Contribution of *trans*-18:1 Acids from Dairy Fat to European Diets

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Twelve commercial samples of French butter, purchased in October–November, and 12 other samples, purchased in May–June, were analyzed with particular attention to their *trans*-octadecenoic acid contents. The isomeric fatty acids were quantitated by a combination of gas–liquid chromatography (GLC) of total fatty acids as isopropyl esters on a polar capillary column (CPSil 88) and of silver nitrate-impregnated thin-layer chromatography followed by GLC of the pooled saturated (used as internal standards) and *trans*-octadecenoic acid fractions. Autumn butters contained 3.22 ± 0.44% *trans*-octadecenoic acids (relative to total fatty acids), whereas those collected during the spring contained 4.28 ± 0.47% ($P < 0.01$). Minimum and maximum values for the two sets of butters were 2.46 (autumn) and 5.10% (spring), respectively. The annual mean value for the *trans*-octadecenoic acid content in all butter samples was 3.8% of total fatty acids (*ca.* 2% for the *trans*-11 18:1 acid). This value allows calculation of the daily individual intake of *trans*-octadecenoic acids from dairy products by populations of member states of the European Economic Community (EEC). It varies from 0.57 g (Portugal) to 1.66 g (Denmark). The mean value for the twelve countries of the EEC is 1.16 g/person/d, which is close to data published for the United States. In France, the consumption of *trans* octadecenoic acids from dairy fat is higher than that from margarines (*ca.* 1.5 vs. 1.1 g/person/d).

KEY WORDS: Butter, dairy fat, fatty acid composition, gas–liquid chromatography, isopropyl esters, milk, *trans*-octadecenoic acids.

It is generally assumed that the major contribution to the daily intake of *trans*-unsaturated fatty acids by humans is industrial partially hydrogenated fats and oils. Ruminant milk and meat fat would contribute to this intake at a comparatively more moderate level. This is certainly true for the United States' population, for whom it has been estimated that 90–95% of *trans* fatty acids in the tissues come from partially hydrogenated vegetable fats and oils (1). But there is some doubt as to whether this situation exists in other countries, for example, in some of the member states of the European Economic Community (EEC).

Most studies conducted with different animal species have concluded that *trans*-octadecenoic acids do not exert any major adverse effect on health. However, recent studies (2,3) with human volunteers have demonstrated that partially hydrogenated soybean oils produced significantly higher serum cholesterol and low-density lipoprotein (LDL) cholesterol, while reducing high-density lipoprotein (HDL) cholesterol, than did *cis*-unsaturated acid-containing diets. In these studies, the mean daily per capita intake of *trans* fatty acids was 33 (2) or 24 g (3). Because some data indicate that such effects are dose-dependent (3), we believe that it may be useful to evaluate the true mean amount of *trans* fatty acids ingested every day, with accuracy and reliability. Such evaluations may also be of some help for further epidemiological studies.

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In the present report, we have focused our attention on the *trans*-octadecenoic acid content of bovine milk fat, because literature data are confusing about this point. Bovine milk is the main source of ruminant milk fat, except perhaps for some Mediterranean countries, where the consumption of ewe and goat cheese may be too large to be negligible. In the literature, varying values of 3 (1,4), 5 (5,6) or even 7% (7) have been used to estimate the mean daily intake of *trans*-octadecenoic acids originating from dairy fats. These mean contents lie well inside the extreme values of less than 1% for winter butterfat (8) to 8–9% for summer butterfat (8–10). Seasonal variations of the content of *trans*-octadecenoic acids in dairy fats, linked to the feeding conditions of the cattle, have been recognized for several decades (9). However, superimposed on these natural variations are spurious variations of analytical origin. deMan and deMan (11) have observed that values of *ca.* 2 or 7% could be obtained for the same sample of butterfat, depending only on the method employed [uncorrected infrared (IR) absorptions at 970 cm^{-1} of methyl esters and triglycerides, respectively]. With a few samples, Smith *et al.* (12) also observed that results obtained by IR spectroscopy could be twice those determined by gas–liquid chromatography (GLC) coupled with argentation thin-layer chromatography (Ag-TLC). Consequently, data given by different authors (using different methods) are to be taken and compared with great caution. Mean values of the *trans*-octadecenoic acid content of dairy fat used in the literature (1,4–7) to estimate *trans* acid consumption appear to be chosen in a quite arbitrary manner. Thus, there is an evident need for more accurate, homogeneous and direct determination of the *trans*-octadecenoic acid content of dairy fats. Seasonal variations of the *trans* content must be taken into account in these determinations.

