Near Infrared Spectral Patterns of Fatty Acid Analysis from Fats and Oils[1]

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A near infrared (NIR) spectral pattern of oil contains information about fatty acid composition, because NIR absorption bands around 1600–1800 nm and 2100–2200 nm are due to the straight carbon chain and *cis* double bonds, respectively. This study was undertaken to build a foundation for the rapid determination of the fatty acid composition in oil by an NIR method. First, NIR spectra of pure triglycerides were measured and characterized. Fatty acid compositions could be estimated roughly by comparing the spectra of fats and oils (butter fat, pig milk fat, soybean oil and palm oil) with those of pure triglycerides. Secondly, the NIR spectra of these fats and oils were reconstructed by summation of the triglyceride spectra, which are multiplied by factors corresponding to the fatty acid composition of the sample determined by gas chromatography. The calculated spectra agree with the originals, especially for that of soybean oil. However, in order to reconstruct spectra precisely, it may be necessary to reevaluate the loading weight of each triglyceride, which was equal in this study.

KEY WORDS: Fatty acid composition, near infrared, spectroscopy.

Recently, near infrared (NIR) spectroscopy has been recognized as a powerful analytical technique. NIR methods allow rapid determination of various constituents in agricultural and food products (1,2). However, it has been considered an empirical method because calibration equations have to be obtained prior to routine analyses. Therefore, a qualitative approach is necessary for it to gain a rightful place in the analytical methods as a rational spectroscopic technique.

Measuring NIR spectra of various fatty acids, Holman and Edmondson (3) showed that the absorptions at 1680, 2150 and 2190 nm might be assigned to vibration of C-H bonds bound to *cis*-unsaturation, and Murray (4) found that fatty acids have characteristic spectral patterns around 1700 to 1730 nm. Thus, NIR spectral patterns, especially in the wavelength ranges of 1600–1800 nm and 2100–2200 nm, contain information about *cis*-unsaturation as well as about the carbon chain itself. Since they can be considered a reflection of the fatty acid moieties in oil, we have suggested that NIR spectra of oil may represent their fatty acid composition, and we have used this information to detect foreign fat adulteration in dairy products (5). Panford and deMan (6) studied the influence of fatty acid composition on wavelength selection for measuring oil content of oilseeds by NIR. However, there are few studies on determining fatty acid composition by NIR. This work was undertaken to develop a foundation for the rapid determination of fatty acid composition in fats and oils by NIR spectroscopic method. First, NIR spectra of pure triglycerides were measured and examined; then the NIR spectral patterns of oils were reconstructed by combining those of the triglycerides; and, finally, the agreement between original and calculated spectra was examined.

MATERIALS AND METHODS

Materials. Four kinds of fats and oils were analyzed: two fats extracted by the Röse-Gottlieb method (7) were from commercially available butter (purchased from Snow Brand Co.) and from pig milk (produced in the National Institute of Animal Industry, Tsukuba, Japan); the other two commercially available oils were soybean oil (purchased from Wako Pure Chemicals, Osaka, Japan) and palm oil (purchased from Yuro Chemicals, Tokyo, Japan).

The triglycerides purchased from Sigma Chemical Co. (St. Louis, MO) were also analyzed: tributyrin (abbreviated as C4:0), tricaproin (C6:0), tricaprylin (C8:0), tricaprin (C10:0), tripalmitolein (*cis*-C16:1), triolein (*cis*-C18:1), trilinolein (*cis*-C18:2), trilinolenin (*cis*-C18:3) and trilinoelaidin (*trans*-C18:2). These were used without further preparation.

Chemical measurements. The fatty acid composition of fats and oils were analyzed with gas chromatography (GC) after methyl- (8) or isopropyl-esterification (9). GC analyses of the fatty acid methyl esters were carried out according to the previous report (5). GC conditions for determining the fatty acid isopropyl esters, *i.e.*, for butter fat, were slightly changed as follows: 2 m × 3 mm i.d. Pyrex column packed with 10% uniport HP coated on 80/100 mesh DEGS (Gasukuro Kogyo Co., Tokyo, Japan); 230°C injection temperature; 30 mL/min carrier gas flow; column temperature programmed 100°C to 210°C at 8°C/min, then holding for 46 min at 210°C. Each sample was analyzed twice and the averages of duplicate measurements were calculated.

Physical measurements. A near infrared spectroscopic analyzer (InfraAnalyzer 500, Bran and Luebbe, Germany) was used to measure NIR transflectance at wavelengths from 1600 to 2300 nm at 1-nm intervals. Sample presentation was carried out at room temperature in an aluminum sample holder, British cup (Bran and Luebbe), and covered with a slide glass according to the previous report (5,10).

