Use of NIR Spectroscopy for Estimation of FA Composition of Soy Flour

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ABSTRACT: The feasibility of NIR spectroscopy for the determination of FA composition in soy flour was examined. NIR spectra were obtained for a small amount of soybean powder (about 8 mg) in a modified single-grain cup using an NIR instrument by scanning the wavelengths from 1100 to 2500 nm at 2-nm intervals. The relationship between the NIR spectral patterns of soybean powder and the FA compositions was examined: As the linoleic acid ratio increased, the NIR absorbance at 1708 nm, where the linoleic acid moiety has an absorption band, became stronger downward in the second-derivative NIR spectra. The correlation coefficients between the standardized NIR readings at 1708 nm and the linoleic acid ratio or the oleic acid ratio in the FA composition of soy flour were –0.853 and 0.877, respectively. A rough estimation of the linoleic acid moiety or oleic acid moiety in soy flour could be successfully carried out with even a very small amount of soy flour according to the NIR spectral pattern due to the wavelength assignments of moieties.

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KEY WORDS: Analysis, fatty acid composition, *Glycine max* L., linoleic acid, near infrared, oleic acid, second derivative, soybean, spectroscopy.

Soybean (*Glycine max* L.) is a major oilseed crop. Soybean FA composition is an important consideration for breeding programs. One goal of breeding projects is to reduce the level of linoleic acid. However, the conventional method for FA determination is labor intensive. It includes milling, oil extraction, chemical reaction, and GC analysis. A simple and rapid method for the determination of FA composition is necessary for screening soybean varieties (1). Furthermore, in this case, an individual seed is sometimes to be selected from many samples to be tested, and because of this situation, an analysis of a very small sample is demanded.

NIR spectroscopy is recognized as one of the most powerful and useful tools in agricultural and food analysis. NIR has also been used for the analysis of soybean constituents (2,3). FA composition is an important index of fat or oil quality for nutritional classification. Therefore, if NIR can be used for the analysis of FA composition, it would become an even more useful technique. NIR analysis has been carried out using a statistical method, multiple linear regression analysis (4). However, in this report, we took another approach. The feasibility of NIR spectroscopy for estimating FA composition in the soybean was examined according to spectral assignment. Since the NIR spectral patterns were reflected by the FA compositions, the absorption bands of *cis*-unsaturation and the carbon chain length of the FA moieties in oil appear in the NIR wavelength region, especially around the 1600–1800 nm region (5–10).

MATERIALS AND METHODS

Samples. Thirty-one varieties were cultivated, harvested, and collected from various areas of Japan in 1999. They were Ohsodenomai, Kariyutaka, Kitamusume, Toyokomachi, Toyohomare, Toyomusume, Hayahikari, Yuhzuru, Otofuke'ohsodefuri, Suzumaru, Ohsuzu, Miyagishirome, Ryuhou, Suzuyutaka, Enrei, Ohtsuru, Tamahomare, Akishirome, Tachinagaha, Tama'urara, Nattoshouryu, Hatayutaka, Nakasen'nari, Hou'en, Ayakogane, Tamamasari, Sayanami, Suzukogane, Fukuyutaka, Murayutaka, and Ichihime. These samples were sent to our research center and milled by a Cyclone Sample Mill (Udy Corporation, Fort Collins, CO) through a screen ($\phi = 1.0$ mm). All the powdered samples were packed in a sealed polyethylene bag (Unipack; Seisan Nihon Co., Tokyo, Japan) and stored at 5°C until analyzed.

Chemical measurements. The oil was extracted from about 2 g of sample powder with diethyl ether by the Soxhlet method (Soxtec System HT 1043 Extraction Unit; Tecator, Hoganas, Sweden). The extracted oils were then used for the 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 for determining the FAME were according to the previous report (9). Each sample was analyzed twice. The average of duplicate measurements was calculated. Samples were normalized to 95% for major FA components: palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids.

