Identification and Quantitation of *trans*-9,*trans*-12-Octadecadienoic Acid Methyl Ester and Related Compounds in Hydrogenated Soybean Oil and Margarines by Capillary Gas Chromatography/Matrix Isolation/Fourier Transform Infrared Spectroscopy

Magdi M. Mossoba,*,† Richard E. McDonald,‡ Jo-Yun T. Chen,† David J. Armstrong,‡ and Samuel W. Page†

Division of Contaminants Chemistry, Food and Drug Administration, Washington, D.C. 20204, and Division of Food Chemistry and Technology, Food and Drug Administration, Cincinnati, Ohio 45226

The processing of vegetable oils by hydrogenation leads to the isomerization of cis unsaturated fatty acids. The resulting *trans*-9,*trans*-12-18:2 isomer is of concern because it was reported to disrupt the metabolism of essential fatty acids and to influence the synthesis of prostaglandins and other cell regulators. Geometric (cis or trans) and positional isomers in catalytically hydrogenated soybean oil and margarines have been separated as fatty acid methyl esters (FAMEs) by capillary gas chromatography (GC) using a polar cyanopropylpolysiloxane liquid-phase column. This paper demonstrates that the unique matrix isolation/Fourier transform infrared (MI/FT-IR) spectral bands due to the =C—H stretch and the out-of-plane deformation vibrations are highly characteristic of the number and configuration of the double bonds in FAME isomers. By using GC/MI/FT-IR, C_{18} FAME isomers from hydrogenated soybean oil and margarines were identified, and the presence of four *trans*, *trans*-diene isomers in one of the hydrogenated soybean oil test samples is reported. FAMEs containing trans double bonds were quantified. The amounts of the *trans*-9,*trans*-12-18:2 isomer found in the margarines investigated were low (0.3%).

The nature and levels of the different essential fatty acid constituents of vegetable oils and margarines are important considerations in selecting or recommending diets for the general population. *trans*-9,*trans*-12-Octadecadienoic (linoelaidic) acid (*t*9,*t*12-18:2) is one of several unsaturated geometric isomers of octadecadienoic acid that are generated during the partial hydrogenation of vegetable oils and in the processing of margarines. The *t*9,*t*12-18:2 isomer, which is not found naturally in vegetable oils, was shown to interfere with lipid metabolism (Anderson et al., 1975; Holman et al., 1983; Privett et al., 1977) and with the biosynthesis of prostaglandins (Hwang and Kinsella, 1979). To assess the significance of such adverse physiological effects, analytical methodology was needed to isolate and conclusively identify the *t*9,*t*12-18:2 isomer.

Although many methods for the determination of fatty acid isomers have been reported (Ackman, 1987; AOAC, 1984; Gildenberg and Firestone, 1985; Kobayashi, 1980; Marchand and Beare-Rogers, 1982; Sebedio et al., 1987), the nature and levels of the trans isomers in hydrogenated oils and other food products have not been adequately addressed. The lipid contents of margarines and other foods were recently determined by Slover et al. (1985); however, conflicting literature reports (Jackson et al., 1980; Marchand and Beare-Rogers, 1982; Sahasrabudhe and Kurian, 1979) about the levels of the *t*9,*t*12-18:2 isomer in certain margarines further suggested the need for analytical methodology that can conclusively determine the trans isomer composition of food products containing hydrogenated oils.

While infrared spectroscopy has long been used to distinguish between cis and trans isomers and to determine the *total* trans content of fatty acids in oils (AOAC, 1984), the infrared analysis of individual isomers in complex mixtures of unsaturated fatty acids could be achieved only with gas chromatography/Fourier transform infrared spectroscopy (GC/FT-IR). An excellent example of the application of this technique is the analysis of cyclic fatty acid mixtures isolated from heated linseed and sunflower oils (Sebedio et al., 1987). However, catalytic hydrogenation of edible oils usually generates low levels of trans isomers (less than 1%); greater instrumental sensitivity than that of GC/FT-IR is required to determine these levels. This sensitivity was recently provided by GC/matrix isolation/FT-IR (GC/MI/FT-IR) (Bourne et al., 1984; Holloway et al., 1988; Mossoba et al., 1988, 1989; Reedy et al., 1985) in which the GC effluent was cryogenically trapped in real time, for subsequent analysis by FT-IR. Thus, individual fatty acid isomers can be separated by capillary GC, trapped in a microscopic solid argon matrix at 11 K, and extensively analyzed by FT-IR.