Conditions to obtain the second-derivative spectrum were as follows. Two nm between output points, 2 nm in moving average, 12 nm per derivative segment, and 12 nm between derivative segments. Thus, the range of the second-derivative spectrum became 1619 to 2281 nm by 2 nm, owing to IDAS software (Bran and Luebbe).

A range of 1600–2200 nm was used in analysis for raw spectra, and 1619–2201 nm for second-derivative spectra,

[1]Part of this study was presented at the 3rd International Conference on Near Infrared Spectroscopy on June 28, 1990, Brussels, Belgium.

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because the information about fatty acid composition is concentrated in these regions, as mentioned above.

Reconstruction of NIR spectra. Binary files of the spectral data were converted to ASCII files by IDAS software to carry out further data processing with software developed at our laboratory. Since trilaurin (C12:0) and longer saturated triglycerides do not melt at room temperature, the NIR spectrum of *trans*-C18:2 was used as a substitute for that of C18:0, and those NIR spectra of C12:0 to C16:0 were estimated by interpolation between that of C10:0 and *trans*-C18:2, *i.e.*, the spectral value at each wavelength was calculated as follows:

$$(\text{C12:0}) = (\text{C10:0})\times 3/4 + (\textit{trans}\text{-C18:2})\times 1/4$$

$$(\text{C14:0}) = (\text{C10:0})\times 1/2 + (\textit{trans}\text{-C18:2})\times 1/2$$

$$(\text{C16:0}) = (\text{C10:0})\times 1/4 + (\textit{trans}\text{-C18:2})\times 3/4$$

The estimated NIR spectrum of each oil (butter fat, pig milk fat, soybean oil and palm oil) was calculated by summation of each triglyceride spectrum, which was multiplied by a factor corresponding to the fatty acid composition of the oil sample obtained by GC. Since levels of spectral values were different between original and calculated spectra, the latter were modified further—the level of the calculated spectrum at the starting point (1600 nm or 1619 nm) was made equal to that of the original spectrum, and the maximum or minimum value around 1710–1725 nm was corrected to that of the original spectrum:

$$(B_x)_{\text{modified}} = (B_x - B_s) \times (A_s - A_m)/(B_s - B_m) + A_s$$

where $(B_x)_{\text{modified}}$ is the modified value of B_x, B_x is a calculated spectral value at wavelength x, B_s is a calculated spectral value at the starting wavelength (1600 or 1619 nm), B_m is a maximum (raw spectra) or minimum (second-derivative spectra) of the calculated spectral value around 1720 nm, A_s is an original spectral value at the starting point, and A_m is a maximum (raw spectra) or minimum (second-derivative spectra) of an original spectral value around 1720 nm. No other conversions, except parallel transference and magnification along the ordinate axis, were carried out. The differences between calculated and original spectra also were obtained.

RESULTS AND DISCUSSION

NIR spectra of triglycerides. Figure 1 shows the raw NIR spectra of pure triglycerides. In Figure 1 (A and B), except for C4:0 and C6:0, the NIR spectra of saturated and *trans*-unsaturated triglycerides show the same trend, *i.e.*, they have extrema at *ca.* 1725, 1760 and 2130 nm. Since C12:0 to C18:0 appear to have the same trend, their NIR spectra were estimated from those of C10:0 and *trans*-C18:2. As for the region around 1800 nm, the spectra rise when saturated, although they do not rise when unsaturated. This range seems to contain information about the degree of saturation of fatty acid moieties. In Figure 1C, as the degree of *cis*-unsaturation increased, the maximal peak around 1725 nm (*cis*-C18:1) shifts to the lower wavelength—1717 nm (*cis*-C18:2), and further to 1712 nm (*cis*-C18:3). The peaks at 1660 nm and 2145 nm are also due to *cis*-unsaturation. Because Holman and Edmondson (3) measured fatty acids and not triglycerides, their peaks assigned to *cis*-unsaturation were at slightly longer wavelengths (*ca.* 10–20 nm) than ours.

Figure 2 shows the second-derivative NIR spectra of pure triglycerides. In Figure 2 (A and B), except for C4:0 and C6:0, the NIR spectra of saturated and *trans*-unsaturated triglycerides, also show the same trend—troughs were observed at 1725, 1761 and 2125 nm. According to Osborne *et al.* (2), the second-derivative spectrum has a trough corresponding to each peak in the raw spectrum. Further, in its second-derivative spectrum a linear background can be eliminated, and peaks can be resolved that overlap substantially in the raw NIR spectrum, *i.e.*, absorption bands at 1661 and 2117 nm appeared in the second-derivative spectrum. In addition, the troughs at 2143 and 2177 nm became clearer than in the raw spectra. In Figure 2C, the higher the degree of *cis*-unsaturation, the more the minimal trough shifted to lower wavelength—from 1725 (*cis*-C18:1) *via* 1713 (*cis*-C18:2) to 1709 (*cis*-C18:3) nm.

