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Estimation of Fatty Acid Composition in Soybean Powder by Examining Near Infrared Spectroscopic Patterns

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1. Introduction

Soybean (*Glycine max* L.) is one of the major oilseed crops. The fatty acid composition in oilseeds is an important consideration for breeding programs (Daun, 1998). Proper levels of unsaturated fatty acid moieties in vegetable oils have been recognized as good nutritional characteristics for health. One goal of breeding projects is the alteration of the fatty acid composition, for example, the reduction of the level of linoleic acid moiety. Then, its analytical method is of an important concern. However, the conventional method for the fatty acid composition is very time-consuming and much labor intensive. It includes milling samples, oil extraction from it, chemical-reaction, and gas-chromatographic (GC) or high performance liquid-chromatographic analysis. A simple and rapid method for the determination of the fatty acid composition is necessary for screening soybean varieties on the demands in breeding projects, where useful individuals are selected from a lot of samples to be tested. 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 on a very small amount of sample is also demanded.

Near infrared (NIR) spectroscopy has been recognized as one of the most powerful analytical tools (Osborne et al., 1993a; Panford, 1988; Osborne, 2007), and is widely used for the simple and rapid analyses of various agricultural and food products. NIR spectroscopy has also been used for the analysis of soybean constituents (Sato et al., 1994b; 2008). The fatty acid composition is an important index of fat or oil quality from nutritional point of view. Therefore, if NIR spectroscopy can be used for the analysis of the fatty acid composition, it would become an even more useful technique for soybean analysis. NIR analyses for the fatty acid composition have been carried out using a statistical method, i.e., a multivariate analysis (Pazdermik et al., 1997; Roberts et al., 2006; Patil et al., 2010). However, in this chapter, the author took another approach. The feasibility of NIR spectroscopy for estimating the fatty acid composition in soybean oil was examined according to the spectral patterns or assignments. Since the NIR spectral patterns were reflected by the fatty acid compositions, the absorption bands of cis-unsaturation and the carbon chain length of the fatty acid moieties in oil appear in the NIR wavelength region, especially around the 1600-1800 nm region (Sato et al., 1990, 1991, 1994a, 1995ab, 1998,

2002abc, 2003; Karen et al., 1994). In this region, the absorption band around 1720 nm is shifted to the shorter wavelength when the unsaturation in the fatty acid moieties increases, and it is shifted to the longer wavelength when the carbon chain length increases. Then, the author examine this wavelength range to determine the fatty acid composition in oil.

2. Materials and methods

2.1 Samples

The thirty-one samples were used in this study, and they 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 (Sato et al., 2002a). The other twenty-nine samples were also used for this study, and they were cultivated, harvested, and collected from various areas of Kyushu, the southern part of Japan, in 2000. Their varieties were Fukuyutaka, Suzuotome, Kiyomidori, L-star, and Sachiyutaka (Sato et al., 2002b).

These samples were sent to our laboratory and were milled by a Cyclone Sample Mill (Udy Corporation, Colorado, USA) through a screen (φ=1.0mm). Plural whole seeds, a single seed, and soybean powder were measured. Peeled single soybean seeds, a piece of a particle cut from a soybean seed with a knife, and a small amount of powder obtained by a drill (a rotary blade: φ=1.0mm) were also prepared from the seeds of the samples collected in 2000. There were no damages on its hypocotyl. All the samples were packed in a sealed polyethylene bag (Unipack; Seisan Nihon Co., Tokyo, Japan), and stored at 5°C until being analyzed.

2.2 Chemical measurements

The fatty acid composition was determined by the conventional GC method: The oil was extracted from about 2 g of sample powder with diethyl ether by the Soxhlet method using an instrument: Soxtec System HT 1043 Extraction Unit (Tecator, Sweden). The extracted oils were then used for the GC analysis after trans-esterification with sodium methoxide/methanol solution according to the conventional method (Chikuni et al., 1989).

The gas chromatograph was equipped with an FID detector (GC-17A, Shimadzu Co., Kyoto, Japan) (Sato et al., 2002a). Each sample was analyzed twice. The average was used for the following analyses. As for the calculation of the fatty acid composition, the sum of the percentages of the major five fatty acid moieties, palmitic (C16:0) + stearic (C18:0) + oleic (C18:1) + linoleic (C18:2) + linolenic (C18:3), was converted to 95.0%.

2.3 Near infrared spectroscopic analysis

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 on each sample a few times. The average spectra were calculated, and the analysis was carried out. The sample presentation methods for the NIR measurements were depending upon the sample types as follows:

1. whole plural soybean seeds (about 60g) in a whole grain cell (Photo 1-a)) on a rotating drawer.

2. a whole single soybean seed, a husked single one, or a crashed particle in a single grain cup (center hole diameter = 24 mm, Bran+ Luebbe Co.) (Photo 1-b)).

3. a small amount of soybean powder in a modified single grain cup (Photo 1-c)) : a small amount of soybean powder (about 8 mg) was taken into a modified single-grain cup (center hole diameter = 20 mm, Bran+ Luebbe Co.) with a small spatula, and the cup was jiggled slightly until the surface of the flour was arranged to the same or the horizontal level in the center of the cup, then the NIR spectra were measured. This cup was developed for measuring NIR spectra of a small particle and was successfully used for the analysis of the fatty acid composition of a single rapeseed as previously reported (Sato et al., 1998). It has a smaller and deeper hole in it, and it has a sharper parabolic curve to the base than a normal one. This cup was also used for a small amount drilled soybean powder.