NIR. An InfraAlyzer 500 (Bran+Luebbe GmbH, Norderstedt, Germany) was used to measure the NIR reflectance spectra in the wavelength range of 1100 to 2500 nm at 2-nm intervals. By using a small spatula, a small amount of soybean powder (about 8 mg) was poured into a modified single-grain cup (INF-B333-01, center hole diameter = 20 mm, Bran+Luebbe Co.), the cup was jiggled until the flour was arranged in the center of the cup, and then the NIR spectra were measured. This cup was developed for measuring NIR

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*To whom correspondence should be addressed. E-mail: sato@affrc.go.jp spectra of a small particle and was successfully used for the analysis of FA composition of a single-grain rapeseed as previously reported (9). It has a smaller and deeper hole in it a sharper parabolic curve to the base than a normal one.

Mathematical treatment of NIR spectral data for standardization. The parameters for calculating 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, these NIR spectra were standardized in order to make the comparison easier; the spectral value was 0.0 at 1600 nm and −1.0 at the minimum, 1724 nm. The second-derivative mathematical treatment and the standardization of NIR spectral data were carried out as reported in previous articles (8,9).

RESULTS AND DISCUSSION

The NIR spectra of a small amount of soybean powder in a modified single-grain cup were clearer because the modified cup allowed for a better collection of the reflected light owing to the sharper parabolic curved surface. The single-grain cup itself has a very slight absorption around 1400 nm, but it does not affect the absorption bands due to oil.

Three varieties of soybeans were adopted as examples for the explanation. Their FA compositions are described in Figure 1. They were characteristic, especially in the ratios of oleic acid (C18:1) and linoleic acid (C18:2) moieties. In the original NIR spectra, the difference due to FA composition could not be detected. Also, in the second-derivative NIR spectra calculated with the default parameters, the spectral difference could not be detected. By using the parameters described in the Materials and Methods section, a clearer difference was obtained, as shown in Figure 1. As the percentage of linoleic acid moiety increased, the absorption band around 1708 nm was stronger downward in the second-derivative NIR spectra because the spectral phase reversed in the second-derivative spectra.

For comparison, the second-derivative NIR spectral value at 1724 nm was arranged to the same as shown in Figure 1. As the percentage of linoleic acid moiety (C18:2) increased, the spectral value around 1708 nm, where the linoleic acid moiety (C18:2) has effects because of its broad absorption band and larger deviations, became slightly stronger downward in the second-derivative NIR spectra.

The correlation between the percentage of FA moiety and the standardized reading at 1708 nm is shown in Figure 2. Figure 2A shows the relationship of the percentages of

	palmitic C16:0	stearic C18:0	oleic C18:1	linoleic C18:2	linolenic C18:3
Sample I	11.38	2.76	15.80	56.01	9.05
Sample II	10.32	2.59	23.34	50.07	8.67
Sample III	12.12	2.25	28.61	44.51	7.51

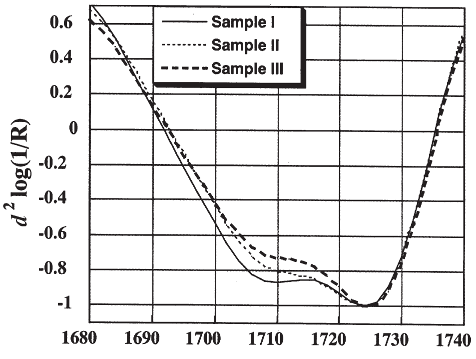

FIG. 1. FA composition of soy flour samples (%) and 1680–1740 nm wavelength region of the standardized second-derivative NIR spectra using modified parameters of a small amount of soy flour poured into a modified single-grain cup. R = reflectance.

