# Determination of *trans* Fatty Acids and Fatty Acid Profiles in Margarines Marketed in Spain

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**ABSTRACT:** Two gas chromatography (GC) procedures were compared for routine analysis of *trans* fatty acids (TFA) of vegetable margarines, one direct with a 100-m high-polarity column and the other using argentation thin-layer chromatography and GC. There was no difference ($P > 0.05$) in the total *trans* 18:1 percentage of margarines with a medium level of TFA (~18%) made using either of the procedures. Both methods offer good repeatability for determination of total *trans* 18:1 percentage. The recoveries of total *trans* isomers of 18:1 were not influenced ($P > 0.1$) by the method used. Fatty acid composition of 12 Spanish margarines was determined by the direct GC method. The total contents of *trans* isomers of oleic, linoleic, and linolenic acids ranged from 0.15 to 20.21, from 0.24 to 0.99, and from 0 to 0.47%, respectively, and the mean values were 8.18, 0.49, and 0.21%. The mean values for the ratios [*cis*-polyunsaturated/(saturated + TFA)] and [(*cis*-polyunsaturated + *cis*-monounsaturated)/(saturated + TFA)] were 1.25 ± 0.39 and 1.92 ± 0.43, respectively. Taking into account the annual per capita consumption of vegetable margarine, the mean fat content of the margarines (63.5%), and the mean total TFA content (8.87%), the daily per capita consumption of TFA from vegetable margarines by Spaniards was estimated at about 0.2 g/person/d.

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**KEY WORDS:** Argentation thin-layer chromatography, capillary gas chromatography, fatty acid composition, hydrogenated oil, *trans* fatty acids, vegetable margarines.

The fatty acid composition of triglycerides has a direct effect on the physical, chemical, and biological properties of dietary fats. The high concentration of unsaturated fatty acids of the *cis* configuration is mainly responsible for the low melting point found in natural vegetable oils. One of the tools that industry uses to give fats and oils the desired functionality for specific products is partial hydrogenation. Approximately one-third of all edible fats and oils in the world are hydrogenated (1). During partial hydrogenation, some double bonds of fatty acids are saturated, but part of the *cis* double bonds are isomerized into their *trans* form. In the past, formation of *trans* fatty acid (TFA) isomers was considered an advantageous side reaction because TFA have higher melting points and greater stability than their *cis* isomers (2,3). But several epidemiological and clinical studies on the health effects of TFA published in the last few years indicate that TFA intake may be a risk factor for cardiovascular disease (4,5). *Trans* isomers have been reported to raise serum cholesterol levels in low-density lipoproteins (6).

Margarines and shortenings, the latter being preferentially consumed in processed foods, are among the main TFA sources in the diets of several Western countries (7–9). TFA also are found in milk and some meats because polyunsaturated fatty acids in foodstuffs for ruminants are partially biohydrogenated by rumen microorganisms (10). Recently, in the review by Craig-Schmidt (11), important variations in TFA intake of different countries were found to be due mainly to differences in the composition of margarines and other industrially hydrogenated fats as well as in the pattern of fat consumption.

A variety of analytical methods for determination of TFA content already exists. Infrared spectroscopic analysis (12) and Fourier transform infrared spectroscopy (13,14) are used for rapid determination of *trans* unsaturation of edible fats. Capillary gas chromatography (GC) has been the standard tool for the separation of fatty acids; the combination of GC with argentation thin-layer chromatography (Ag-TLC) (15,16) or with high-performance liquid chromatography (17,18) is often recommended (especially in milk fat TFA analysis). However, these preseparation methods are time consuming and therefore not applicable on a routine basis (19). Recently, Ratnayake (20) reviewed the literature on TFA analysis and concluded that detailed information on the fatty acid composition of partially hydrogenated vegetable oils can be obtained by GC analysis alone.

This paper describes the results of a study on fatty acid composition and TFA content by direct GC of several vegetable margarines available in the Spanish market. The validation of the direct GC method using a high-polarity 100-m column was checked against the Ag-TLC/GC method, which is tedious and time-consuming.

