

Fatty Acid Composition of Spanish Shortenings with Special Emphasis on *trans* Unsaturation Content as Determined by Fourier Transform Infrared Spectroscopy and Gas Chromatography

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ABSTRACT: A Fourier transform infrared spectroscopic procedure was used to analyze 34 edible fats (22 shortenings and 12 vegetable margarines) as neat fats (IR_{NF}) to determine their total *trans* fatty acid (TFA) content. The sloping baseline was corrected with a reference spectrum based on a nonprocessed olive oil. The calibration was done using seven partially hydrogenated fats with an individual TFA content previously determined by the combination of gas chromatography (GC) with argentation thin-layer chromatography. Taking into account the different absorptivities of various *trans* isomers, different correction factors were calculated using the calibration standards (0.83 and 1.71 for single *trans* bonds in both diethylene and triethylene and for *trans*, *trans*-diethylene fatty acids, respectively) and applied to calculate the total TFA of samples. Moreover, the samples were converted to their methyl esters and reanalyzed following the same procedure (IR_{FAME}). Differences in TFA content of fats were not found when a *t*-test was used to compare the results obtained by IR_{NF} vs. either IR_{FAME} or GC, suggesting that IR of neat fats could be used, thus avoiding the need to prepare sample solutions in organic solvents and to prepare fatty acid methyl esters. The mean TFA content (determined by IR_{NF}) of a representative group of Spanish shortenings (22 samples) that varied widely in terms of fat sources, processes, and purposes (bakery, sandwiches, ice cream, coatings, chocolate coverings) was $6.55 \pm 11.40\%$, although more than 54% contained $<3\%$ of TFA. Fatty acid composition of shortenings by direct GC using a 100-m polar cyanopolysiloxane capillary column indicated that the mean *trans*-18:2 isomer content was 0.58%, ranging from 0.9 to 3.4%. Small amounts of *trans*-18:3 isomers ($<0.3\%$) were observed in 18 of the 22 shortenings studied; the maximal value was $<2\%$. The mean value of the fraction saturated + TFA of shortenings was high ($59.95 \pm 12.73\%$), including two values higher than 83%.

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It has been suggested that a high consumption of some *trans* fatty acids (TFA) formed by the partial hydrogenation of oils and fats to obtain the right physical characteristics of margarines

and shortenings moderately increases the risk of coronary heart disease (1). In 1993, Boatella *et al.* (2) reported that the contribution of bakery products to the total TFA daily intake in Spain was low because of their low TFA content ($1.6 \pm 1.6\%$, when analyzing 83 bakery products). These results contrast with those from other Western countries where bakery and snack foods and other commercial products made with partially hydrogenated vegetable oils are important contributors to total TFA consumption. Although it has been reported that the TFA content of margarines and shortenings has been reduced in recent years in several countries (3–6), Parcerisa *et al.* (7) found, in 1999, a much higher TFA level ($6.5 \pm 4.2\%$) when analyzing 15 Spanish bakery products. On the other hand, it is interesting to know if, in reducing the TFA content of edible fats, some manufacturers again used tropical fats that are more detrimental, from a health point of view.

For some time, infrared spectroscopy (IR) has been a part of the official methods for determination of *trans* unsaturation content. Some of the basic problems found in the use of IR have been improved with the use of Fourier transform infrared spectroscopy (FTIR). Many modifications have been proposed to improve the accuracy of IR procedure, and some of them have been included in the official methods (8). However, the *trans* calibration material proposed (methyl elaidate in CS_2) may not appear to be the most suitable because partially hydrogenated fats and oils commonly contain a wide range of 18:1, 18:2, and 18:3 *trans* and *cis* isomers that may not absorb to the same extent on IR (9). The use of a mixture of fatty acid methyl esters (FAME) with a known *trans* content derived from a partially hydrogenated vegetable oil has been recommended by Ratnayake and Pelletier (10). Moreover, the use of oil samples as calibration material leads to better accuracy in reproducing the baseline of unknown samples with these calibration standards.

