A Rapid and Simple Approach to Identify Different Sunflower Oil Types by Means of Near-Infrared Reflectance Spectroscopy

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ABSTRACT: The potential of near-infrared reflectance spectroscopy (NIRS) to perform an easy and rapid classification of different sunflower oil types was investigated. A total of 118 oil samples showing large variation in their fatty acid compositions were analyzed by both NIRS and gas–liquid chromatography (GLC). They were classified into five classes, characterized by (i) high palmitic acid content (>29%), (ii) high palmitic acid in high oleic acid background (>27 and >51%, respectively), (iii) high stearic acid content (>22%), (iv) high oleic acid content (>83%), and (v) standard oil type. Second-derivative transformation and scatter corrections were applied to the original log (1/*R*) spectra, and the correlation coefficients between NIRS spectral information and GLC fatty acid values were studied to identify the wavelengths with the best discriminating ability. The use of the spectral data at 2134 nm permitted all the samples with high levels of total saturated fatty acids (>29%, classes i, ii, and iii) to be discriminated from the samples with standard levels (<22%, classes iv and v). The use of a second wavelength, 2192 nm, led to a further separation of the samples with high $C_{18:1}$ content within each group (classes ii and iv, respectively). Therefore, an accurate discrimination of four of the five sunflower oil types was achieved by using the spectral information at two wavelengths exclusively. The oil samples belonging to classes i and iii could not be separated with this approach, which was explained on the basis of the small spectral differences observed between the two classes.
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KEY WORDS: NIRS, near-infrared reflectance spectroscopy, oleic acid, palmitic acid, spectral characterization, stearic acid, sunflower oil.

Considerable advances in the modification of seed oil quality have been made in recent years by using conventional breeding methods. Moreover, the availability of modern biotechnological approaches has speeded up the development of new oil types (1) and major advances in this field are expected in the next years (2). The increase in variability will demand fast, cost-effective, and easy-to-handle methods to discriminate between different oil types.

Oils are commonly classified after separating and identifying fatty acids or triglycerides by chromatographic methods. These methods are very accurate but also costly and time consuming, and they require the use of toxic, flammable pollutant reagents and gases. Other classifying methods such as the iodine value test are tedious and less accurate than chromatographic procedures (3).

Near-infrared reflectance spectroscopy (NIRS) is a powerful analytical technique for determining a wide range of constituents in agricultural and food products. NIRS analyses are very fast, cost-effective, and provide a safe working environment. The technique was initially developed for quantitative determinations, which requires the development of calibration equations to correlate NIRS spectral information with chemical information provided by a primary analytical method (4). Nevertheless, quantification is not always needed and simpler qualitative approaches are frequently enough for many applications, avoiding the complex calibration process (5).

Recent studies have demonstrated the potential of qualitative analysis based on NIRS spectra to discriminate between oils from different vegetable sources (3,6,7) or to identify adulterants in oils (8). Most of these studies were based on the application of multivariate statistical methods on the complete spectral information (6–8). Multivariate methods are rather complex and require extensive computing facilities. However, Bewig *et al.* (3) followed a simpler approach based on the use of a few discrete wavelengths, obtaining an acceptable discrimination of oils from four vegetable sources (canola, soybean, peanut, and cottonseed oils). A further step in the characterization of the NIRS technique to discriminate between different fatty acid profiles is to study its potential to perform a qualitative discrimination between different oil types from the same vegetable source.

A recent study (9) showed that the fatty acid composition of different sunflower oil types (e.g. oils with high palmitic, high stearic, or high oleic acid contents) can be accurately determined by NIRS, provided that calibration equations are previously developed. The objective of this study was to develop an easier qualitative way to discriminate between different sunflower oil types by means of simple spectral characterization.

MATERIALS AND METHODS

Samples. A total of 118 samples of sunflower oil with a large range of variation in their fatty acid compositions were used.

