

# *Trans*-18:1 Acids in French Tub Margarines and Shortenings: Recent Trends

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**ABSTRACT:** The fatty acid composition of twelve French tub margarines and three industrial shortenings was established with particular attention to their *trans*-18:1 acid content. Four of the twelve margarines (including two major brands, with 60% of market share) were devoid of *trans* isomers, one contained less than 2% *trans*-18:1 acids, whereas the seven others had a mean content of  $13.5 \pm 3.6\%$  *trans* isomers. Four years ago, no margarines with 0% *trans*-18:1 acids could be found. It is deduced that the recent Dutch and American studies on possible effects of *trans* acids on human health (serum cholesterol, heart disease risks) may have had some influence on French margarine manufacturers. Presently, an average French tub margarine contains only 3.8% of *trans*-18:1 acids instead of 13% four years ago. To protect brand names, some manufacturers have replaced partially hydrogenated oils with tropical fats or fully hydrogenated oils. On the other hand, two of the three shortenings had high levels of *trans*-18:1 acids: 53.5 and 62.5%. This last value, obtained for a sample of hydrogenated arachis oil, seems to be one of the highest values ever reported for edible hydrogenated oils. In this sample, *trans*-18:1 plus saturated acids accounted for 85% of total fatty acids. This would indicate that shortening producers and users are not yet aware of recent dietary recommendations, probably because these products are not easily identifiable by consumers in food items, in contrast to margarines.

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Since the discovery of the industrial process of partial hydrogenation of oils by Normann, a German researcher, at the beginning of the century, the use of chemically hardened fats for human consumption always has been a matter of debate among nutritionists (1). Until recently, little evidence was found concerning any adverse effects of these modified fats on human health. Since 1990, however, several papers have appeared that cast some doubt on the harmlessness of partially hydrogenated oils, more particularly of their *trans* acids. Dutch studies (2,3) have shown that ingestion of relatively large amounts of such geometrically modified unsaturated acids by human volunteers may impair the distribution of

cholesterol among plasma lipoproteins, in such a way that they might increase heart disease risks. This potentiality was further supported by epidemiological studies from Harvard University (4,5), in which a significant positive correlation was found between margarine consumption and increased heart disease risks. In the latter studies, the range of *trans* acid consumed was in the range of that found in European countries (1,6), and far less than in the Dutch studies.

Although these results are still controversial, they are a cause for concern, and it is useful to determine whether the *trans* studies have had any impact on margarine producers. Four years ago, we analyzed the *trans*-18:1 acid content of French margarines and related products. Although we did not fully publish our results, we were unable to find 0%-*trans* margarines. The mean content at that time was around 13% (7). We know that French margarine producers are aware of and concerned about the *trans*-acid problem. Thus, we decided in this study to conduct a second investigation of the *trans*-acid content of present commercial margarines, after the publication of the Dutch and American studies. Our results clearly show that the major French margarine producers (subsidiaries of two multinational companies) have considerably lowered the *trans*-acid content in their products, and that a trend toward 0%-*trans* margarines is evident.

## MATERIALS AND METHODS

**Samples.** Twelve different samples of tub margarines (Table 1), including major national brands, were purchased from several local supermarkets in Bordeaux, France. Shortenings were kindly provided by an industrial food manufacturer who wishes to remain anonymous. All samples were mixtures of vegetable oils, either in their native state or hydrogenated (fully or partially).