On the other hand, the correction of the sloping baseline directly by subtracting as reference sample a nonhydrogenated oil having only *cis* double bonds or its methyl esters, proposed by some authors (11–13) to resolve the background interferences produced by the overlapping of the *trans* band with other features in the IR spectrum (which reduces the accuracy of the quantitation, especially at low TFA levels), is still not included in the official methods.

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In the present work, we used seven partially hydrogenated oil samples [with an individual TFA content previously analyzed by a combination of GC with argentation thin-layer chromatography (TLC)] as calibration material to determine by FTIR the total *trans* unsaturation content of 34 edible fat samples using the neat fat directly. To test the validity of this IR procedure against another standard IR method, the same samples were converted to their FAME and reanalyzed following the same procedure. Finally, the fatty acid profiles obtained with a direct GC analysis of FAME using a 100-m polar cyanopolysiloxane capillary column of a representative group of shortenings marketed in Spain were discussed.

EXPERIMENTAL PROCEDURES

Samples and standards. Commercial shortenings ($n = 22$) marketed in Spain (15 supplied by two shortening-producing factories and 7 supplied by different bakery industries) were used. According to the information supplied, 19 shortenings were vegetable oils and fats and 3 were animal fats. Of the vegetable shortenings, 10 samples are used for biscuits and bakery, 4 to fill cakes, 2 for chocolate coating, 2 for ice cream, and 1 to spread on sandwiches. The fatty acid composition of these samples was determined by direct GC. The total TFA content was determined by FTIR, using two different methods: analysis of neat fat and of FAME.

Also, 12 different samples of Spanish vegetable margarines, whose fatty acid composition as determined by direct GC had been previously published (14), were utilized to determine their total *trans* unsaturation contents by the same FTIR procedures as in the shortening samples.

All the samples were melted at 50–70°C and the oily layer decanted. The oil phase was filtered through a Buchner funnel, with anhydrous Na_2SO_4 and vacuum pump, and stored frozen until analysis.

For quantitative determinations of total FAME in GC, an anhydrous soy–maize oil blend with a certified fatty acid composition (reference material CRM-162, obtained from the Commission of the European Communities, Brussels, Belgium) was used.

A nonprocessed olive oil (virtually TFA-free) and an equimolar mixture of three saturated triglycerides (trimyristine, tripalmitin, and tristearin) were used as reference standards in IR. For the calibration of IR *trans* C=C bonds, seven partially hydrogenated oil samples having total *trans* contents [determined by GC with prior separation of the *cis* and *trans* fractions by argentation TLC as described by Alonso *et al.* (14)] in the 0.73–23.94% TFA range, which had served as test materials in the research project SMT4-CT97-2144 of the European Union, were used as neat fat and as FAME.

Preparation of FAME for GC analysis. The procedure was based on the method proposed by Christopherson and Glass (15). About 100 mg of shortening oil was weighed to 0.1 mg and dissolved in 3 mL of hexane. Methanolic potassium hydroxide (2 N) (0.1 mL) was then added, and the mixture was stirred for 1 min and left to rest for 15 min. Next, the hexane

layer was separated, and 0.2 μL of hexane fraction was injected in the GC.

Preparation of FAME for FTIR analysis. The procedure was the same as for GC analysis, but after transesterification, 0.05 mL glacial acetic acid was added, and the organic phase was washed twice with an aqueous NaCl solution (50 g/L). The ester solution was dried over anhydrous Na_2SO_4 , filtered, and the solvent removed in a vacuum evaporator (16).

GC. GC analysis of FAME was performed on an Autosystem GC (PerkinElmer Co., Beaconsfield, United Kingdom) equipped with a flame-ionization detector. Analyses were performed with a CP Sil 88 column (100 m \times 0.25 mm i.d.) containing 100% cyanopropyl siloxane, stationary phase, film thickness 0.20 μm (Chrompack, Middelburg, The Netherlands). The column is operated isothermally at 180°C, with hydrogen carrier gas pressure at 1.2 kg/m². The split ratio was 1:50, and the injector and detector temperatures were 250°C.

