Nondestructive Near-Infrared Reflectance Spectroscopy of Sesame (Sesamum indicum L.) Components by Single Seed Analysis

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Sesame (Sesamum indicum L.), is one of the most important of the oilseed plants, and the improvement of its components is an important goal in breeding. For screening sesame cultivars and lines with improved components, a simple and rapid determination method is necessary. Genetic resources and cultivars, or lines, are precious in the breeding project. The demand for nondestructive analysis of a small sample, such as single seed analysis, is strong for the breeding based on the composition of specific constituents. The seeds need to be analyzed nondestructively for use in breeding and cultivation after the analysis and selection. We previously reported that the amounts of the major constituents and the fatty acid (FA) composition in plural sesame seeds could be successfully estimated by simple, rapid and nondestructive analysis using near infrared (NIR) spectroscopy (Sato et al., 2003; Sato et al., 2004). In this report, the nondestructive evaluation of sesame composition by using NIR spectroscopy was investigated for analysis of a small sample, i.e., single seed.

Materials and Methods

1. Samples and Chemical Measurements
The samples and the methods of chemical measurements were the same as those used in the previous report (Sato et al., 2003; Sato et al., 2004).

2. Near Infrared Spectroscopy
An intact single sesame seed was placed in the hole at the bottom of an improved single grain cup (hole diameter 20 mm, (Bran+Luebbe (B+L) GmbH, Norderstedt, Germany)) as reported previously (Sato et al., 1998). The NIR reflectance spectrum of an individual seed was measured without a glass lid. An InfraAlyzer 500 (B+L) was used to collect their NIR reflectance spectra in the wavelength range from 1100 to 2500 nm at 2 nm steps. For each sample, five individuals were measured, and the average spectrum was calculated and was used for the NIR analysis. The methods of obtaining the calibration equations and the evaluation were similar to those in the previous report (Sato et al., 2004).

Results and Discussion

Figure 1 shows the original NIR spectra of sesame seeds with a yellowish-brown seed coat (Masekin, an example) and a black seed coat (Iwateguro). The overall reflectance spectra of sesame seeds with yellowish-brown, dark-brown or white seed coat seemed to be the same as those with a yellowish-brown seed coat. On the other hand, the black seeds had a different reflectance spectrum. These two typical NIR spectra were obviously different in the range from 1100 to 1400 nm. The spectrum for one single seed

Fig. 1. The original NIR spectra of plural seeds and a single seed of sesame (Masekin: yellowish-brown coat; Iwateguro: black coat).
had a pattern to that for plural seeds, but its variation range was very small in both cases (yellowish-brown seed coat and black seed coat). However, using these two types of NIR spectra together, multiple linear-regression analyses between NIR spectral data and the chemical data were carried out.

Figure 2 shows the correlation between the values estimated by the NIR method applying calibration equations developed for plural sesame seeds to the spectral values of a single seed and the chemical data: correlation coefficient (r), standard error of prediction (SEP), mean-corrected SEP (mc-SEP) and bias. The regression lines were biased and skewed. However, the contents of the constituents might be roughly estimated from the NIR data except for the palmitic acid and the stearic acid proportion, whose variations were small.

Table 1 shows the multiple linear regression analysis of the values obtained by the NIR method applying the calibration equations developed for a single seed to the spectral values of a single seed, and the correlation between the values estimated by the NIR method and the chemical method. The calibration process was developed for single-seed analysis: the calibration
equations, the correlation coefficient (r), and the standard error of calibration (SEC). These calibrations provided the best prediction. According to Osborne and Fearn (1993), moisture has an absorption band around 1940 nm, oil has an absorption band around 1700-1800 nm, 2100-2200 nm, and 2300-2400 nm, and protein has an absorption band around 1980, 2050 and 2180 nm. The selected wavelengths for moisture used for the correction or reverse correlation were 1694, 1786 and 2354 nm derived from oil; and, those for oil used for the correction or reverse correlation were 1910 and 2014 nm derived from moisture or protein. The wavelength for protein reflecting the chemical structures of the constituents and were under their influence: 2130 nm, or were used for the correction or reverse correlation: 1658, 1770 and 2442 nm derived from oil. The wavelength for FA were reflected the chemical structures of the constituents: 1700 nm and so on.

Figure 3 shows the correlation between the values estimated by the NIR method developed for a single seed and the chemical data. The coefficient of the correlation between the values estimated by the NIR method and the chemical method (r) and the standard error of the prediction (SEP) was 0.931 and 0.415 % for moisture, 0.906 and 1.486 % for oil, 0.885 and 0.972 % for protein, 0.348 and 0.766 % for palmitic acid proportion, 0.789 and 0.391 % for the stearic acid proportion, 0.836 and 1.270 % for oleic acid proportion, and 0.853 and 1.455 % for the linoleic acid proportion, respectively. The bias and the skew were drastically improved by using the calibrations developed for a single seed analysis. The SEPs were also improved. The contents of the constituents might be fairly estimated except for the palmitic acid proportion, whose variations were small. These calibrations are useful because the contribution ratio, the square of correlation coefficient, exceeds 60 %, except for the palmitic acid proportion.

The present findings indicate that the contents of the major constituents and FA composition in the sesame seed could be successfully estimated by a simple, rapid, and nondestructive method for breeding selection, irrespective of coat color by the NIR method. Further, for a single seed analysis, the NIR spectra data for a single seed were compared with the chemical data and analyzed.

References
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Table 1. Multiple linear regression analysis of the values obtained by the NIR method applying the calibration equations developed for a single seed to the spectral values of a single seed, and the coefficient correlation between the values estimated by the NIR method and chemical method.

| Calibration   | Calibration equation                                                                 | r   | SEC  |
|---------------|--------------------------------------------------------------------------------------|-----|------|
| Moisture      | 5.456 +140.604*d2L(1358)+122.372*d2L(1694)+208.897*d2L(1786)+612.431*d2L(2354)       | 0.922 | 0.489 |
| Oil           | 47.936 +2206.146*d2L(1286)+8077.092*d2L(1582)+1429.898*d2L(1806)+502.12*d2L(1910)+1037.239*d2L(2014) | 0.924 | 1.302 |
| Protein       | 19.988 +363.749*d2L(1658)+1310.065*d2L(1770)+1090.023*d2L(2130)+1246.508*d2L(2442)  | 0.892 | 0.810 |
| Palmitic acid | -0.581 +1535.238*L(1556)−1811.68*L(1568)+291.426*L(1640)                           | 0.492 | 0.540 |
| Stearic acid  | 7.06 −178.501*L(1700)+358.891*L(1868)−320.288*L(1888)+1363.675*L(1996)−1242.712*L(2000)−541.866*L(2364)+732.221*L(2372)−174.33*L(2424) | 0.885 | 0.294 |
| Oleic acid    | 12.85 +4922.612*L(1700)−6833.522*L(1704)−7092.406*L(1716)+9182.861*L(1720)−195.613*L(1944) | 0.847 | 1.306 |
| Linoleic acid | 57.434 −1994.11*L(1700)+10446.434*L(1712)−8667.844*L(1720)+186.063*L(2076)        | 0.866 | 1.349 |

r : Coefficient of correlation between the values estimated by the chemical and NIR methods.
SEC : Standard error of calibration.
L(1556) : raw spectral data at 1556 nm.
d2L(1358) : second derivative spectral data at 1358 nm.
Fig. 3. The correlation between the values estimated by the NIR method applying the calibration equations developed for a single seed to the spectral values of a single seed and the chemical data.