Characterization of Edible Oils, Butters and Margarines by Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance

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The combination of attenuated total reflectance (ATR) and mid-infrared spectroscopy (MIRS) with statistical multidimensional techniques made it possible to extract relevant information from MIR spectra of lipid-rich food products. Wavenumber assignments for typical functional groups in fatty acids were made for standard fatty acids: Absorption bands around 1745 cm^{-1}, 2853 cm^{-1}, 2954 cm^{-1}, 3005 cm^{-1}, 966 cm^{-1}, 3450 cm^{-1} and 1640 cm^{-1} are due to absorption of the carbonyl group, C-H stretch, =CH double bonds of lipids and O-H of lipids, respectively. In lipid-rich food products, some bands are modified. Water strongly absorbs in the region of 3600–3000 cm^{-1} and at 1650 cm^{-1} in butters and margarines, allowing one to rapidly differentiate the foods as function of their water content. Principal component analysis was used to emphasize the differences between spectra and to rapidly classify 27 commercial samples of oils, butters and margarines. As the MIR spectra contain information about carbonyl groups and double bonds, the foods were classified with ATR-MIR, in agreement with their degree of esterification and their degree of unsaturation as determined from gas–liquid chromatography analysis. However, it was difficult to differentiate the studied food products in terms of their average chainlength.

KEY WORDS: Attenuated total reflectance, butter, chainlength, fatty acid composition, margarine, mid-infrared spectroscopy, oil, principal component analysis, unsaturation, water.

Many spectroscopic techniques, such as nuclear magnetic resonance (NMR), pulsed NMR (1), near-infrared spectroscopy and mid-infrared spectroscopy (MIRS) (2), have been used to study lipids. MIRS is a fast, reliable and accurate method for their characterization. Its first application has been the determination of fat and moisture in dairy products. The fat content is usually estimated from the strong absorption bands of the carbonyl group at 1745 cm^{-1} (νC=O), and of the CH_2 group at 2870 cm^{-1} (νC-H) (3,4). MIRS is used for the quantitation of fatty acid's unsaturation. The main absorption band for measuring *trans* unsaturation is 966 cm^{-1} (δC-H) (5,6), whereas *cis* unsaturation can be observed at 3008 cm^{-1} (νC-H) or at 1650 cm^{-1} (7). Several other applications of MIRS, such as the determination of fat oxidation, fat crystallinity or the identification of hydroxyl groups, have been reported (8,9).

Rough assignments of the infrared (IR) bands of purified lipids in nonabsorbent solvents are given in tables (see Ref. 10). These assignments were determined only on pure fatty acids, and no precise assignments have been given for natural lipid-rich products, such as edible oils, margarines or butters. Moreover, many MIR spectral studies have been conducted in transmission mode. In transmission mode, the sample has to be dissolved in a solvent before the spectrum can be recorded. Such procedures are time-consuming, and these methods are difficult to apply for routine analysis. The attenuated total reflectance (ATR) technique, the basic theory of which has been described in the literature (11,12), enables the acquisition of MIR spectra with no need for further sample preparation (7). Fourier transform infrared (FTIR) and ATR have only been applied for determination of fat and moisture contents in butter by Van de Voort *et al.* (13).

The purpose of the present study was to precisely assign the absorption bands of MIR spectra of edible lipids, recorded in ATR mode. The spectra of lipid-rich foods are compared with those of pure fatty acids and esters and related to the fatty acid composition as determined by gas chromatography. As the differences between spectra are often minor, they were emphasized by means of principal component analysis (14,15).

EXPERIMENTAL PROCEDURES

Sample collection. The sample collection included several chemical standards, edible oils, butters and margarines. The nine lipid standards (Table 1) were purchased from Sigma Chemical Co. (St. Louis, MO). Twenty-seven fat-rich commercial products were obtained from local supermarkets to span a wide range of composition. The sample collection included 14 different edible oils (olive, OI: two samples; corn, MA: two; sunflower, SU: one; peanut, PE: two; walnut, WA: one; grapeseed, GR: three; rapeseed, RA: two; "dietetic" oil, DI: one). The dietetic oil was composed of 50% corn and 50% sunflower oil. Nine butters and four margarines were also studied (unsalted butter with 15% water, UN: one sample; salted butter with 15% water, SA: five; low-fat butter, 48% water, LF: three; margarine, 19% water, MR: four samples).

Analysis of fatty acid composition with gas-liquid chromatography (GLC). The lipid fraction of butters and margarines was first extracted with chloroform/methanol (2:1) by the method of Folch *et al.* (16).