IR spectroscopy. IR spectra were recorded using a Perkin-Elmer 1725X spectrophotometer equipped with a DTGS detector. For each spectrum, 64 scans were usually accumulated at a spectral resolution of 2 cm^{-1} , and the signals obtained were fed into a microcomputer for storage, display, plotting, and processing. The sample chamber was purged with dry, carbon dioxide-free air during the data collection to avoid spectral contributions from atmospheric gases. Spectral contributions from residual water vapor were removed using a set of water vapor spectra recorded under the same conditions.

For IR analysis of solid samples as neat fat, these were prewarmed to about 70°C and then placed in the IR cell at the appropriate temperature to maintain the samples in the liquid state while scanning the spectra. A cell with NaCl windows and a pathlength of 28 μm was placed in the cell insert. All samples were analyzed by measuring the spectral profiles in the 1050–850 cm^{-1} region that includes the 967 cm^{-1} band characteristic of *trans* C=C bond. The cell was cleaned with chloroform and acetone at the end of each analytical run. Either the spectrum of the nonprocessed olive oil or that of the equimolar mixture of saturated triglycerides, used as reference standards, was directly subtracted from those of the *trans* analytes in order to correct the sloping baseline of the 967 cm^{-1} band. For IR analysis of samples as methyl ester derivatives, with the same type of windows, the reference standards were the FAME of the olive oil.

FTIR quantitation was based on the measurement of the subtracted spectral profile between 1050 and 850 cm^{-1} , where the intensity of the 967 cm^{-1} band is correlated with the isolated *trans* content. Calibration was carried out using the seven partially hydrogenated oil calibration samples on the following basis: if we take c_1, c_2, \dots, c_n as the weight percentages of the individual TFA components [determined by GC with prior separation of the *cis* and *trans* fractions by argentation TLC as described by Alonso *et al.* (14)], the absorbance, A , of a neat fat is given by the equation $A = \sum \epsilon_i b c_i$, ($i = 1, \dots, n$), where b and ϵ_i are the pathlength of the IR cell and absorptivity of the i component, respectively. On the other hand, ϵ_i can be expressed as a function of ϵ_1 , $\epsilon_i = k_i \epsilon_1$,

whereby the above equation becomes $A = \epsilon_1 b \sum k_i c_i$. Obviously, $\sum k_i c_i$ is the total *trans* concentration of component 1 that would generate the absorbance A , i.e., the total *trans* content of any oil sample is expressed as its corresponding virtual content of component 1. Although in the following equation, $A = \epsilon bc$, ϵ and c can be conceived as the average absorptivity and total *trans* content of a oil sample, respectively, this simple equation is not valid for a linear plot of absorbance against total *trans* content of the standards because this would indicate that ϵ is constant for a series of oil samples, and this is not the case.

Only in the case where $c_1 \gg \sum c_i$ ($i = 2, \dots, n$) is the concentration c_1 measured by IR (simplified equation $A = \epsilon_1 b c_1$) very near that of the corresponding weight percentage found by GC, and spectroscopic calibration with only one component (methyl elaidate or trielaidin) may be suitable. Consequently, before the calibration of fats containing several *trans* components, it is necessary to know k_i in the above general equation $A = \epsilon_1 b \sum k_i c_i$. In this way this IR method can be applied to oil sample series with varying proportions of *trans* components. By considering partially hydrogenated oil samples used for calibration, whose compositions in individual TFA were known, we obtained through linear equation systems $k_2 = 0.83$ for single *trans* bonds in diethylene and triethylene fatty acids, and $k_3 = 1.71$ for *trans,trans*-dienes. The resulting calibration equation is given by $A = \epsilon_1 b (c_1 + 0.83c_2 + 1.71c_3)$, where c_1 , c_2 , and c_3 are the weight percentages of mono-*trans*-monoethylenic fatty acids (c_1), mono-*trans*-diethylenic and mono-*trans*-triethylenic fatty acids (c_2), and *trans,trans*-diethylenic acids (c_3). All samples were analyzed for *trans* content by application of the partial least squares technique. Spectral manipulation of data (subtraction and quantitation) was done using the SpectraCalc program (Galactic Industries Corporation, Salem, NH).