Fatty acid composition by GC method. Table 1 shows fatty acid compositions (wt. %) of butter fat, pig milk fat, soybean oil and palm oil. These were chosen as representative fats and oils from animal and plant origin. They have characteristic fatty acid compositions; milk fat consists mainly of oleic acid and saturated acid moieties, pig milk fat has a bit more unsaturated fatty acids, soybean oil consists of mainly unsaturated fatty acids (linoleic acid, etc.), and palm oil has mainly saturated ones.

Compared with the results of Iverson and Sheppard (11,12), in our experimental conditions, the values of 4:0 (abbreviation for fatty acid moiety) in butter fat were underestimated. The values of 8:0 and 10:0 were slightly overestimated. Those of 6:0, 12:0 to 18:0 and 16:1 to 18:3 were at the same level as found by Iverson and Sheppard. As for pig milk fat, fatty acid moieties other than 14:0 and 16:1 (which were underestimated a little) were at the same level as described by Christie (13). However, the Table shows the general characteristics of the fatty acid compositions of these fats and oils. Strictly speaking, these values for unsaturated fatty acids are not necessarily equivalent to the degree of *cis*-unsaturation, but are reflected by it, because almost all unsaturated fatty acids of biological origin contain only *cis* bonds (14).

Reconstruction of NIR spectra of fats and oils. So far, the NIR spectra of pure monoacyl triglycerides were measured. Fats and oils mainly consist of triglycerides, but most molecules contain different fatty acid moieties. To simplify the situation, the authors supposed that fat was a mixture of pure monoacyl triglycerides and that there were no interactions between those moieties. By means of the NIR spectra of pure triglycerides, the reconstruction of fats and oils were carried out. Figures

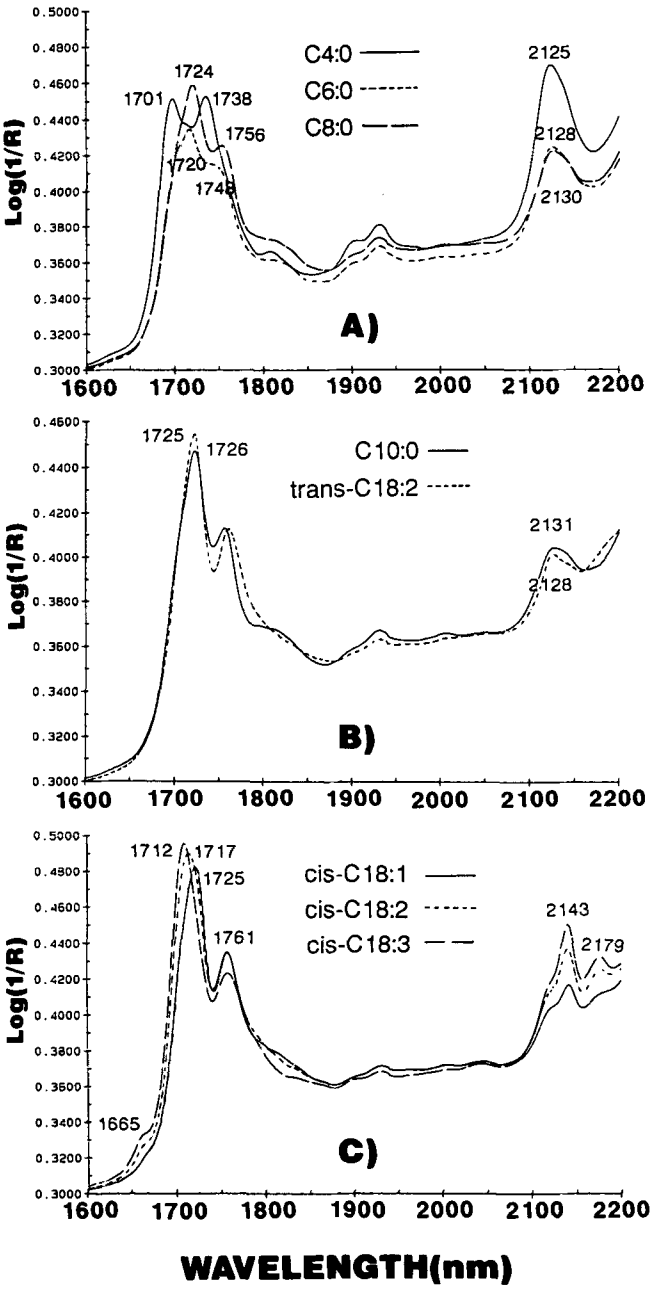

FIG. 1. Near infrared raw spectra of triglycerides. A, tributyrin (C4:0), tricaproin (C6:0) and tricaprylin (C8:0); B, tricaprin (C10:0) and trilinoelaidin (*trans*-C18:2); and, C, triolein (*cis*-C18:1), trilinolein (*cis*-C18:2) and trilinolenin (*cis*-C18:3).

