New Estimation Method for Fatty Acid Composition in Oil Using Near Infrared Spectroscopy

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The absorption bands of cis-unsaturation and the carbon chain length of the fatty acid moieties in oil appear in the near infrared (NIR) wavelength region, especially around 1600–1800 nm. Using this region, a new estimation method for fatty acid composition analysis is proposed. Because the differences of the original NIR spectra are miniscule even in this region, the second derivative NIR spectra were examined in order to estimate the fatty acid composition in oil exclusively from the spectral patterns obtained. The parameters for calculating the second derivative NIR spectra were examined to make the spectral difference clearer. In any parameter, the absorption band was shifted to the shorter wavelength region when the unsaturation in fatty acid moieties increased, and it was shifted to the longer wavelength region when the carbon chain length increased. When the parameters were correct, this NIR method can estimate the fatty acid composition roughly, but simply, easily, and sometimes nondestructively.

Key words: analysis; fatty acid composition; near infrared; second derivative; spectroscopy

Recently, near infrared (NIR) spectroscopy has been recognized as one of the most powerful and useful analytical techniques for estimating the contents of some major constituents in agricultural and food products.1-3) However, in the NIR method, multiple linear regression analyses or multivariate analyses were mainly done using the spectral data with the referenced chemical data.

In this report, the spectral pattern might be especially noticeable. Fatty acid composition is an important nutritional index for fats and oils. In the NIR spectra, especially in fats and oils, spectral patterns give a rough estimation of fatty acid composition because the absorption bands of cis-unsaturation and the carbon chain length of fatty acid moieties appear around the 1600–1800 nm region of the wavelength.4) Using this region, a new estimation method for fatty acid composition analysis is proposed. However, since the differences of the original or raw NIR spectra are miniscule even in this region, second derivative NIR spectra are used for this purpose.5-8)

The problem lies in identifying what parameters are best to obtain a clearer difference. The default parameters were used for powdered-type samples because the NIR method was conventionally developed for them, and those for calculating the second derivative were not examined for the extraction of information from the NIR spectra. In this report, the parameters for calculating the second derivative NIR spectra were changed and examined in order to estimate the fatty acid composition from the NIR spectra alone.

Materials and Methods

Samples. Since oils or fats consist primarily of triglycerides or methyl esters of glycerine, the following pure methyl esters were used as representatives of fatty acid moieties for measuring the standard NIR spectra: the methyl esters of oleic (C18:1), linoleic (C18:2), linolenic (C18:3), erucic (C22:1), palmitic (C16:0), and stearic (C18:0) acids (Sigma Co., MO, USA). Two individual rape varieties, Michinokunatane and Bounty, cultivated in the farms of the author’s research center (located in Nishigoshi, Kumamoto-ken, Japan) were also adopted as examples of methyl ester mixtures in this study: varieties with high or low levels of erucic acid, and their chemical measurements were in the previous report.7)

Near infrared spectroscopy. The standard samples were scanned with an NIR instrument with a syrup cup (Bran+Luebbe (B+L) GmbH, Norderstedt, Germany) for a liquid sample covered with nonreflection glass (Fig. 1a) and a single-grain cup (B+L) in which a small amount of solid sample was placed in the center of the hole (Fig. 1b). An IntraAlyzer 500 (B+L) was used to measure the NIR transfectance or transreflectance spectra in the wavelengths from 1100 to 2500 nm at 2-nm intervals. The spectral data obtained were converted to the American Standard Code for Information Interchange (ASCII) files and were then used for the cal-

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Calculation of second derivative NIR spectra. The parameters examined included the moving average (MA), the size of the derivative segments (SEG), and the gap between the derivative segments (GAP). These were converted to the corresponding number of data points. The outline of the calculation of the second derivative NIR spectra is described in Fig. 2. This is a moving-average segment convolution (MASC) method. First, the raw spectral data were input in the variables s1[x]; then, using MA, the moving average s2[x] was obtained. Using SEG, the sum of the derivative segment s3[x] was obtained; then, using GAP and s3[x], the second derivative s4[x] was calculated. The corresponding wavelength equaled \[ \text{original wavelength} + \text{MA} + \text{SEG} + \text{GAP} \]. The algorithm above is mainly based on the IDAS software (B+L).