4. a standard amount of soybean powder (about 3g) in a standard cell (Photo 1-d)) .

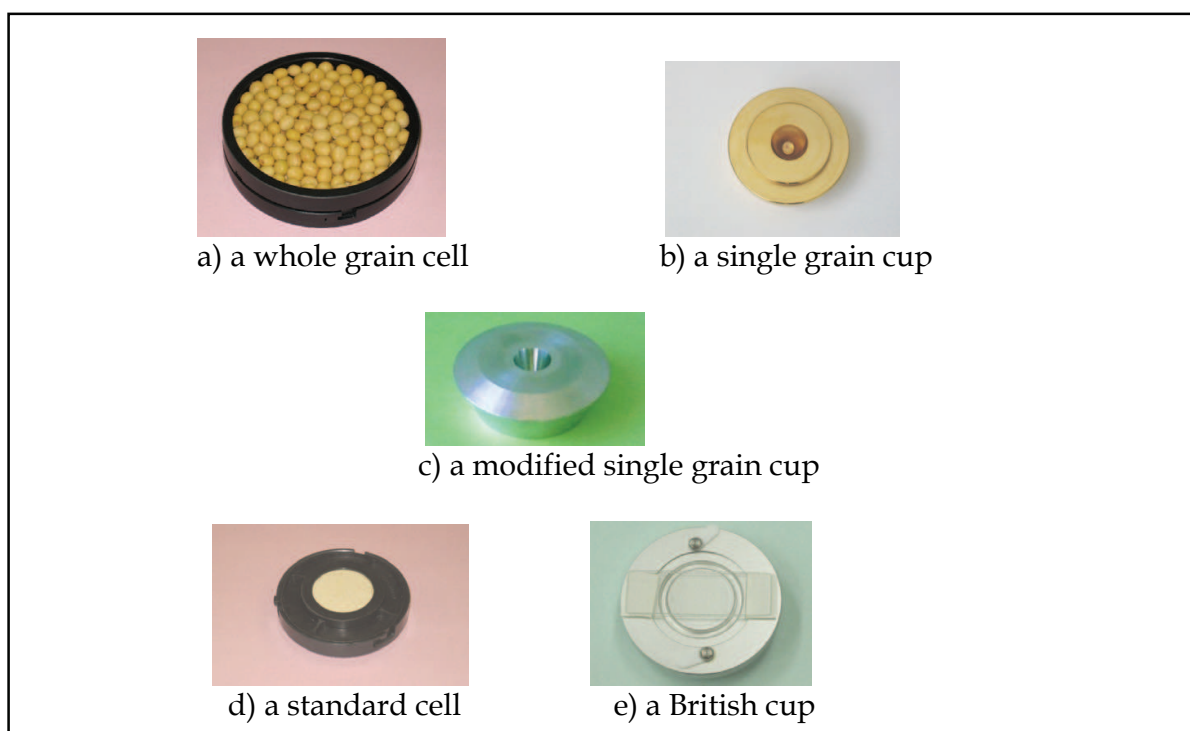5. extracted oil sandwiched between two slide glasses on a British cup (Photo 1-e)).

a) a whole grain cell   b) a single grain cup

c) a modified single grain cup

d) a standard cell   e) a British cup

Photo 1. The sample presentation methods for the NIR measurements

2.4 Mathematical treatment on NIR spectral data for the calculation of the second derivative spectra and that of the standardization.

The NIR wavelength range from 1600 to 1800 nm includes the information concerning the carbon chain length and the unsaturation of the fatty acid moieties (Sato et al., 1990, 1991, 1994, 1995ab, 1998, 2002abc, 2003). However, in the original NIR spectra, the difference due to the fatty acid composition could not be detected, and the second derivative spectra were calculated. The default parameters for calculating the second derivative spectra were as follows: the size of the moving average was 24 nm; the size of the derivative segments was 24 nm; and the gap between the derivative segments was 30 nm. However, with this condition,

the differences of the spectral characteristics do not appear. In order to strengthen this information and to make their differences clearer, the following parameters for calculating the second derivative spectra were adopted: 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. Then, since the intensity level of the obtained second derivative spectral values were different, these NIR spectral data were further standardized in order to make the comparison easier by making the spectral value at 1600 nm as 0.0 and the spectral value at the minimum around 1724 nm as -1.0 (Sato et al., 2002c) as in the following equation:

The corrected or standardized 2nd NIR spectral data at xxxx nm

= (-1)*(d2L(xxxx) - d2L(1600))/ (d2L(around 1724) - d2L(1600))