Isolation of *trans*-monoenoic acids by Ag-TLC and their subsequent analysis by GLC in the presence of an internal standard are generally considered both tedious and time-consuming. However, these procedures appear to be well adapted to accurately quantitate these isomeric fatty acids. To be conclusive, data provided by these means must be quantitative. This implies that all fatty acids, including the shorter ones, should be actually taken into account. For quantitative evaluations, convenient correction factors have to be applied to raw data provided by the integrator linked to the flame-ionization detector, unless such correction factors are avoided by a judicious choice of the alcohol used for esterification of fatty acids. As starting material, we have used butters, the fatty acid compositions of which are representative of dairy fat triglycerides. To take into account the seasonal variations of the *trans* content, butters were purchased in both spring and autumn. In this study, we assume that the mean *trans* content of French butters does not differ significantly from that of butters of other European countries. This assumption is based on the fact that cattle feeding varies little from one country to another. For comparison purposes, we have also analyzed several samples of margarines and margarine-like foods, including major national brands. This study was necessary because data concerning the *trans* unsaturation content of French butters and margarines are ancient and particularly scarce (6

samples of butters and 5 samples of margarines analyzed about 30 years ago) (10,13).

EXPERIMENTAL PROCEDURES

Samples. Twenty-four samples of fresh butter, including two samples of low-calorie butters with 42 and 61% butterfat, were purchased in local supermarkets during two periods, October–November 1992 and May–June 1993. Most of the samples were major national brands and are thus representative of French butters. For comparison purposes, 17 samples of margarines and margarine-like foods (low-calorie margarines) were also purchased and analyzed for their *trans*-octadecenoic acid content. Pure 16:0, 18:0 and *trans*-9 18:1 acids were from Sigma Chemical Company (St. Louis, MO).

Preparation of fat solutions. Eight hundred to 850 mg of butter or margarine, or a portion of low-calorie butter or margarine containing the same amount of fat, were dispersed in 10 mL isopropanol under magnetic stirring. Hexane (15 mL) was added, followed by a sufficient amount of anhydrous Na_2SO_4. A portion (2.5 mL) of the resulting suspension was withdrawn with an all-glass syringe and filtered into a Teflon-lined, screw-capped tube through a disposable microfiltration unit (Millex-GV, 0.2 μm pore size; Millipore, Molsheim, France) (14,15).

Preparation of fatty acid isopropyl esters (FAIPE). Isopropanol (1.8 mL) and 0.25 mL concentrated H_2SO_4 were added to the clear filtrates. The tubes were tightly capped and vigorously shaken, and the reaction was allowed to proceed in a boiling waterbath for 1 h (16). At the end of the reaction, the tubes were cooled, and distilled water (5 mL) was added. The tubes were vortexed for *ca.* 30 s and allowed to stand for 1 min or so. The upper phase was withdrawn and replaced by an equal volume of hexane. After vortexing and standing a second time, the upper phase was withdrawn and pooled with the first one. A third extraction was performed in the same manner (16).

Ag-TLC. FAIPE were fractionated by TLC on silica-gel plates impregnated with $AgNO_3$. Commercial precoated plates (DC-Vertigplatten Kieselgel H; Merck, Darmstadt, Germany) were dipped in a 5% (wt/vol) $AgNO_3$ solution in acetonitrile for 20 min, partially air-dried and activated at 120°C for 30 min (17). The developing solvent was the mixture hexane/diethyl ether/acetic acid (90:10:1, vol/vol/vol). At the end of the chromatographic runs, the plates were briefly air-dried and sprayed with a 0.2% (wt/vol) ethanolic solution of 2′,7′-dichlorofluorescein. After examination of the plates under ultraviolet light, the bands corresponding to the saturated and *trans*-monoenoic acids were scraped off in an aluminum foil, and the gel from the two bands was transferred into the same test tube. To the gel were added successively 1.5 mL methanol, 2 mL hexane and 1.5 mL of a 5% (wt/vol) aqueous solution of NaCl (17). Thorough mixing followed each addition. After standing for *ca.* 1 min, the hexane phase was withdrawn almost quantitatively and concentrated under a light stream of N_2 in a waterbath (40°C). The residue was dissolved in a small volume of hexane and used as such for further GLC analyses. Some *cis*-monoenoic acid fractions were treated in the same way for identification purposes.

GLC. Analyses of FAIPE by GLC were carried out on a Carlo Erba 4130 chromatograph, fitted with a flame-ionization detector and a split injector and coupled to an LT 430 temperature programmer (Carlo Erba, Milano, Italy). Separations were performed on a CP Sil 88 fused-silica capillary column (50 m × 0.25 mm i.d., 0.20 μm film; Chrompack, Middelburg, The Netherlands). For the separation of total FAIPE prepared with butterfat, the column was operated at 65°C for 6 min, the temperature was then increased at a rate of 5°C/min up to 185°C and left at this point until the end of the analysis. When saturated plus *trans*-monoenoic acids had to be analyzed, the column was operated either under the preceding conditions (for identification purposes) or isothermally at 160°C (for quantitative determinations). In both cases, the temperature of the detector and the injector was 250°C, and the inlet pressure of the carrier gas (helium) was 100 kPa. Quantitative analyses were performed with an SP 4290 integrator (Spectra Physics, San Jose, CA). *t*-Test was applied for statistical evaluations.

