Geometrical Isomers of Linolenic Acid in Low-Calorie Spreads Marketed in France

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The fatty acid compositions of 20 samples of low-calorie spreads marketed in France have been examined by gas-liquid chromatography (GLC) of their isopropyl esters on a fused silica capillary column coated with 100% cyanopropyl polysiloxane. Spreads containing linolenic acid at a level of 2.3% or higher (5 out of the 20 samples under study) also contain *trans*-18:3 isomers. These were identified, after fractionation of their isopropyl esters by thin-layer chromatography (TLC) on silica-gel plates impregnated with $AgNO_3$, by GLC on two capillary columns of different polarities and comparison of their equivalent chain lengths with those of authentic standards. Identifications were supported by GLC/mass spectrometry of the dimethyl esters resulting from ozonolysis in BF_3/methanol of the monoenes isolated by $AgNO_3$-TLC after hydrazine reduction of 18:3 isomers. 9*c*,12*c*,15*t*-18:3 and 9*t*,12*c*,15*c*-18:3 were found to be the most abundant 18:3 isomers in the spreads, with small amounts of 9*c*,12*t*,15*c*-18:3. These isomers occurred in the relative proportions 52–55, 41–42 and 4–6%, respectively. These proportions are independent of the origin of the sample. The tentatively identified 9*t*,12*c*,15*t*-18:3 also occurred in some instances. In 2 of the spreads, total geometrical isomers of linolenic acid accounted for 0.9–1% of the total fatty acids (up to 28% of the total 18:3n-3 fraction). The presence of 18:3n-3 geometrical isomers in the spreads is likely due to rapeseed or soybean oils that were deodorized under rather harsh conditions before these were blended with other fats or oils. Partial hydrogenation of these oils may also contribute to accumulation of the same linolenic acid isomers in the spreads.

KEY WORDS: Fatty acid composition, gas-liquid chromatography, isopropyl esters, linolenic acid, low-calorie spreads, *trans* isomers.

Most of the unsaturated fatty acids present in living tissues and foods contain double bonds that have the *cis* configuration. Except for fat from bovine and ovine milk and flesh, which contributes to some extent to the daily intake of *trans* isomers in humans, the bulk of such isomers comes from ingestion of partially hydrogenated vegetable oils (1). Much research has been devoted to the study of potential physiological effects of *trans*-octadecenoic acids (and particularly of elaidic acid) on the organism. With few exceptions (2), there is apparently no compelling evidence that these fatty acids may promote any deleterious effect (3). A similar conclusion has been drawn concerning the mono-*trans* isomers of linoleic acid (3). On the other hand, the all-*trans* isomer of linoleic acid inhibits microsomal Δ 6 desaturases and hence interferes with the metabolism of n-6 fatty acids (4). Fortunately, this isomer is practically absent in partially hydrogenated oils. *Trans* isomers of linolenic acid occur in minor quantities in deodorized or partially hydrogenated soybean oils (5,6). These isomers have also been detected in fried oils (7,8). Recent *in vivo* studies have shown that one of the three mono-*trans* isomers of linolenic acid (9*c*,12*c*,15*t*-18:3) is desaturated and elongated to higher polyunsaturated fatty acids (9,10) (mainly *trans* n-3 eicosapentaenoic and docosahexaenoic acids) which are incorporated into rat tissue lipids (9,10). This isomer is also readily incorporated as such in phospholipids (10) with a particular affinity for the mitochondrial-specific phospholipid cardiolipin (11). Little is known about the physiological effects of these geometrical isomers. Recently, it has been shown that *trans* n-3 eicosapentaenoic and docosahexaenoic acids exhibit different inhibitory effects on arachidonic acid metabolism in human platelets as compared to their respective *cis* isomers (12). However, *trans* isomers of linolenic acid have not yet been detected in human tissues (13,14).

In the present study, we have examined the fatty acid composition of several low-calorie spreads marketed in France that contain 20 to 65% fat. The fat was composed of vegetable oils (hydrogenated or not), of dairy fats, or of mixtures of both. In some of these samples, we have identified *trans* isomers of linolenic acid.

EXPERIMENTAL PROCEDURES

Samples and chemicals. Twenty different commercial items of low-calorie spreads containing 20 to 65% fat were purchased from local retail markets in May and October 1989 and stored at −20°C. All solvents were redistilled before use. Pure fatty acids were from Sigma (St. Louis, MO) or from Fluka (Buchs, Switzerland). The mixture of *trans* isomers of linolenic acid (as methyl esters) was prepared and characterized by Grandgirard *et al.* (15) and has been used previously for another study (11). Its composition is: 4.5% 9*c*,12*c*-18:2; 31.5% 9*c*,12*c*,15*t*-18:3; 4.6% 9*c*,12*t*,15*c*-18:3; 14.0% 9*t*,12*c*,15*c*-18:3; 4.6% 9*t*,12*c*,15*t*-18:3 and 40.3% 9*c*,12*c*,15*c*-18:3.