3. Results and discussion

3.1 A general NIR method

NIR spectra have information of constituents, and the information is extracted from the NIR data using a computer, i.e., the relationship between NIR spectral values and the contents of the constituents or the property values is calculated using the statistical techniques of the multivariate analysis by comparing their variations with a computer. On the other hand, as for a colorimetric method (Fig. 1-A)), the dilution series of some fixed quantity or concentration of the target substance is prepared as a standard in advance. Next, a target substance in the sample is extracted, refined, and is made in the chemically same condition as this dilution series. Then, they are compared at the same unit. The substances both in the dilution series and in the extract are compared with the colorimetry. Also in the NIR method, there is surely a similar procedure for moisture content analysis (Osoborne & Fearn, 1986). However, as for the general NIR method (Fig. 1-B)), the contents values and the spectral values are compared directly without any chemical treatments but with the mathematical conversions for emphasizing to make the relationship linear. The data are compared directly at the remained different unit of their own, or in the chemically different situation. The fluctuations of information both in the chemical data and in the NIR spectra are compared by a computer. The purpose of the NIR method is not only making the calibration equations (Fig. 2, Step I), but using them on the mother population to obtain individual numerical values (Fig. 2, Step II) for the decision-making (Fig. 2, Step III) such as the screening varieties in breeding projects, the quality control in the producing plant, the sample selection or rejection and so on. If you stop at the step I, you are suspected to only „make up the cover story". An NIR method is that this grand design of Fig. 2 is made work well. The NIR method is the tool for practical use. One's demand of the precision of the analysis depends upon one's purpose: sometimes precise, and sometimes rough. The relationship is one of the following things; a direct, an indirect, and a pseudo one. If you can take a risk judging from the SEP, RPD and so on, you can use any calibration equations you developed, sometimes not for an official use, but for your internal or private use. This might be the use in the unintended direction by a founder of NIR analysis. Genuine spectroscopists dislike this situation. However, you can use them with your self-responsibility. The statistics describes the events happened in the past. Then, if it grasps the principle, it can predict the events that happen in the future?! In general, when using an instrument, at least the zero-adjustment calibration is needed. As for the NIR method, the degree of the calibration
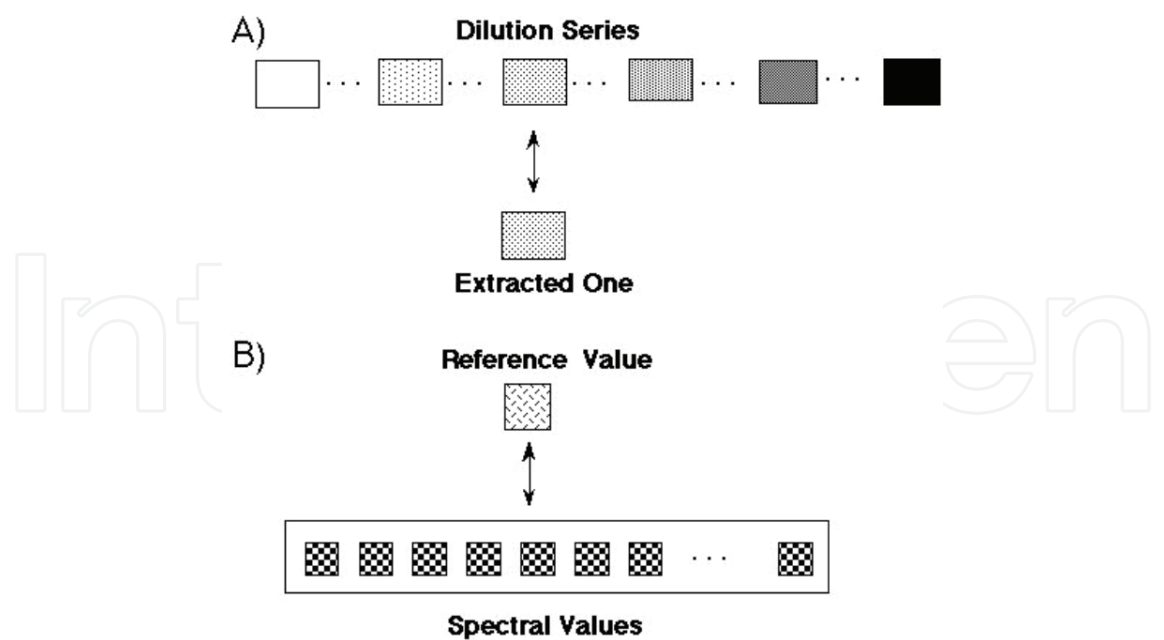
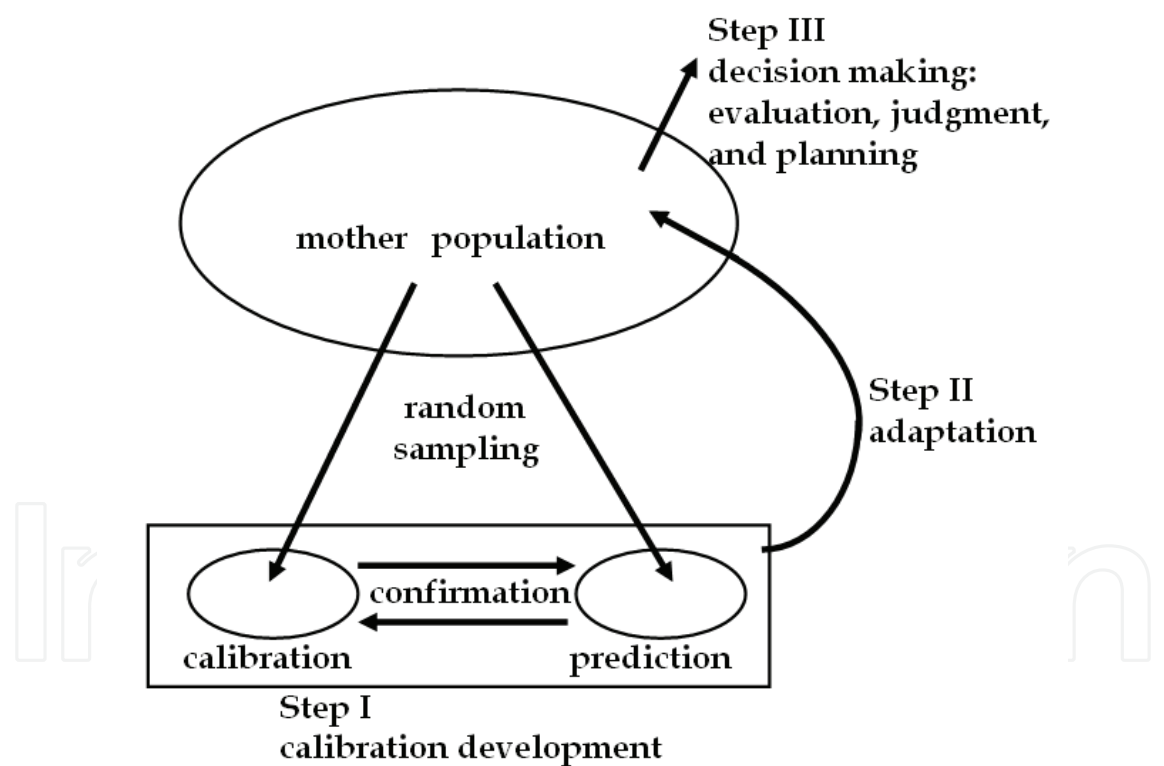Fig. 1. Comparing A) colorimetric method vs. B) NIR method.