The fatty acid composition of oils and margarines was determined by GLC of the methyl esters. These esters were prepared by direct transmethylation of lipids by boron trifluoride in methanol, as described by Morrison and Smith (17). The separation of the fatty acid methyl esters (FAME) was achieved according to Leseigneur-Meynier and Gandemer (18).

The short-chain fatty acids of butter were too volatile to be properly analyzed as methyl esters and thus were determined by GLC of their isopropyl esters. Fatty acid isopropyl esters (FAIPE) were prepared with isopropanol/hexane/H_2SO_4 (36 N) (2:1.1:0.2, vol/vol/vol) at 100°C during 1 h [Wolff and Castera-Rossignol (19)]. FAIPE were separated on a Hewlett-Packard (Boeblingen, Germany) Model 5890 Series II gas chromatograph, fitted with a capillary column (60 m × 0.32 mm i.d.) coated with 0.25 μm DB 225 (J&W Scientific, Folsom, CA), in the splitless injection mode. After remaining 3 min at 40°C, the oven

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TABLE 1

Description of Lipid Standards

Compound name	Shorthand description	Sigma reference[a]	Sample label
Caprylic acid (99% purity)	8:0	C2875	CA
Oleic acid (99% purity)	18:1*c*	O3879	OL
Linoleic acid (99% purity)	18:2*c*	L1376	LL
Linolelaidic acid (99% purity)	18:2*t*	L2126	LI
Linolenic acid (98% purity)	18:3*c*	L2376	LN
Palmitoleic acid (99% purity)	16:1*c*	P9417	PA
Linoleic acid ethyl ester (99% purity)	20:2*c*	L1751	LE
Lauric acid methyl ester (99.5% purity)	13:0	L4875	LM
Heptanoic acid methyl ester (99% purity)	8:0	H6754	HM
Capric acid propyl ester (99% purity)	13:0	C8259	CP

[a]Sigma Chemical Co., St. Louis, MO.

temperature was raised to 190°C at the rate of 10°C/min, remained steady for 10 min and was raised again by 10°C/min to 210°C for 5 min. The head pressure of the hydrogen carrier gas was 1.5 bar, and injector and detector temperatures were set at 250°C.

FAME and FAIPE, detected with flame-ionization detectors, were identified by comparison of their retention times with those of standard fatty acid mixtures. The proportion of each fatty acid was calculated from the integrated area of each peak and expressed as a percentage of the area of all peaks. According to Wolff and Fabien (20), The FAME and the FAIPE have response factors close to 1 in the length range of the studied fatty acids (from C:16 to C:20 for the FAME and from C:4 to C:20 for FAIPE). It was therefore not necessary to apply any conversion factors when estimating the proportion of fatty acids from the peak areas.

The iodine index (g I_2/100 g lipid) was calculated from the fatty acid composition according to Wolff (21). The average chainlength (AVL) was calculated from molar fatty acid composition.

MIR ATR spectra. The MIR spectra were obtained with a Fourier transform spectrometer IFS25 (Bruker, Wissemburg, France), equipped with a DTGS detector and a horizontal ATR accessory. The samples were placed in contact with the ATR element (ZnSe crystal, 45° ends) at room temperature according to Harrick (11). Each spectrum was collected at 2 cm^{-1} resolution from 4000 to 680 cm^{-1} and was the result of 200 scans. Each spectral acquisition required 200 s. A triangular apodization was used. Between measurements, the crystal was cleaned with ethanol and hexane. Three spectra were collected for each sample. The spectral collection therefore included 108 spectra (36 samples in triplicate).

Mathematical processing and statistical analysis. The baseline of the spectra was first corrected by using the instrument software (*BaseLine* command of the program "Spectrafile-IR"; Heyden & Son, Köln, Germany). After this correction, the average intensities still differed markedly from one spectrum to another. To reduce these differences, the data were modified in accordance with Downey *et al.* (22) by means of the following formulae (Equations 1 and 2):

$$c_i = l_i/\text{norm} \quad [1]$$

$$\text{norm} = \sqrt{\sum_{j=1}^{N} l_j^2} \quad [2]$$

where c_i is the corrected value of absorbance at wavenumber i; l_j is the baseline-corrected absorbance value at wavenumber j; and N is the number of data points for each spectrum.

Principal component analysis (PCA) is commonly used in near-infrared spectroscopy for emphasizing the differences between spectra and identifying the most important absorption bands (14,15). In this work, PCA was applied on the corrected spectra in the range of 4000–1560 cm^{-1}, at 4 cm^{-1} intervals. The variables were the 610 spectral data, and the observations were the samples. The spectra of lipid standards were selected as "principal" or "active" observations and were involved in the assessment of the PCA eigenvectors. The spectra of oils, butters and margarines were used as additional observations and were projected in the PCA space defined by the eigenvectors.