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TABLE 1
Classification of 118 Samples of Sunflower Oil into Five Classes According to Their Fatty Acid Composition, Number of Samples Within each Class, and Fatty Acid Composition Indicated by the Average, Minimum, and Maximum Values

Class	*n*	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$
High $C_{16:0}$[a]	11	32.0	6.9	2.0	9.7	47.4
		29.3–35.8	6.0–8.5	1.7–2.2	9.1–10.4	40.6–50.1
High $C_{16:0}$/high $C_{18:1}$	11	30.6	8.0	1.9	55.6	3.6
		27.1–35.2	6.8–8.6	1.7–2.2	51.7–59.4	3.0–4.4
High $C_{18:0}$	18	6.9	0.0	24.8	16.7	51.3
		6.4–7.7		22.6–28.5	13.7–19.7	47.3–55.2
High $C_{18:1}$	18	4.6	0.0	3.9	88.2	2.9
		3.9–7.6		2.9–5.3	83.9–90.5	1.9–4.1
Standard	60	6.3	0.0	9.4	40.0	44.1
		4.9–9.6		3.9–15.1	20.3–54.9	34.1–64.4

[a]The samples of this class showed an average $C_{16:2}$ content of 1.8%. Fatty acids are expressed as percentage of the total fatty acids.

They were chosen from a set of oil samples used in a previous study on quantitative analysis of the fatty acid composition of sunflower by NIRS (9). The samples were classified into five classes according to their fatty acid profiles (Table 1).

Oil samples with high palmitic acid ($C_{16:0}$) and standard oleic acid ($C_{18:1}$) contents were obtained from plants of the mutant line CAS-5 (10). Samples with high $C_{16:0}$ content in a high $C_{18:1}$ background were obtained from the mutant line CAS-12 (11). Samples with elevated stearic acid ($C_{18:0}$) content were obtained from the mutant line CAS-3 (10). High $C_{18:1}$ samples were extracted from seeds of several high oleic acid lines (12). The class with standard fatty acid composition included samples with sums of saturated fatty acid under 22% and with less than 55% $C_{18:1}$. They were selected from different inbred lines developed at the Institute for Sustainable Agriculture of Córdoba, and also from the mutant lines CAS-4 and CAS-8, with a slight increase of $C_{18:0}$ content (10).

Oil extraction. The seeds were husked manually and ground with an IKA A10 grinder (Janke & Kunkel GmbH & Co. KG, Staufen, Germany). For oil extraction, 1 g meal was placed into a vial and 5 mL diethyl ether was added. The vial was shaken periodically for 5 h and then the solvent was evaporated.

Gas–liquid chromatography. The fatty acid composition of the oil was analyzed by methyl esterification (13) followed by GLC on a Perkin-Elmer Autosystem gas-liquid chromatograph (Perkin-Elmer Corporation, Norwalk, CT) equipped with a 2-m-long column packed with 3% SP-2310/2% SP-2300 on Chromosorb WAW (Supelco Inc., Bellefonte, PA). The oven, injector, and flame-ionization detector were held at 195, 275, and 250°C, respectively. The carrier gas was nitrogen at a flow of 20 mL min^{-1}. The analysis time was 12 min.

NIRS analyses. Glass fiber disks (Millipore, ref. AP4004705) were impregnated with 2–3 drops of oil, placed into an standard NIRS cup, and scanned on a monochromator NIR Systems model 6500 (NIR Systems, Inc., Silver Springs, MD). The reflectance spectra (log 1/*R*) from 400 to 2500 nm were recorded at 2 nm intervals.

Management of NIRS spectral information. Different procedures to characterize spectral differences associated with the fatty acid composition of the oil, e.g., calculations of average spectra, difference spectra, and standard deviations among spectra, were carried out with the software package ISI version 3.10 (Infrasoft International, Port Matilda, PA). In all cases, and according to previously reported results (3,9,14), second-derivative transformation, De-trend, and standard normal variate (SNV) scatter correction (15) were previously applied to the log (1/*R*) spectra.