Fat extraction. Fat was extracted according to Wolff and Castera-Rossignol (16). Depending on its fat content, about 2 g (65%), 3 g (41%) or 5 g (20%) of the sample were dispersed with a magnetic stirrer in 10 mL of isopropanol in a Teflon beaker. Hexane (15 mL) was then added to the stirred suspension, followed by a sufficient amount of anhydrous Na_2SO_4. In some instances, it was necessary to add anhydrous Na_2SO_4 before pouring hexane in order to avoid the formation of large starch or gelatine clumps. The suspension was then filtered and purified on a column (2 cm i.d.) equipped with a sealed-in coarse fritted disk protected by a filter paper disk and containing a lower layer (2 cm height) of anhydrous Na_2SO_4 and an upper layer (2 cm height) of Celite 545 (Prolabo, Paris, France). The two layers were separated by a second filter paper

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disk. The whole system was washed with hexane-isopropanol (3:2, v/v) (17) before use. A supplementary portion of this solvent mixture (75 mL) was progressively used for the quantitative transfer of the suspension onto the column and for the complete elution of fat. The purified lipid solution was collected in a 100-mL volumetric flask at a rate of 1.5–2 mL/min.

Preparation of fatty acid isopropyl esters (FAIPE). FAIPE were prepared according to Wolff and Fabien (18). Aliquots of the lipid solutions (2.5 mL, generally containing 25–30 mg fat) were transferred into Teflon-lined screw-capping tubes. Isopropanol (1.7 mL) was added and then 0.25 mL pure H_2SO_4. The solutions were thoroughly mixed and the tubes were tightly capped. The reaction was performed in a boiling water-bath for 1 hr with occasional shaking. To insure that no evaporation occurred during this step, the tubes were weighed before and after the reaction. After cooling the tubes under tap water, distilled water (5 mL) was added and the tubes were vigorously shaken. After standing for about 1 min, the upper layer containing FAIPE was withdrawn and replaced by an equal volume of hexane for a second extraction. A third extraction was performed in the same way. Occasionally, a fourth extraction was made and analyzed by gas-liquid chromatography (GLC) to insure that no FAIPE was left in the lower phase. If necessary, anhydrous Na_2SO_4 was added to the pooled upper phases before these were stored at 4°C for further analyses. Methyl esters of the mixture of 18:3n-3 isomers and of authentic fatty acids were transformed similarly into isopropyl esters.

Fractionation of 18:3 and 18:1 isomers. FAIPE were separated according to the number and geometry of their double bonds by thin-layer chromatography (TLC) on $AgNO_3$-impregnated silica-gel plates ($AgNO_3$-TLC). Commercial precoated plates (DC Vertigplatten Kieselgel H, Merck, Darmstadt, Germany) were soaked in a 5% (w/v) $AgNO_3$ solution in acetonitrile for 30 min and activated for 30 min at 120°C. The migration solvent used to separate linolenic acid geometrical isomers was a mixture of hexane-diethyl ether-acetic acid (85:15:1, v/v/v). A mixture of the same solvents in different proportions (90:10:1, v/v/v) was used to isolate *trans*-monoenoic acids. After drying, the plates were sprayed with a 0.2% solution of 2′,7′-dichlorofluorescein in 96° ethanol and viewed under ultraviolet light. The bands were carefully scraped off in an aluminum foil. The silica gel was then transferred into a test tube and FAIPE were extracted in the following way: 1 mL methanol was added to the gel, followed by 2 mL hexane, 1 mL of a 1% NaCl solution in water and 0.1 mL acetic acid. Thorough mixing followed each addition. After decantation, the upper phase was removed and replaced by 2 mL hexane. A third extraction was performed in the same way. The pooled extracts were evaporated to dryness under a stream of N_2, and FAIPE were dissolved in a small volume of hexane for further analyses. In order to quantitate *trans*-octadecenoic acid isomers, the band containing these fatty acids was scraped together with saturated fatty acids. 16:0 and 18:0 were then used as internal standards for quantitative analyses by GLC. A complementary study conducted with mixtures of 16:0, 18:0, 9*t*-18:1 and 9*c*-18:1 isopropyl esters of known compositions has shown that the method was quantitative (Wolff, R.L., unpublished data).

Gas-liquid chromatography (GLC). Quantitative analyses of FAIPE by GLC were carried out on a Carlo Erba 4130 chromatograph equipped with a flame ionization detector, a split injector and an LT 410 temperature programmer (Carlo Erba, Milano, Italy). A fused silica capillary column coated with 100% cyanopropyl polysiloxane (CP™Sil 88, 50 m × 0.33 mm i.d., 0.24 μm film; Chrompack, Middelburg, Holland) was used with helium as carrier gas (inlet pressure: 0.8 kg/cm²). It was temperature-programmed from 65°C (held at this temperature for 4 min) to 175°C at 7.5°C/min and maintained at this point until completion of the analysis. Both injector and detector were kept at 250°C. Quantitative analyses were performed with an SP 4290 integrator (Spectraphysics, San Jose, CA). Equivalent chain lengths (ECLs) were determined according to Ackman (19) by using the same column operated isothermally at 175°C and saturated FAIPE (16:0, 18:0 and 20:0) as standards. Alternatively, a DB-Wax capillary column (30 m × 0.25 mm i.d., 0.2/μm film; J & W Scientific Inc., Folsom, CA) was used and operated isothermally at 190°C with helium as carrier gas (inlet pressure: 1.5 kg/cm²).

Structural determination of the 18:3 geometrical isomers. FAIPE prepared from sample A1 were converted to fatty acid methyl esters with BF_3/methanol (20). The 18:3 isomers were then isolated by high-performance liquid chromatography (HPLC) on a semi-preparative C18 column (Lichrosorb, 7 mm i.d., 25 cm, Merck) with a mixture of acetonitrile-acetone (90:10, v/v) at a flow rate of 4 mL/min. Peaks were detected with a Waters R410 differential refractometer (Waters Assoc., Milford, MA). Purity of the 18:3 fraction was checked by GLC. This fraction was further submitted to hydrazine reduction as previously described (21). The reaction was allowed to proceed for 3 hr until a mixture rich in 18:0 and 18:1 isomers and poor in 18:2 and 18:3 compounds was obtained. The resulting 18:1 isomers (representative of the ethylenic bonds in the original 18:3 mixture) were fractionated by $AgNO_3$-TLC with toluene as developing solvent. *Cis* and *trans* monoenes (localized and extracted as described above) were then submitted to ozonolysis in BF_3/methanol (7). The resulting dimethyl esters (DME) were further identified by GLC/mass spectrometry (GC/MS) under conditions recently described (22).