**Preparation of fat solutions.** An aliquot of margarine (containing ca. 800 mg fat) weighed in a 50-mL Teflon beaker was dispersed in 10 mL of isopropanol with an Ultra-Turrax T 25 equipped with an S 25 N 10 G shaft (Janke & Kunkel GmbH & Co. KG, Staufen, Germany). Hexane (15 mL) was added and the suspension was dispersed a second time. Anhydrous Na<sub>2</sub>SO<sub>4</sub> was added and the suspension was dispersed a third time. An aliquot (2.5 mL) of the supernatant was withdrawn with a 5-mL all-glass syringe and filtered into a Teflon-lined, screw-capped 100 × 18 mm Pyrex tube through a disposable

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**TABLE 1**  
Margarine Composition as Indicated on the Label

Sample	Oils
1	Corn germ, palm, rapeseed, palm kernel
2	Sunflower, palm, palm kernel, safflower
3	Sunflower, palm
4	Rapeseed, palm, palm kernel, soybean, sunflower
5	Sunflower, palm kernel, palm, safflower
6	Sunflower, palm, rapeseed
7	Sunflower, rapeseed, coconut, palm kernel
8	Sunflower, palm, rapeseed
9	Sunflower, coconut, palm kernel
10	Sunflower, palm, palm kernel
11	Sunflower, palm <sup>a</sup> , rapeseed
12	Sunflower, palm, palm kernel

<sup>a</sup>Most probably palm kernel or coconut oil; see the 12:0 acid content in Table 2.

microfiltration unit (Millex-GV, 0.2  $\mu\text{m}$  pore size; Millipore, Molsheim, France) (8).

**Preparation of fatty acid isopropyl esters (FAIPE).** FAIPE were prepared essentially as described previously (6,7). Isopropanol (1.8 mL) and 250  $\mu\text{L}$  of concentrated  $\text{H}_2\text{SO}_4$  were added to the clear fat solutions, prepared in duplicate. The tubes were tightly capped and vigorously shaken, and the reaction was allowed to proceed at 100°C for 1 h. At the end of the reaction, the tubes were cooled, and 5% aqueous NaCl (5 mL) was added. The tubes were vortexed for *ca.* 30 s and allowed to stand for *ca.* 1 min. The upper phase was withdrawn and replaced by an equal volume of pure hexane. After vortexing a second time, the upper phase was withdrawn and pooled with the first one. A third extraction was performed in the same manner. To prepare FAIPE with shortenings, two drops of the melted fat were introduced into a Teflon-lined screw-capped tube to which were added 1.5 mL of hexane, 2.8 mL of isopropanol, and 250  $\mu\text{L}$  of concentrated  $\text{H}_2\text{SO}_4$ . The tubes were then handled as for margarines.

**Silver-ion thin-layer chromatography (AG-TLC) fractionation of FAIPE.** FAIPE (typically 0.5 mL of the FAIPE solutions) were fractionated by TLC on silica-gel plates (20  $\times$  10 cm) impregnated with  $\text{AgNO}_3$ . Commercial precoated plates (DC-Vertigplatten Kieselgel H; Merck, Darmstadt, Germany) were dipped in a 5% (wt/vol)  $\text{AgNO}_3$  solution in acetonitrile for 20 min, partially air-dried and activated at 120°C for 20 min. The developing solvent was hexane/diethyl ether (90:10, vol/vol). At the end of the chromatographic runs, the plates were air-dried and sprayed with a 0.2% (wt/vol), 95% ethanolic solution of 2',7'-dichlorofluorescein. The plates were viewed under ultraviolet light. When *trans*-18:1 acids had to be quantitated, the bands corresponding to the saturated ( $R_f = 0.8$ ) and *trans*-monoenoic acids ( $R_f = 0.7$ ) were scraped off together into a test tube. To the gel was added successively 1.5 mL of methanol, 2 mL of hexane, and 1.5 mL of a 5% (wt/vol) aqueous solution of NaCl. Thorough mixing followed each addition. After standing for *ca.* 1 min, the upper hexane phase was withdrawn and concentrated under a stream of  $\text{N}_2$  in a waterbath (40°C). The residue was dissolved in a

small volume of hexane (generally 200  $\mu\text{L}$ ) for subsequent analyses.

