Nondestructive Near-Infrared Reflectance Spectroscopic Analyses of the Major Constituents of Sesame (Sesamum indicum L.) Whole Seeds with Different Coat Color

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Sesame (Sesamum indicum L.) is one of the most important oilseed plants, and has also gained considerable attention as an alternative crop to rice in Japan. The genetic improvement of the constituents of sesame is an important subject (Tashiro, 1989). However, the conventional method of determining the contents of the major constituents is time-consuming and laborious. A simple and rapid method of determination is necessary for screening sesame varieties and lines. A nondestructive method of analysis of seeds is needed so that the sample seeds can be used for breeding after analyses and selection.

Near-infrared (NIR) spectroscopy is an important tool in the fields of agriculture and food analyses (Norrís, 1987; Murray, 1990; Osborne and Fearn, 1993; Barton and Kays, 2001; Shenk et al., 2001; Kawano, 2002). NIR is also used for analyzing the fatty acid composition in sesame seeds (Sato et al., 2003) and for identifying their geographic origin by their contents (Kwon et al., 1998). The major constituents are important indices of seed quality for nutritional classification, and their quantification by NIR analyses would be valuable. Here, we report the feasibility of NIR spectroscopy for the nondestructive estimation of the major constituents of sesame seeds, irrespective of their coat color sesame seeds had.

Materials and Methods

1. Samples

Thirty kinds of samples were collected as genetic resources in Myanmar by the Seed Bank Project of Japan International Cooperation Agency (JICA). Fifty-two other samples of Japanese varieties and lines were obtained from 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 Kyushu Okinawa Region (KONARC, Kumamoto, Japan) for chemical and NIR analyses. The samples were the same as those used in the previous report (Sato et al., 2003).

2. Chemical Measurements

The moisture content was measured by a vacuum oven drying method (98 °C for 5 hours) using a vacuum oven (LCV-242, Espec, Osaka). The oil content was determined by the Sohxlet method using a Soxtec, System HT 1043 Extraction Unit (Tecator Co., Ltd., Sweden). The total N content was determined by the Dumas method with a nitrogen-analyzer (rapid N, Elementar Analysensysteme GmbH, Germany), with an oxygen supply of 170 ml/min to a sample of 150mg amount, and it took five minutes for analysis of a sample. The protein content was calculated by multiplying the obtained total nitrogen percentage by the protein factor: 5.30 (The Japanese Society for Food Science and Technology, 1996), and expressed as moisture-free basis.

3. Near-Infrared Spectroscopy

Intact sesame seeds were fully packed into a space of a single-grain cup (hole diameter = 24 mm, (Bran + Luebbe (B+L) GmbH, Norderstedt, Germany)). They were covered with a glass lid so that the surface of the sample would be arranged smooth. 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. 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. Using IDAS software (B+L), multiple linear-regression analysis was carried out between the NIR spectral data and the chemical data. When the first and second derivative NIR spectra were calculated, the default parameters were used.

Results and Discussion

Fig. 1 shows the original NIR spectra (1-a) and the second-derivative NIR spectra (1-b) of sesame with a yellowish-brown seed coat (Masekin, an example) and sesame with a black seed coat (Iwateguro as an example). The overall spectra for sesame with yellowish-brown, dark-brown and white-coated seeds seemed to be the same as those with a yellowish-brown seed coat. On the other hand, the black seeds had a different spectrum. These two typical NIR spectra were obviously different in 1100 to 1400 nm (Fig. 1). However, using these two types of NIR spectra together, multiple linear-regression analyses were carried out.