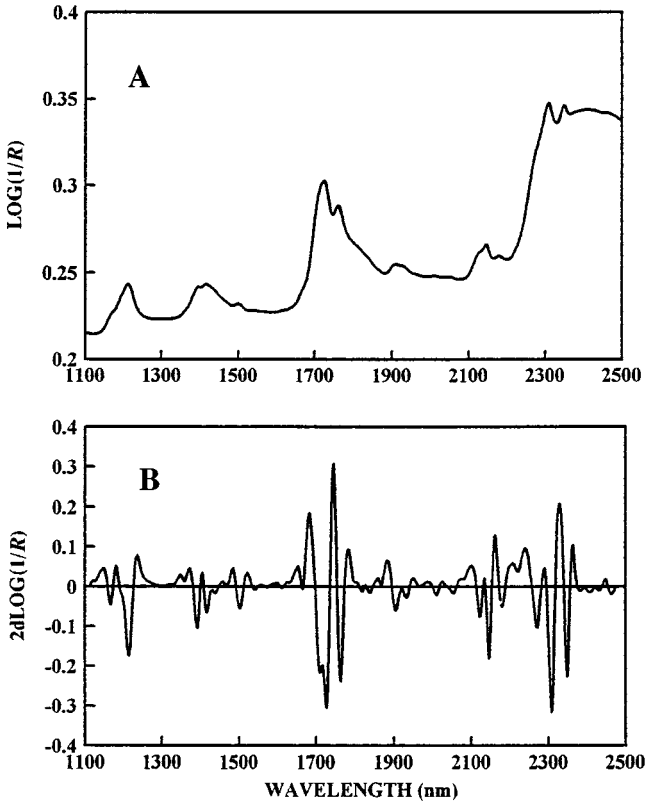

FIG. 1. Average spectrum of the 118 oil samples analyzed by NIRS: (A) original log (1/*R*) spectrum; (B) after second-derivative transformation and scatter corrections.

RESULTS AND DISCUSSION

Figure 1 shows the average spectrum (log 1/*R*) of the 118 samples included in the study and the corresponding transformed spectrum after second derivative and scatter corrections. Characteristic oil absorption bands (9,16) can be observed in the regions from 1150 to 1250 nm, 1350 to 1450 nm, 1650 to 1800 nm, 2100 to 2220 nm, and 2250 to 2380 nm. Absorption peaks were sharper in the second derivative-transformed spectrum than in the original log (1/*R*).

The objective of this study was to identify a few discriminating wavelengths which could provide a rapid classification of the different oil classes. Some algorithms based on the determination of Mahalanobis distances between all pairs of spectral data clusters have been proposed to assist in wavelength identification (5), and this was the approach followed by Bewig *et al.* (3) in the discrimination of oils from different vegetable sources. However, we decided to follow a simpler method based on the determination of the correlation coefficients between the transformed spectral value at each wavelength and the fatty acid values determined by GLC (Fig. 2). For all the fatty acids the highest (positive) correlation coefficient corresponded to a wavelength in the 2100 to 2200 nm region. They were 2188 nm for $C_{16:0}$ ($r = 0.72$), 2124 nm for $C_{18:0}$ ($r = 0.62$), 2138 nm for $C_{18:1}$ ($r = 0.99$), and 2194 nm for $C_{18:2}$ ($r = 0.98$). Most of the lowest (negative) correlation coefficients also corresponded to this region, and they were 2134 nm for $C_{16:0}$ ($r = -0.77$), 1798 nm for $C_{18:0}$ ($r = -0.59$), 2192 nm for $C_{18:1}$ ($r = -0.99$), and 2140 nm for $C_{18:2}$ ($r = -0.99$). These wavelengths were tested for their ability to discriminate between different fatty acids levels.

It became clear that some wavelengths produced a good discrimination between samples with high concentration of total saturated fatty acids ($C_{16:0} + C_{18:0} > 29\%$) and samples with standard concentration (<22%). The first group included samples with high $C_{16:0}$ content in both standard and high $C_{18:1}$ backgrounds, and samples with high $C_{18:0}$ content. The best discrimination was found at 2134 nm. The spectral information at this wavelength also showed the lowest (negative) correlation coefficient with the sum of saturated fatty acids (data not shown) and, in consequence, the highest (positive) correlation coefficient with the sum of unsaturated fatty acids. Figure 3 shows the latter relationship. Other wavelengths also produced a good discrimination between classes differing in the levels of saturated fatty acids, most of them located in the region between 2100 and 2200 nm. Some studies have demonstrated that the main spectral changes associated with the increase in the degree of unsaturation are located in this region, because of the presence of absorption bands caused by *cis* double bonds (17–19).