RESULTS AND DISCUSSION

Fifteen out of the 20 samples under study contained less than 0.6% linolenic acid. These samples did not present any peaks between 18:2n-6 and 18:3n-3, where the *trans* isomers of linolenic acid are expected to elute during GLC (6–8). The 5 other samples had a linolenic acid content in the range 2.3–4.6% (Table 1) and showed several unidentified peaks in the chromatographic zone of interest (Fig. 1). The label on these samples indicated that four of these contained dairy fats and vegetable oils (samples A1, A2, A3 and B), with no precision concerning the nature of their physico-chemical treatments. Sample C contained unhydrogenated plus partially hydrogenated soybean oils. Samples A1, A2, and A3 are produced in France by the same manufacturer. Sample B is also a French product, but sample C is imported from Denmark. Products A1, A2, A3 and C represented 54% of the 28,000 tons of low-calorie spreads that were consumed in 1988 in France (23).

Preceding studies (18,24) have shown that the methods

TABLE 1

Fatty Acid Compositions[a] (weight percent of total[b]) of Some Low-Calorie Spreads Marketed in France

Fatty acid[c]	A1	A2	A3	B	C	D	E
4:0	1.20 ± 0.11	1.04 ± 0.08	1.12 ± 0.08	0.82 ± 0.02	—	4.04 ± 0.04	—
6:0	0.78 ± 0.07	0.68 ± 0.03	0.70 ± 0.04	0.52 ± 0.02	—	2.44 ± 0.01	—
8:0	0.90 ± 0.06	0.80 ± 0.04	0.80 ± 0.04	0.38 ± 0.01	—	1.38 ± 0.02	—
10:0	1.25 ± 0.05	1.08 ± 0.04	1.06 ± 0.05	0.82 ± 0.06	—	2.88 ± 0.06	—
10:1	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	—	0.32 ± 0.01	—
12:0	3.71 ± 0.05	3.24 ± 0.13	3.28 ± 0.11	1.37 ± 0.02	0.09 ± 0.02	3.28 ± 0.12	—
14:0	4.63 ± 0.10	4.13 ± 0.17	4.19 ± 0.15	2.52 ± 0.06	0.30 ± 0.03	11.32 ± 0.43	0.32 ± 0.03
i-15:0	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	—	—	0.29 ± 0.01	—
14:1	0.27 ± 0.02	0.26 ± 0.02	0.29 ± 0.05	0.16 ± 0.02	—	1.04 ± 0.04	—
ai-15:0	0.20 ± 0.01	0.23 ± 0.03	0.22 ± 0.02	0.19 ± 0.02	—	0.57 ± 0.03	—
15:0	0.35 ± 0.01	0.38 ± 0.02	0.38 ± 0.01	0.26 ± 0.01	—	1.29 ± 0.07	—
i-16:0	0.07 ± 0.01	0.07 ± 0.00	0.08 ± 0.01	—	—	0.29 ± 0.01	—
16:0	24.40 ± 0.26	23.39 ± 0.03	23.58 ± 0.30	14.09 ± 0.11	16.13 ± 0.75	32.12 ± 0.31	14.53 ± 0.32
t-16:1	0.08 ± 0.01	0.14 ± 0.02	0.13 ± 0.02	—	—	0.19 ± 0.00	—
c-16:1	0.47 ± 0.02	0.45 ± 0.03	0.43 ± 0.03	0.26 ± 0.02	0.07 ± 0.01	1.48 ± 0.03	0.10 ± 0.01
i-17:0	0.09 ± 0.01	0.12 ± 0.01	0.13 ± 0.02	0.09 ± 0.01	—	0.34 ± 0.02	—
ai-17:0	0.14 ± 0.02	0.12 ± 0.00	0.15 ± 0.02	0.12 ± 0.03	—	0.50 ± 0.00	—
17:0	0.24 ± 0.01	0.20 ± 0.01	0.25 ± 0.02	0.20 ± 0.01	0.08 ± 0.01	0.66 ± 0.04	—
17:1	0.07 ± 0.01	0.07 ± 0.01	0.11 ± 0.02	0.08 ± 0.01	—	0.28 ± 0.01	—
18:0	5.47 ± 0.14	5.67 ± 0.15	5.92 ± 0.08	10.04 ± 0.27	5.53 ± 0.17	9.43 ± 0.22	7.73 ± 0.12
c&t-18:1	39.60 ± 0.18	41.78 ± 0.24	40.59 ± 0.57	38.48 ± 0.27	35.36 ± 0.73	22.35 ± 0.60	32.63 ± 0.21
c,t-18:2	0.17 ± 0.01	0.18 ± 0.01	0.15 ± 0.01	0.19 ± 0.02	0.10 ± 0.03	0.15 ± 0.04	0.46 ± 0.06
18:2n-6	11.08 ± 0.19	11.18 ± 0.08	11.05 ± 0.23	25.26 ± 0.15	36.99 ± 0.69	1.23 ± 0.11	43.65 ± 0.23
X_1+X_2[d]	0.47 ± 0.02	0.52 ± 0.03	0.28 ± 0.02	0.26 ± 0.02	0.11 ± 0.01	—	—
X_3+br-20:0	0.09 ± 0.00	0.10 ± 0.01	0.12 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	—	—
X_4	0.36 ± 0.02	0.33 ± 0.03	0.18 ± 0.02	0.21 ± 0.01	0.08 ± 0.01	—	—
18:3n-3	2.32 ± 0.05	2.45 ± 0.04	3.33 ± 0.19	2.81 ± 0.06	4.58 ± 0.14	0.41 ± 0.03	0.31 ± 0.02
20:0	0.34 ± 0.02	0.34 ± 0.01	0.36 ± 0.02	0.31 ± 0.01	0.35 ± 0.04	0.14 ± 0.01	0.14 ± 0.02
conj-18:2	0.16 ± 0.01	0.24 ± 0.00	0.26 ± 0.03	0.16 ± 0.01	—	0.30 ± 0.01	—
20:1	0.48 ± 0.02	0.49 ± 0.03	0.50 ± 0.04	0.10 ± 0.01	0.14 ± 0.01	0.08 ± 0.01	0.07 ± 0.02