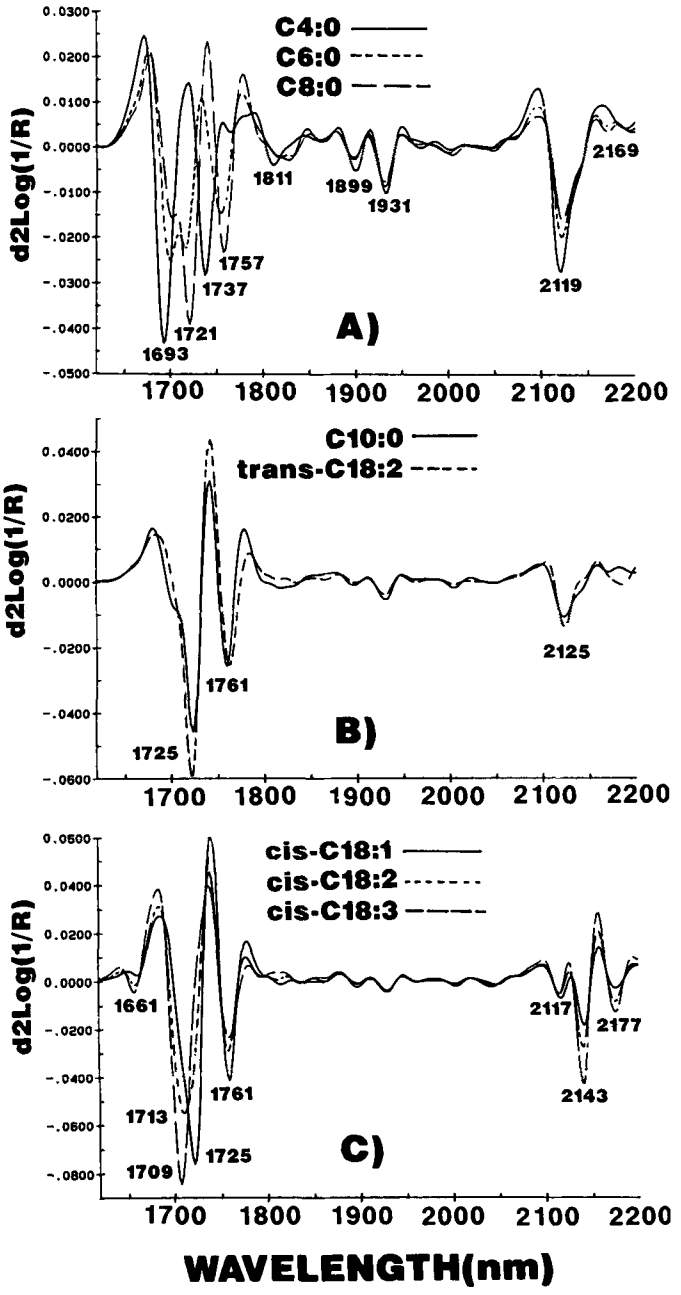

FIG. 2. Near infrared second-derivative spectra of triglycerides. Legends as in Figure 1.

3 through 6 show actual and calculated NIR spectra of butter fat, pig milk fat, soybean oil and palm oil, respectively. The solid lines represent the actual, and the broken lines the reconstructed spectra.

From the original NIR spectra of oils and fats (solid lines in Figs. 3–6) the fatty acid compositions could be estimated roughly. As for soybean oil (Fig. 5), peaks were observed at 1718, 2141 and 2180 nm in the raw spectrum and troughs at 1719, 2141 and 2175 nm in the second-derivative spectrum and, furthermore, a peak and a trough at 1718 and 1719 nm were broader than for other fats and oils. Those things were characteristic for C18:2 (Figs. 1C and 2C). The NIR spectrum of soybean oil seemed to be influenced mainly by the linoleic acid moiety. As for but-

TABLE 1

Fatty Acid Compositions [wt. % (± S.D.)] of Butter Fat, Pig Milk Fat, Soybean- and Palm Oil as Isopropyl or Methyl Esters