Fig. 2. The outline of the NIR method

becomes to the extreme extent. An NIR method is adjusted to the respective official method, and when the latter assumed to be stable and steady, the former demonstrates a very good performance. In NIR spectroscopy, multiplevariate analysis is mainly carried out for the analysis of fatty acid composition (Kovalenkoet al., 2006 ; Roberts et al., 2006). The author takes another approach of not using multivariate analysis but examining the NIR spectral patterns or the assignments as in the following.

3.2 Characteristics of NIR spectra

Firstly, the spectra were examined. Figure 3-a) and 3-b) show the original NIR spectra. As for powdered soybean(Fig. 3-b)), there are absorption bands due to moisture (around 1960 nm), oil (1600-1800, 2120-2170, and 2200-2400m), carbohydrate (around 2100 nm), and protein (around 2180nm). However, as for extracted oil, only the absorption bands due to oil were clear (1600-1800, 2120-2170, and 2200-2400m). On the other hand, as for plural whole seeds (Fig3-a)), oil and protein absorption bands are weak.
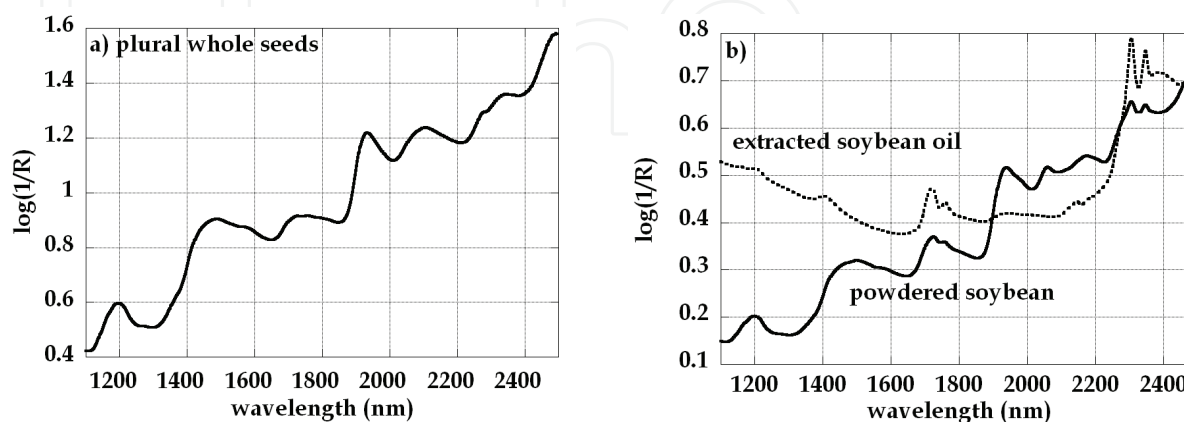
Fig. 3. The original NIR spectra of a) plural whole soybean seeds, and b) powdered soybean and extracted soybean oil.

A single seed, a peeled single seed, and a crashed peeled particle have similar spectral pattern as plural whole seeds, but small fluctuations. A small amount of powdered soybean has similar spectral pattern as a standard amount of soybean powder, but small fluctuations. In the following sections, discussion is proceded with soybean powder because of the clearer appearance in oil or fat absorbance region.