[a]Fatty acids were analyzed as isopropyl esters by GLC on a CP™Sil 88 fused silica capillary column (temperature programming).
[b]Figures are means ± S.D. of results obtained from at least four analyses of isopropyl ester, each isopropyl ester preparation being issued from an independent fat extract of the same sample. Components representing less than 0.07% of total fatty acids are not included.
[c]Fatty acids are listed according to their order of elution from the column. Abbreviations: i, iso; ai, ante-iso; *c, cis; t, trans;* isom, geometrical isomer; conj, *cis,trans* conjugated; X_i, unidentified fatty acids; br, branched.
[d]Values for unknown fatty acids (X_i) were obtained by combining results obtained before and after $AgNO_3$-TLC fractionation.

selected for this work (fat extraction and FAIPE preparation) have no effect on the geometrical conformation of double bonds in fatty acids, independently of their number (up to 6). Thus, the fatty acids we describe here cannot be the result of experimental artefacts. FAIPE were chosen in lieu of fatty acid methyl esters, which are more often used for GLC of fatty acids, because there is practically no need to apply correction factors to transform peak area percentages, as calculated by the integrator, into fatty acid weight percentages (18). This is particularly useful when short chains are present. Chromatograms obtained with FAIPE are otherwise qualitatively similar to those obtained with fatty acid methyl esters (FAME) (Fig. 1).

Table 1 gives the fatty acid compositions of the 5 samples in which unusual peaks were detected. For comparison, the fatty acid compositions of a full-butter fat and a full-vegetable oil low-calorie spread (samples D and E, respectively) without such components are included in Table 1. Table 1 also illustrates the widely differing compositions of fatty acids in low-calorie spreads.

A partial chromatogram showing the resolution between the unknown fatty acids and some near-eluting fatty acids of predictable structure is given in Figure 2. This tracing corresponds to a GLC (with temperature programming) on the CP™Sil 88 column of FAIPE prepared with sample A1. All-*cis*-18:3n-3, 20:0, and 20:1 were characterized before and after $AgNO_3$-TLC fractionation by comparing their retention times with those of isopropyl esters prepared with authentic fatty acids. Tentative identification of the conjugated *cis,trans*-18:2, a well-known component of butterfat (25), is based on the fact that this fatty acid is present only in spreads containing butterfat. Its percentage is higher in full-butterfat spreads (Table 1, sample D) and decreases with the amount of butterfat in the spreads (as determined by their butyric acid content). This fatty acid is absent from spreads made exclusively with vegetable oils (Table 1, sample E). It migrates in the front part (first fifth) of the *cis*-monoene fraction after $AgNO_3$-TLC (results not shown), which is in agreement with other authors' observations (26).

The elution order of all-*cis*-18:3n-3 relative to 20:0 (as isopropyl esters) is different from that obtained when methyl esters are chromatographed under the same conditions on the CP-Sil™88 column. 18:3n-3 Isopropyl ester elutes before (temperature programming; Fig. 1) or almost with (isothermal; Table 2) 20:0 isopropyl ester, but 18:3n-3 methyl ester elutes largely after 20:0 methyl ester. The elution order of methyl esters is in agreement with the supplier's indications (27) and with other authors' findings (6–8,28). The peculiar properties of isopropyl esters as compared to methyl esters are particularly useful in