In the present work, capillary GC separation with a polar cyanopropylpolysiloxane liquid-phase column, coupled with MI/FT-IR spectroscopic analysis, allowed us to unambiguously identify the *t*9,*t*12-18:2 isomer of interest and to readily differentiate it from other C_{18} geometric and positional isomers in complex fatty acid methyl ester (FAME) profiles of margarines and partially hydrogenated soybean oil. GC/MI/FT-IR was also used to quantitate the *t*9,*t*12-18:2 and other trans C_{18} FAMEs in these food products. This paper quantitatively compares trans isomer levels in two test materials obtained by hydrogenating soybean oil to an iodine value of about 95 by using two different catalysts; trans isomer levels in two commercial margarines were also determined.

EXPERIMENTAL SECTION

Instrumentation. Gas chromatographic separations were performed on a Hewlett-Packard Model 5890 instrument (Avon-

† Division of Contaminants Chemistry.
‡ Division of Food Chemistry and Technology.

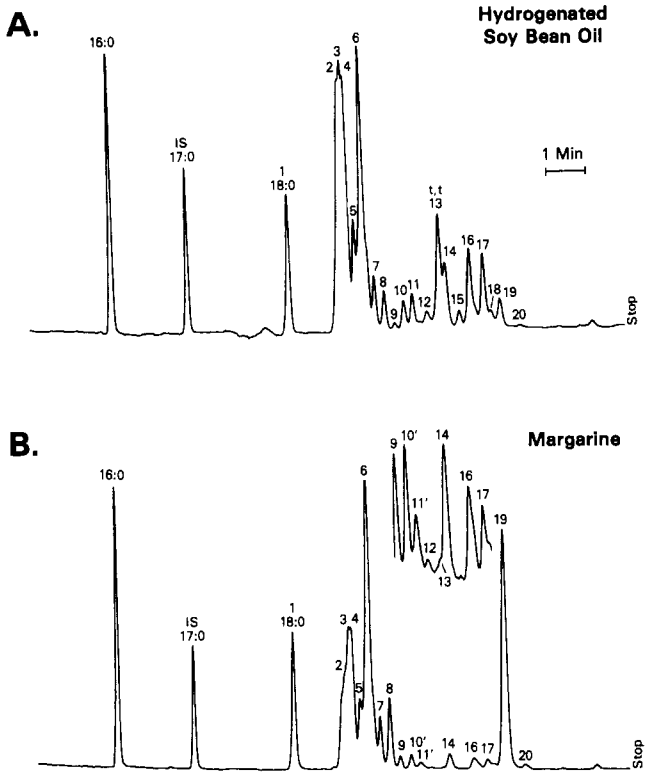

Figure 1. Partial GC/FID chromatograms for (A) hydrogenated soybean oil FAME (sulfur-containing nickel catalyst used in hydrogenation) and (B) margarine FAME (margarine B). Inset shows a ×10 blowup of the FID response for several peaks including 12 and 13. The 17:0 FAME was used as IS. Temperature was ramped to 170 °C.