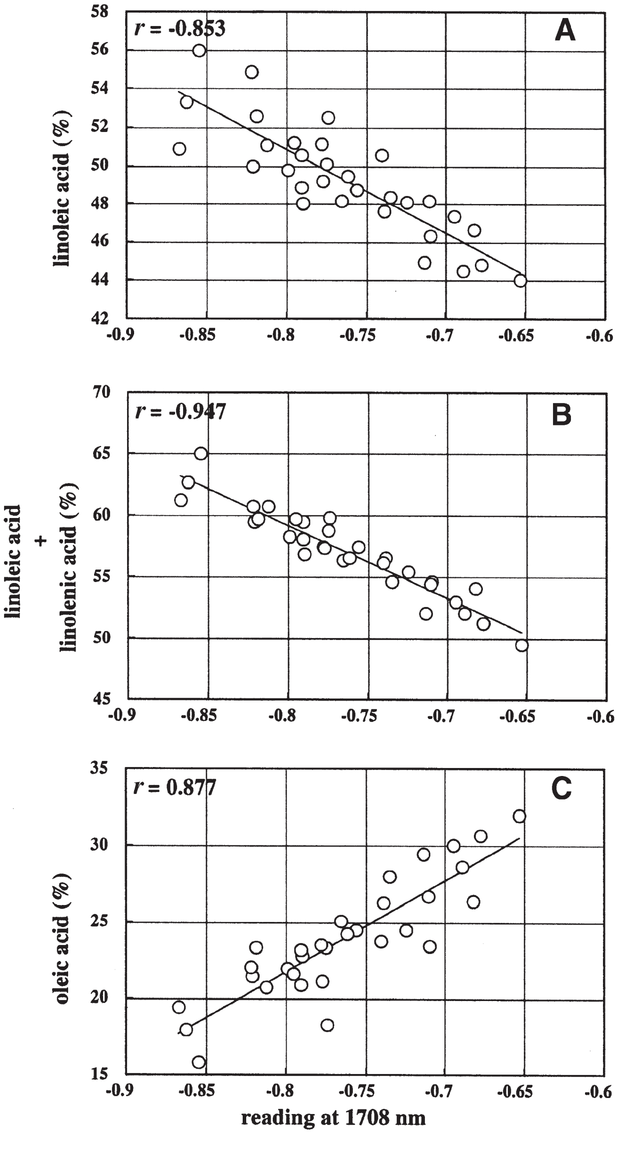

FIG. 2. Correlation between the percentage of FA moiety and the standardized NIR spectral readings at 1708 nm: (A) linoleic (C18:2), (B) linoleic (C18:2) + linolenic (C18:3), and (C) oleic (C18:1)

linoleic acid (C18:2) vs. the readings at 1708 nm; the correlation coefficient was –0.853. The scatter plot shows that the stronger the absorption band at 1708 nm downward, the higher the percentage of the linoleic acid moiety (C18:2), as already mentioned. The results show that a rough estimation of the percentage of the linoleic acid moiety is possible. In this region, the linolenic acid moiety (C18:3) has little influence on the absorption band. There is a tendency for the linolenic acid to increase as the amount of linoleic acid increases. This is why a discrepancy from the regression line emerged when the linoleic acid (C18:2) moiety ratio increased, as shown in Figure 2A. The correlation of linoleic + linolenic acid moieties vs. the readings at 1708 nm was examined (Fig. 2B). The correlation coefficient improved dramatically. The discrepancy from the regression line diminished. Incidentally, the correlation coefficient for the linolenic acid moiety vs. the readings at 1708 nm was –0.638 because its ratio and the variation were small. On the other hand, in Figure 2C the correlation of the oleic acid moiety (C18:1) vs. the readings at 1708 nm is positive.

This method can be used for the selection of varieties with even minutely higher or lower levels of linoleic (or oleic) acid in soybean. The method did not use empirical calibration equations but was instead based on spectroscopic pattern analysis. There is no need to develop a calibration equation.

So far, we have successfully introduced the NIR method for single-seed, or very small amount, analysis of FA composition, such as that in sunflower seed (8) and in rapeseed (9). In this report, we used a very small amount (about 8 mg) of soy flour, and this amount can be sampled several times from a single soybean seed.

Using the NMR method, Yoshida *et al.* (12) successfully carried out FA analysis of an intact single soybean seed, but it took almost 1 h for data accumulation. In our study it took only 1 min. Furthermore, if sampling of a small amount of soybean powder is possible by using a mini-drill so as not to destroy the hypocotyls in a seed, germination ability can be retained, and the method can be adopted for screening of varieties not only with the required FA composition but also with useful properties linked with the FA composition based on the spectral assignments. We have successfully introduced a nearly nondestructive method.

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