RESULTS AND DISCUSSION

Fatty acid composition. The fatty acid compositions of the samples varied greatly, according to their nature and their vegetable or animal origins (Table 2). The butters contained short- and medium-chain fatty acids (from 4 to 12 carbon), whereas the oils and margarines are mainly composed of 16- to 18-carbon fatty acids. The AVLs were about 17.8, 16.8 and 14.4 carbons for oils, margarines and butters, respectively. The oils had remarkably constant AVL. Among the butters, one low-fat sample (LF) presented high AVL values. According to its commercial label, this sample was made of a mixture of butter and sunflower oil. Similarly, the product commercially labelled "sunflower margarine" exhibited the highest AVL among the studied margarines.

The degrees of unsaturation were also different between oils and butters. The main difference appeared in the proportion of 18:2 fatty acids, which was generally higher in vegetable lipids. The assessed iodine index was also representative of the differences between oils and

TABLE 2

Fatty Acid Composition of Butters, Oils and Margarines Determined by Gas-Liquid Chromatography of the Fatty Acid Methyl Esters (oils and margarines) and of the Isopropyl Esters (butters)

Sample	Label	Fatty acid composition (%)			Iodine index (g I_2/100/g lipids)	Average chainlength
		Saturated	Monounsaturated	Polyunsaturated		
Butters						
unsalted	UN	76.5	21.7	1.8	23.6	13.5
salted	SA	75.7	22.5	1.8	21.9	14.3
salted	SA	75.0	24.6	0.5	20.1	13.6
salted	SA	74.0	25.3	0.8	21.0	14.1
salted	SA	74.6	25.1	0.3	20.3	14.1
salted	SA	75.0	24.7	0.3	13.8	14.8
low-fat sunflower	LF	42.4	28.8	28.8	77.7	16.2
low-fat	LF	84.5	15.1	0.4	13.0	13.9
low-fat	LF	80.7	16.3	3.0	18.1	13.3
Oils						
olive oil	OI	11.5	80.6	7.9	86.9	17.8
olive oil	OI	11.3	80.6	8.1	87.1	17.8
corn oil	MA	12.2	30.1	57.7	132.4	17.8
corn oil	MA	12.9	30.0	57.1	130.7	17.8
sunflower oil	SU	11.9	25.8	62.3	135.9	17.8
peanut oil	PE	8.6	59.1	32.3	119.2	17.9
peanut oil	PE	15.7	46.7	37.6	110.0	17.7
walnut oil	WA	10.6	17.3	72.1	156.9	17.8
grapeseed oil	GR	12.0	21.1	66.9	140.4	17.8
grapeseed oil	GR	12.7	21.8	65.5	138.5	17.8
grapeseed oil	GR	10.9	21.4	67.7	142.0	17.9
rapeseed oil	RA	16.4	45.9	37.7	109.8	17.7
rapeseed oil	RA	12.8	56.0	31.2	111.0	17.7
dietetic oil	DI	14.1	28.9	56.9	130.0	17.8
Margarines						
sunflower	MR	16.9	33.4	49.7	120.0	17.8
margarine	MR	55.4	34.5	10.1	48.7	16.1
margarine	MR	48.6	36.5	14.9	59.3	16.6
margarine	MR	52.2	37.4	10.4	52.3	16.9

margarines in comparison with butters. The range of iodine index values of oils was rather large, from 87 g I_2/100 g lipids for olive oil (OI) to 157 g I_2/100 g for walnut oil (WA). Peanut oil and rapeseed oil showed intermediary values, with iodine index around 110 g I_2/100 g. The iodine index of butters generally varied between 13 and 23 g I_2/100 g and that of margarines between 50 and 60 g I_2/100 g. The high values observed for one LF butter (78 g I_2/100 g) and one margarine (120 g I_2/100 g) corresponds to sunflower oil-enriched products.