## EXPERIMENTAL PROCEDURES

*Standard and reagents.* Heptadecanoic, stearic, and elaidic acid methyl esters were supplied by Sigma Chemical Co. (St. Louis, MO); chemical reagents were of analytical reagent grade.

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*Samples.* Twelve different samples of Spanish margarines, including the major national brands, were purchased from several local supermarkets. According to the labels, sample 1 was a mixture of sunflower oil and partially hydrogenated sunflower oil; samples 2, 4, 7, 9, 10, and 11 were mixtures of vegetable oils and hydrogenated vegetable fats; samples 3, 6, and 7 were mixtures of vegetable oils and partially hydrogenated vegetable fats; sample 5 was a mixture of corn oil and partially hydrogenated vegetable fats; and samples 8 and 12 were mixtures of corn oil and partially hydrogenated corn oil. All the samples melted at approximately 50°C. The oil was dissolved in hexane, dehydrated with anhydrous sodium sulfate, filtered through hydrophobic dry paper, and concentrated on a rotatory evaporator at 60–70°C.

*Preparation of fatty acid methyl esters (FAME).* The following procedure was based on the method proposed by Christopherson and Glass (21). About 100 mg of margarine 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 the hexane fraction was injected in the GC. For recovery analysis of the Ag-TLC fractions and direct GC systems, 1 mL of heptadecanoic acid methyl ester (11 mg/5 mL hexane) was added to margarine oil as internal standard before transesterification.

*Ag-TLC.* FAME were fractionated according to the number and geometry of double bonds by TLC following a slightly modified procedure by Precht and Molkentin (16). The TLC glass plates (20 × 20 cm) with silica gel (Merck, Darmstadt, Germany) were soaked in a 20% aqueous solution of silver nitrate (Panreac, Barcelona, Spain) for 16 h, partially air-dried, and activated at 120°C for 30 min. A 100-µL solution of FAME (100 mg/mL) was applied to the activated plates in a narrow band. The plates were developed twice in a saturated chamber in hexane and diethyl ether (9:1, vol/vol) with 15 cm migration. At the end of chromatographic runs, the plates were air-dried and sprayed with a 0.20% (wt/vol) ethanolic solution of 2′,7′-dichlorofluorescein and the bands were visualized under ultraviolet light. The bands corresponding to the saturated and *trans* monoenoic FAME which were previously identified by a mixture of stearic FAME (18:0) and elaidic FAME (*trans*-9-18:1) running in Ag-TLC, were scraped into a flask. The FAME were extracted with 80 mL diethyl ether in four extractions, and the solvent was evaporated in a rotoevaporator under stream of nitrogen. The residue was dissolved in 200 µL of heptane, and the solution was used for the analysis of FAME by GC.

*GC analysis conditions*. 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 × 0.25 mm i.d.) containing 100% cyanopropyl siloxane, stationary phase, 0.20 µm film thickness (Chrompack, Middelburg, The Netherlands). The initial temperature of 175°C was maintained for 28 min, then raised to 210°C at a rate of 1.3°C/min for 10 min. The split ratio was 1:50, and the carrier gas was hydrogen with a head pressure of 1.2 kg/m$^2$. The injector and detector temperatures were 250°C.

For quantitative determinations of total FAME, an anhydrous soy–corn oil blend with a certified fatty acid composition (reference material CRM-162, obtained from the Commission of the European Communities, Brussels, Belgium) was used to determine the response factors. Tentative identification of *trans*-18:2 and *trans*-18:3 isomers was made by comparing the equivalent chain-length values of esters obtained in our samples with those of a reference isomerized linseed oil FAME, which had served as test material in the research project SMT4-CT97-2144 of the European Union. To calculate the total content of *trans*-18:1 isomers, the ratio 18:0 to total *trans*-18:1 was determined in the saturated plus *trans* monoenoic Ag-TLC fraction and related to the 18:0 content of total FAME.

*Statistical analysis*. A *t*-test was used to compare differences between mean values for the total content of *trans*-18:1 isomers measured by the two methods. Variance analysis was run on the recovery data using elaidic acid methyl ester level and method as the main effects.