RESULTS AND DISCUSSION

trans-*Octadecenoic acid content of butterfat.* Table 1 compares the fatty acid composition of a weighed mixture of pure 16:0, 18:0 and *trans*-9 18:1 acids that were transformed into FAIPE, before and after their fractionation by Ag-TLC. In the second case, silica-gel bands containing saturated and *trans*-monoenoic acids were scraped individually and then pooled in the same tube before elution of FAIPE from the gel. Table 1 shows that there is no discrimination in recovery of either saturated or *trans*-monounsaturated FAIPE separated and eluted from the gel according to our procedures. This fully justifies the use of 16:0 and 18:0 acids present in the butterfat itself as internal standards to quantitate *trans*-octadecenoic acids.

Approximately 70 components were taken into account by the integrator coupled with the chromatograph, of which 40 could be properly identified, from 4:0 to conjugated 18:2 acids. The 30 remaining unidentified peaks (referred to as "Others" in Table 2) together accounted for less than 2% of total fatty acids. The chromatographic runs were stopped when peaks with retention times in the

TABLE 1

Comparison of the Proportions of Fatty Acids in a Weighed Mixture of Fatty Acids Before and After Their Fractionation as Isopropyl Esters by Argentation Thin-Layer Chromatography (Ag-TLC)

Fatty acid	% Before Ag-TLC[a] (n = 3)[c]	% After Ag-TLC[b] (n = 2)
16:0	51.7 ± 0.3	51.3 ± 0.5
18:0	19.6 ± 0.1	19.8 ± 0.2
trans-9 18:1	28.7 ± 0.1	28.9 ± 0.5
trans-9 18:1/16:0	0.56	0.56
trans-9 18:1/18:0	1.46	1.46

[a]Proportions as weight percentages of fatty acids after isopropylation.
[b]Proportions as weight percentages of fatty acid isopropyl esters after fractionation by Ag-TLC and pooling of the separated fractions before extraction from the gel according to the sequence of solvents described in the Experimental Procedures section.
[c]Number of determinations.

TABLE 2

Fatty Acid Compositions as Weight Percentages of Total Fatty Acids of Fat from French Butters Collected during October–November and May–June

Fatty acid	Collection period					
	October–November			May–June		
	Mean ± SD[a]	Minimum values	Maximum values	Mean ± SD	Minimum values	Maximum values
4:0	4.29 ± 0.24	3.77	4.62	3.83 ± 0.13*[b]	3.58	4.01
5:0	0.04 ± 0.01	0.02	0.05	0.03 ± 0.01	0.02	0.03
6:0	2.55 ± 0.11	2.33	2.79	2.38 ± 0.08*	2.28	2.54
7:0	0.03 ± 0.01	0.02	0.04	0.03 ± 0.01	0.02	0.03
8:0	1.52 ± 0.08	1.40	1.66	1.42 ± 0.07*	1.35	1.96
9:0	0.04 ± 0.01	0.03	0.05	0.03 ± 0.01	0.03	0.04
10:0	3.28 ± 0.20	2.98	3.62	3.14 ± 0.19	2.90	3.53
10:1	0.35 ± 0.02	0.31	0.37	0.30 ± 0.01*	0.28	0.32
11:0	0.06 ± 0.01	0.05	0.07	0.06 ± 0.01	0.04	0.07
12:0	3.75 ± 0.27	3.42	4.40	3.57 ± 0.26	3.23	4.10
13:0	0.10 ± 0.01	0.08	0.12	0.10 ± 0.01	0.09	0.11
iso 14:0	0.12 ± 0.02	0.10	0.15	0.13 ± 0.01	0.10	0.15
14:0	11.32 ± 0.33	10.73	11.74	11.03 ± 0.36	10.37	11.59
iso 15:0	0.27 ± 0.02	0.24	0.33	0.34 ± 0.04*	0.28	0.43
a-iso 15:0[c]	0.67 ± 0.10	0.57	0.77	0.58 ± 0.04*	0.53	0.68
14:1	0.88 ± 0.07	0.68	0.94	0.91 ± 0.07	0.80	0.98
15:0	1.13 ± 0.08	0.99	1.30	1.15 ± 0.04	1.08	1.24
iso 16:0	0.24 ± 0.03	0.21	0.33	0.27 ± 0.02*	0.25	0.30
16:0	29.27 ± 1.91	24.09	31.46	27.05 ± 1.37*	24.95	29.17
trans-16:1	0.08 ± 0.03	0.04	0.13	0.13 ± 0.02*	0.10	0.16
cis-7 16:1	0.19 ± 0.04	0.11	0.28	0.22 ± 0.02	0.19	0.25
iso 17:0	0.34 ± 0.02	0.30	0.37	0.42 ± 0.02*	0.39	0.45
cis-9 16:1	1.50 ± 0.13	1.31	1.70	1.29 ± 0.07*	1.18	1.37
a-iso 17:0	0.45 ± 0.03	0.41	0.49	0.51 ± 0.02*	0.47	0.54
cis-11 16:1	0.17 ± 0.03	0.12	0.21	0.16 ± 0.01	0.14	0.18
17:0	0.65 ± 0.03	0.58	0.71	0.70 ± 0.04*	0.63	0.78
cis-8 17:1	0.06 ± 0.01	0.03	0.08	0.09 ± 0.02*	0.06	0.13
cis-9 17:1	0.29 ± 0.03	0.23	0.29	0.29 ± 0.02	0.27	0.33
18:0	9.61 ± 0.54	9.05	10.83	10.96 ± 0.69*	10.03	12.47
trans + *cis* 18:1[d]	22.62 ± 1.58	20.81	26.56	24.00 ± 1.03**	22.44	25.72
isom. 18:2[e]	0.68 ± 0.12	0.50	0.89	0.93 ± 0.08*	0.79	1.08
18:2n-6[f]	1.35 ± 0.15	1.13	1.59	1.17 ± 0.15*	0.98	1.46
20:0	0.13 ± 0.01	0.11	0.15	0.15 ± 0.02	0.11	0.18
18:3n-3	0.46 ± 0.09	0.33	0.66	0.60 ± 0.07*	0.48	0.67
20:1	0.12 ± 0.01	0.09	0.13	0.10 ± 0.02	0.08	0.13
conj. 18:2[g]	0.48 ± 0.10	0.37	0.71	0.74 ± 0.16*	0.51	1.03
Others	0.91 ± 0.36	0.32	1.40	1.26 ± 0.31	1.02	1.82