The ranges of the contents were: 3.68-8.07% for moisture, 36.15-49.40% for oil, and 17.68-27.26% for protein (moisture-free basis) as described in Table 1. The Myanmar samples had a higher moisture content than the NICS samples, but whether the storage situation affected the moisture content is uncertain. Yermanos et al. (1972) reported an oil content of 40.4-59.8% in sesame seeds from various world areas, and of 47-54.6% in Japanese sesame lines. El Tinay et al. (1976) reported an oil content of 42.2-52.2% in the exotic type, and that of 41.3-49.6% in the local type, and also reported protein content of 45.0-53.7% in the introduced type, and that of 45.0-60.0% in the local type. Kinman et al. (1954) reported an oil content of 45.29-63.38% (moisture-free) and protein content of 16.69-25.69% (moisture-free) in sesame seeds. The results obtained here were similar to those reported before.

Table 2 describes the results of the calibration process: the calibration equations, the correlation coefficient, and the standard error of calibration (SEC). This calibration 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 1940 nm.

Table 1. Contents of moisture, oil and protein for calibration and prediction sets in 2001.

|                  | Moisture content(%) | Oil content(%) | Protein content(%)* |
|------------------|---------------------|----------------|---------------------|
|                  | Mean   | Min.-Max. | SD   | Mean   | Min.-Max. | SD   | Mean   | Min.-Max. | SD   |
| **Calibration Set** |        |           |      |        |           |      |        |           |      |
| Myanmar (n=20)    | 6.66   | 6.11-7.47 | 0.40 | 43.53  | 37.78-47.36 | 2.07 | 22.87  | 20.42-24.93 | 1.38 |
| NICS (n=35)       | 4.62   | 3.68-5.44 | 0.40 | 45.04  | 36.15-49.40 | 3.71 | 23.49  | 20.69-27.26 | 1.87 |
| **Prediction Set** |        |           |      |        |           |      |        |           |      |
| Myanmar (n=10)    | 6.74   | 5.84-8.07 | 0.69 | 42.87  | 37.64-48.92 | 3.48 | 21.47  | 17.68-24.13 | 2.10 |
| NICS (n=17)       | 4.70   | 3.97-5.33 | 0.40 | 44.87  | 39.77-49.40 | 3.12 | 23.78  | 20.41-26.36 | 1.58 |

NICS : National Institute of Crop Science.
Min.-Max. : Minimum-Maximum.
SD : Standard Deviation.
* Moisture-free basis.
NIR Analyses of Sesame band around 1980, 2050 and 2180 nm. The selected wavelengths reflected the chemical structures of the constituents, and were under their influence, or were used for the correction or reverse correlation: the wavelengths of 1867 nm, 2019 nm and 2411 nm for moisture, that of 1828 nm for oil, and those of 1966 nm and 2122 nm for protein. When using the second derivative spectral values for the analysis of oil content, two wavelengths calibration equations were obtained as described in Table 2 (oil-2 and oil-4 case).

Table 2 also describes the results of the prediction process. Fig. 2 shows the regression of the values estimated by the NIR method with those determined chemically. The correlation coefficient between the NIR method vs. the reference method (r) and the standard error of the prediction (SEP) was 0.958 and 0.318% for moisture, 0.913 and 1.431% for oil, and 0.916 and 0.830% for protein, respectively.

The performance of NIR analysis of intact seed or meal we obtained was similar to that obtained by other investigators: Sato et al. (1999) conducted the nondestructive analysis of major constituents in rapeseeds by the NIR method and reported an SEP of 0.446% for moisture content, and 1.103% for oil content. Velasco et al. (1999) reported the nondestructive analysis of oil content in a single rapeseed by the NIR method for oil content (r² = 0.97 in validation). Velasco et al. (2002) also conducted nondestructive analysis of protein content in a single rapeseed by the NIR method and reported an SEP of 0.72-0.81% for some kinds of rapeseeds.

The present findings indicate that the contents of the major constituents in the sesame seeds could be successfully estimated for a simple, rapid and nondestructive breeding selection, irrespective of coat color by the NIR method. Previously, we successfully...
estimated the fatty acid composition in sesame by nondestructive analysis (Sato et al., 2003). Evaluation of a method of a single seed analysis by this NIR method is underway. Prediction of other constituents by using the NIR method will be more useful for sesame breeding.

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