## RESULTS AND DISCUSSION

Figure 1 shows a partial chromatogram of FAME from an unfractionated margarine oil. Separation of positional isomers of 18:2 and 18:3 using the direct GC method with the 100-m CP Sil-88 capillary column was very efficient. Although separation of *cis* and *trans* isomers of 18:1 was not completely resolved because of minor coelution of *trans*-18:1 (mainly *trans*-15-18:1) with *cis*-18:1 (16), the data in Table 1 showed no difference ($P > 0.05$) between the mean total *trans*-18:1

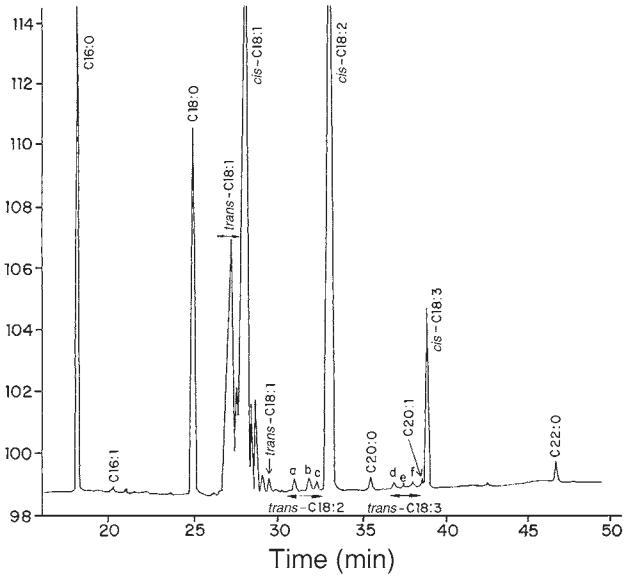


**FIG. 1.** Partial gas chromatogram of fatty acid methyl esters prepared from partially hydrogenated margarine oil. Tentative identification of peaks (*trans* isomers of 18:2 and 18:3): (a) 18:2 9*t*,12*t*; (b) 18:2 9*c*,12*t*; (c) 18:2 9*t*,12*c*; (d) 18:3 9*c*,12*c*,15*t*; (e) 18:3 9*c*,12*t*,15*c*; (f) 18:3 9*t*,12*c*,15*c*.

percentages obtained using the direct GC and the Ag-TLC/GC methods. This was probably because the most important result of hydrogenation of vegetable oils in quantitative terms was *trans*-9-18:1, although some hydrogenated oils (i.e., soybean and canola oils) can also contain substantial amounts of *trans*-10-18:1 and *trans*-11-18:1. Other authors (22,23) indicate that the quantification of *trans*-18:1 in vegetable margarines was underestimated using direct GC with a 50-m CP Sil-88 column. In this sense, Ratnayake (20) reported that the 100-m capillary columns provided the best resolution of geometrical and positional 18:1 isomers with reasonable analysis time.

On the other hand, although other edible fats (mainly partially hydrogenated fish oils) have substantial amounts of isomers of *trans*-16:1 that overlap with $C_{17}$ fatty acids, they are found in very low percentages in vegetable margarines [0.04% of total fatty acids (24)].

The repeatability of the comparative results for total *trans*-18:1 percentage using the two analytical methods was tested on a margarine oil with medium TFA content (~18%). Table 1 shows the individual mean values and the standard deviations for the total *trans*-18:1 percentages obtained from five replicate determinations. Under our conditions, both methods offered good repeatability [coefficient of variation (CV) = 1.37 and 1.19%, respectively, for the direct GC and the Ag-TLC/GC methods].

For recovery analysis, known amounts of approximately 50 and 100% elaidic acid methyl ester with respect to initial amount of total *trans*-18:1 acids were added to a margarine oil sample in which total *trans*-18:1 content had been determined previously using heptadecanoic acid methyl ester as internal standard. Table 2 shows the amounts of elaidic acid methyl ester added to the margarine oil sample and the recovery percentages determined by both methods. Although the recovery percentages were higher ($P < 0.001$) with the lowest amounts of added elaidic acid methyl ester, differences between the two methods were not found ($P > 0.1$). Ulberth and Henninger (14) and Ali *et al.* (25) have reported elaidic acid methyl ester recovery levels similar to this study using Ag-TLC/GC and infrared spectroscopy methods, respectively.