[a]Mean ± SD of 12 samples.
[b]Values with one or two asterisks are significantly different ($P < 0.01$ or $P < 0.02$, respectively) from corresponding values for autumn butters (*t*-test).
[c]Values for a-iso 15:0 and 14:1 acids, not separated during gas–liquid chromatographic analysis of total fatty acid isopropyl esters (FAIPE), are obtained by combining results of analyses of total FAIPE and of analyses of saturated plus *trans*-monoenoic FAIPE, and using 15:0 acid as internal standard.
[d]Sum of all peaks corresponding to *cis* or *trans* 18:1 acids, or to mixtures of these.
[e]Group of several peaks that do not correspond to either saturated, *cis* or *trans*-monoenoic acids and that are supposed to be *cis* and/or *trans* isomers of methylene and/or nonmethylene-interrupted octadecadienoic acids. May contain trace amounts of 19:0 acid.
[f]18:2n-6 and 18:3n-3 acids are the all-*cis* isomers.
[g]Conjugated 18:2 acid(s).

neighborhood of 20:3n-6 acid were eluted off from the column (at *ca.* 48 min). According to Strocchi and Holmann (18), peaks eluting after this fatty acid represent less than 1% of total fatty acids from butterfat. Consequently, data obtained in the present study take into account more than 99% of butterfat fatty acids.

The use of FAIPE instead of fatty acid methyl esters (FAME) is interesting for several reasons. First of all, there is no solvent evaporation step, and thus no loss of the more volatile fatty acid esters. All short chains, including butyric acid isopropyl ester, are quantitatively recovered from the reactional mixture after three extractions with hexane (16). Second, butyric acid isopropyl ester is completely separated from the solvents (hexane and isopropanol) by GLC (Fig. 1), and the corresponding peak area is accurately computed by the integrator. Finally, there is no need to apply correction factors to transform peak area percentages into fatty acid weight percentages. Figure 2 shows the theoretical conversion factors (relative to 18:0 acid) needed to transform peak area percentages into fatty acid weight percentages. These factors have been calculated according to the formula established by