After this first separation of two groups based on the level of total unsaturation, it was found that a large number of

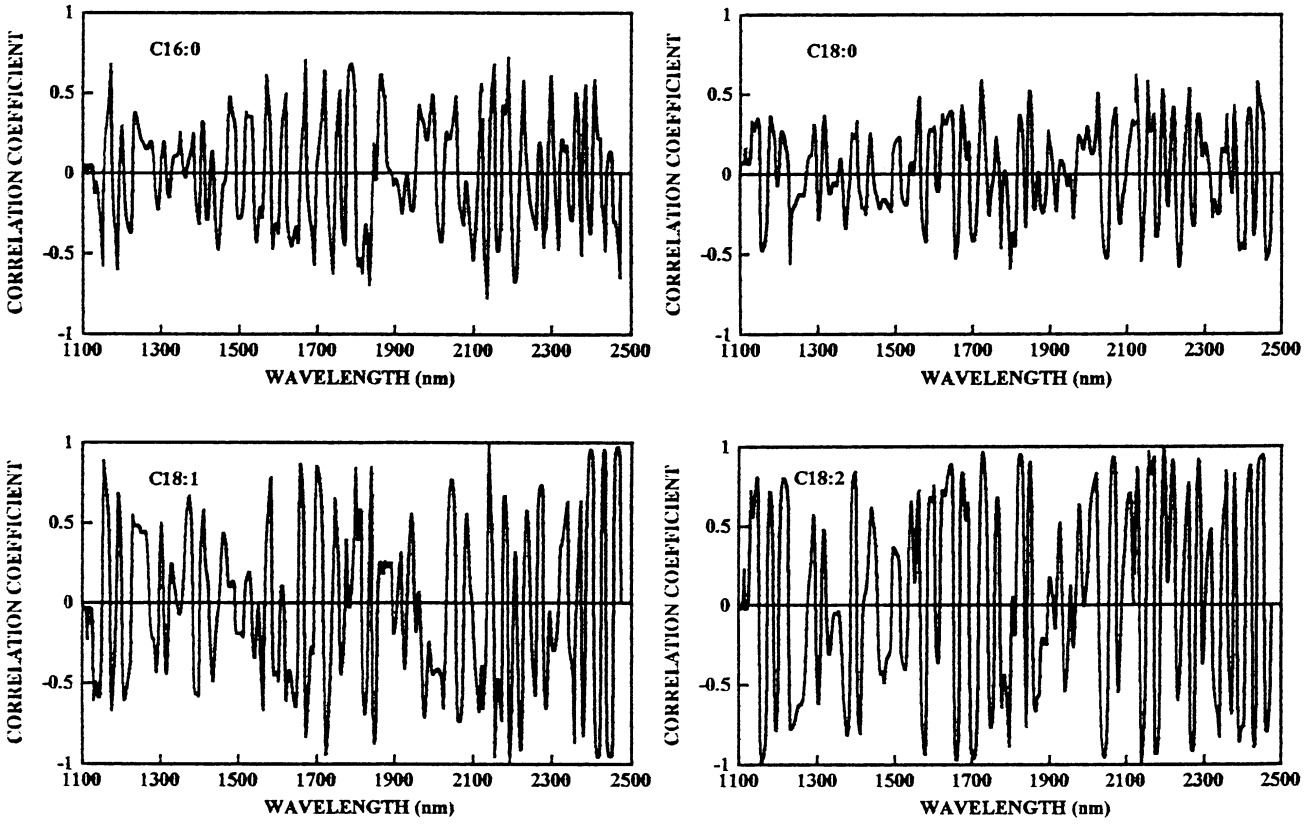

FIG. 2. Correlation coefficients between the corrected spectral data (second derivative + scatter corrections) and fatty acids values in 118 oil samples of sunflower.

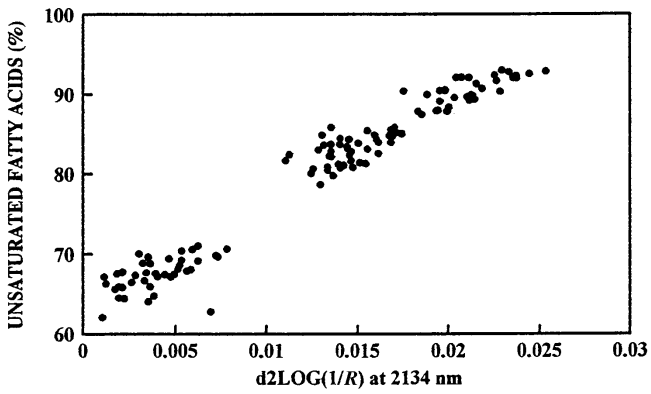

FIG. 3. Relationship between corrected spectral data (second derivative + scatter corrections) at 2134 nm and sum of unsaturated fatty acids ($C_{16:1} + C_{16:2} + C_{18:1} + C_{18:2}$).