**Gas-liquid chromatography (GLC) of FAIPE.** FAIPE were analyzed on a Carlo Erba 4160 chromatograph, fitted with a flame-ionization detector (FID), a split injector, and an LT 430 temperature programmer (Carlo Erba, Milano, Italy). Separations were obtained on a CP Sil 88 fused-silica capillary column (50 m  $\times$  0.25 mm i.d., 0.20  $\mu\text{m}$  film; Chrompack, Middelburg, The Netherlands). For the separation and quantitation of total FAIPE, the column temperature was maintained at 135°C for 6 min, and then increased at 5°C/min to 185°C and maintained at this point until the end of the chromatographic runs (45 min). When saturated plus *trans*-monoenoic acids had to be analyzed for the quantitative determination of *trans*-18:1 acids, the column was operated under the same conditions. The inlet pressure of the carrier gas (helium) was 120 kPa. The detector and injector were held at 250°C. *Trans*-18:1 acids were quantitated using 16:0 and 18:0 acids as internal standards (6,7).

## RESULTS AND DISCUSSION

**Tub margarines.** The fatty acid composition of a representative collection of French tub margarines is summarized in Table 2. Four of the twelve samples were devoid of *trans*-18:1 acids, whereas four years ago we were unable to find such *trans*-free margarines (7). One sample contained only 1.5% of *trans*-18:1 acids (less than in butterfat). The *trans*-18:1 acid content in the seven other samples varied from 7.2 to 17.6%, with a mean of  $13.5 \pm 3.6\%$ . This is a similar value to that established four years ago (7).

Incidentally, we wish to insist on the methodology used in the present study. If one compares results obtained by direct, single-step GLC of FAIPE, and results obtained by coupling Ag-TLC and GLC of FAIPE, one finds that direct GLC gives *trans*-18:1 acid values that are 12–28% lower than the second method used in the present study (results not shown). Although popular, direct GLC, even on highly efficient columns, is inappropriate for the quantitation of *trans*-18:1 acids, generally giving underestimates.

On average, French tub margarines typically contain one-third saturated plus *trans*-18:1 acids, one-fourth *cis*-monoenoic acids, and two-fifths *cis*-polyunsaturated acids (Table 3). However, the range for these acids varies widely. For each of the main nutritional classes, the maximum values are approximately twice the minimum values (Table 3). The 0%-*trans* margarines (samples 5, 7, 9, and 11) are similar in composition to other margarines in regard to the main nutritional categories of fatty acids: same mean content of saturated, *cis*-monoenoic, and *cis*-polyunsaturated acids. This demonstrates that there is no need to incorporate partially hydrogenated oils in margarines, since hydrogenated oils can be replaced by palm, palm kernel, or coconut oils, either in their native state, or after complete hydrogenation. In 0%-*trans* margarines, there is a significantly higher level of 12:0 and 14:0 acids than in other margarines, but not of 16:0 acid. Palm