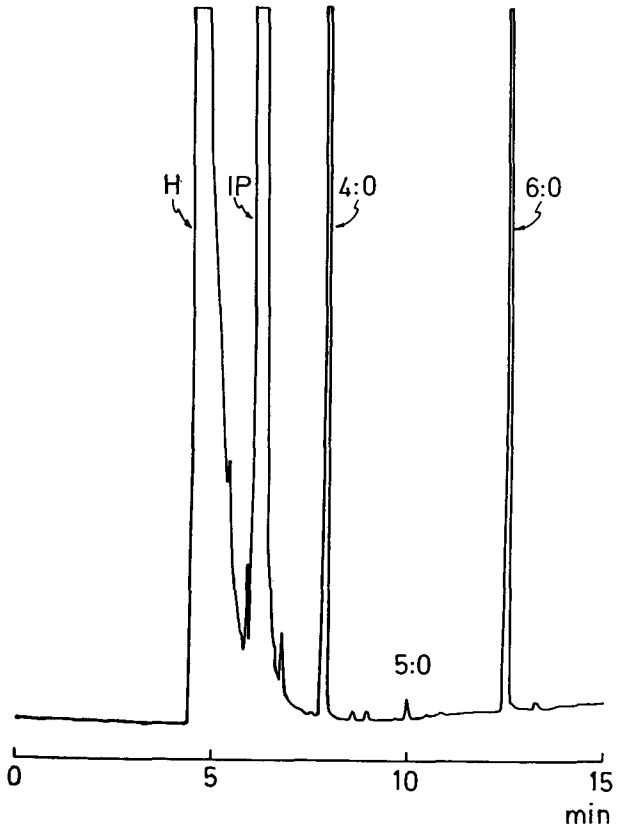

FIG. 1. Partial chromatogram of fatty acid isopropyl esters prepared with butterfat, showing the habitual resolution obtained between the solvents (H, hexane; IP, isopropanol) and butyric acid isopropyl esters. Analysis on a CP Sil 88 capillary column with temperature programmed from 65°C (6 min) to 185°C at a rate of 5°C/min.

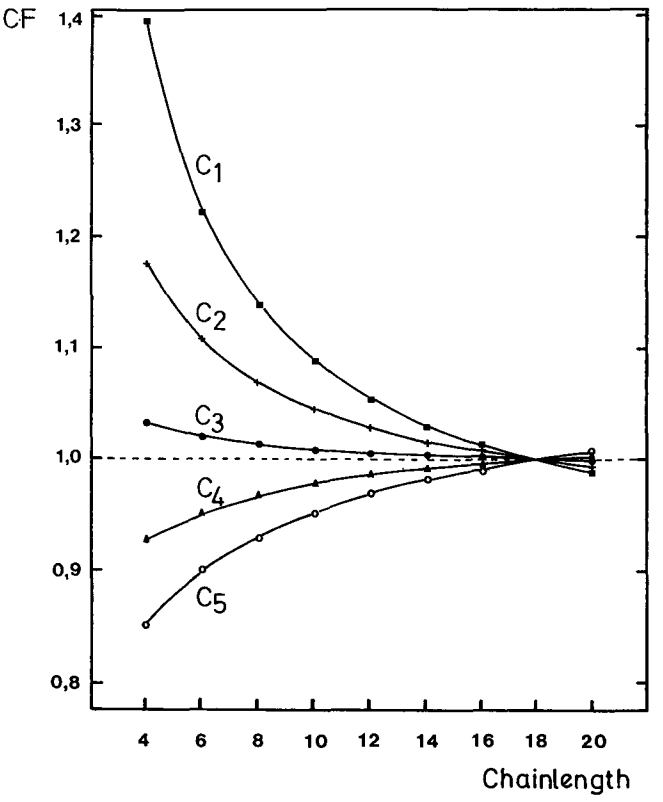

FIG. 2. Conversion factors (CF) used to transform peak area percentages into fatty acid weight percentages as a function of the chainlength of saturated fatty acids and of the number of carbon atoms (C_1 to C_5) of the alcohol used to esterify the fatty acids.

Wolff and Fabien (16), and are based on the principles of Ackman and Sipos (19). The curves shown in Figure 2 indicate that esters of C_3-alcohols have conversion factors close to 1, independently of the chainlength of the acids. No other alcohols give such results. For example, the conversion factor relative to 18:0 acid for butyric acid isopropyl ester is only 1.03, instead of 1.39 for the corresponding FAME (16). So there is no need to apply conversion factors in current practice to transform peak area percentages into fatty acid weight percentages.

After fractionation of FAIPE by Ag-TLC, *trans*-monoenoic acids were generally separated from their *cis* counterparts by a distance of *ca.* 1 cm. Thus, there is no overlap between the two fractions. The gas-liquid chromatographic pattern of *trans*-octadecenoic acids isolated from butterfat (Fig. 3) is relatively constant. There is little variation in the proportions of the different parts of this profile with the season (Table 3). The main component is the *trans*-11 18:1 acid (vaccenic acid) (20–23). It represents slightly more than one-half of total *trans*-octadecenoic acids (Table 3), which is in good agreement with literature data (20–23).