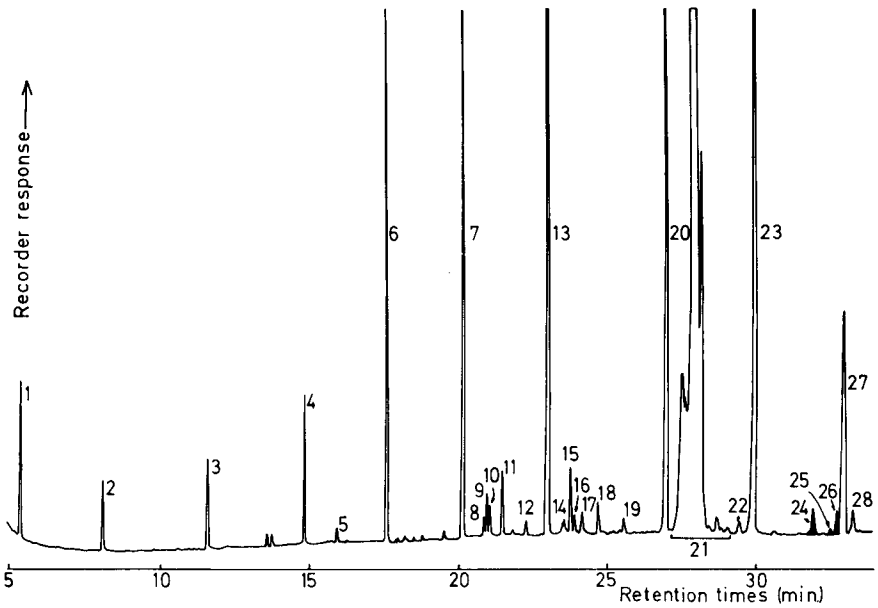

FIG. 1. The C_4 to C_{20} region of a chromatogram of FAIPE prepared with fat extracted from a sample of low-calorie spread (sample A_1). Analysis on a CP™Sil 88 with temperature programming. Identification of peaks: 1, 4:0; 2, 6:0; 3, 8:0; 4, 10:0; 5, 10:1; 6, 12:0; 7, 14:0; 8, *iso* 15:0; 9, 14:1; 10, ante-*iso* 15:0; 11, 15:0; 12, *iso* 16:0; 13, 16:0; 14, *trans* 16:1; 15, *cis* 16:1; 16, *iso*-17:0; 17, ante-*iso* 17:0; 18, 17:0; 19, 17:1; 20, 18:0; 21, *trans* and *cis* 18:1; 22, *cis*-9,*trans*-12 18:2; 23, *cis*-9,*cis*-12 18:2; 24, 25, 26, unidentified 18:3 isomers; 27, *cis*-9,*cis*-12,*cis*-15 18:3; 28, 20:0.

this study, because *trans* isomers of linolenic acid cannot be confused with 20:0 or 20:1 isomers, a situation that occurs when methyl esters are used (6–8,28).

Some unidentified components (noted X_1, X_2, X_3, and X_4 in Fig. 2) were observed between 18:2n-6 and 18:3n-3 only in those spreads having a percentage of all-*cis*-linolenic acid equal to or higher than 2.3% of total fatty acids, and probably containing rapeseed or soybean oil. In some instances, components X_1 and X_3 were absent or too small to be detected (shoulder X_1) or adequately quantitated (peak X_3); when this occurred, peak X_4 was fused with the leading edge of the peak corresponding to all-*cis*-18:3n-3, but it was still distinguishable as a shoulder (Fig. 3). Quantitation of these isomers was then realized by combining results of analyses by GLC performed before and after $AgNO_3$-TLC fractionation (see below).

Peak X_3 was first believed to be a branched 20:0 because it was initially found in the saturate fraction after $AgNO_3$-TLC (Fig. 2). However, a peak having the same retention time was also found together with X_2 and X_4 in a fraction (Rf = 0.22) migrating just ahead of all-*cis*-18:3n-3 (Rf = 0.18) after separation by $AgNO_3$-TLC. In spite of their closeness, these fractions were practically not cross-contaminated, as could be judged by GLC (Fig. 2). When compared with a mixture of well-defined *trans* isomers of linolenic acid, components X_2, X_3, and X_4 have the same retention times (CP™Sil 88 and DB-Wax columns; isothermal) (Table 2) as do 9*c*,12*c*,15*t*-18:3, 9*c*,12*t*,15*c*-18:3 and 9*t*,12*c*,15*c*-18:3, respectively. These chromatographic data strongly suggest that components X_2, X_3, and X_4 are probably mono-*trans* isomers of linolenic acid, component X_3 occurring in some instances (mainly in sample C) in admixture with a (tentatively identified) branched 20:0. Identification of 18:3 isomers is supported by the following data. Monoenes obtained after hydrazine treatment of the whole 18:3 fraction (isolated by HPLC) were resolved into 3 bands upon $AgNO_3$-TLC fractionation. Their Rf values were 0.32 (band 1), 0.39 (band 2) and 0.48 (band 3). Analysis by GC/MS of DME resulting from oxidative cleavage of monoenes in band 1 indicates that these are mainly (88% of the mixture) constituted of C_9, C_{15} (major) and C_{12} DME (minor). As band 1 had the same Rf as oleic acid methyl ester, it is concluded that it contains *cis*-monoenes. In the same way, components in band 2 led to the formation of C_{12} DME (major) and C_9 and C_{15} DME (minor) deriving from *cis*-18:1 isomers. Band 3 had the same Rf as elaidic acid methyl ester and contained 3 main isomers (95% of the fraction). These isomers were in the proportion 49.1 (first eluting peak), 9.9 (second peak) and 41% (third peak) and gave mainly C_9, C_{15} (major) and C_{12} DME (minor) after ozonolysis in BF_3/methanol. However, some other minor peaks were also present (all other DME ranging from C_6 to C_{15}). We thus conclude that the main unknown components X_2, X_3 and X_4 are true geometrical isomers of linolenic acid. However, we cannot exclude the presence of small amounts of some positional isomers that would be masked by the main geometrical isomers during GLC. Identification of X_2 (9*c*,12*c*,15*t*-18:3), X_3 (9*c*,12*t*,15*c*-18:3n-3) and X_4 (9*t*,12*c*,15*c*-18:3n-3) exactly corresponds to identification established by Ackman *et al.* (5) and Grandgirard *et al.* (7) for artefacts detected in deodorized or heated rapeseed, soybean and linseed oils. It should be emphasized that none of these isomers could be de-