**TABLE 2**  
Composition of Fatty Acids (wt% of total) in French Tub Margarines

Fatty acid	Sample <sup>a</sup>											
	1	2	3	4	5	6	7	8	9	10	11	12
6:0	— <sup>b</sup>	trace <sup>c</sup>	—	0.08	trace	—	0.23	—	0.20	—	trace	—
8:0	0.03	0.35	0.02	0.85	0.64	0.07	2.82	—	1.60	0.02	0.44	0.09
10:0	0.03	0.28	0.03	0.61	0.43	0.05	2.17	0.02	1.23	0.03	0.37	0.07
12:0	0.26	3.72	0.15	4.94	5.74	0.51	20.87	0.06	11.73	0.08	4.97	0.96
14:0	0.34	1.36	0.32	1.94	1.99	0.54	7.09	0.27	4.28	0.21	1.65	0.48
16:0	15.64	11.46	11.94	9.80	10.20	15.76	7.11	13.13	6.69	10.84	9.46	9.46
16:1	0.11	0.08	0.09	0.07	0.07	0.11	0.07	0.07	0.06	0.06	0.05	0.06
17:0	0.06	0.05	0.05	0.05	0.04	0.05	0.03	0.04	0.04	0.05	0.04	0.04
18:0	4.83	8.06	6.61	7.52	8.41	6.23	6.79	5.93	6.40	6.72	8.48	7.75
<i>cis</i> -18:1	26.57	20.30	21.98	32.38	20.39	30.25	22.26	27.42	18.69	21.37	20.34	25.45
<i>trans</i> -18:1	13.60	1.53	15.53	17.62	—	7.23	—	13.52	—	16.61	—	10.57
18:2 <i>iso</i> . <sup>d</sup>	0.31	0.15	0.63	1.40	0.06	0.15	0.27	0.60	0.15	0.56	0.08	0.46
18:2 <i>n</i> -6 <sup>e</sup>	37.11	52.12	41.53	21.57	51.60	37.16	28.00	38.08	48.38	42.02	52.92	43.34
18:3 <i>n</i> -3	0.45	0.10	0.26	0.08	trace	0.88	1.60	0.26	0.09	0.45	0.16	0.28
20:0	0.40	0.26	0.26	0.42	0.22	0.32	0.24	0.40	0.25	0.25	0.23	0.28
20:1	0.18	0.12	0.11	0.35	0.09	0.21	0.29	0.16	0.10	0.09	0.14	0.11
Others <sup>f</sup>	0.26	0.06	0.49	0.32	0.12	0.48	0.16	0.04	0.11	0.64	0.67	0.60

<sup>a</sup>Sample numbers refer to Table 1.

<sup>b</sup>Not detected.

<sup>c</sup>Trace amounts (less than 0.02%).

<sup>d</sup>Geometrical and/or positional isomers (*iso.*) of 18:2*n*-6 acid.

<sup>e</sup>18:2*n*-6 and 18:3*n*-3 acids are the natural all-*cis* isomers.

<sup>f</sup>Include 22:0 acid in some instances.

oil (like palm kernel oil) is practically present in all brands (Table 1), but the content of 16:0 acid remains reasonable (equal to or less than 15% of total fatty acids).

Four margarine brands, indicating on their label the presence of hydrogenated oils (samples 5, 7, 9, and 11), are devoid of *trans*-18:1 acids, probably because one of their component oils was fully hydrogenated. On the other hand, the other brands also mention the presence of partially hydrogenated oils, and they contain *trans*-18:1 acids (Table 2). Consequently, a simple reading of the label does not indicate the presence or absence of *trans*-18:1 acids.

With one exception (sample 4), linoleic acid geometrical and/or positional isomers are less than 0.6% of total fatty acids. We did not detect geometrical isomers of  $\alpha$ -linolenic acid, since with the exception of samples 6 and 7,  $\alpha$ -linolenic acid contents were generally less than 0.5%.

Two brands analyzed (samples 5 and 7) represent *ca.* 60% of the market share of tub margarines (9). They are now devoid of *trans*-18:1 acids. Four years ago, sample 5, the most popular brand in France, contained *ca.* 11% of *trans*-18:1 acids (results not shown). Sample 7 is a new product. Because tub margarines represent about 60% of all household margarines (stick and tub margarines) (9), this would imply that

more than one-third of margarines now sold in France are free of *trans*-18:1 acids. Surprisingly, the major French producer who sells the *trans*-free margarine brand (sample 5) continues to manufacture another popular brand that contains *ca.* 17% of *trans*-18:1 acids (sample 4).

The mean *trans*-18:1 acid content for samples other than samples 5 and 7 is  $9.6 \pm 7.0\%$ . Assuming an equivalent market share for each of these minor brands implies that the mean *trans*-18:1 acid content in French tub margarines is 3.8%, compared to *ca.* 13% four years ago. Accordingly, the average *trans*-18:1 acid content of French tub margarines is in the range of *trans*-18:1 acid levels in ruminant milk fats, beef meat fat, and tallow (6,7). However, an important difference between ruminant fats and tub margarines is the greater variability of the *trans*-18:1 acid content in the latter (0–17.6% instead of 1.5–6%). If one assumes that consumers are loyal to a given brand of margarine, some will unwittingly ingest variable amounts of *trans*-18:1 acids, whereas others will consume little or no *trans*-18:1 acids from household tub margarines. Moreover, there is a considerable regional heterogeneity in margarine consumption in France. Household purchases of margarine is 4.5 times higher in the north than in the southwest or the southeast regions of the country (10). One can therefore estimate that the consumption of *trans*-18:1 acids from household tub margarines can vary from 0 to 1.3 g/person/day, depending on the region and the margarine brand consumed. The problem is complicated further by the difficulty in establishing the real fate of household margarines because part of them are used for frying with unaccountable losses, whereas a portion is directly consumed either as such or in homemade foods. So, wastage and, consequently, con-