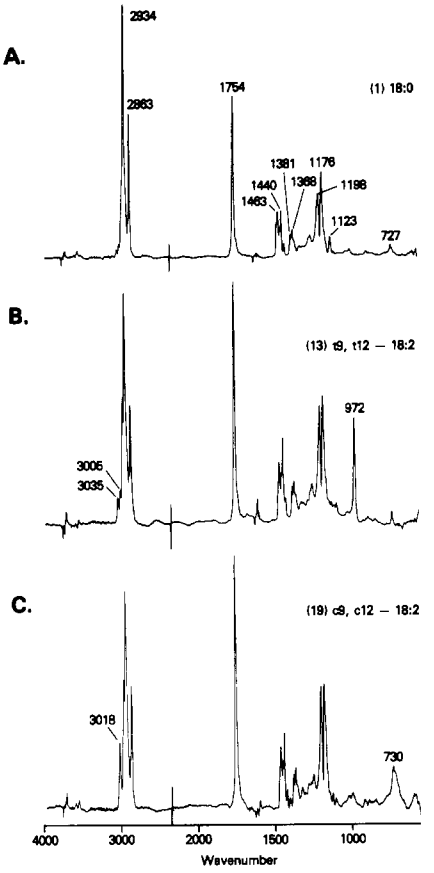

Figure 2. MI/FT-IR spectra at 4-cm^{-1} resolution acquired by coadding 300 scans (2 min 43 s) for (A) 18:0, (B) *t*9,*t*12-18:2, and (C) *c*9,*c*12-18:2 FAMEs. Unique features are found in each spectrum, e.g., at 1123 cm^{-1} in (A), at 3035, 3005, and 972 cm^{-1} in (B), and at 3018 and 730 cm^{-1} in (C).

dale, PA) equipped with a flame ionization detector and a Hewlett-Packard 3392A integrator. A CP-Sil-88 capillary column (Chrompack, Inc., Bridgewater, NJ), 50 m × 0.22 mm (i.d.), with a 0.19-μm stationary-phase film was used. Helium containing 1.5% argon (Matheson Gas Products) at approximately 27 cm/s linear velocity was used as the carrier gas, and helium (99.995%) at 30 mL/min was used as the makeup gas to the detector. The injection and detector temperatures were 250 and 300 °C, respectively. The carrier gas mixture was purified by using an Alltech Associates Hydro-Purge II and a heated Supelco gas purifier filter. A splitless injection mode using a 10-μL Hamilton 701N syringe was followed; injections of about 1 μL were made 6 s after the start of a run for a total of 15 s. The injector was purged 0.5 min after the start of a run. The initial column oven temperature was 75 °C with a 2-min hold, followed by a 20 °C/min increase to 170 or 200 °C, and the oven was held at this temperature until the analysis was complete. A column effluent split ratio of 50:50 was calculated from the flame ionization detection (FID) response factors (area counts per nanogram injected) obtained with the column directly connected to the detector, and subsequently to the GC/MI interface. Therefore, during an analysis, half the amount of analyte injected was trapped on the MI collection disk, and the other half was measured by FID. A Sirius 100 FT-IR spectrometer (Mattson Instruments, Madison, WI) equipped with a Cryolect matrix isolation unit was used. The Cryolect system has been described previously (Bourne et al., 1984; Reedy et al., 1985); 300 analyte interferograms were coadded (2 min 43 s at 4-cm^{-1} resolution), and the background was usually collected (300 scans) before or after the analyte peak.

Materials. Refined unhydrogenated soybean oil was obtained from a local distributor. Two nickel catalysts were obtained from a commercial source. One catalyst contained 20% nickel, and the other contained 20% nickel and 1% sulfur. Two commercial margarines from different manufacturers were purchased from a local food store.

Hydrogenation and Esterification. Soybean oil was hydrogenated by using 0.1% catalyst in a 1-gal pressure vessel reactor (Autoclave Engineers, Erie, PA) at 1000 rpm and 138-kPa hydrogen pressure. By using the nickel catalyst, the oil was hydrogenated at 150 °C for 15 min to an iodine value of 95

Table I. FAME Bands Observed by MI/FT-IR for 18:0

assignment	posn, cm^{-1}
CH_3 asym str	2961 w[a]
CH_2 asym str	2935 s
CH_3 sym str	2880 w
CH_2 sym str	2863 s
ester C=O str	1754 s
CH_2 scissors	1463 m
CH_3 sym scissors	1381 w
ester sym C—O str	1176 m
CH_3 in-plane rock	1123 w
CH_2 rock	727 w

[a] Key: s = strong; m = medium; w = weak.

Table II. Normalized Intensity of Important Bands (cm^{-1}) for C_{18} FAMEs Relative to the Ester Carbonyl Stretching Vibration

FAME	=C—H str,[a] trans, 3035	=C—H str, cis 3018	=C—H str, cis 3009	aliphatic, C—H str, 2935	C=O, str, 1754	CH def, trans, 972
18:0				1.60	1.00	
*t*9-18:1	0.07			1.29	1.00	0.23
*c*9-18:1			0.17	1.21	1.00	
*t*9,*t*12-18:2	0.10			0.96	1.00	0.43
*c*9,*c*12-18:2		0.26		0.89	1.00	
*c*9,*c*12,*c*15-18:3		0.44		0.74	1.00	

[a] Intensity of the second *trans*-=C—H stretching band at 3005 cm^{-1} was not included since it overlapped two adjacent bands.