Assignment of ATR-MIR spectral bands from lipid standards. The infrared ATR spectra of four fatty acids, differing by their unsaturation, are shown in Figure 1. Good-quality spectra that present a high signal-to-noise ratio were obtained within a short time. Several peaks are common to every fatty acid. The highest is at about 1708 cm^{-1}. It is characteristic of the carbonyl stretch of the carboxyl group of the free acid. In the region 3000 to 2800 cm^{-1}, the absorption of the C-H group can be observed. Two distinct bands are visible at about 2920 and 2845 cm^{-1}. The first band results from the asymmetrical stretching mode of the two C-H bands of the methylene group (νas CH_2). The second arises from the symmetrical stretching (νs CH_2), in which the two C-H bonds extend and contract in phase. The absorption bands at 2962 and 2872 cm^{-1} are attributed to asymmetrical (νas CH_3), and symmetrical stretching (νs CH_3), respectively. Fatty acid dimers display large, broad O-H stretching absorption bands in the region of 3500 to 2500 cm^{-1}.

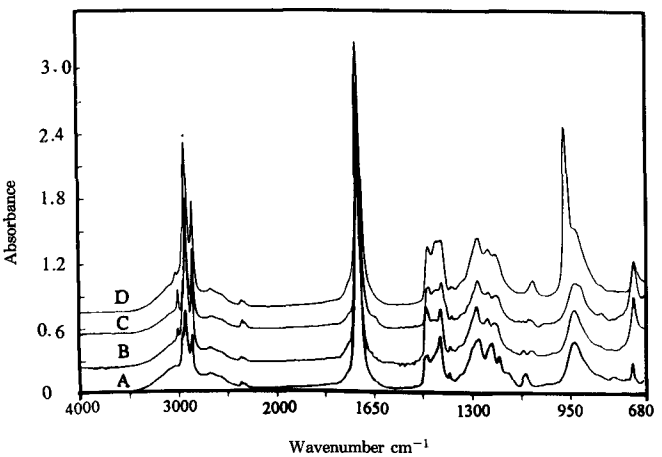

FIG. 1. Mid-infrared spectra: A, caprylic acid (C8:0); B, oleic acid (18:1*c*); C, linoleic acid (18:2*c*); D, linolelaidic acid (18:2*t*).

In all spectra, absorption bands associated with CH_2 and CH_3 deformations occur between 1500 to 1400 cm^{-1}. These bands are ambiguously assigned, except for the band at 1460 cm^{-1}, which corresponds to δs CH_2 groups. At the lower frequencies, the band near 720 cm^{-1} is assigned to the CH_2 group in rocking mode.

The regions from 1320 to 1210 cm^{-1} and 1420 to 1390 cm^{-1} are representative of C-O stretching and O-H

TABLE 3

Characteristic Attenuated Total Reflectance Infrared Absorption Band of Oil

Wavenumber[a]	Group	Type of vibration	Remarks
3450*m	O-H	str.	intermolecular bonded (water)
3005 w	C-H	sym. str.	-CH=CH-(*cis* olefin)
2953 m	C-H	asym. str.	aliphatic (-CH_3)
2922 s	C-H	asym. str.	aliphatic (-CH_2)
2853 s	C-H	sym. str.	aliphatic (-CH_2)
1743 s	C=O	str.	ν(C=O) ester
1640*m	O-H	def.	δ(O-H) water
1462 m	C-H	scissoring	aliphatic (-CH_2)
1377 w	C-H	sym. def.	aliphatic (-CH_3)
1238 m	C-H	out-of-plane bend	aliphatic (-CH_2)
1162 s	C-O	str.	ν(C-O) ester
1025**vw	C-O-C	str.	ν(C-O-C) ester
966 vw	C-H	out-of-plane bend	*trans* (-CH=CH-)
722 m	C-H	rocking	aliphatic (-CH_2)

[a]Abbreviations: s, strong; m, medium; w, weak; vw, very weak; * water; ** ν(C-O-C) of unsalted butter and salted butter, asym., asymmetrical; def., deformation; str., stretching; sym., symmetrical.

bending, respectively. The O-H group also absorbs at about 936 cm^{-1} (O-H out-of-plane bending). These assignments are summarized in Table 3.

The characteristic 3005 and 3011 cm^{-1} bands of unsaturated fatty acids, visible in the spectra of oleic and linoleic acids result from the *cis*=CH stretch bands. The spectrum of linolelaidic acid shows the stretching of the CH of the *trans* double bonds at 3025 cm^{-1} and its deformation at 966 cm^{-1}. The intensities of the *trans* and *cis*=CH stretch bands are directly proportional to the number of these bonds.

Spectra of fat-rich food products. The spectra of olive oil, butters and margarine are shown in Figures 2-4, respectively. In comparison with free fatty acids, several differences can be observed. The esterification of fatty acids causes a shift of the strong absorption band of the C=O group from 1708 to 1744 cm^{-1}. The C-O ester band appears at 1160 cm^{-1}. It is clearly distinct from the C-O of the carboxyl groups of free fatty acids. On the whole spectrum, the C-H bands are not modified. The broad band of the O-H groups, found for pure free fatty acids in the region of 3000 is no longer present. In the same way, the fatty acid O-H bands at 1410 and 936 cm^{-1} become weak.