Statistical analysis. Student's *t*-test was used to determine whether there were significant differences between the *trans* percentages obtained by IR as neat fat and as FAME or by GC.

RESULTS AND DISCUSSION

Total *trans* content of edible fat samples determined by IR. To improve the accuracy of the standard IR procedures aimed at determining the total *trans* unsaturation content of fat samples (as neat fat or as FAME), a multiple-point calibration method was used. Because the various *trans* isomers of the edible fats do not absorb to the same extent, seven partially hydrogenated oil calibration samples having individual *trans* contents previously determined using GC-TLC were used. The correction factors k_i , which related the total *trans* unsaturation determined by IR ($\sum k_i c_i$) to the weight percentages (c_i) of the component TFA measured by GC-TLC, were calculated as indicated in the Experimental Procedures section and were found to be 0.83 and 1.71 for single *trans* bonds in both diethylene and triethylene fatty acids and for *trans,trans*-diethylene, respectively. These values were similar to those (0.84 and 1.74)

obtained by Ratnayake *et al.* (17) for single *trans* bonds in diethylene fatty acids and for *trans,trans*-diethylenic fatty acids, respectively, and show that the mono-*trans* triene absorb to the same extent as mono-*trans* diene. On this basis, the calibration plot of absorbance, A , against $\sum k_i c_i$ is included in Figure 1. The high correlation coefficient indicates that these two variables (A and $\sum k_i c_i$) are sufficiently linear for routine analysis over the given range.

As the spectral backgrounds generated by the two reference standards assayed (a nonhydrogenated olive oil and a mixture of saturated synthetic triglycerides) were practically coincident, the analytical results obtained in both cases were considered to be equivalent (percentage *trans* differences not higher than 0.02%).

Figure 2A shows a plot of the values of total *trans* unsaturated content determined by IR analysis of the samples as neat fat (IR_{NF}) vs. the TFA weight percentages obtained by GC and corrected with their respective absorptivities. The obtained regression equation ($n = 33$) includes all the samples analyzed, excepting one with a TFA content (70%, as determined by GC) higher than the maximum used in the calibration range (24%). The equation of the line reflects a good concurrence between this IR method and the GC method. Student's test was applied to see how different these two types of percentage *trans* values were. In this connection, the test revealed that these methods provided values that were not significantly different ($P < 0.8$).

When this IR procedure was used, *trans* FTIR values determined as FAME (IR_{FAME}) were in close agreement with those obtained as neat fat (IR_{NF}) (Fig. 2B). This is supported not only by the correlation equation ($IR_{FAME} = 1.0430 \times IR_{NF} - 0.2530$, $R = 0.9930$, $SD = 0.9538$), but also by Student's test, which in this case gives a sufficient low probability ($P < 0.50$) for the two methods to be considered significantly different. Thus, the use of the IR_{NF} method for determination of total TFA is proposed, because it is a far more direct method than the standard one used for methyl ester samples. Moreover, the method applied here has been successful for oil samples of varying TFA contents (0.17–20.21% *trans*-18:1 isomers, 0.02–1.27% *trans*-18:2

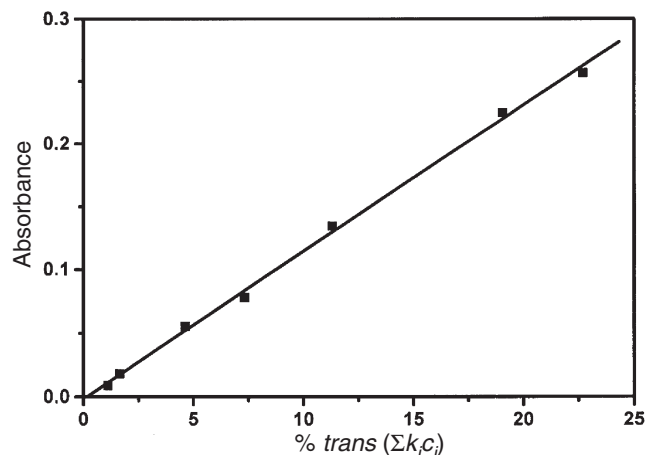


FIG. 1. Calibration plot of absorbance against % *trans* ($\sum k_i c_i$). Linear regression equation: $A_{NF} = 0.0116 \sum k_i c_i - 0.0010$. $R = 0.9989$, $SD = 0.0050$.