3.3 Examination of the conditions of calculating the second derivative spectra.

Figure 4 shows the default second derivative spectra of soybean powder in the standard cup. It was calculated with the default parameter condition. A part of the spectra concerned (1600 – 1800 nm) were also shown in Fig. 5. Their fatty acid compositions are described also in Fig. 5. No differences appeared. On the other hand, Figure 6 and 7 shows the second derivative spectra calculated with the other parameter condition. In this case, the differences were clearer. Their fatty acid compositions are described also in Fig. 7. They were characteristic especially in the ratio of oleic acid moiety (C18:1) and linoleic acid moiety (C18:2). Using the parameters described above, a clearer difference was obtained as shown in Fig. 7. Further, in order to make the spectral patterns clearer and to arrange the fluctuation level of the absorption to the same, the standardized second derivative NIR spectral values were calculated (Fig.8). As the percentage of the linoleic acid moiety increased, the absorption band around 1708 nm was stronger downward in the corrected second derivative NIR spectra, because the spectral phase was reversed in the second derivative spectra.

As for a small amount of soybean powder analysis, the clear NIR spectra of it were obtained using a modified single-grain cup, because the modified cup had been improved for a better collection of the reflection light from a small amount of sample due to the sharper parabolic curved surface than normal single seed cup. A single-grain cup itself has a very small artificial effect around 1400 nm, but it does not affect the absorption bands due to oil. As for a small amount analysis, its spectra have small fluctuations, but have similar characteristics. Further, standardization makes them clearer.
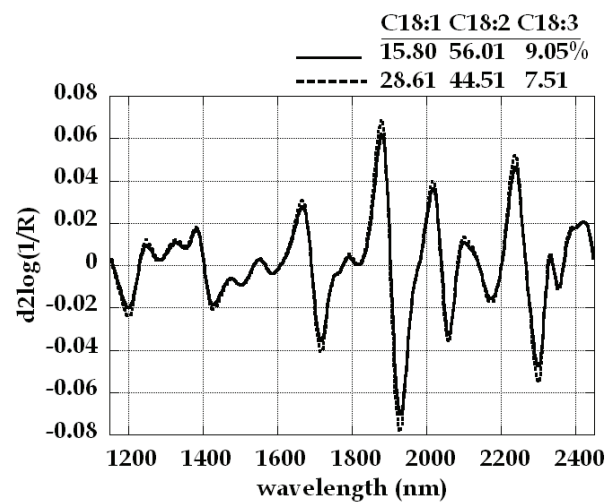
Fig. 4. The default second derivative NIR spectra of soybean powder in the standard cup.
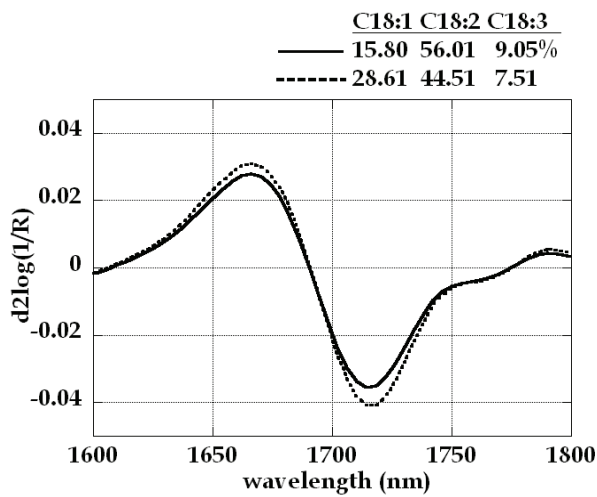

Fig. 5. A part of the wavelength region (1600-1800nm) of the default second derivative NIR spectra (Fig.4).
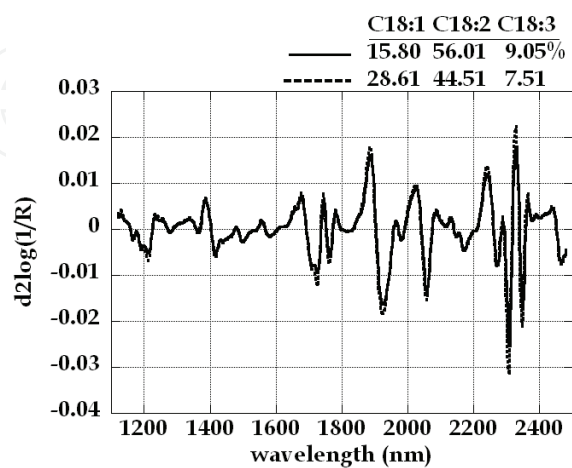