Table III. Characteristic Bands for C_{18} FAMEs Observed at 4-cm^{-1} Resolution

FAME	GC peak	band position, cm^{-1}						
saturated								
18:0	1					1123		727 vw[a]
trans-monoenes								
t-18:1	2–5	3033			3003	1126	971	728 vw
cis-monoenes								
c-18:1	6–9			3010		1124		728 br
trans,trans-dienes								
t,t-18:2	10–12	3035			3005		971	730 vw
*t*9,*t*12-18:2	13	3035			3005		972	730 vw
cis/trans-dienes								
NMI-*c/t*-18:2[b]	14, 15	3034 sh		3010			972	730 br
*c*9,*t*12-18:2	16		3018 br				971	730 br
*t*9,*c*12-18:2	17		3018 br				971	730 br
cis,cis-dienes								
*c*9,*c*12-18:2	19		3018					730 br
c,c-18:2	20		3017					729 br
cis,cis,cis-triene								
*c*9,*c*12,*c*18-18:3	21		3019					727 br

[a] Key: vw = very weak; br = broad; sh = shoulder. [b] NMI = non-methylene interrupted.

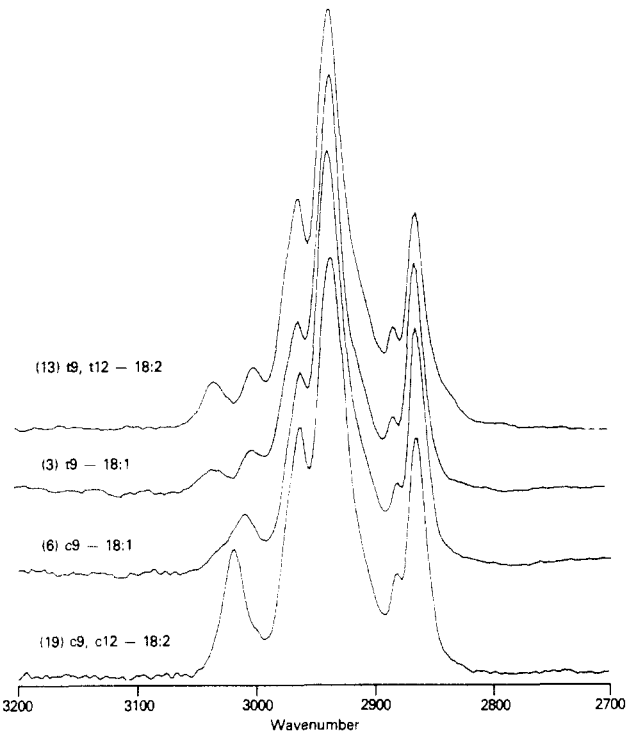

Figure 3. Expanded C–H stretch region observed for several FAMEs, showing subtle spectral differences due to the cis or trans nature of the double-bond configuration.