**TABLE 3**  
Minimum, Maximum, and Average Content of Fatty Acids (wt % of total) by Nutritional Categories in French Tub Margarines

Fatty acid category	Minimum	Maximum	Mean $\pm$ SD
Saturated + <i>trans</i> -18:1	25.64	47.35	33.56 $\pm$ 6.50
<i>Cis</i> -monoenes	18.85	32.80	24.19 $\pm$ 4.46
<i>Cis</i> -polyunsaturated	21.65	53.08	39.91 $\pm$ 13.82

sumption, will be different depending on the culinary use of margarines.

**Shortenings.** Margarines and shortening also are consumed *via* industrially processed foods (snacks, pastries, confectionery). In contrast to the United States, shortenings are not sold in France for household use, but only for industrial purposes. In France, it is estimated that shortening consumption is about one-half the total consumption of margarine (*ca.* 3.8 kg/person/year). It is assumed that processed foods that contain margarines or shortenings are more uniformly consumed throughout the country than household margarines, for which a greater disparity is observed. These foods would contribute, to a greater extent, to the total consumption of *trans*-18:1 acids, together with milk fat and other ruminant fats. It is estimated that a minimal consumption of *trans*-18:1 acids from these sources is in the range 2–2.5 g/person/day. Consequently, the range of *trans*-18:1 acids consumed by the French population would be 2–3.8 g/person/day, with a mean of *ca.* 2.8 g/person/day (6).

We were unable to find data on the production and consumption of shortenings in France, because most of these products are imported. To our knowledge, their fatty acid composition never has been described in the literature. The three samples we analyzed were provided by a manufacturer who used to employ these shortenings in health foods. As shown in Table 4, two of the three samples contain *trans*-18:1 acids in amounts higher than 50%, up to 62.5% of total fatty acids in one case (partially hydrogenated arachis oil). If *trans*-18:1 acids and saturated acids are summed, a total of 85% is reached for sample B (Table 4). Even more surprising, this shortening is marketed by the same manufacturer who recently launched the major 0%-*trans* margarine brand (sample 5). This attitude may be explained by the recent attention of dieticians and nutritionists on margarines, and the desire of manufacturers to protect their brand image. The problem of *trans* acids is not as acute in France as in North America, and margarines retain a good image. However, some producers have anticipated some eventual side effects of the Dutch (2,3) and American (4,5) conclusions on the possible health effects of *trans*-18:1 acids and on the possibility of regulations that could force them to include the *trans* fatty acid content in margarine labels. It can be concluded that these studies have had a major impact on several French margarine manufacturers. Whether this 0%-*trans* trend will continue and expand to other products remains to be seen, and continuous surveys must be performed.

On the other hand, shortenings are hidden in a large number of different food items where they cannot be identified by consumers. In this case, there is no brand image to protect and the producer remains anonymous. Except for the unavoidable minimum consumption of *trans*-18:1 acids from ruminants, consumers who wish to limit their *trans* acid consumption can now purchase 0%-*trans* margarines, but they also should avoid processed foods containing partially hydrogenated oils. Unfortunately, as shown in the present study, it is difficult for the consumer, and also for nutritionists and dieticians, to se-