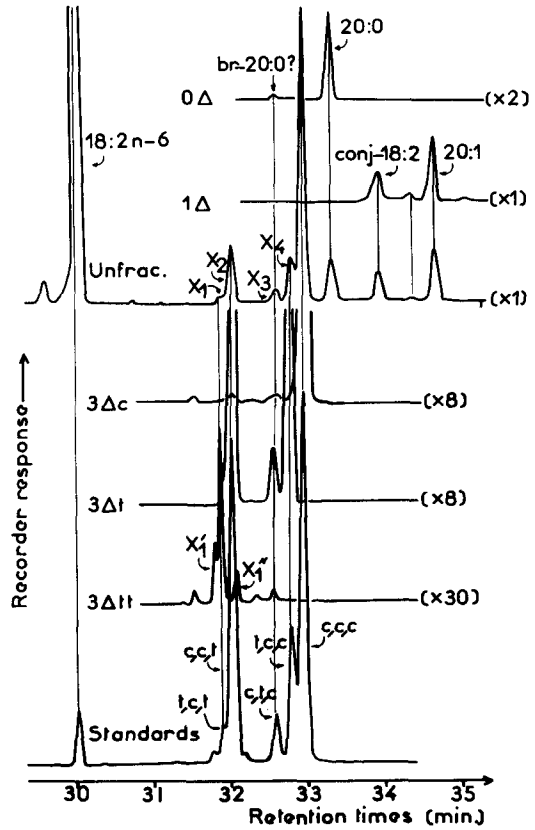

FIG. 2. Partial chromatograms of FAIPE before and after $AgNO_3$-TLC fractionation. FAIPE were prepared with fat extracted from sample A1 and analyzed on the CP™Sil 88 column with temperature programming. 0 Δ, saturates; 1 Δ *c*, *cis* monoenes (including *cis,trans* conjugated 18:2); unfrac., unfractionated FAIPE; 3 Δ *c*, all-*cis* trienes (linolenic acid); 3 Δ *t*, mono-*trans* isomers of linolenic acid; 3 Δ *t,t* di-*trans* isomers of linolenic acid; standards, mixture of well-characterized geometrical isomers of linolenic acid (configuration of double bonds are given in the order Δ 9, Δ 12 and Δ 15). Values between parentheses at the right of chromatograms indicate the approximate concentration of the samples relative to unfractionated FAIPE.

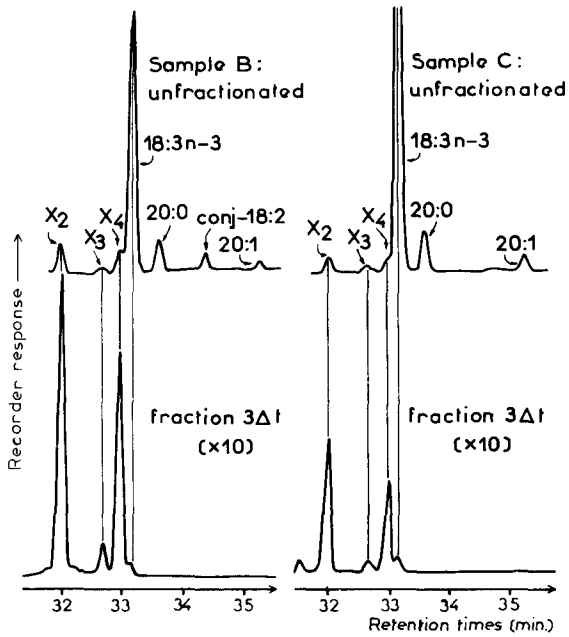

FIG. 3. Partial chromatograms of FAIPE prepared from samples B and C and showing the mono-*trans* isomers of linolenic acid (X_1, X_2 and X_3) before (unfractionated) and after (fraction 3 Δ *t*) $AgNO_3$-TLC fractionation. Analyses are performed with the CP™Sil 88 column (temperature programming). Values between parentheses at the right of chromatograms indicate the approximate concentration of the samples relative to unfractionated FAIPE.

TABLE 2

Equivalent Chain Lengths (ECLs) of Some Geometrical Isomers of Linolenic Acid Isopropyl Esters

Isomer	ECL on CP™Sil 88	ECL on DB-Wax
9*c*,12*c*,15*t*-18:3	19.76	19.24
9*c*,12*t*,15*c*-18:3	19.92	—[a]
9*t*,12*c*,15*c*-18:3	19.96	19.45
9*c*,12*c*,15*c*-18:3	20.02	19.31

[a]Not resolved from 9*t*,12*c*,15*c*-18:3.

tected by GLC of the pooled 3 Δ fractions isolated by $AgNO_3$-TLC from FAIPE prepared with authentic butterfat, even after a 30-fold concentration of the sample before injection (results not shown).

Component X_1 on the leading edge of peak X_2 (Fig. 2) was found in a fraction having an Rf value of 0.27 after $AgNO_3$-TLC separation. When a concentrated aliquot of this fraction was analyzed by GLC, 2 supplementary peaks (X_1' and X_1'' in Fig. 2) were observed. From their migration characteristics on $AgNO_3$-TLC (7,29), and taking in account the number of isomers in the fraction, we suppose that the 3 peaks may correspond to the di-*trans* isomers of linolenic acid. The major peak is probably 9*t*,12*c*,15*t*-18:3, the di-*trans* isomer which preferentially accumulates upon heat-treatment (steam deodorization or frying) of oils containing linolenic acid (5,7). On a CP™Sil 84 column, component X_1 was well resolved from the main X_2 peak and could be quantitated: it represents *ca.* 7% of total *trans*-18:3 isomers in sample A1. However, this column did not allow the separation of components X_3 and X_4 (results not shown).