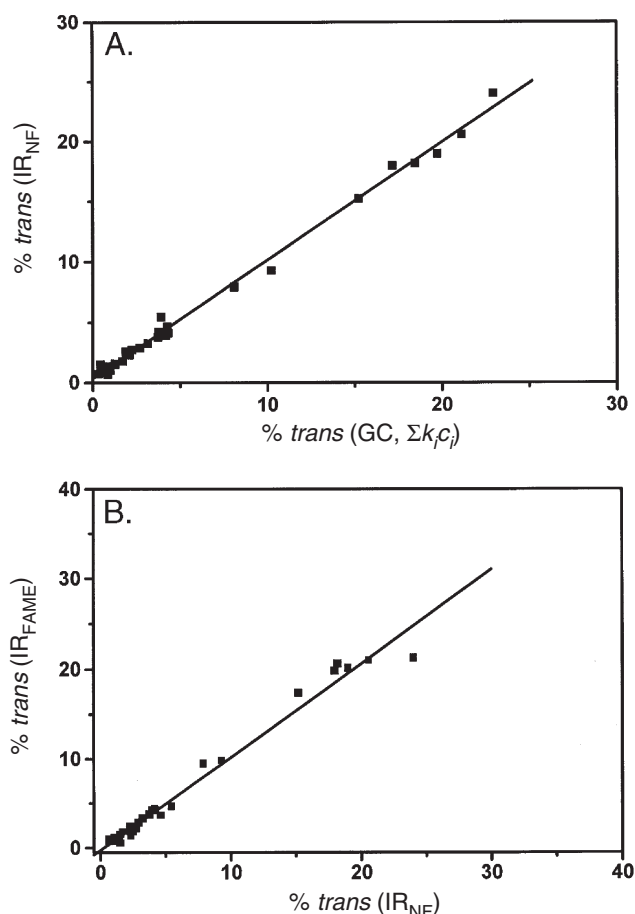


FIG. 2. (A) Plot of % *trans* values of neat fats (NF) predicted by the infrared (IR) method vs. the % *trans* values obtained by gas chromatography (GC). Linear regression equation: % *trans* (IR_{NF}) = 0.9801% *trans* (GC, Σk_c_i) + 0.0361. $R = 0.9974$, $SD = 0.4997$. (B) Correlation of % *trans* (IR_{FAME}) vs. % *trans* (IR_{NF}). Linear regression equation: % *trans* (IR_{FAME}) = 1.0430 % *trans* (IR_{NF}) - 0.2530. $R = 0.9930$, $SD = 0.9538$. FAME, fatty acid methyl esters.

isomers, 0.01–0.70% *trans*-18:3 isomers, 0.01–0.24% *trans,trans*-18:2 isomers). Ulberth and Henninger (18) have also demonstrated that the results obtained by analysis of TFA of fats as triglycerides (dissolved in CS₂) compare favorably with those obtained using methyl esters provided that the calibration mixtures with trielaidin were used. However, these authors express a preference for direct measurement on FAME, which are liquid samples and consequently do not need to be dissolved in CS₂. Mossoba *et al.* (12) and Sedman *et al.* (13), using attenuated total IR reflectance, avoided the step of dissolving the triglyceride samples in CS₂ by warming them and recording their spectra in the liquid state. Our results suggest that the combined use of a *trans*-free unprocessed oil to baseline correction and partially hydrogenated oils having known *trans* contents as standards for calibration permits the analysis of margarines and shortenings as neat fat by FTIR without special accessories. Moreover, the potential availability of certified hydrogenated vegetable oils with a known content of TFA for quantitation purposes obviates the need to prepare standards for calibration.