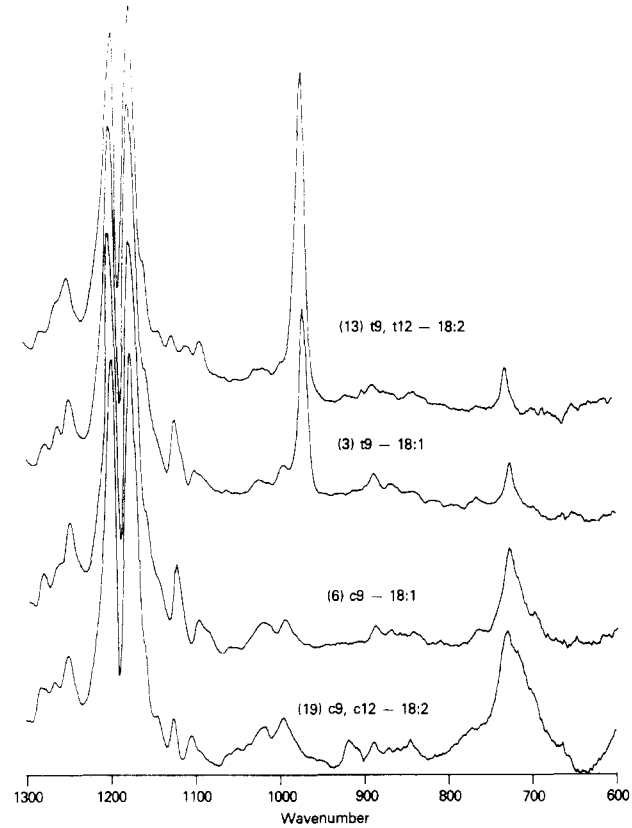

Figure 4. Expanded spectral region observed for several FAMEs, showing the sharp band at 972 cm^{-1} due to the CH out-of-plane deformation vibration only in isomers with trans double-bond configuration (upper two spectra). The trans band for FAME 13 is more intense than that for FAME 3; see Table II.

(test material 6A). The oil was also hydrogenated at 175 °C for 4 h to an iodine value of 96 by using the sulfur–nickel catalyst (test material 7H). FAMEs were prepared by transesterification with boron trifluoride/methanol according to the official AOAC method (1984, Sections 28.056–28.059), and iodine values were determined by the official AOAC procedure (1984, Sections 28.023–28.024). FAMEs of the fat in two commercial margarines were also prepared.

Standard and Test Sample Preparation and Analysis. A FAME standard isomer mixture (K110) and 17:0, *t*9-18:1, and *t*9,*t*12-18:2 FAME standards were obtained from Alltech Associates (Deerfield, IL). All solutions were prepared in distilled-in-glass isooctane (Burdick & Jackson Laboratories, Inc., Muskegon, MI). A standard working stock solution containing *t*9-18:1 and *t*9,*t*12-18:2, each at 44.0 ng/μL, was prepared from the neat standards; this solution was diluted to prepare GC/MI/FT-IR calibration standards. A 17:0 FAME internal standard (IS) stock solution at 1 mg/100 μL was prepared from the solid standard, and aliquots from this IS stock solution were mixed with the standard working stock solution. The test sample solutions were prepared from transesterified margarine, transesterified soybean oil, or hydrogenated, transesterified soybean oil and contained aliquots of the IS stock solution. Test sample solutions were diluted as needed in order for the components present in large amounts (i.e., *t*9-18:1) to fall within the calibration range. Calibration plots were generated of milliabsorbance (monitored at 2935 cm^{-1} for the 17:0 IS and at 972 cm^{-1} for the standard trans FAMEs) vs nanograms injected. The level of trans isomers in test sample solutions was calculated from the observed trans band absorbance, the regression line equations describing the calibration plots for the *t*9-18:1 and *t*9,*t*12-18:2 FAME standards with one and two trans double bonds, respectively, and the nanogram amounts of IS added.