	Butter fat[a]	Pig milkfat	Soybean oil	Palm oil
4:0	1.84 (±0.01)	—	—	—
6:0	2.28 (±0.00)	—	—	0.60 (±0.02)
8:0	4.89 (±0.32)	—	—	8.19 (±0.10)
10:0	4.41 (±0.07)	—	—	5.78 (±0.19)
12:0	3.51 (±0.46)	—	—	43.28 (±1.06)
14:0	11.43 (±0.14)	2.95 (±0.06)	—	17.31 (±0.49)
16:0	29.58 (±0.81)	31.39 (±0.42)	10.30 (±0.41)	10.05 (±0.17)
16:1	3.02 (±0.16)	9.23 (±0.03)	0.06 (±0.01)	—
18:0	10.70 (±0.08)	4.96 (±0.12)	3.39 (±0.23)	3.18 (±0.34)
18:1	23.27 (±1.08)	36.12 (±0.53)	24.24 (±0.51)	6.18 (±0.31)
18:2	2.60 (±0.35)	10.60 (±0.08)	53.79 (±0.36)	2.11 (±0.27)
18:3	1.43 (±0.62)	0.98 (±0.24)	6.90 (±0.32)	—

[a]Isopropyl esterification.

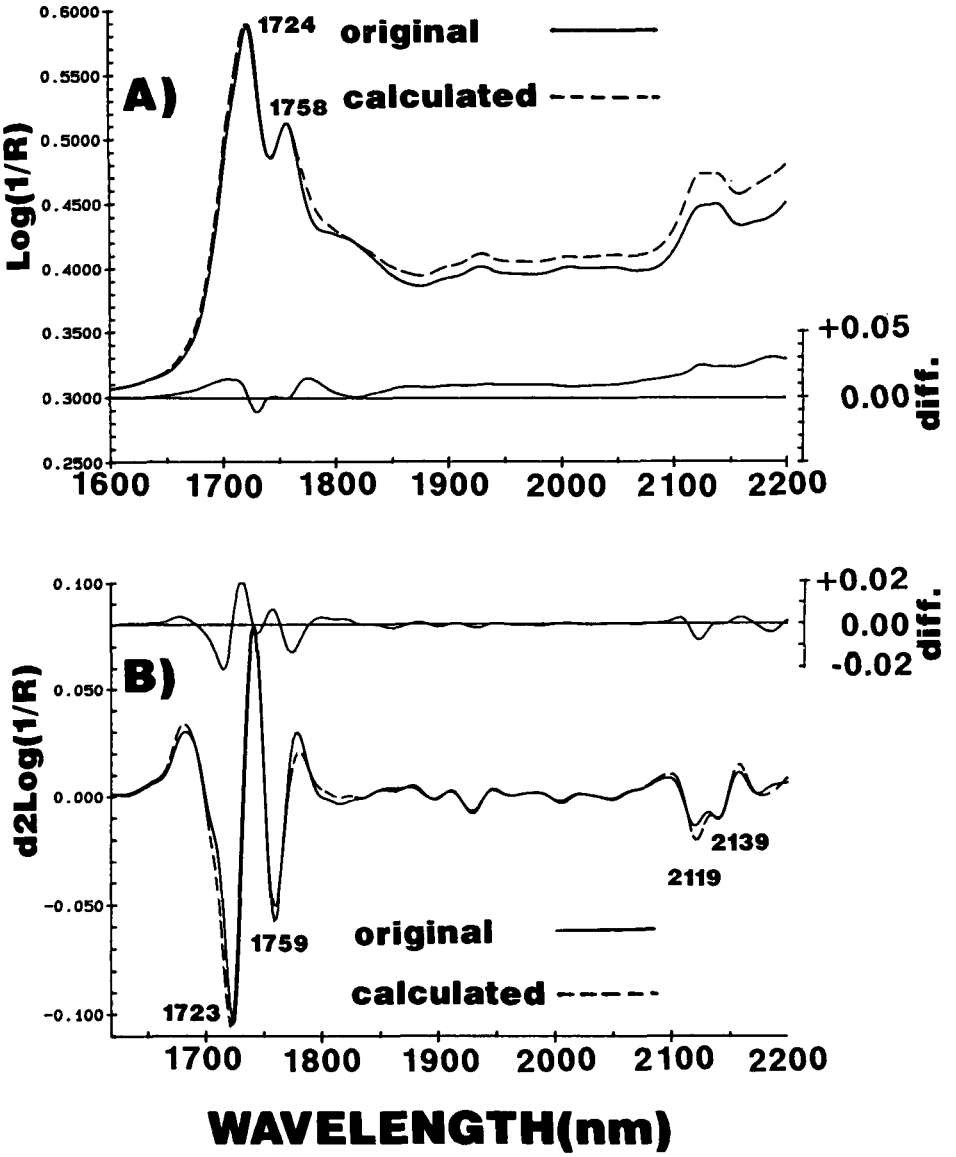

FIG. 3. Near infrared spectra of butter fat. A, raw spectra; B, second-derivative spectra, original (solid line) and calculated (broken line). Diff., difference between calculated and original spectra.