One characteristic of the spectra of the studied foods is due to the presence of water in butters and margarines (15-48% water). Water strongly absorbs in the region 3600-3000 cm^{-1} and at about 1650 cm^{-1}. These bands are the most intense in LF margarines and butters that contain 19 to 48% water (Fig. 3b). Differences due to the fatty acid composition appear to be weak in first analysis. The spectra of LF butters and margarines (Figs. 3a and 4) differ from the spectra of oils, unsalted butter and salted butter (Figs. 1 and 3b) by a weak band at 1025 cm^{-1}, which is assigned to the *trans* C-O-C group. The main assignments of spectra oils, butters and margarines are given on Table 3.

PCA. PCA was performed on the full set of 108 spectra to attribute the spectral variations. The four first principal components made up 99.9% of the total sum of

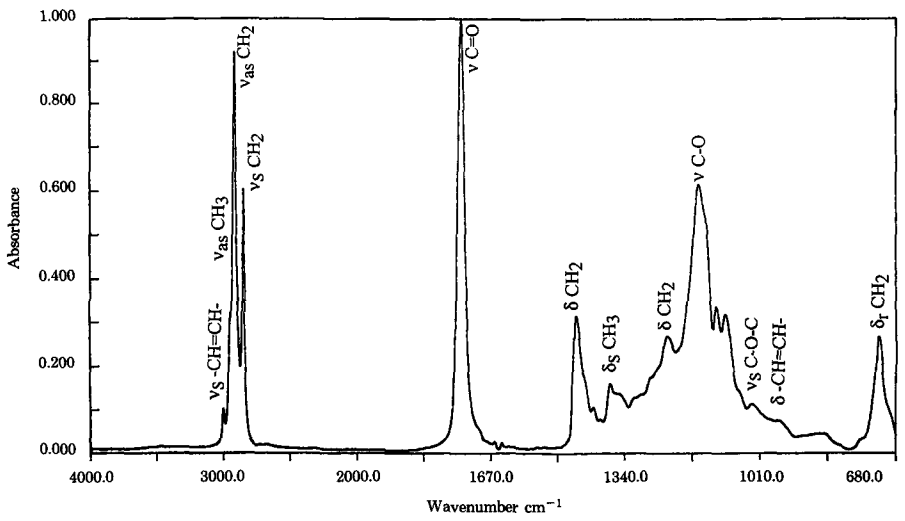

FIG. 2. Mid-infrared absorbance spectrum of olive oil from 4000 to 680 cm^{-1}.

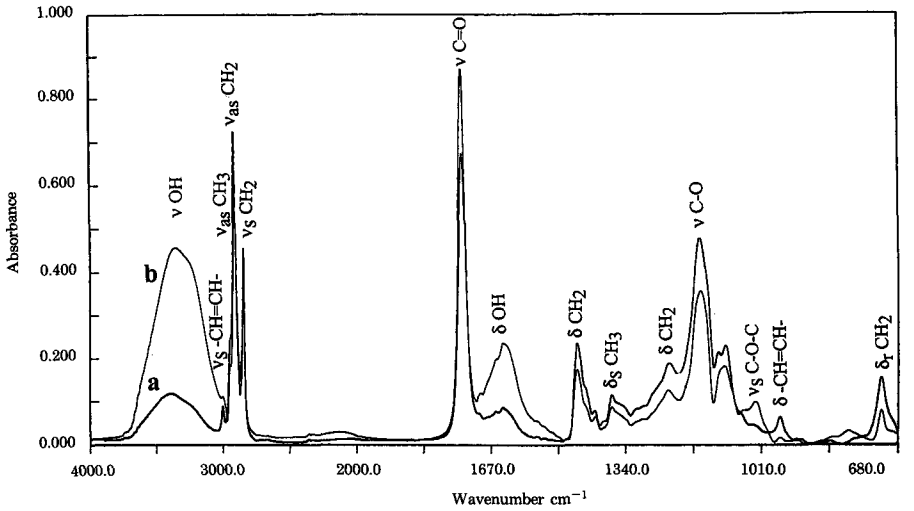

FIG. 3. Mid-infrared absorbance spectrum from 4000 to 680 cm^{-1} of (a) salted butter, (b) low-fat butter.