Mathematical treatment of NIR spectral data for standardization. The NIR spectral region of 1600–1800 nm was especially noteworthy in this examination because the variation of the fatty acid moieties in this region was more pronounced than in the region of 2100–2200 nm, where only a degree of unsaturation could be detected. However, since the intensity level of the original spectral values was different, the spectra were then standardized to make it easier to compare them to the obtained second derivative NIR spectral patterns: the level of the starting point at 1600 nm was made to be 0.0, and the minimal value around 1708–1730 was corrected to –1.0, according to a previous report.4,5)

Results and Discussion

Effects of degree of unsaturation of the moieties

In the second derivative NIR spectra, the absorption bands go downward as the intensity increases, i.e., their troughs correspond to the peaks of the original spectra. First, the parameters were set: the MA was 2 nm, the SEG was 6 nm, and the GAP was changed (Figs. 3a–3c). Incidentally, when using smaller parameters, a noise problem occurred, and it became difficult to examine the spectra. In Fig. 3a, the absorption band was shifted to the shorter wavelength region in the second derivative NIR spectra as the unsaturation in the moieties increased. The inclination was similar in other cases. However, when the GAP was 6 nm, C18:1 and C18:0 had a shoulder in each spectrum around 1710 nm, and C18:2 also had a shoulder around 1720 nm, as shown in Fig. 3a. Furthermore, the shift difference was small. The absorption bands of C18:2 and C18:3 were similar. As the GAP increased, the shoulders gradually disappeared, as shown in Figs. 3b and 3c. Furthermore, the shift difference between C18:2 and C18:3 became bigger and clearer. However, in Fig. 3c, the shift difference between C18:1 and C18:2 grew slightly smaller. The bigger GAP produced a smoother effect.
When the MA was 4 nm, the SEG 12 nm, and the GAP 12 nm, the shoulders disappeared, and, furthermore, the shift difference grew larger and clearer. In this case, the shift difference between C18:1 and C18:2 became clearer than in other cases. For fats and oils, the difference between C18:1 and C18:2 is especially important from a nutritional point of view.

The parameters were then set: the MA was 16 nm, the SEG was 24 nm, and the GAP was changed (Figs. 3e–3g). The most notable difference was the shift increase, shown in Figs. 3e–3g, because of the smoother effect produced by the bigger parameters. The default parameters of IDAS software (MA 24 nm, SEG 24 nm, and GAP 30 nm) produced similar results. When the MA and the SEG were bigger, the GAP produced no effects on clarifying the difference.

Effects of carbon chain length of the moieties

The parameters were first set: the MA was 2 nm, the SEG was 6 nm, and the GAP was changed (Figs. 4a–4c). When the GAP was 6 nm, the absorption band was shifted to the longer wavelength region in the second derivative NIR spectra, as the carbon chain length increased. However, C16:0, C18:0, C18:1, and C22:1 had shoulders in the absorption bands near 1710 nm, as shown in Fig. 4a. As the GAP increased, the shoulders disappeared.

The parameters were then set: the MA was 16 nm, the SEG was 24 nm, and the GAP was changed (Figs. 4e–4g). The absorption band was shifted to the longer wavelength region in the second derivative NIR spectra as the carbon chain length increased. The only notable point was the shift difference in each case. Smoother effects could also be observed. The default parameters produced these same results. When the MA and SEG were bigger, the GAP had no effects.
Comparing Figs. 3b and 3d, the separation of C18:2 and C18:3 was a little clearer, and the absorbance bands of the latter are more symmetrical. Comparing Figs. 4b and 4d, the separation of C18:1 and C22:1 was a little clearer, and the absorbance bands of the latter are more symmetrical. When the MA was 4 nm, the SEG 12 nm, and the GAP 12 nm, the shoulders in the absorption bands also disappeared with the results described above. These parameters gave a good result for the deconvolution.