Fig. 6. The second derivative NIR spectra, calculated with the other parameter condition, of soybean powder in the standard cup.
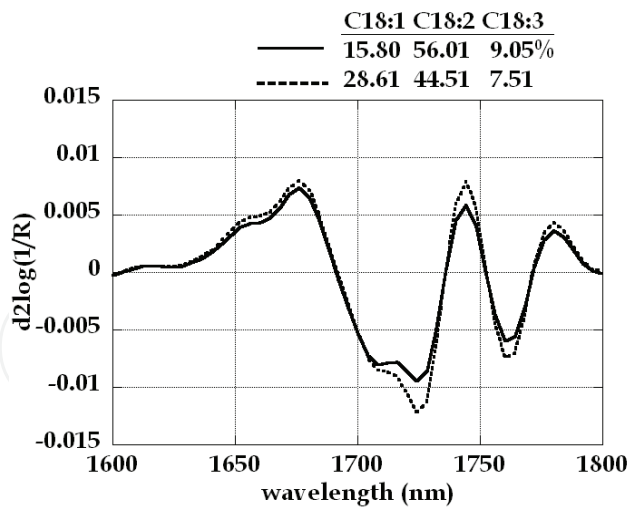
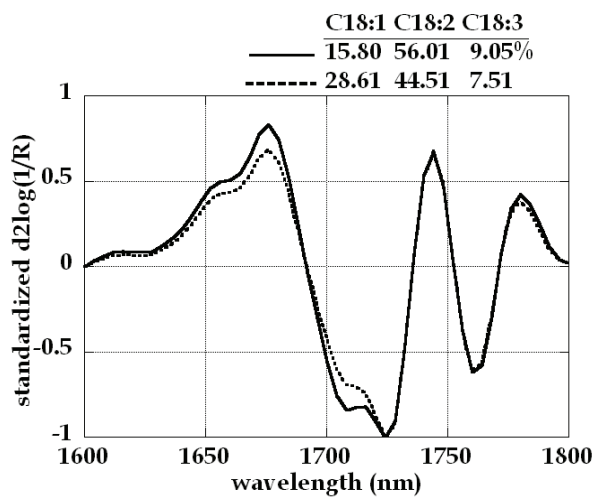Fig. 7. A part of the wavelength region (1600-1800nm) of the other second derivative NIR spectra calculated with the other parameter condition (Fig.6).

Fig. 8. The standardized second derivative NIR spectra of powdered soybean.
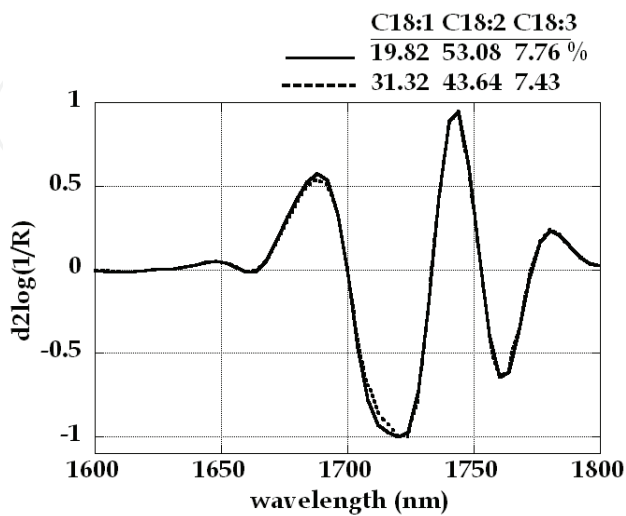
Fig. 9. The standardized second derivative NIR spectra of extracted oil.

As for the NIR spectra of extracted oil, Two varieties were adopted as examples for the explanation. Their fatty acid compositions are described in Fig. 9. They were characteristic especially in the ratio of oleic acid moiety (C18:1) and linoleic acid moiety (C18:2). Using the other parameters, a clearer difference was obtained as shown in Fig. 9. The spectral NIR pattern was different from that of soybean powder. However, 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 was reversed in the second derivative spectra.

3.4 Relationship between the NIR spectral patterns and the fatty acid composition.

The standardized spectral value at 1708nm is adopted as an index of linoleic acid moiety in oil for further discussion. As for soybean powder in the standard cup, which was harvested in 1999, the correlation between the percentage of the fatty acid moiety and the standardized reading at 1708 nm was shown in Fig. 10. Figure 10-a) shows the relationship of the percentages of linoleic acid moiety (C18:2) vs. the readings at 1708 nm, and the correlation coefficient was -0.803. The scattering plot shows: the stronger the standardized second derivative spectral value at 1708 nm downward, the higher the percentage of the linoleic acid moiety (C18:2) as mentioned above. The results showed that the rough estimation of the percentage of the linoleic acid moiety (C18:2) was possible. However, in this region, linolenic acid moiety (C18:3) has a little influence on the absorption band. There is a tendency for the linolenic acid (C18:3) to increase as the amount of linoleic acid (C18:2) increases. This is why a discrepancy from the regression line emerged when the linoleic acid (C18:2) moiety ratio increased as shown in Fig. 10-a). Then, the correlation of the percentages of linoleic (C18:2) + linolenic acid moiety (C18:3) vs. the readings at 1708 nm was examined (Fig. 10-b)). The correlation coefficient improved dramatically. The discrepancy from the regression line diminished. Incidentally, the correlation coefficient between the linolenic acid moiety (C18:3) vs. the readings at 1708 nm was -0.717, because its ratio and the variation were small. On the other hand, in Fig. 10-c), the correlation of the percentages of oleic acid moiety (C18:1) vs. the standardized readings at 1708 nm was shown. This shows the reverse correlation.
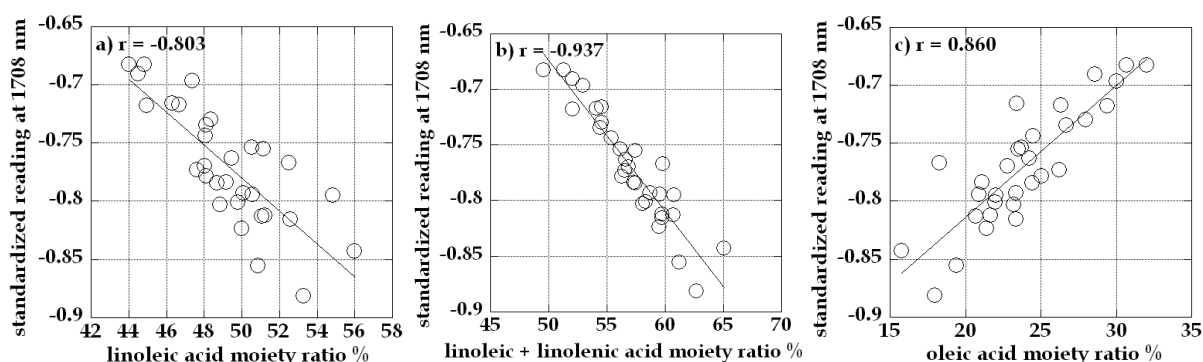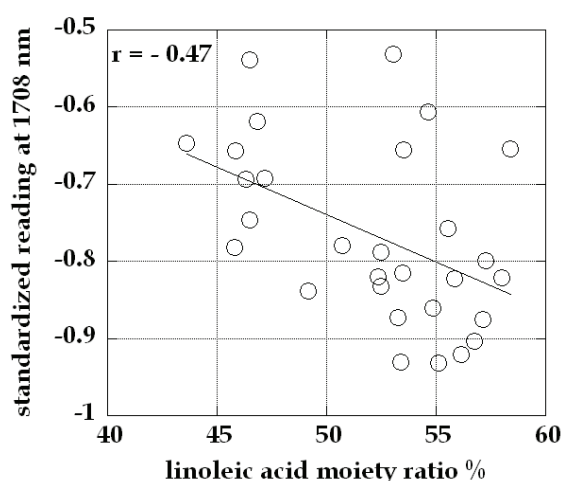