The distribution of TFA measured by FTIR as neat fat in a representative group of Spanish shortenings (22 samples) that varied widely in terms of fat sources, processes, and purposes was as follows: 45.4% were between 1 and 3%; 27.3% between 3 and 5%; 18.2% were higher than 5% and only 9.1% were lower than 1%. The mean TFA content was 6.55% ± 11.40%, which is similar to (and more variable than) those obtained by Parcerisa *et al.* (7) in bakery products marketed in Spain (6.5 ± 4.2%) or by Ovesen *et al.* in 1996 (6) and 1998 (19) in Danish bakery shortenings (6.8 ± 3.1% and 6.7 ± 2.3% on 34 and 38 brands, respectively) and much lower than those obtained by Bayard and Wolff (4) in France (from 26 to 62%) and by Henninger and Ulberth (5) in Austria (12.1%). The mean value of TFA also seems to be lower than that of Spanish vegetable margarines (8.87%) (14). This is surprising in that when compared within a single country, the TFA contents of margarines are usually lower than those of shortenings (4,6,19).

Fatty acid composition of Spanish shortenings determined by GC. The amount of *trans*-18:1 isomers (as a percentage of TFA determined using direct GC) in Spanish shortenings showed a wide range of variation: from 0.4 to 69% with a mean value of 6.30%, although more than 63% of shortenings contained <3% of *trans*-18:1. It is well established that direct GC, even on long polar capillary columns, does not give access to all *trans*-18:1 isomers (4,5,20). Under our experimental conditions, separation of *cis* and *trans* isomers of 18:1 was not complete, but only for the coelution of *trans*-15-18:1 with *cis*-18:1. However, the content of *trans*-15-18:1 in the partially hydrogenated vegetable oils (that constituted 86% of the shortening samples studied in the present work) is low [0.9–3.4% of total *trans* fatty acids, Wolff *et al.* (20)]. In this way, the results obtained by Alonso *et al.* (14), using either direct GC or argentation TLC combined with GC using the same analytical conditions as in this work, showed no difference in the total *trans*-18:1 percentage of vegetable margarines.

The mean *trans*-18:2 isomer content was 0.58%, ranging from 0 to 3.4%. Small amounts of *trans*-18:3 isomers (<0.3%) were observed in 18 of the 22 shortenings studied; the maximal value was <2%. Two shortenings (for sandwich spreads and for chocolate coatings) were virtually *trans* free (<1% of total TFA content), but their total saturated fatty acid contents were high and markedly different (48 and 96%, respectively).

As a whole, the fatty acid composition (Table 1) of the collection of Spanish shortenings was highly variable; this high heterogeneity is related to the differences in the sources of fat, processes, and purposes (bakery, sandwiches, ice cream, chocolate coatings, etc.). They are grouped into two categories based on their palmitic acid content. Six of the 22 shortenings had more than 40% of 16:0 (which is characteristic of palm oil), presenting a relatively homogeneous composition and a relatively low content of the different *trans* isomers.

There was another group of nine shortenings with <40% palmitic acid (8.7–39.7%) and <8.5% of stearic acid with

similar *cis*-18:2 (15.54 ± 5.41%) and total saturated fatty acid (54.60 ± 7.45%) contents but with different profiles in the saturated fraction (12:1, 4:4, and 30.7% as mean values of 12:0, 14:0, and 16:0), which suggests that they contained substantial but variable amounts of coconut oil. In fact, the sum of contents of lauric, myristic, and palmitic acids of the shortenings belonging to both groups was on average 45.5%; this is quite striking and is much higher than the 28.7% reported by Ovesen *et al.* (19) in Danish shortenings. The total *trans* content in this group is low (2.1%). The third group, with <40% palmitic acid and >8.5% stearic acid (which includes the samples of animal origin), has a relatively high content of total *trans* isomers (16.8%). In addition, it was more heterogeneous, presenting high and variable TFA, 12:0 and 18:0 contents, and low *cis*-18:2 content. This generally indicates the existence of two types of shortenings in the market: (i) shortenings obtained from tropical fats, mainly coconut oil, and (ii) shortenings obtained by partial hydrogenation of vegetable fats or by addition of animal fats and/or coconut oil. Given that saturated fatty acids, especially palmitic, lauric, and myristic, raise blood cholesterol (21), from a health point of view there would be little to gain from achieving the right physical characteristics with these saturated fatty acids instead of TFA. At present there are other options in shortening manufacturing, ranging from modification of the hydrogenation process, blending with a stearic-rich hard fat (22) and interesterification of vegetable oil blends (23), to the application of genetic engineering to produce oils that do not require hydrogenation (24).