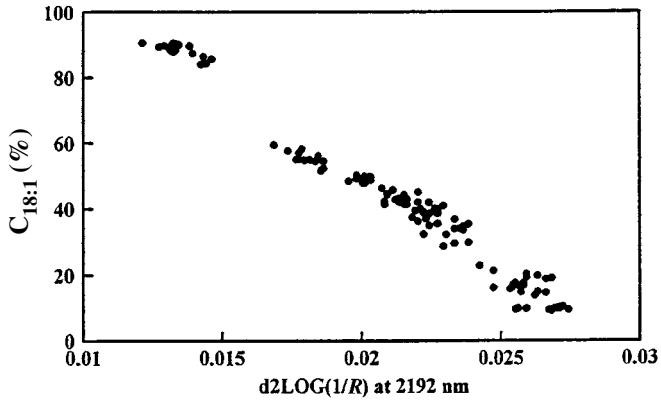

FIG. 4. Relationship between corrected spectral data (second derivative + scatter corrections) at 2192 nm and $C_{18:1}$ content.

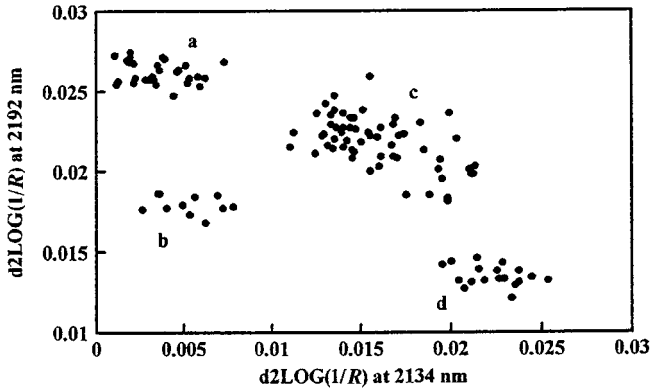

FIG. 5. Corrected spectral data (second derivative + scatter corrections) at 2134 vs. 2192 nm: a, samples with high levels of saturated fatty acids (>29%) in low oleic acid background (<22%); b, samples with high $C_{16:0}$ (>27%) in high oleic acid background (>51%); c, samples with standard fatty acid composition; d, samples with high $C_{18:1}$ content (>83%).

wavelengths permitted the classification of the samples according to their $C_{18:1}$ content. The best discrimination was found at 2192 nm (Fig. 4). This wavelength showed the lowest (negative) correlation coefficient with $C_{18:1}$ content. The use of this wavelength together with the aforementioned 2134 nm permitted a clear classification of the oil samples within four groups (Fig. 5). The samples with high levels of saturated fatty acids (>29%) were separated in two groups, corresponding to the samples with standard $C_{18:1}$ levels (group *a* in Fig. 5) and to the samples with high $C_{18:1}$ background (>51%, group *b*). The samples with standard levels of saturated fatty acids were also classified in two groups, characterized by standard $C_{18:1}$ content (<55%, group *c*) and high $C_{18:1}$ content (>83%, group *d*), respectively. The groups *b*, *c*, and *d* in Figure 5 corresponded to three different oil types defined in Table 1. However, group *a* included two different oil types, which could not be separated by using these two wavelengths. Both of them contained similar high levels of saturated fatty acids in a low $C_{18:1}$ background, but they differed for the principal saturated fatty acid, $C_{16:0}$ in one oil type (>29%) and $C_{18:0}$ in the other (>22%). None of the remaining wavelengths initially detected in the analysis of correlation coefficients between spectral data points and fatty acid values permitted a clear discrimination between the two classes.