Fig. 10. The scattering plots between the standardized second derivative NIR spectral reading at 1708nm vs. a) linoleic acid moiety ratio, b) linoleic + linolenic acid moiety ratio, and c) oleic acid moiety ratio. (soybean powder from samples cultivated in 1999).

On the other hand, as for a small amount of soybean powder from samples cultivated in 1999, the correlation coefficiencts between the standardized second derivative NIR spectral reading at 1708nm vs. linoleic acid moiety ratio, linoleic + linolenic acid moiety ratio, and

oleic acid moiety ratio were -0.853, -0.947, and 0.877, respectively. In spite of a small amount analysis, clear results were obtained due to the standardization.

3.5 Relationship between the NIR spectral patterns and the fatty acid composition of the other materials harvested in 2000.

The correlation coefficients between the standardized second derivative NIR spectral reading at 1708 nm vs. oleic acid moiety ratio, linoleic acid moiety ratio, linolenic acid moiety ratio, and linoleic + linolenic acid moiety ratio (soybean samples harvested in 2000) described in Table 1. When a seed coat was remained, the correlation coefficients of plural whole seeds and a single seed were not good. Figure 11 shows the scattering plots between the standardized second derivative NIR spectral reading at 1708 nm vs. linoleic acid moiety ratio for whole plural seeds. The characteristics mentioned above were poorly appeared, and then no good correlation was obtained as described in Tables 1 and as shown in Fig.11.

Fig. 11. The scattering plots between the standardized second derivative NIR spectral reading at 1708 nm vs. linoleic acid moiety ratio for whole plural seeds. There is no good correlation.

The correlation coefficients showed: the stronger the absorption band at 1708 nm downward, the higher the percentage of the linoleic acid moiety as mentioned above. The results showed that the rough estimation of the percentage of the linoleic acid moiety (C18:2) was possible. However, in the case of whole plural seeds in a rotating cup, the inclination was not good enough.

The variations of the samples harvested in 2000 were smaller than those in 1999: the smaller cultivated areas and the less varieties, and then their correlation coefficients were better for the powder analysis. Figure 12 shows the two year data combined. However, the regression lines were closely coincident.

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

So far, the authors have successfully introduced the NIR method for single-seed, or very small amount, analysis of fatty acid composition, such as that in a sunflower seed (Sato et al., 1995b) , in sesame seed (Sato et al., 2003), and in a rapeseed (Sato et al., 1998). In this

samples	oleic acid moiety ratio	linoleic acid moiety ratio	linolenic acid moiety ratio	linoleic + linolenic acid moiety ratio
whole plural seeds in a rotating cup	0.48	-0.47	-0.38	-0.48
whole single seed in a mormal single grain cup	0.59	-0.53	-0.56	-0.58
whole single seed in a special single grain cup	0.48	-0.40	-0.57	-0.46
pealed single seed in a single grain cup	0.87	-0.83	-0.73	-0.86
standard amount of powder, obtained by a ultracentrifugal mill in a standard cup	0.97	-0.96	-0.71	-0.97
small amount of powder, obtained by a ultracentrifugal mill in a single grain cup	0.97	-0.96	-0.69	-0.98
crashed particles in a single grain cup	0.81	-0.79	-0.64	-0.82
small amount of powder, drilled from a seed in a modified single grain cup	0.86	-0.85	-0.63	-0.87
extracted oil in a British cup	0.98	-0.95	-0.76	-0.98