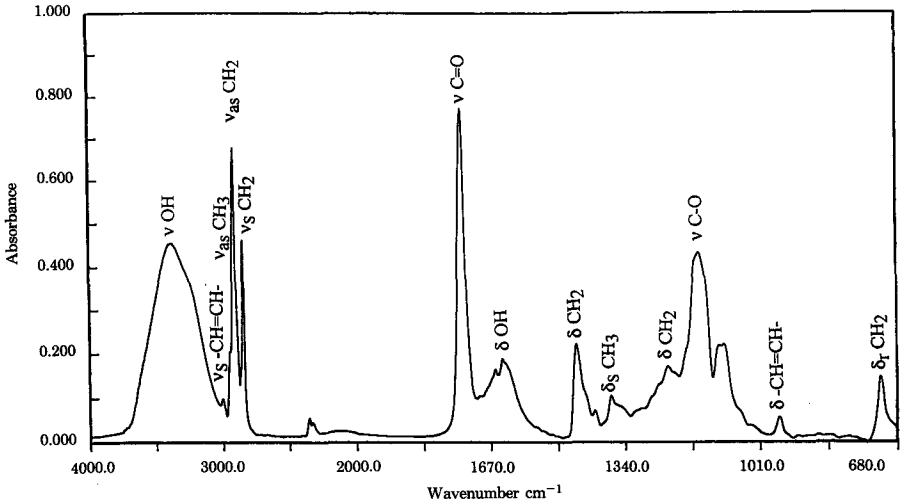

FIG. 4. Mid-infrared absorbance spectrum of margarine from 4000 to 680 cm^{-1}.

squares. The biplot of PC scores 1 and 2 (94.9% of the total sum of squares) is given in Figure 5.

On axis 1, three groups of spectra were clearly separated, from negative to positive values of the first scores: free fatty acids, fat-rich food products and esterified free fatty acids. The first score was therefore related to the concentration of ester function in the samples. The fat-rich food products and the fatty acid ester standards, which both showed a C=O absorption band at 1740 cm^{-1}, were separated on the PCA biplot according to the intensity of this band.

The axis 2 seems to be representative of CH_2 and C=O concentration. Fatty acid standards were arranged according to the number of CH_2 in their chain, in the following order: caprylic acid (6 CH_2), linolenic acid (10 CH_2), linoleic acid and palmitoleic acid (12 CH_2), oleic acid (14 CH_2). All the commercial oils, butters and margarines were regrouped in the 12 to 14 CH_2 area of the axis.

In the region around 1740 cm^{-1}, the first eigenvector (Fig. 6) showed a shape similar to the first derivative of the spectra. Such a shape is representative of the displacement of the C=O absorption band from 1740 to 1707 cm^{-1}. These bands were already visible by direct examination of the original spectra and correspond to the function ester and acid, respectively. The second eigenvector is shown on Figure 7. The bands characteristic of CH_2 grouping (2913 and 2853 cm^{-1}) were represented by negative peaks, whereas the bands of C=O (1755 and 1707 cm^{-1}) were associated with positive ones. This second eigenvector therefore emphasized the negative correlation between the concentrations of the CH_2 and C=O groups. This opposition was also visible on Figure 5, where CA (caprylic acid) and HM (heptanoic acid methyl ester) were

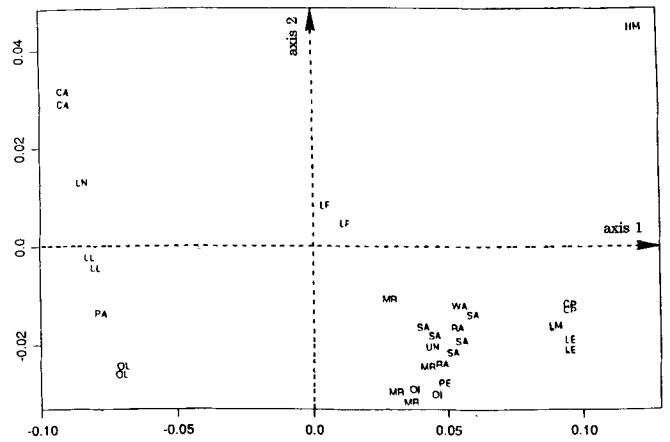

FIG. 5. Principal component analysis map of the spectral collection (plan 1-2). Axis 1 is related to the concentration of ester function in the samples. Axis 2 seems to be representative of CH_2 and C=O concentration. Abbreviations: HM, heptanoic acid methyl esters; CA, caprylic acid; LN, linolenic acid; LF, low-fat; LL, linoleic acid; PA, palmitoleic acid; OL, oleic acid; MR, margarine; WA, walnut oil; SA, salted butter; RA, rapeseed oil; UN, unsalted butter oil; OI, olive oil; PE, peanut oil; CP, capric acid propyl ester; LM, lauric acid methyl ester; LE, linoleic acid ethyl ester.