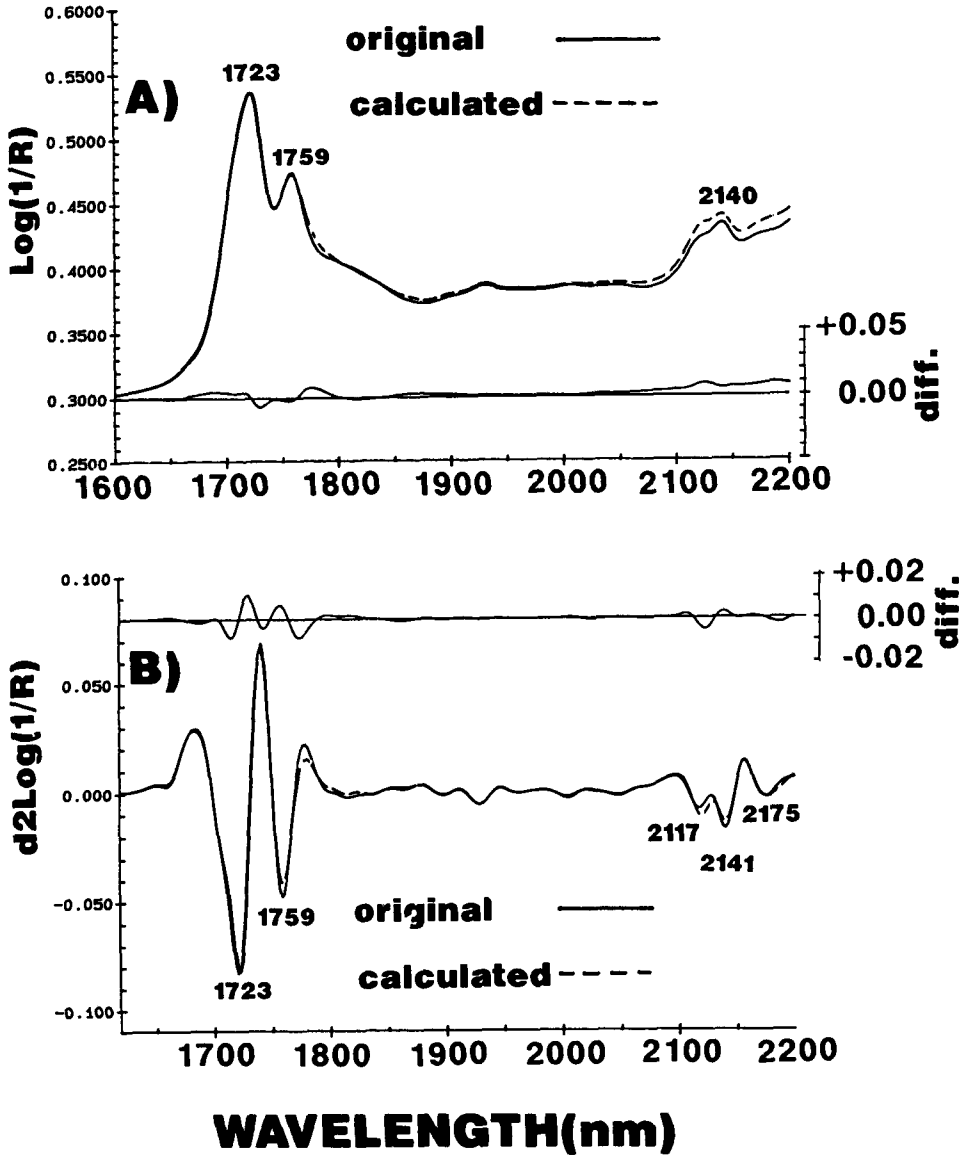

FIG. 4. Near infrared spectra of pig milk fat. Legends as in Figure 3.

ter fat (Fig. 3), peaks were observed at 1724 and 2140 nm in the raw spectrum and troughs at 1723 and 2139 nm in the second-derivative spectrum, and they are similar to those of saturated fatty acid moieties and C18:1. Butter fat was estimated to have mainly oleic acid and saturated fatty acid moieties. Pig milk fat and palm oil have the extrema at the same wavelengths as butter fat. However, the 2140 peak of pig milk fat (Fig. 4) is a little sharper than that of butter fat, which indicates that the former seems to have more unsaturated fatty acid moieties than the latter. On the other hand, in palm oil spectra (Fig. 6) there was a weak peak at 2140 nm, and this indicates that it has little unsaturated fatty acid. These findings were confirmed by the data of Table 1.

The actual and calculated spectra show the same trends (Figs. 3-6), *i.e.*, both spectra had the extrema at the same wavelengths. The agreements were excellent, especially for soybean oil. The NIR spectra could be reconstructed satisfactory.