Table 1. The correlation coefficients between the standardized second derivative NIR spectral reading at 1708 nm vs. oleic acid moiety ratio, linoleic acid moiety ratio, linolenic acid moiety ratio, and linoleic + linolenic acid moiety ratio (soybean cultivated in 2000).
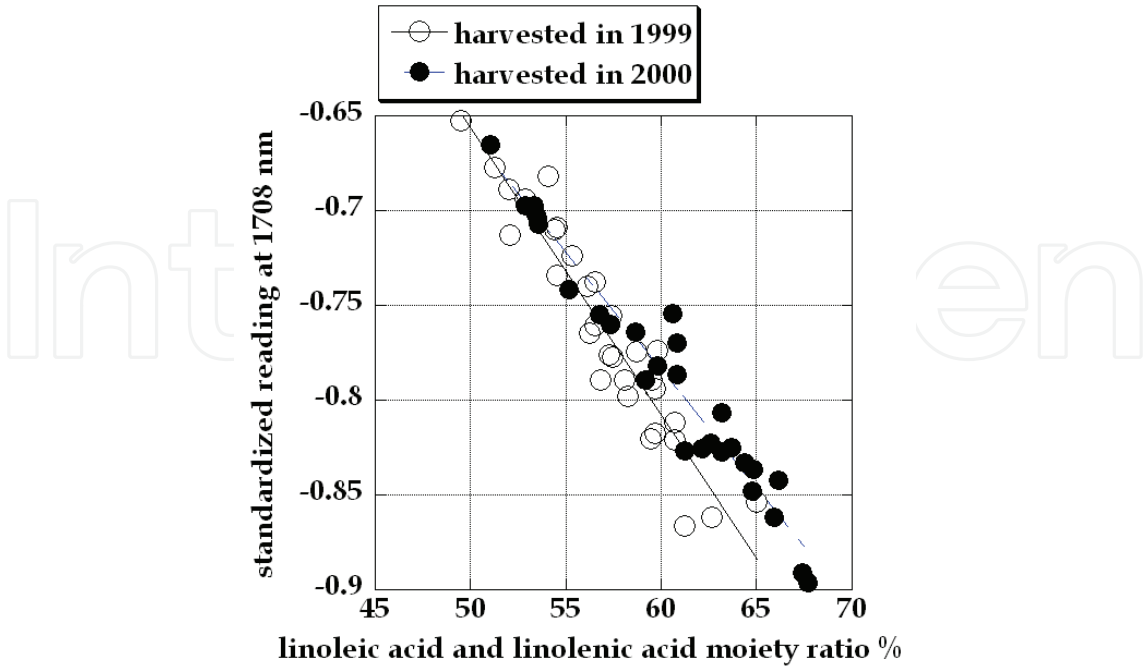

Fig. 12. The two year data combined for a small amount of powder in a modified single grain cup analysis case.

report, the authors 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 nuclear magnetic resonance method, Yoshida *et al*. (1989) successfully carried out the fatty acid analysis of an intact single soybean seed. However, it took almost one hour for accumulations. On the other hand, it took only one minute in the authors' case. Further, when a Fourier transform type instrument is used instead of a scan type instrument, the measurement time becomes faster. Furthermore, if sampling of a small amount of soybean powder is possible using a mini-drill without destroying a hypocotyl in a seed, germination ability can be retained, and the method can be adopted for screening of the varieties not only with the required fatty acid composition but also with useful properties linked with the fatty acid composition based on the spectral assignments. The authors have successfully introduced a nearly nondestructive method.

4. Conclusions

The near infrared (NIR) spectroscopy has been recognized as a powerful analytical tool and has been widely used for estimating the contents of the constituents or the property of various agricultural products. However, the NIR method is thought to be an empirical method using multivariate statistical analyses. In this chapter, the author took another approach based on the NIR spectral pattern analysis to develop a simple and rapid method for the determination of the fatty acid composition in soybean oil. The NIR wavelength range from 1600 to 1724 nm includes the information concerning the unsaturation and the chain length of fatty acid moiety. First, the author examined the conditions of the mathematical treatment for the conversion of the original spectral data to the second derivative one in order to make this information clearer. Then, the NIR spectral data was standardized to compare easily by making the spectral value at 1600 nm as 0.0 and the spectral value at the minimum around 1724 nm as -1.0. The correlation coefficient between the corrected second derivative NIR readings at 1708 nm and the percentage of linoleic acid moiety in soybean oil was good enough for a rough estimation of it even with a very small amount of powder. Using this technique, the authors examine the determination of fatty acid composition of other oilseeds: (husked) sunflower, rapeseed and sesame without developing the calibration equations. These findings give theoretical bases to NIR technology. Further, if this nondestructive method will be used as a marker of the property linked with the fatty acid composition, it will develop its use in the new fields.

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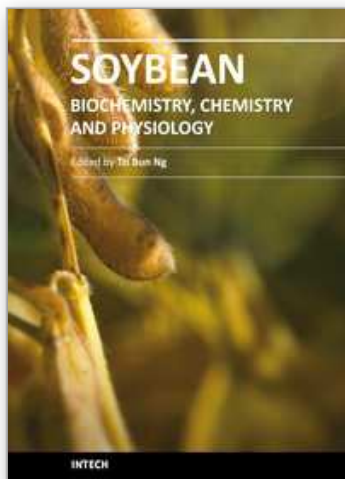
Soybean - Biochemistry, Chemistry and Physiology

Edited by Prof. Tzi-Bun Ng

Soybean is an agricultural crop of tremendous economic importance. Soybean and food items derived from it form dietary components of numerous people, especially those living in the Orient. The health benefits of soybean have attracted the attention of nutritionists as well as common people.

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