The detailed fatty acid compositions of butterfat from autumn and spring butters are presented in Table 2. Some small but significant variations are observed between the two sets of data. Short fatty acids, such as 4:0, 6:0, 8:0

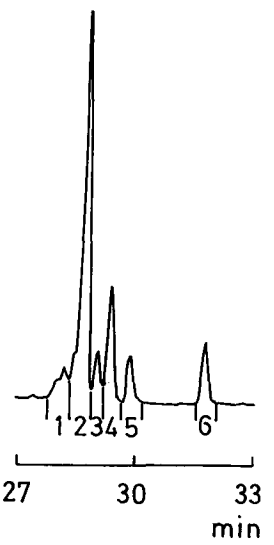

FIG. 3. Characteristic profile of *trans*-octadecenoic acid isopropyl esters isolated by argentation thin-layer chromatography and analyzed on a CP Sil 88 capillary column (160°C; helium pressure, 100 kPa). The proportions of parts 1–6 are given in Table 3. Tentative identification of peaks according to Reference 20: 1, *trans*-6 to *trans*-9; 2, *trans*-10 plus *trans*-11: 3, *trans*-12 18:1; 4, *trans*-13 plus *trans*-14 18:1, 5, *trans*-15 18:1; 6, *trans*-16 18:1.

TABLE 3

Proportions (as percentages of total) of the Different Parts of the Gas–Liquid Chromatographic Profile of *trans*-Octadecenoic Acids Isolated by Argentation Thin-Layer Chromatography from Fatty Acid Isopropyl Esters Prepared with Butterfat

Peak number[a]	Tentative identification[b]	Proportions	
		Autumn	Spring
1	*trans*-6 to *trans*-9	9.6 ± 1.3	7.2 ± 0.8
2	*trans*-10 + *trans*-11	50.4 ± 3.9	58.2 ± 3.8
3	*trans*-12	7.2 ± 0.7	6.2 ± 0.7
4	*trans*-13 + *trans*-14	17.2 ± 1.6	15.4 ± 1.7
5	*trans*-15	6.6 ± 0.6	5.7 ± 0.6
6	*trans*-16	9.0 ± 1.2	7.3 ± 0.9

[a]Peak numbers refer to Figure 3.
[b]According to Reference 20.

TABLE 4

***trans*-Octadecenoic Acid Content (as weight percentages relative to total fatty acids) of French Butters Purchased in Autumn and in Spring**

Sample[a]	*trans*-18:1/16:0[b]	*trans*-18:1/18:0	Mean[c]
A1	3.46	3.31	3.39
A2	2.46	2.46	2.46
A3	3.50	3.53	3.52
A4	3.49	3.48	3.49
A5	3.28	3.23	3.25
A6	2.92	2.98	2.95
A7	3.16	3.49	3.33
A8	2.67	2.65	2.66
A9	3.03	2.95	2.99
A10	2.92	2.90	2.91
A11	4.03	3.80	3.92
A12	3.74	3.78	3.76
Mean ± SD	3.22 ± 0.45	3.21 ± 0.43	3.22 ± 0.44
S1	4.49	4.53	4.52
S2	4.57	4.64	4.61
S3	4.45	4.66	4.56
S4	4.83	4.36	4.61
S5	5.01	5.18	5.10
S6	4.02	4.04	4.03
S7	3.65	3.69	3.67
S8	4.52	4.64	4.58
S9	3.41	3.52	3.47
S10	4.29	4.27	4.28
S11	4.08	4.16	4.12
S12	3.76	3.95	3.86
Mean ± SD	4.26 ± 0.48	4.30 ± 0.47	4.28 ± 0.47

[a]Samples A and S correspond to autumn and spring butters, respectively.
[b]Percentages of *trans*-octadecenoic acids are calculated relatively to 16:0 or 18:0 acids used as internal standards.
[c]Mean values of percentages calculated using 16:0 and 18:0 acids as internal standards.

and 10:1 acids, are significantly ($P < 0.01$) lower in spring butters than in autumn butters. On the other hand, 18:0, *cis* plus *trans* 18:1, 18:2 (n-6, nonconjugated and conjugated isomers) and 18:3 acids are higher in spring than in autumn. This may be linked to the grazing conditions of part of the cattle during spring.

Table 4 gives the *trans*-octadecenoate content of autumn and spring butterfats. First of all, there is no difference between mean values for *trans* fatty acids when either 16:0 or 18:0 acid is used as the internal standard. Second, it is clear that spring butterfats contain a higher amount of *trans*-octadecenoic acids than do autumn butterfats (4.28 ± 0.47% vs. 3.22 ± 0.44%, respectively; $P < 0.01$). For butterfats from the two seasons, the lowest and highest values are 2.46 (autumn) and 5.10% (spring), respectively (Table 4). So, the *trans*-octadecenoic acid content may double throughout the year. According to some studies (11,24), the *trans* contents observed in October–November and in May–June butters are representative of, respectively, the lowest and the highest values observed throughout the year. Values for *trans* fatty acids are apparently maintained for six months in spring and summer and for about four months in autumn and winter (11,24).