However, strictly examining the agreement between original and calculated spectra, the following items should be pointed out. For fats and oils that contain significant ratios of saturated fatty acids, slight differences were observed at the ranges around 1700 and 2120 nm, *i.e.*, the amounts of saturated fatty acids were overestimated a little. In this study, the loading weight of each triglyceride spectrum was equal. Therefore, it might be necessary to reevaluate each triglyceride loading weight. In addition,

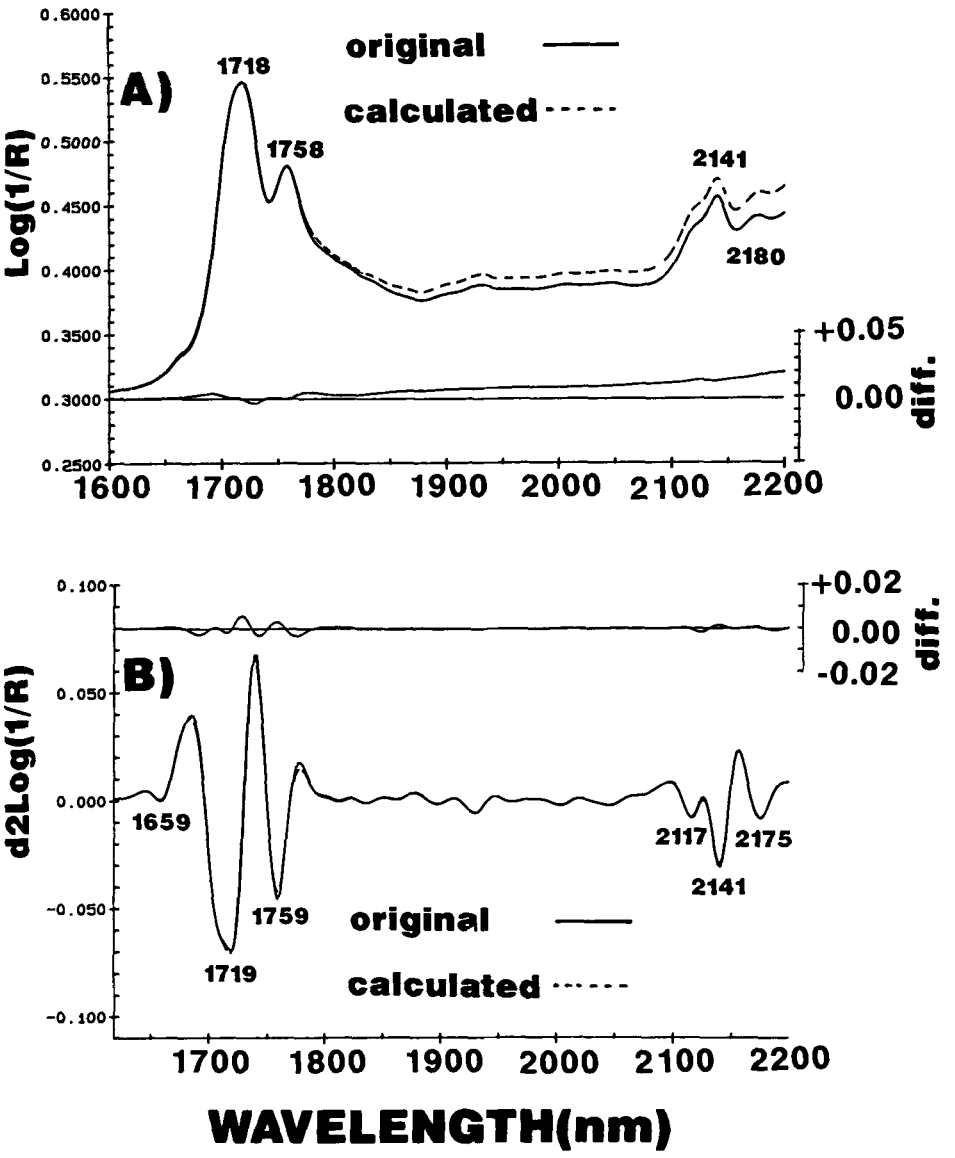

FIG. 5. Near infrared spectra of soybean oil. Legends as in Figure 3.

the calculated NIR spectral pattern around 1800 nm did not agree with the original one, *i.e.*, the former did not project as much as the latter. Because the authors used *trans*-C18:2 instead of C18:0 for estimated NIR spectra, this is another differing region between saturated and unsaturated fatty acid moieties, as shown in Figure 1. It is necessary to use these regions to extract information about saturated moieties. To reconstruct NIR spectra patterns more precisely, the robust NIR spectra should be obtained in advance, *e.g.*, by standardized sample presentation. For example, a specially designed cell that allows constant sample temperature and thickness should be installed. To eliminate experimental fluctuations, it may be necessary to subject NIR spectra to some mathematical treatment, such as normalization.