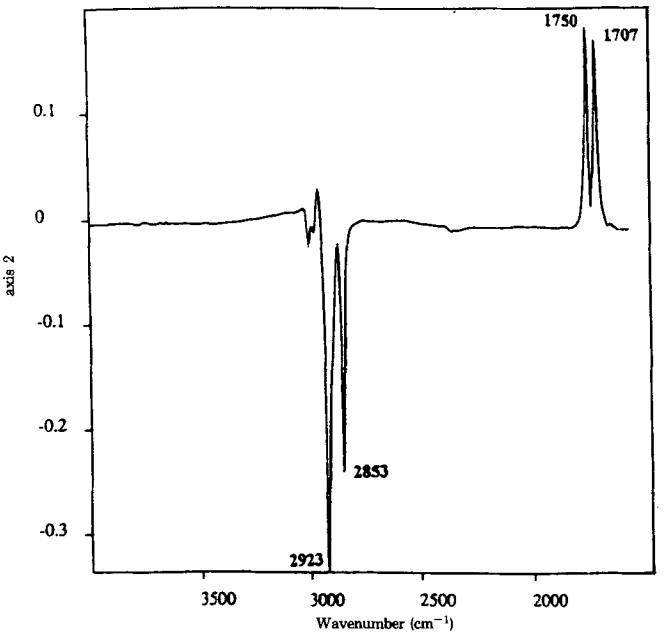

FIG. 7. Second eigenvector of principal component analysis.

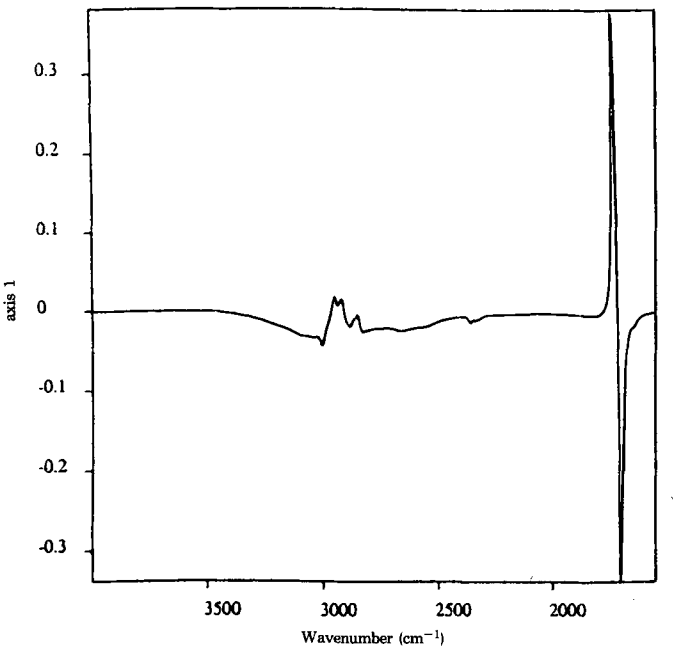

FIG. 6. First eigenvector of principal component analysis.

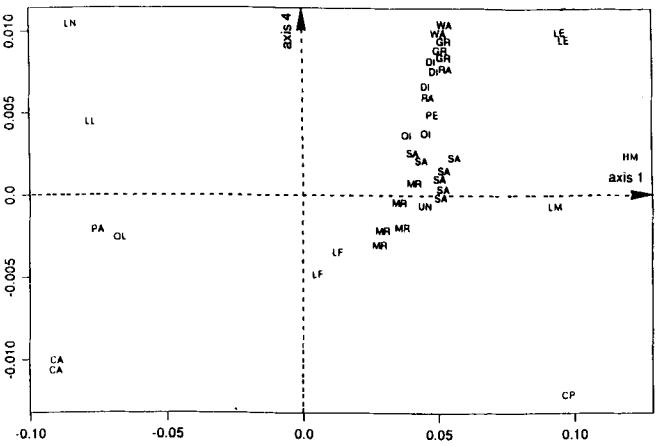

FIG. 8. Principal component analysis map of the spectral collection (plan 1-4). See Figure 5 for other abbreviations. GR, grapeseed oil; DI, dietetic oil.

opposite to OL (oleic acid) and LE (linoleic acid ethyl ester). CA and HM had shorter carbon chains than OL and LE.