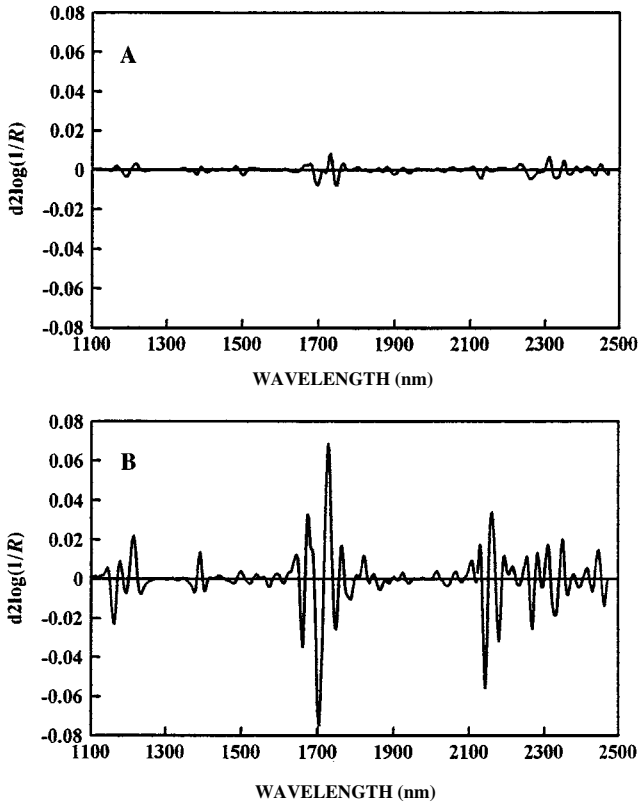

FIG. 6. Difference spectra between the following classes: (A) high $C_{16:0}$ in low $C_{18:1}$ background and high $C_{18:0}$; (B) high $C_{16:0}$ in low $C_{18:1}$ background and $C_{16:0}$ in high $C_{18:1}$ background. Difference spectra were calculated from the corrected (second derivative + scatter corrections) average spectra for each class.

A further attempt to separate the samples from both unresolved classes through a discrete wavelength approach was done by determining their spectral differences. To that end, the average spectra of both classes were calculated and the difference spectrum between the two average spectra was computed (Fig. 6A). This was compared with the difference spectrum between two clearly separated classes, high $C_{16:0}$ and high $C_{16:0}$ in a high $C_{18:1}$ background (Fig. 6b). The results revealed very small spectral differences between the samples characterized by high levels of saturated fatty acids in low $C_{18:1}$ background. The further use of some of the wavelengths for which the differences were higher permitted a partial separation of the classes, but no wavelength was found to produce a complete discrimination. It was therefore concluded that it is impossible to discriminate samples with high $C_{16:0}$ content in low $C_{18:1}$ backgrounds from samples with high $C_{18:0}$ content through a qualitative approach based on the use of two wavelengths. Such discrimination can be successfully achieved by using different whole spectrum approaches, e.g., principal component and discriminant analysis (L. Velasco, B. Perez-Vich, and J.M. Fernandez-Martinez, unpublished results), and multivariate calibration (9).

Figure 7 shows the most significant regions of the average spectra of the five oil classes included in this study. The samples of two classes were markedly different from the rest. They included the samples with high $C_{18:1}$ contents (>83%) and the samples with high $C_{16:0}$ contents (>27%) in high $C_{18:1}$ backgrounds (>51%), respectively. The common denominator of both classes was a very low $C_{18:2}$ content, with average values of under 4%, as compared with average values of over 40% in the other three classes (Table 1). These spectral differences, together with the lack of discrimination between the high $C_{16:0}$ (low $C_{18:1}$) and high $C_{18:0}$ classes, suggest that differences in the degree of unsaturation of sunflower oil are more easily detected by NIRS than differences in the length of the fatty acid chain. The reason for this may be the small variation for fatty acid chain length in sunflower oil, since previous studies have reported large spectral differences associated with the variation of the chainlength of fatty acids (17,18). Furthermore, the discrimination of $C_{22:1}$ content in mustard seeds has been associated with such differences (20). This may also be the explanation for the high weight of the spectral region between 2100 and 2200 nm in the initial correlation study and for the good discrimination of different sunflower oil types with wavelengths of this region, since the spectral range between 2100 and 2200 nm is closely associated with the degree of *cis* unsaturation (17–19).