Although samples A contain relatively high percentages of *trans* isomers of linolenic acid (Table 1), their *trans*-octadecenoic acid content is quite low: between 2.4 and 2.8% of total fatty acids. These samples contain butterfat, the proportion of which can be deduced from their butyric acid content: between one-quarter and one-third of total fat. Butters, analyzed at the same time these spreads were purchased, had a *trans*-octadecenoic acid content of 2–3% (results not shown). However, the *trans*-octadecenoic acid content of butterfat is known to be dependent on the nutritional status of the cattle and to be subject to seasonal variations. In France, it varies from 2 to 8%, mainly as a function of the linoleic acid content in the feed (30). This means that the major part of *trans*-octadecenoic acids in samples A is attributable almost

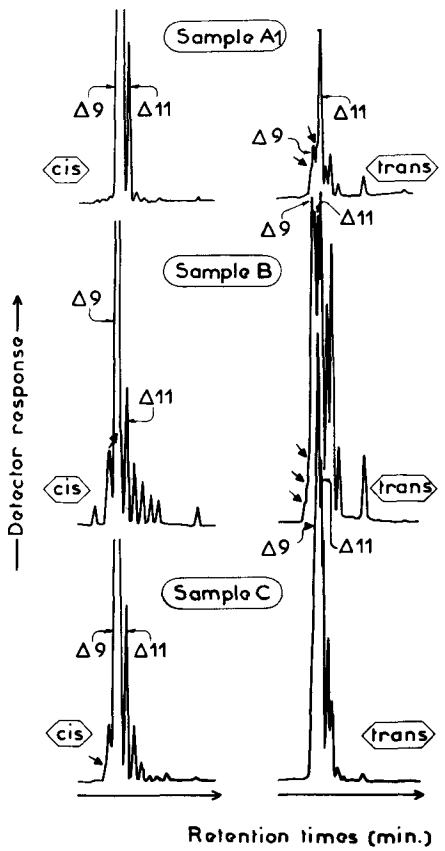

FIG. 4. Chromatograms of *cis*- and *trans*-octadecenoic acids (as FAIPE) isolated by $AgNO_3$-TLC from samples A1, B and C. Analyses are performed with the CP™Sil 88 column (temperature programming). 9*c*-, 11*c*-, 9*t*- and 11*t*-18:1 were identified by using authentic standards. Short black arrows indicate shoulders corresponding to partially resolved isomers.

solely to their butterfat fraction. This hypothesis is supported by the fact that the major *trans*-octadecenoic acid in spreads A is 11*t*-18:1 (Fig. 4), the prominent *trans* isomer found in butterfat (31). The fatty acid profile of *trans*-octadecenoic acids isolated from the spreads was otherwise exactly the same as that isolated from authentic butterfat (results not shown, identical to those presented in Fig. 4). Moreover, the *cis*-octadecenoic acid fraction contains mainly 9*c*- and 11*c*-18:1 in the proportions 96/4 (Fig. 4). It is, therefore, concluded that samples A do not contain partially hydrogenated vegetable oils. The *trans* isomers of linolenic acid they contain must originate from treatments other than hydrogenation of their constitutive oils. From the ratio between linoleic and linolenic acid, it is deduced that rapeseed oil rather than soybean oil is present in the spreads. Further examination of data for samples A in Table 1 indicates that these spreads probably contain palm oil and coconut or palm kernel oils. This is evidenced by their high 12:0, 14:0 and 16:0 levels. As these vegetable fats are devoid of linolenic acid, they cannot be responsible for the presence of linolenic acid geometrical isomers in the spreads.

Samples B and C contain the same 3 major isomers of linolenic acid as samples A (Fig. 3) but they are present in lower percentages (Table 1). *Trans*-octadecenoic acids (analyzed by GLC after $AgNO_3$-TLC fractionation) account for 18.4 ± 0.5% of total fatty acids in sample B, with at least 10 distinguishable isomers (Fig. 4). The *cis*-octadecenoic acid fraction showed 11 detectable components (Fig. 4), with 9*c*-18:1 representing about 77% of the total fraction. This value is slightly overestimated due to the presence of another isomer (probably 10*c*-18:1) in the trailing edge of the main Δ 9 peak. Thus, spread B undoubtedly contains oils that have been partially hydrogenated. This was not mentioned on the label. Sample C contains a somewhat lower percentage of *trans*-octadecenoic acids (11.6 ± 0.5%) with fewer isomers (Fig. 4). The *cis*-octadecenoic acid fraction also contains a smaller percentage of isomers different from oleic and *cis*-vaccenic acids (Fig. 4). This indicates that the constitutive hydrogenated oil present in spread C has been hydrogenated to a lesser degree than in spread B. Alternatively, the hydrogenated oil may have been diluted with native oil. This explanation would be in better agreement with the label, which indicates that the spread contains native and hydrogenated soybean oils.

TABLE 3

Relative Proportions of the Major Geometrical Isomers of Linolenic Acid in Some Low-Calorie Spreads

	A1 (n=3)	B (n=3)	C (n=1)
9*c*,12*c*,15*t*-18:3[a]	52.5 ± 1.8	52.0 ± 0.6	54.5
9*c*,12*t*,15*c*-18:3	5.3 ± 1.5	5.7 ± 0.1	4.4
9*t*,12*c*,15*c*-18:3	42.2 ± 0.8	42.3 ± 0.6	41.1
Total *trans*-isomers[b]	0.9	0.5	0.2
9*c*,12*c*,15*c*-18:3[b]	2.3	2.8	4.6
Percent of *trans*-isomers relative to total 18:3n-3	28	15	4

[a]Probably containing minor amounts of di-*trans* isomers.
[b]Percentages relative to total fatty acids.