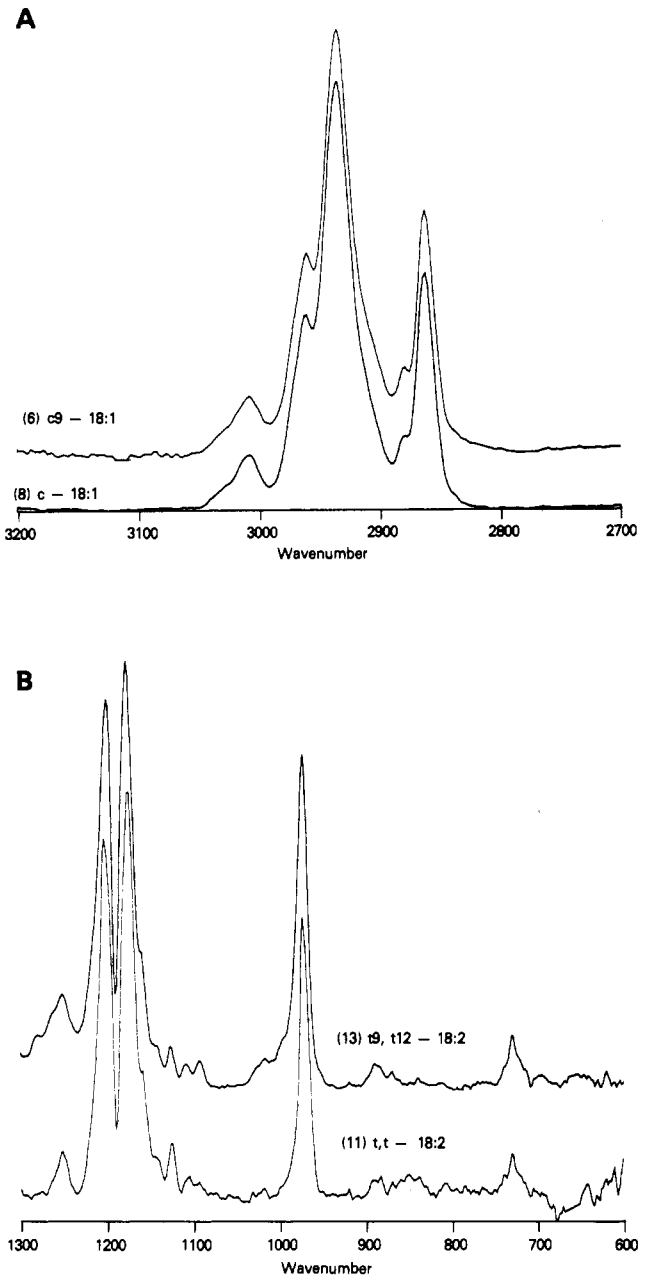

Figure 5. (A) Expanded C–H stretch region showing that both isomers 6 and 8 are *cis*-monoene FAMEs. (B) Expanded spectral region showing similar features for two *trans,trans*-diene FAME isomers (11 and 13) found for hydrogenated soybean oil mixture 7H.

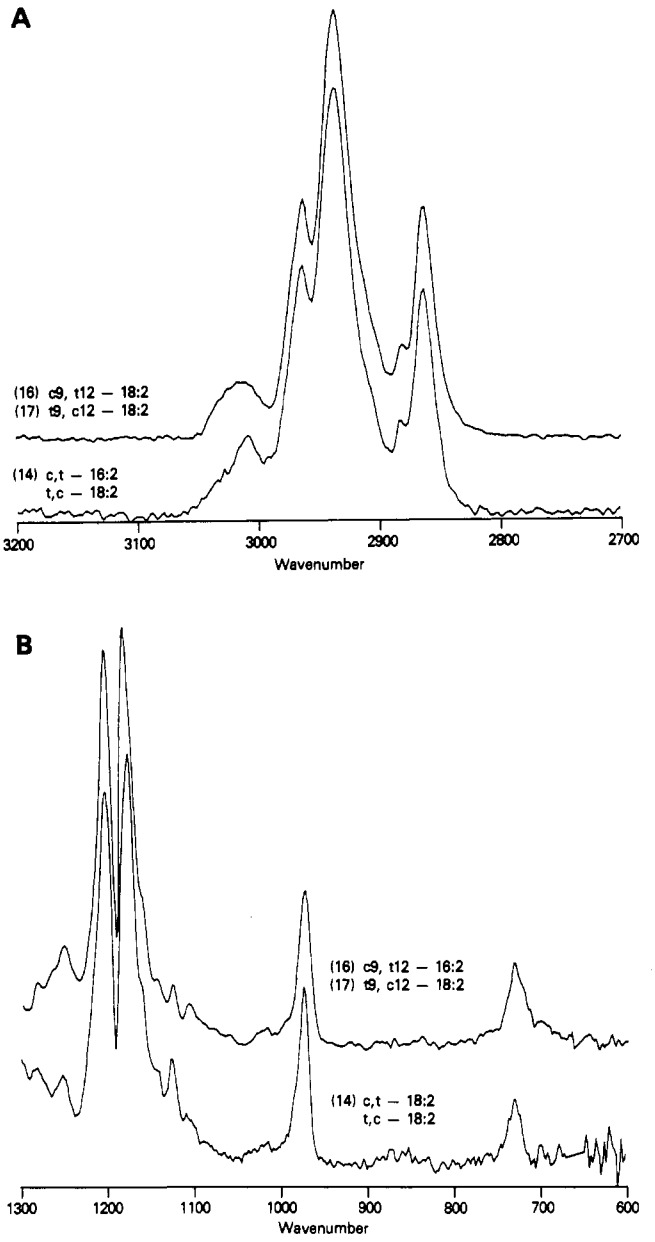

Figure 6. Expanded spectral region observed for FAME isomers 14 and 16 or 17 (A) showing the presence of cis character (subtle, yet significant differences in band position (3018 cm^{-1} (top) and 3010 cm^{-1} (bottom)) and band shape are readily found) and showing the presence of trans character at 971 cm^{-1} (B).