The representation of PC scores 1 and 4 (89.7% of total sum of squares) is given in Figure 8. Axis 4 is related to the degree of unsaturation. The fatty acid standards were arranged in the following order: CA (saturated fatty acid), OL and palmitoleic acid (monounsaturated), LE (diunsaturated), linolenic acid (triunsaturated). The same arrangement was observed with the samples of edible fats. For example, WAs, which had the highest percentage of double bonds (72.1% polyunsaturated fatty acid), were placed opposite to LF butters (0.4% polyunsaturated fatty acid) and OIs which had the highest proportion of OL (one double bond). In the same way, the margarines (sunflower, margarine, LF) and butters (unsalted, salted) that had the highest percentage of saturated fatty acids were close to the HM and lauric acid methyl ester (LM), which contain only saturated fatty acids.

The fourth eigenvector (Fig. 9) tallied with the interpretation given above and gave rise to significant positive peaks at 3010 cm^{-1} that were assigned to =CH stretching vibrations.

In this PCA map, the samples were not separated as function of their water content because the spectra of the

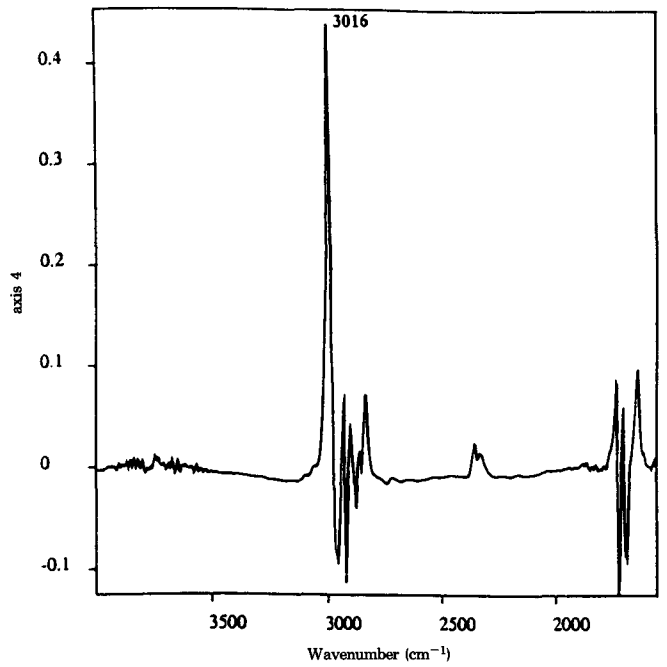

FIG. 9. Fourth eigenvector of principal component analysis.

pure lipid standards, which do not contain water, were selected as principal observation. Therefore, the PCA map does not include water as a variable.

Comparison of ATR-MIR with GLC analyses. The combination of ATR spectroscopy with PCA allows the differentiation lipid-rich food products as function of some particularities of their fatty acid composition.

The samples can be roughly classified according to their degree of unsaturation, determined by GLC, along axis 4 of the PCA. For example, the highly unsaturated WA is opposite to the most saturated LF butters. However, the GLC classification is not strictly respected for all samples, and further experiments are needed to study the possibility to quantify lipid unsaturation with ATR.

Axis 1 of PCA clearly separates the lipids according to their C=O concentration, which is not the case with GLC analysis performed on total lipids. It was impossible to classify the food samples as a function of the length of their constituting fatty acids. In effect, the fatty acids are distributed along axis 2 according to their CH_2 numbers. Thus, the carbons involved in double bonds in unsaturated fatty acids are not taken into account on axis 2. The combination of the information contained in axis 2 and 4 (unsaturation) would be needed to calculate chain-length.

It is difficult to precisely compare the results obtained with GLC and ATR-MIR. The first method, which is a reference method to evaluate fatty acid composition of lipids, gives detailed results about the nature and the percentages of the constitutive fatty acids. However, it involves long procedures, which have to be performed carefully all along the analytical procedure. On the contrary, the spectral analysis can be routinely performed on intact food samples to obtain a rapid classification. The information obtained with this method is more global than that obtained with GLC. For example, if an average degree of unsaturation is obtained by MIRS, information about the water content, the presence of *trans* fatty acids and that of nonesterified fatty acids can also be found.

We have shown here that the combination of ATR spectroscopy with statistical multidimensional techniques made it possible to extract relevant information from MIR spectra of lipids. This method allows rapid classification of lipid-rich foods according to their degree of unsaturation. The PCA maps allow a direct appreciation of the similarities between edible fats.

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