It was therefore concluded that under the given conditions, the direct GC method does not appreciably underestimate the total TFA content of vegetable margarines when compared with the Ag-TLC/GC method. Both methods offer comparable results in terms of repeatability and recovery in the determination of main TFA percentages. Hence, direct GC may be considered advantageous for routine analysis of TFA in vegetable margarines.

*Fatty acid composition of Spanish margarines.* Fatty acid composition of a representative collection of Spanish margarines is shown in Table 3. All the margarines were mixtures of a number of oils in their native state with fat and oils hydrogenated or partially hydrogenated. As a consequence, the amounts of *trans*-18:1 isomers in the samples ranged from 0.15 to 20.21%, with a mean value of 8.18%. These results are comparable to those reported for French margarines by Bayard and Wolff (22), who found a mean value of 8.02%. However, a wide range of *trans*-18:1 contents in the margarines of different countries has been reported. Thus, the mean content in Danish margarines (some of them containing fish oil) was only 3.4% (26), whereas Ratnayake *et al.* (9), who examined about 100 samples of Canadian margarines (in which canola oil is the primary oil used) obtained a high mean content of *trans*-18:1 isomers (20.3%).

The *trans*-18:2 isomer contents found in our study were low (<1%), ranging from 0.24 to 0.99%. Improvements in condi-

**TABLE 1**
**Comparison and Repeatability of Values for Total *trans* 18:1 Fatty Acids in Margarine by Direct GC and Ag-TLC/GC Methods**

| | Total *trans* 18:1 (% methyl ester)[b] | | | | | |
|---|---|---|---|---|---|---|
| Method[a] | 1 | 2 | 3 | 4 | 5 | Mean ± SD |
| Direct GC | 17.25 | 17.21 | 17.48 | 17.65 | 17.77 | 17.47 ± 0.24 |
| Ag-TLC/GC | 17.43 | 17.57 | 17.89 | 17.72 | 17.94 | 17.71 ± 0.21 |

[a]GC, gas chromatography; Ag-TLC, argentation-thin-layer chromatography.
[b]Mean value of two injections.

**TABLE 2**
**Recovery of Total *trans* 18:1 Fatty Acid Methyl Esters from Margarine Oil Sample by Direct GC and Ag-TLC/GC Methods**

| | | Amount[b] added | | Recovered[c] measured | | |
|---|---|---|---|---|---|---|
| Method | Initial[a] amount | A | B | A | B | Recovered (% of calculated) |
| Direct GC | 14.34 | 7.12 | 14.18 | 21.06 | 27.19 | 98.14/95.34 |
| Ag-TLC/GC | 14.53 | 7.12 | 14.18 | 21.35 | 27.48 | 98.61/95.72 |

[a]Mean value of five independent determinations expressed as mg of total *trans* 18:1/100 mg of oil. Replicate injections. See Table 1 for abbreviations.
[b]Elaidic acid methyl ester (mg). (A) amount added ~50% initial amount; (B) amount added ~100% initial amount.
[c]Mean value of three independent determinations expressed as mg of total *trans* 18:1 replicate injections. (A) amount added ~50% initial amount; (B) amount added ~100% initial amount.

