, 2176, 2308, and 2348 nm in the second-derivative spectra (Fig. 9B). These wavelengths were similar to those of whole-grain seeds. The second-derivative math treatment made the absorption characteristics of fat molecules more distinctive for the single grain. In Figure 9C, the single-grain cell had artifacts at 1400 nm. However, it had no effect on the absorption region of oil.

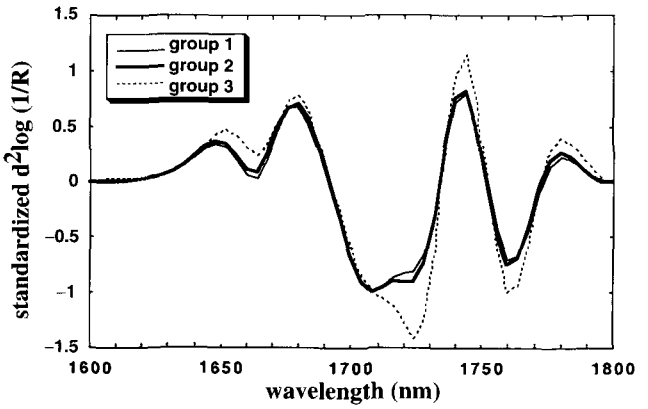

FIG. 10. The 1600–1800 nm wavelength region of standardized, second-derivative near-infrared spectra of manually-husked, single-grain sunflower seed.

Figure 10 shows the 1600–1800 nm wavelength region of standardized, second-derivative NIR spectra. The patterns obtained correspond to three levels of linoleic acid. The same inclination as in Figure 7 could be observed.

The difference between L(1716) and L(1724) also was calculated as an index for the relative concentration of the fatty acids. Figure 11 shows the correlation between [L(1716)–L(1724)] and the percentage of linoleic acid. The correlation coefficient for (group 1 + group 2 + group 3) was –0.971, –0.905 for (group 1 + group 2) and –0.847 for (group 1). As for single-grain seed, NIR spectra reflected the fatty acid composition well. These results indicate that the fatty acid composition can be estimated with a small amount of sample, as small as a single grain.

The correlation for the extracted oil was the best, because there was a stable relationship, even if a smaller region was considered. Correlation for machine-husked whole grains was better than for the manually-husked single grain. The oil extraction for GC analysis was carried out on a number of whole grains, not on each single grain. Therefore, the fatty acid variation was somewhat averaged. On the other hand, with a single grain, the variation of the individuality of the single grain still remains. This is the explanation for lower correlation.

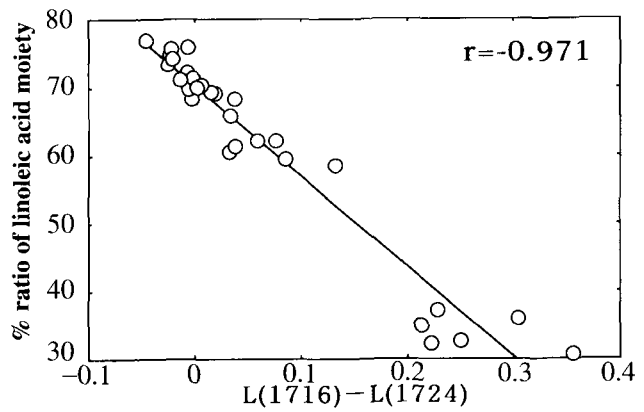

FIG. 11. Correlation between the percentage of linoleic acid and the difference between the spectral values at 1716 and at 1724 nm, [L(1716) – L(1724)], for manually-husked, single-grain sunflower seed.

Kaffka *et al.* (12) and Robertson *et al.* (13) tried to use the NIR method for analysis of the major constituents in husked ground sunflower seed powder. The authors also examined the fatty acid composition for husked whole-grain seed by NIR spectroscopy. Combination of all these trials will further improve sunflower oil quality.

The capability of the NIR method was clarified for the analysis of the fatty acid composition of sunflower seeds. Especially for a manually husked single-grain seed, nondestructive analysis could be successfully carried out, while the germination possibility could be maintained. These results also can be adopted for determining the fatty acid composition of oilseeds in general, and one can choose the method depending on the sample type or the purpose, i.e., from liquid to solid.

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