Our values allow calculation of the mean annual content of *trans*-octadecenoic acids in butters, which is 3.8% of total fatty acids. About one-half of these isomers (*ca.* 2%) is *trans*-11 18:1 acid. Although many reports have dealt with the *trans*-fatty acid content of dairy fat, surprisingly few data obtained by GLC combined with Ag-TLC procedures similar to ours are available in the literature. A composite sample of Italian butters contained 5.34% *trans*-octadecenoic acids (18). Homer (25), who analyzed Finnish butters, found that summer and winter butters (number of samples not reported) contained 4.9 and 3.1% of *trans*-octadecenoic acids, respectively. Our values are in fairly good agreement with these figures. This would indicate that there is little variation, if any, linked to the geographical situation of the countries, provided analytical procedures are the same. Smith *et al.* (12) analyzed 5 brands of United States butter and found a single value of 1.8% for each of the 5 samples. Enig *et al.* (26) analyzed 3 samples of United States butters and found values ranging from 3.1 to 3.8%, but these values were overestimates because components with chain-lengths less than C-12 were not taken into account in their study. Lund and Jensen (20) analyzed one sample of Danish summer butter and found 6.5% *trans* octadecenoates. However, this value is probably an overestimate also, because the capillary columns used in this study were operated at 180°C, and short chains were certainly not taken into account under such conditions. Finally, Åkesson *et al.* (27) found 2.7% *trans* octadecenoates in a single sample of Swedish butter. Most of the other *trans* fatty acid determinations were performed by IR absorption measurements at about 970 cm^{-1}, and thus concern total isolated *trans* double bonds. However, it should be emphasized that measurements of *trans* unsaturation by IR absorption are not accurate when the *trans*-unsaturation content is below 15% (28). A few determinations were by Ag-TLC and charring followed by densitometry (21) or by column chromatography on $AgNO_3$-impregnated silica gel followed by weighing of the *trans*-monoenoic acids (10). IR absorption determinations should give results differing by less than 1% from GLC/Ag-TLC determinations, due to the fact that fatty acids containing one or more isolated *trans* double bonds are essentially composed of *trans*-octadecenoic acids in dairy fats (18). If one considers that one-half of the nonconjugated isomers of 18:2n-6 acid may contain one *trans* ethylenic bond, *trans* unsaturated fatty acids other than 18:1 acid isomers should be less than 0.5% of total fatty acids. However, in-depth experimental comparisons of the levels of isolated

trans double bonds in butterfat by IR absorption measurements and by GLC/Ag-TLC procedures have not yet been performed.

The seasonal variations of *trans* unsaturation in milkfat have been studied by several authors. However, all of these studies were based on IR absorption measurements. From Gray's work (24), a mean annual value of 5.53 ± 0.92% (maximum, 7.31%; minimum, 4.52%) can be calculated for New Zealand milkfat (17 samples), a value that is close to that calculated for Danish butters (5.19 ± 1.44%, 90 samples; maximum, 8.5%; minimum, 3.0%) (29). Canadian butters (11) show a slightly lower value (4.6%, 13 samples; maximum, 5.7%; minimum, 4.0%) and Australian milkfats (30) a slightly higher one (6.01%, 116 samples; maximum, 7.64%; minimum, 4.27%). Older data, published for Italian dairy fats, are probably overestimates (8.84 ± 1.10%, 48 samples; maximum, 12.4%; minimum, 7.0%) (31). The mean value determined in the present study is significantly lower than the preceding ones. This is undoubtedly due to differences in methodologies with IR absorption measurements giving higher *trans* unsaturation values than GLC/Ag-TLC procedures (11). Slight differences in cattle feeding habits from one country to another are probably of minor importance.

Consumption of trans*-octadecenoic acids from butterfat by people from EEC countries.* Values concerning the apparent human consumption of dairy products, expressed as kilograms of whole milk used to manufacture all dairy products consumed in one year, are published by the French center CNIEL (Interprofessional National Center for Dairy Economics) (32). Data for each of the EEC countries are given. Therefore, the quantity of dairy fats consumed in each country is accessible by simply multiplying these consumption data by the mean fat content of whole milk, 3.7%. To get access to the daily per capita intake of *trans*-octadecenoic acids, the daily per capita consumption of dairy fats is multiplied by 0.95 to take into account the proportion of fatty acids in triglycerides, and by the mean content of *trans*-octadecenoic acids as determined in the present study (3.8%). Some wastage of dairy fats may occur, but we were unable to find any evaluation of this parameter in the literature. However, it can reasonably be assumed that wastage of dairy products is low, and we will neglect it in our calculations. The value established for French butters is extended to other EEC countries, as mentioned earlier, because cattle breeding and feeding habits vary little from one country to another. Results obtained in this way are displayed in Table 5. From these data, it appears that the *trans*-octadecenoic acid consumption is highly dependent on the country, because northern countries consume higher amounts of dairy products than southern countries. The maximum value is reached in Denmark (1.66 g/d), while the minimum value is observed in Portugal (0.57 g/d). A mean value for all EEC countries of 1.16 g/d can be established. Corresponding data for the United States are around 0.8 g/d (33). If bovine meat fat is taken into account, the per capita availability of *trans* acids is then 1.4 to 1.5 g/d (4). Values for the individual consumption of *trans* fatty acids from dairy fats of 1.6–1.7 g/d and 1.5 g/d have been published for former West Germany (8) and for The Netherlands (5), respectively. However, the authors who established these values used a mean content of *trans*-octadecenoic acids for dairy fats of 5%. If our value of 3.8% is used, these values are reduced to 1.25 and 1.14 g/d, respectively, which are in good agreement with values for these countries shown in Table 5.