**TABLE 3**
**Fatty Acid Composition (% total fatty acids) in Spanish Margarines**

| | Sample[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fatty acid | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 8:0 | — | 1.04 | 0.35 | 0.34 | 0.36 | 0.68 | — | — | 0.13 | 0.37 | 0.46 | — |
| 10:0 | — | 0.86 | 0.32 | 0.32 | 0.29 | 0.57 | — | — | 0.15 | 0.38 | 0.41 | — |
| 12:0 | 0.10 | 7.84 | 4.44 | 4.82 | 4.34 | 4.62 | 0.18 | — | 1.22 | 5.05 | 5.31 | 0.34 |
| 14:0 | 0.16 | 2.99 | 1.59 | 1.78 | 1.59 | 1.93 | 0.30 | — | 0.60 | 1.81 | 1.86 | 0.17 |
| 16:0 | 7.87 | 18.45 | 10.97 | 12.10 | 14.01 | 16.23 | 16.94 | 11.26 | 14.42 | 12.56 | 11.79 | 11.17 |
| 16:1 | 0.09 | 0.08 | 0.09 | 0.10 | 0.10 | 0.09 | 0.09 | 0.10 | 0.08 | 0.07 | 0.08 | 0.10 |
| 18:0 | 7.09 | 6.34 | 8.61 | 9.32 | 7.66 | 7.26 | 7.16 | 4.76 | 6.87 | 9.01 | 9.21 | 5.06 |
| *trans* 18:1 | 17.47 | 2.06 | 0.15 | 0.35 | 0.64 | 9.75 | 7.40 | 19.54 | 20.21 | 0.32 | 0.41 | 19.81 |
| *cis* 18:1 | 25.59 | 20.75 | 20.51 | 19.28 | 23.31 | 21.89 | 24.50 | 28.24 | 25.81 | 18.87 | 17.46 | 29.05 |
| *trans* 18:2 | 0.85 | 0.24 | 0.25 | 0.29 | 0.24 | 0.41 | 0.37 | 0.91 | 0.78 | 0.32 | 0.27 | 0.99 |
| *cis* 18:2 | 39.52 | 35.21 | 49.15 | 46.76 | 44.09 | 32.08 | 37.50 | 33.89 | 25.24 | 46.42 | 47.98 | 31.86 |
| *trans* 18:3 | — | 0.26 | — | 0.37 | 0.19 | 0.10 | 0.47 | 0.06 | 0.29 | 0.40 | 0.35 | — |
| *cis* 18:3 | 0.24 | 3.25 | 2.73 | 3.68 | 2.50 | 3.67 | 4.19 | 0.57 | 3.47 | 3.65 | 3.60 | 0.77 |
| 20:0 | 0.29 | 0.28 | 0.31 | 0.26 | 0.41 | 0.31 | 0.38 | 0.39 | 0.31 | 0.31 | 0.29 | 0.42 |
| 20:1 | 0.11 | 0.11 | 0.10 | 0.10 | 0.10 | 0.09 | 0.11 | 0.16 | 0.11 | 0.10 | 0.11 | 0.14 |
| 22:0 | 0.62 | 0.24 | 0.43 | 0.13 | 0.17 | 0.32 | 0.41 | 0.12 | 0.29 | 0.35 | 0.41 | 0.12 |

[a]Mean value of two independent determinations. Sample 1, sunflower oil and partially hydrogenated sunflower oil; samples 2, 4, 7, 9, 10, 11, mixtures of vegetable oils and hydrogenated vegetable fats; samples 3, 6, 7, mixtures of vegetable oils and partially hydrogenated vegetable fats; sample 5, mixture of corn oil and partially hydrogenated vegetable fats; samples 8 and 12, mixtures of corn oil and partially hydrogenated corn oil.

tions of the hydrogenation process have reduced the amount of *trans,trans*-dienes in modern margarines, often to values near zero (27). Small amounts of *trans*-18:3 isomers (<0.5%) were also observed in 9 of the 12 margarines studied; the occurrence of these isomers is mainly associated with the presence of physically refined or deodorized oils in mixtures (28).

Five margarines (samples 3, 4, 5, 10, and 11) were virtually TFA-free (<1% of total TFA content) and had similar and relatively high contents of lauric (4.79 ± 0.36%), myristic (1.72 ± 0.11%), and palmitic (12.28 ± 1.06%) acids. As suggested by Bayard and Wolff (22), the reason for this could be that the partially hydrogenated oils in some margarines can be replaced by palm or coconut oils in their native state. The highest concentrations of linoleic acid (>44%) were found in the five margarines last cited. The mean TFA level of the other seven semisoft margarines (with an 18:2 content from 20 to 40%) found in this study was considerably higher (14.55%) than that reported in Danish margarines [1.2% (26)]. On the other hand, margarines 1, 8, and 12, which were made from sunflower or corn oils and did not include mixtures with tropical fats, had the highest TFA contents (from 18.3 to 20.8%).