**TABLE 4**  
**Composition of Fatty Acids (wt% of total) of Three French Shortenings**

Fatty acid	Sample <sup>a</sup>		
	A	B	C
12:0	0.09	0.03	0.19
14:0	0.39	0.10	0.65
16:0	11.09	11.25	29.20
<i>trans</i> -16:1 <sup>b</sup>	0.12	0.11	trace
<i>cis</i> -16:1	0.03	—	0.06
17:0	0.04	0.03	—
18:0	8.36	6.98	7.04
<i>trans</i> -18:1	53.53	62.49	26.05
<i>cis</i> -18:1	19.54	13.66	30.42
isom. 18:2 <sup>c</sup>	5.21	0.90	1.94
18:2n-6	0.47	0.17	4.15
20:0	0.59	1.17	0.30
<i>trans</i> -20:1 <sup>b</sup>	0.42	0.11	—
22:0	—	2.70	—
Saturated + <i>trans</i>	74.63	85.07	63.43

<sup>a</sup>A, partially hydrogenated soybean oil; B, partially hydrogenated arachis oil; C, mixture of partially hydrogenated palm, rapeseed, and soybean oils.

<sup>b</sup>Isolated in the *trans*-monoenoic fraction after silver-ion thin-layer chromatography.

<sup>c</sup>Geometrical and/or positional isomers of 18:2n-6 acid.

lect a 0%-*trans* margarine through a simple reading of the product label.

*Trans*-18:1 acids from ruminant fats and partially hydrogenated oils. Although we did not try to establish a complete isomer distribution profile of *trans*-18:1 acids in margarines and shortenings, we have calculated the proportion of the *trans*-16 isomer relative to total *trans*-18:1 isomers isolated by Ag-TLC. This 18:1 isomer is readily separated from all other 18:1 isomers by GLC (6,7) and it can be accurately quantitated. In a previous work based on literature data (6), it was estimated that this isomer should account on average for *ca.* 0.5% of total *trans*-18:1 isomers present in a partially hydrogenated oil. In the present study, we could experimentally establish that the mean proportion of the *trans*-16 18:1 isomer relative to total *trans*-18:1 acids in margarines containing partially hydrogenated oils and in shortenings is  $0.8 \pm 0.5\%$  ( $n = 11$ ; results not shown). In milk fat, the corresponding value is 8.1% ( $n = 60$ ), with seasonal variations being taken into account (7,11). In European countries, milk fats represent *ca.* 90% of the *trans*-18:1 acid intake from all ruminant fats (1,6). If one takes into account other ruminant fats (beef meat fat and tallow) which contain a significantly lower proportion of *trans*-16 18:1 isomer (6), the mean proportion of the *trans*-16 18:1 isomer relative to total *trans*-18:1 isomers becomes 7.8%. Human milk lipids are a good source to estimate the quantities of *trans*-18:1 acids ingested daily from hydrogenated oils and ruminant fats (6,12). To estimate the respective contributions of each of the two sources to the daily intake of *trans*-18:1 acids, we have established the following equation:

$$7.8 \times X + 0.8 \times Y = Z \quad [1]$$

where  $X$  stands for the proportion of *trans*-18:1 acids from ruminant fats,  $Y$  for that from partially hydrogenated oils, and  $Z$  for the proportion, as weight percent, of the *trans*-16 18:1 isomer relative to total *trans*-18:1 isomers in human milk.  $Z$  must be determined experimentally. Because  $X + Y = 1$ , Equation 1 becomes:

$$Y = 1.1 - Z/7 \quad [2]$$

When applied to the milk of French women, for which  $Z = 4.9\%$  ( $n = 10$ ) (6), Equation 2 gives  $Y = 0.4$ . This means that the proportion of *trans*-18:1 acids from partially hydrogenated oils is 40%, and consequently that from ruminant fats is 60%. The same conclusions were reached using consumption data (6). If the absolute consumption of *trans*-18:1 acids from ruminant fats is known [such data have been published for European countries (6,7)], then the absolute consumption of *trans*-18:1 acids from partially hydrogenated oils also is known.

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