Single seed of rapeseed as an example of mixtures of esters
Using the data from rapeseed as an example of mixtures of esters, the following analyses were done. These two varieties had characteristic fatty acid composition, as described in Fig. 5a. First, the parameters were set: the MA was 2 nm, the SEG was 6 nm, and the GAP was increased (Figs. 5a–5c). When the GAP was 6 nm, the amount of C18:2 and the existence of C22:1, which are characteristics of the absorption bands, were observed (Figs. 5a, 5b). However, the shoulders of the absorption bands of C18:1 and C18:2 appeared in this case, as shown in Fig. 3a. Because the shoulders strengthen the absorption bands inappropriately, these parameters should not be used. When the GAP was 24 nm, the shift difference was the only change, as shown in Fig. 5c. This indicates that the fatty acid composition between these two varieties differed; however, the nature of the difference was not identified.

The parameters were then set: the MA was 16 nm, the SEG was 24 nm, and the GAP was increased. In these cases, the only change was the shift difference. When using the default parameters, the results were nearly identical. This indicates that the fatty acid compositions of the samples were different; however, it was not clear how they were different.
When the parameters were set with the MA at 4 nm, the SEG at 12 nm, and the GAP at 12 nm (Fig. 5d), the characteristics of the absorption bands could be clearly seen in the amounts of the C18:1 and C18:2 and the existence of C22:1, i.e., the variety Michinoku-natane had lower C18:2 and C18:1 and higher C22:1, while the variety Bounty had higher C18:2 and C18:1 and no C22:1. The former had a weaker absorption band around 1710 nm, and an absorption band at 1730 nm had shifted to a slightly longer wavelength. Furthermore, in this case, the shoulders of the absorption bands of C18:1 and C18:2 disappeared, as shown in Fig. 3d, and the shift differences between C18:2 and C18:1 were clearer. Fatty acid composition is also an important breeding index, and these results produced useful information for the selection of varieties. In practice, the author obtained a good correlation coefficient ($r = -0.858, n = 60$) between the NIR spectral values and the ratio of linoleic acid by using this information.\(^7\)

**Discussion**

McClure et al.\(^9\)–\(^11\) and Morimoto et al.\(^12\) examined the parameters for calculating the second derivative NIR spectra in order to resolve overlapping bands by using synthetic bands. They found that the effects of the derivatives were band improvement and background elimination. They also noted that the bigger the GAP was, the lower the resolving power, the greater the shift, and the broader the band. This research produced the same results. However, the difference was that the synthetic bands used were 30 nm or 40 nm apart at the center of the bands. On the other hand, in this case, the differences were that the center wavelength in the second derivative NIR
spectra of the absorption bands of C18:3, C18:2, C18:1, and C18:0 were 1708, 1712, 1724, and 1730 nm in Fig. 3d and the absorption bands of C18:1, C22:1, C16:0, and C18:0 were 1724, 1726, 1728, and 1730 nm in Fig. 4d. The differences were 4 to 22 nm in the former case and 2 to 6 nm in the latter. Morimoto et al.\textsuperscript{12} also reported that bands that were too closely overlapped could not be resolved and that for two simple band spectra, the GAP must be less than half the bandwidth in order to resolve two peaks. McClure et al.\textsuperscript{9–11} and Morimoto et al.\textsuperscript{12} aimed at a perfect deconvolution of the bands. Morimoto et al.\textsuperscript{12} suggested that the resolution ability might be dependent on the number of constituents as well as the kinds involved. However, there was no need for achieving a perfect deconvolution. In this author's case, the absorption bands were successfully detected, and the characteristics of the fatty acid composition could be estimated from only the second derivative NIR spectra without using chemical data.

With any parameters, the absorption band was shifted to the shorter wavelength region in the second derivative NIR spectra as the unsaturation in fatty acid moieties increased, and the absorption band was shifted to the longer wavelength region as the carbon chain length in them increased. When the parameters were not correct, a shoulder appeared in the absorption band, and the shift was small even when a pure single standard sample was examined. The correct parameters were with the MA at 4 nm, the SEG at 12 nm, and the GAP at 12 nm for estimating fatty acid composition. With the NSAS software (FossNIRSystems, ML, USA), a 12 nm SEG and a 12 nm GAP might be used. The method mentioned above was independent of the population and instrumental differences because empirical calibration equations were not used; however, it was based on spectroscopic pattern analysis. These results can be adapted for other products containing fats and oils. Then, a new estimation method of the fatty acid composition analysis of fats, oil or oilseed was proposed. This method can estimate it roughly, but simply, rapidly and sometimes nondestructively.

References