Table 4 shows the minimum, maximum, and mean values of fatty acids of Spanish margarines grouped by nutritional significance. The *cis*-polyunsaturated fatty acid fraction, which is the most valuable from a nutritional point of view, ranged from 28.71 to 51.88%, with a mean value of 41.84%. This value was slightly higher than those reported by Tsanev *et al.* (29) and Bayard and Wolff (22) in Bulgarian and French margarines, with mean values of 36.30 and 39.91%, respectively. With respect to the saturated plus *trans*-18:1 fatty acid fraction, which is thought to raise blood cholesterol (6), the mean value for all the Spanish brands studied here was 34.33%. This is comparable to the French margarines (33.56%) studied by Bayard and Wolff (22). The indexes most commonly used to express the nutritional value of edible fats were modified to include TFA with the saturated fatty acid fraction: [*cis*-polyunsaturated/(saturated + TFA)] and [(*cis*-polyunsaturated + *cis*-monounsaturated)/(saturated + TFA)], giving respective values of 1.25 ± 0.39 and 1.92 ± 0.43.

Although it has been reported that the TFA content of edible fats (margarines and shortenings) has been reduced during recent years in several countries (9,22,23,26), this trend has not been confirmed in our country. In a study conducted in 1989 on six Spanish vegetable margarines, Coll and Gutiérrez (30) reported a slightly lower level for total TFA (6.68%) than was found in the present work (8.87%). However, the total content of the main saturated fatty acids ($C_{12}$, $C_{14}$, and $C_{16}$) (30 ± 12.9%) reported by Coll and Gutiérrez (30) was noticeably higher than the mean content of 17.5 ± 5.34% observed in the present case. When the values reported by Coll and Gutiérrez (30) were applied to the indexes proposed above, values were noticeably lower (0.75 and 1.28, respectively) than in the present case. Boatella *et al.* (31) recently found a mean TFA content of 10.8% (with high variability, CV = 80%) in 47 Spanish margarines (origin not specified).

Data supporting the relation between TFA intake and cardiovascular risk usually have been obtained with intakes higher than those that occur in everyday life. On the other hand, there is a considerable difference in the intake pattern

**TABLE 4**
**Composition of Fatty Acids (% total fatty acids) by Nutritional Categories in Spanish Margarines**

| Fatty acid category | Minimum | Maximum | Mean ± SD |
|---|---|---|---|
| Saturated + total *trans* | 27.42 | 45.27 | 35.03 ± 5.60 |
| *cis*-Monounsaturated | 17.46 | 29.05 | 22.94 ± 3.75 |
| *cis*-Polyunsaturated | 28.71 | 51.88 | 41.84 ± 8.12 |

of food containing TFA for different countries. The mean annual per capita consumption of vegetable margarine (food service plus household margarines) in Spain fell from 1.5 kg in 1990 to 1.06 kg in 1995 (32), although there was considerable regional variation. Taking the mean fat content (calculated from label declarations) of the margarines studied in this work (63.5%; the majority contained from 60 to 80%; only one product contained 40%) and the mean TFA content, the daily per capita consumption of TFA from vegetable margarines by Spaniards during the last 6 yr could be estimated at about 0.2 g/person/d. This value is much lower than those that have been reported in other European countries: 1.0–1.1 g/person/d in France (33) and between 1.5 (1991/1992) and 0.6 (1995) g/person/d in Austria (23). By taking into account that the total TFA intake in Spain is 2.1 g/d (34), the relative contribution of vegetable margarines is low (near to 10%). Moreover, there has been a slight decrease in total margarine intake in Spain over the last three years (Nielsen, A.C., personal communication).

In conclusion, although the mean intake of TFA by Spanish people was low, it seems that there could be a considerable range of intake among consumers to judge by the high variation in TFA of the margarines studied (CV = 96%) and the different regional patterns of intake. The high variation in TFA content would indicate that not all margarine producers follow the same dietary guidelines. On the other hand, it is very difficult for consumers to judge the nutritional value of a margarine from label indications.

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