A previous study (3) demonstrated that a classification of different vegetable oils (canola, soybean, peanut, and cottonseed) could be successfully achieved by a discrete wavelength approach, by using a four-wavelength discriminant equation,

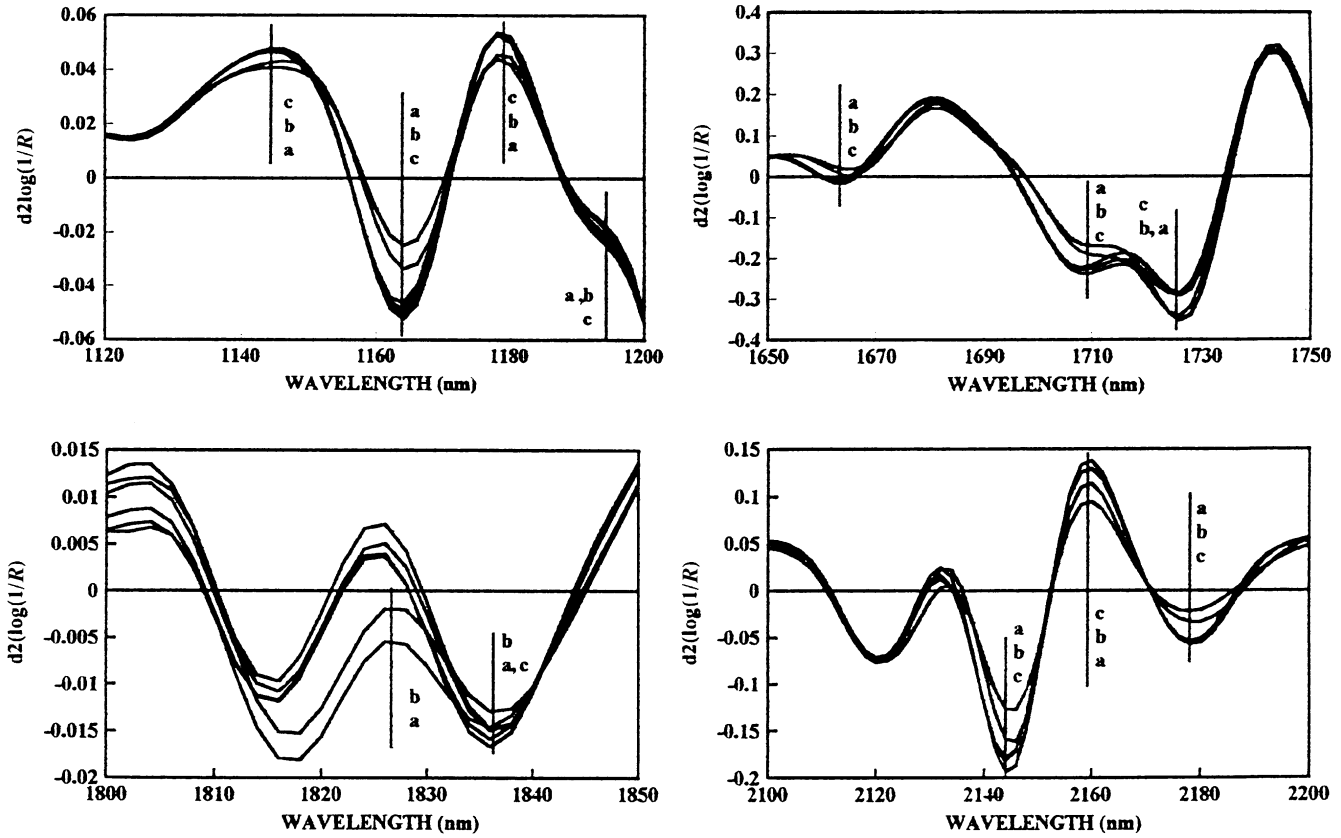

FIG. 7. Average spectra of the five classes defined in Table 1 within the ranges from 1120 to 1200 nm, 1650 to 1750 nm, 1800 to 1850 nm, and 2100 to 2200 nm: a, samples with high $C_{16:1}$ (>27%) in high oleic acid background (>51%); b, samples with high $C_{18:1}$ content (>83%); c, rest of the classes.

or even using only the information at two wavelengths, 1800 and 2110 nm. The results of this study demonstrate that such a discrete wavelength approach is also useful to discriminate different oil types from the same vegetable source. The use of only two wavelengths permitted an accurate classification of most of the oil types currently available in sunflower, and the discrimination only failed to separate high $C_{16:0}$ and high $C_{18:0}$ samples with similar composition in unsaturated fatty acids. This approach permits an easy and rapid characterization of sunflower oil samples analyzed by NIRS without developing calibration equations or performing complex multivariate analyses. These results suggest that simpler and cheaper discrete filter instruments might be of great utility for the rapid characterization of the fatty acid composition of oils.

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