TABLE 5

Mean Daily Per Capita Consumption of Milkfat and of *trans*-Octadecenoic Acids Present in Milkfat by People from the twelve Countries of the European Economic Community (EEC)

Country	kg Milk/year[a]	g Fat/d	g *trans*-18:1/d[b]
France	399	40.4	1.46
Germany	365	37.0	1.37
Italy	289	29.3	1.08
The Netherlands	304	30.8	1.14
Belgium–Luxemburg	360	36.5	1.35
United Kingdom	302	30.6	1.13
Ireland	358	36.3	1.34
Denmark	446	45.2	1.66
Greece	190	19.3	0.71
Spain	162	16.4	0.61
Portugal	153	15.5	0.57
EEC (12 countries)	311	31.5	1.16

[a]Evaluation of the quantity of whole milk (containing 3.7% fat) used to manufacture all dairy products consumed in one year. Values are from the CNIEL (Ref. 32).

[b]Values obtained by multiplying the daily consumption of milkfat by 0.95 (proportion of fatty acids in triglycerides) and by 0.038 (mean content of *trans*-18:1 acids in total fatty acids of milkfat).

To compare the intake of *trans*-octadecenoic acids from dairy fat with that from margarines, it is necessary to evaluate a mean content of *trans*-octadecenoic acids in margarines. From the analyses of seventeen samples of French margarines (hard and soft) and margarine-like foods, a mean content of 13.3 ± 6.8% *trans*-octadecenoic acids (minimum, 3.0%; maximum, 27.5%) is established (detailed data not reported). From the study of Druckrey *et al.* (34) on Danish margarines (30 samples), a mean content of 7.4 ± 4.4% *trans*-octadecenoic acids can be evaluated. Depending on the authors. Swedish margarines present a mean content of 12.0 ± 5.5% (10 samples) (27) or 13% (cited in Ref. 35). Margarines from Great Britain (36) would have a higher mean content of total *trans* unsaturation, 22.2 ± 13.8% (18 samples; true amount of *trans* 18:1 acids not reported), a value close to those determined in U.S. margarines and margarine-like foods [Smith *et al.* (12), 16.2 ± 3.1%, 5 samples; Enig *et al.* (26), 18.4 ± 5.7%, 40 samples; Ottenstein *et al.* (37), 20.4 ± 9.6%, 7 samples, Perkins *et al.* (38), 18.0 ± 7.5%, 12 samples; Slover *et al.* (39), 18.8 ± 5.1%, 63 samples]. Heckers *et al.* (6) used the value 10.8% for German margarines. From all of these values, it would appear that the content of *trans*-octadecenoic acids in margarines may vary widely from one country to another. But variations may also result from the selection of the kinds of margarines under study, hard or soft, for example. Another parameter that can affect the *trans* content is the method used to determine this content. For example, IR determination of the *trans* content in a margarine sample containing partially hydrogenated fish oils will give results higher than the true amount of *trans* octadecenoic acids. Consequently, a mean value of *trans*-octadecenoic acids in European margarines, based on literature data, cannot be easily determined.

The annual per capita consumption of margarines in France, including low-calorie margarines, is 3.9 kg (32).

This value is the sum of food service and household margarines. The market share of low-calorie margarines (mainly containing 41% fat) is low (less than 20%) as compared to that of margarines containing 82% fat. A mean content of fat in margarines and margarine-like foods of 75% thus appear to be a reasonable evaluation. These data, together with the mean content of *trans*-octadecenoic acids in margarines determined in this study, allow calculation of the daily per capita consumption of *trans*-octadecenoic acids from margarines by French people, 1.0–1.1 g/person/d. Consequently, the mean daily intake of *trans* octadecenoic acids from dairy fat (1.5 g/person/d, Table 5) is higher than that from margarines in France.

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