The proportions of the 3 mono-*trans* isomers found in 3 of the 5 samples are given in Table 3. Values for these components were obtained after $AgNO_3$-TLC fractionation. Calculation of their total percentages relative to the sum of all linolenic acid isomers was made in the following way. Because 9*c*,12*c*,15*t*-18:3 (plus 9*t*,12*c*,15*t*-18:3) is uncontaminated and well resolved when unfractionated FAIPE are analyzed, this fatty acid was used as an internal reference to establish the true percentages (relative to total fatty acids) of the 2 other isomers. Because the 9*c*,12*t*,15*c* isomer was mixed with a branched 20:0 and the 9*t*,12*c*,15*c*-18:3 isomer was poorly separated from the all-*cis*-isomer, GLC of total FAIPE gives over- or underestimated values, respectively. Corrections were thus made for these 2 isomers. It is quite clear that the *trans* isomers present an almost identical profile, independent of their origin. Although their total percentages relative to total octadecatrienoic acids vary from 4 to 28%, 9*c*,12*c*,15*t*-18:3 (containing some 9*t*,12*c*,15*t*-18:3) is always the major isomer (range, 52.0–54.5%), followed by the 9*t*,12*c*,15*c* isomer (range, 41.1–42.3%). Both of them are present in considerably higher amounts than the 9*c*,12*t*,15*c* isomer (range, 4.4–5.7%). These values compare

well with values reported by Ackman *et al.* (5) for rapeseed deodorized in the laboratory. In this experiment, 9*c*,12*c*,15*t*-18:3 and 9*t*,12*c*,15*c*-18:3 plus 9*c*,12*t*,15*c*-18:3 represented 45.4–48.6% and 51.4–54.6% of their total, respectively (5).

As demonstrated by Ackman *et al.* (5), steam-vacuum deodorization of linolenic acid-containing oils (rapeseed and linseed oils) induces geometrical isomerization of double bonds in positions 9 and 15 mainly. Similar findings were reported by Grandgirard *et al.* (7) for heated rapeseed and soybean oils. However, these results contrast with the observations of Perkins and Smick (6) who have established that the main isomers that accumulate after partial hydrogenation of soybean oil are 9*t*,12*t*,15*c*-18:3n-3 (proportion relative to total *trans*-isomers: 51%) and 9*t*,12*c*,15*c*-18:3n-3 (33%). According to Devinat *et al.* (32), a 29% level of isomerization of linolenic acid can be reached if the oil is steam-deodorized at a minimum temperature of 240°C for at least 4 hr. However, Ackman *et al.* (5) have obtained a similar degree of isomerization under milder conditions (2 hr at 230°C). Taking into account the above-mentioned observations and data reported in this study, it seems logical to attribute the presence of geometrical isomers of linolenic acid in spreads A to steam-deodorization of rapeseed oil under rather harsh conditions. On the other hand, geometrical isomers of linolenic acid in spreads B and C may have originated from deodorization or from partial hydrogenation of their constitutive linolenic acid-containing oils. However, it has been reported that partial hydrogenation leads to the appearance of high proportions of 9*t*,12*t*,15*c*-18:3 (6), a component which elutes before 9*c*,12*c*,15*t*-18:3 by GLC on highly polar columns (28). In Perkins and Smick's study (6), *trans*-monoenes resulting from hydrazine reduction of 18:3n-3 geometrical isomers were present in the approximate relative proportions (determined by triangulation of the corresponding peaks on the chromatogram in Fig. 6 from Ref. 6): 9*t*-18:1, 51%; 12*t*-18:1, 13%; 15*t*-18:1, 36%. These proportions are close to ours (49.1, 9.9, and 41%, respectively), but our conclusion relative to the identification of linolenic acid *trans*-isomers is quite different. The fact that the 12*t*-18:1 isomer is low in both studies clearly indicates that any 18:3n-3 isomer initially containing a 12*t*-double bond prior to hydrazine treatment must be low too. In our mind, the accumulation of 9*t*,12*t*,15*c*-18:3 as a major isomer of linolenic acid following partial hydrogenation (6) has not been fully demonstrated. Consequently, the absence of 9*t*,12*t*,15*c*-18:3 in spreads B and C does not indicate that their rapeseed or soybean oils were not hydrogenated. Alternatively, it is possible that partial hydrogenation and deodorization may modify linolenic acid in the same manner. This would explain why spreads A, which do not contain partially hydrogenated oils, present the same isomers in the same relative proportions as do samples B and C.

The observations made in this study have been extended to some other processed foods containing soybean or rapeseed oils (Wolff, R.L., unpublished results). All-*cis*-18:3n-3 acid is now known to be an essential fatty acid (33) and a potential precursor of higher polyunsaturated fatty acids. These are incorporated into membrane phospholipids, particularly in the central nervous system (33). Thus, nutritionists tend to recommend its consumption. This is especially the case for infant food formulas. However, it does not appear that artefacts originating from refining processes (steam-vacuum deodorization) have been taken into account in these recommendations. No data concerning their effect on health status are presently available. We believe that these artefacts should be taken into account when dietary guidelines or accurately designed experiments are developed.

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