Table 2 shows the minimal, maximal, and mean values of fatty acids of Spanish shortenings grouped by nutritional sig-

TABLE 2
Composition of Fatty Acids (% total fatty acids) by Nutritional Categories in Spanish Shortenings

Fatty acid category	Minimum	Maximum	Mean ± SD
Saturated + total <i>trans</i> ^a	45.91	98.17	59.95 ± 12.73
<i>Cis</i> -monounsaturated ^b	2.52	38.01	26.24 ± 8.93
<i>Cis</i> -polyunsaturated ^b	0.42	26.57	13.20 ± 7.50

^aDetermined by a Fourier transform infrared spectroscopic procedure to analyze edible fats as neat fats.

^bDetermined by direct GC. See Table 1 for other abbreviation.

nificance. The *cis*-polyunsaturated fatty acid fraction, which is the most valuable from a nutritional point of view, is highly variable (ranging from 0.4 to 26.7% with a mean value of 13.2%). This value was much lower than that reported in a recent study by Alonso *et al.* (14) for Spanish vegetable margarines (42%). The saturated + TFA fraction (determined by IR_{FAME}) attained a very high mean value (59.95 ± 12.73%), including values over 83% in two cases. The mean values of the indexes most commonly used to express the nutritional value of edible fats [*cis*-polyunsaturated/(saturated + TFA)] and [(*cis*-polyunsaturated + *cis*-monounsaturated)/(saturated + TFA)] were, respectively, 0.22 and 0.66. Although these values should be carefully considered because of the high heterogeneity of the samples, they are much lower than those obtained by Alonso *et al.* (14) for Spanish vegetable margarines (1.25 and 1.92, respectively) but very similar to those calculated from the data obtained by Parcerisa *et al.* (7) for bakery products.

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TABLE 1
Fatty Acid Composition (wt% total fatty acids, mean ± SD) of Spanish Shortenings Determined by Direct GC

	>40% Palmitic acid	<40% Palmitic acid	
		<8.5% Stearic acid	>8.5% Stearic acid
<i>n</i>	6	9	7
Fatty acid			
8:0	0.07 ± 0.04	1.04 ± 0.37	1.18 ± 1.71
10:0	0.08 ± 0.05	0.93 ± 0.29	1.26 ± 1.57
12:0	0.90 ± 0.69	12.07 ± 5.35	11.51 ± 19.50
14:0	1.13 ± 0.27	4.40 ± 1.54	5.55 ± 6.31
16:0	40.96 ± 1.04	30.7 ± 12.92	19.79 ± 11.20
16:1	0.12 ± 0.01	0.26 ± 0.46	0.73 ± 0.82
18:0	7.67 ± 1.51	5.07 ± 0.99	13.73 ± 4.58
<i>trans</i> -18:1 isomers	3.06 ± 1.26	1.30 ± 1.03	15.50 ± 24.70
<i>cis</i> -18:1	30.27 ± 1.80	26.1 ± 15.13	21.32 ± 13.43
<i>trans</i> -18:2 isomers ^a	0.30 ± 0.29	0.44 ± 0.36	0.90 ± 1.15
<i>cis</i> -18:2	13.68 ± 1.14	15.54 ± 5.41	6.93 ± 8.92
<i>trans</i> -18:3 isomers ^a	0.15 ± 0.03	0.32 ± 0.32	0.38 ± 0.66
<i>cis</i> -18:3	0.97 ± 0.26	1.22 ± 0.67	0.46 ± 0.44
20:0	0.34 ± 0.03	0.29 ± 0.05	0.31 ± 0.12
20:1	0.09 ± 0.01	0.12 ± 0.07	0.22 ± 0.29
22:0	0.11 ± 0.05	0.14 ± 0.04	0.19 ± 0.13

^aIncludes all the isomers that have at least one *trans* double bond. GC, gas chromatography.

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