In this study, the determination of fatty acid composition of fats and oils by NIR was not examined by multiple linear regression analysis, but from a qualitative point of view. For NIR analysis, we only had to extract the fat, *i.e.*, there was no need for further chemical reaction. This means that it is not necessary to worry about loss of volatile compounds produced by chemical reactions. The NIR method makes it possible to analyze fatty acid composition simply, rapidly and non-destructively.

An NIR spectroscopic method may become an entirely different analytical technique by collecting NIR spectra of various fats and oils as a library, measuring the NIR spectrum of a sample of oil or fat, and by using the library to generate the spectrum that best agrees with the sample.

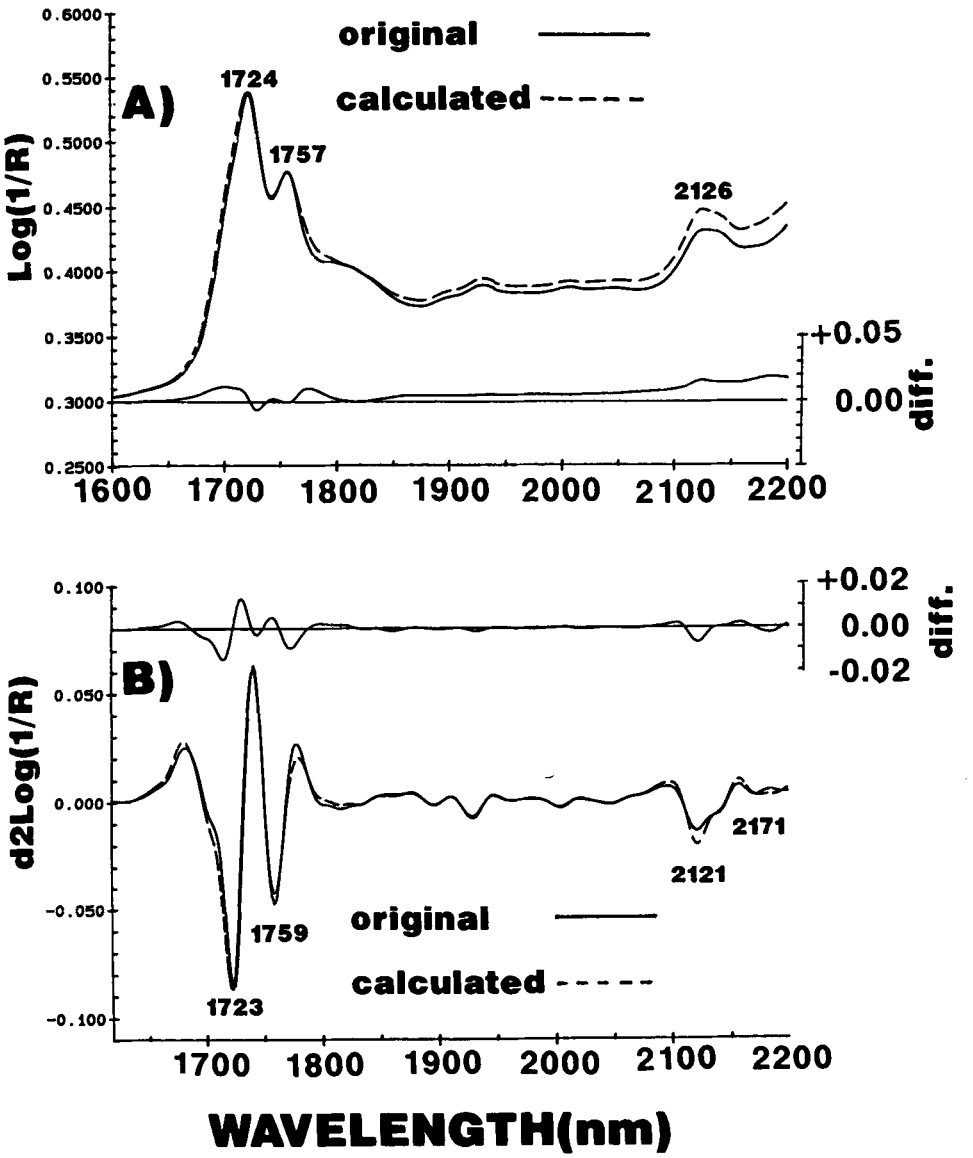

FIG. 6. Near infrared spectra of palm oil. Legends as in Figure 3.

ACKNOWLEDGMENT

We thank the staff of the Swine Management Section, Department of Animal Breeding and Genetics, National Institute of Animal Industry (Tsukuba), for producing pig milk. We also thank Dr. Takashi Ide of our Institute for Gas Chromatographic Data.

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[Received July 8, 1991; accepted August 11, 1991]