The weight percent of each trans FAME in the test sample was calculated as wt % = $10^{-4}(w/W)(V/v)$, where w is the nanogram weight of analyte present in the injected aliquot, corrected for recovery, v is the microliter volume of the injected aliquot taken from the test sample solution, V is the milliliter volume of the test sample solution, and W is the gram test sample weight calculated from the microliter volume of transesterified test material multiplied by its density (0.88 g/mL; Formo, 1979).

RESULTS AND DISCUSSION

The characteristic MI/FT-IR bands that allow the differentiation of the FAME isomers are usually 1 order of magnitude weaker than the maximum absorbances found in such spectra. In order to quantitate the trans isomers of concern, GC conditions for separating FAME isomers had to be optimized first.

Table IV. Recovery of FAMEs, Based on GC/MI/FT-IR Determination of 17:0 Internal Standard

test matl	obsd mabs[a]	ng found	ng added	rec, %
6A1[b]	39.4	19.8	20.0	98.9
6A2	37.3	18.7	20.0	93.7
6A3	40.0	20.0	20.0	100.0
7H	12.8	6.60	6.67	99.3
margarine A	44.2	22.1	23.0	96.3
margarine B	51.3	25.7	26.0	98.7
av				97.8
RSD, %				2.4

[a] Milliabsorbance at 2935 cm^{-1}. [b] 6A1, 6A2, and 6A3 are replicate test samples prepared from test material 6A.

Performance Verification. The performance of the gas chromatograph with the capillary column connected to the flame ionization detector was characterized by using the K110 FAME standard solution. The splitless injec-

Table V. Quantitation of Trans FAMEs from Transesterified Margarines and Hydrogenated, Transesterified Soybean Oil

		observed mabs				GC/MI/FT-IR quantitation, ng injected				total ng[a]				weight %			
	GC	soybean oil[b]		margarine		soybean oil		margarine		soybean oil		margarine		soybean oil		margarine	
trans isomer	peak	S-Ni	Ni	A	B	S-Ni	Ni	A	B	S-Ni	Ni	A	B	S-Ni	Ni	A	B
*t*9-18:1	3	9.6	11.0	9.9	11.3	42.9	48.8	44.2	50.2	43.2	48.8	45.9	50.9	29.5	11.1	18.2	17.8
t,t-18:2	10	5.0				14.9				15.0				3.4			
	11	6.0				17.9				18.0				4.1			
	12	3.2		0.5	0.5	9.6		1.7	1.7	9.7		1.7	1.7	2.2		0.3	0.3
*t*9,*t*12-18:2	13	13.3		0.5	0.5	39.7		1.7	1.7	40.0		1.7	1.7	9.1		0.3	0.3
NMI-*c,t*-18:2	14	4.3	1.4	1.0	1.0	21.9	9.1	7.5	7.5	22.0	9.1	7.7	7.6	5.0	2.1	1.5	1.7
*c*9,*t*12-18:2	16	5.6	1.2	0.7	0.8	26.5	8.3	6.2	6.6	26.7	8.3	6.4	6.7	6.1	1.9	1.3	1.5
*t*9,*c*12-18:2	17	5.5	1.2	0.7	0.7	26.0	8.3	6.2	6.2	26.2	8.3	6.4	6.3	6.0	1.9	1.3	1.4

[a] Corrected for recovery. [b] S-Ni refers to hydrogenated soybean oil mixture 7H; Ni refers to hydrogenated soybean oil mixture 6A.

Table VI. Variability in GC/MI/FT-IR Quantitation of FAMEs

	replicate,[a] wt %			
FAME	6A1	6A2	6A3	RSD, %
3	11.6	11.2	11.1	2.3
14	2.3	2.2	2.1	4.5
16	2.1	2.0	1.9	5.0

[a] 6A1, 6A2, and 6A3 are replicate test samples prepared from test material 6A.

tion mode was used, and the effect of the final temperature (T_f) of the temperature program on the retention time and peak shape was documented. In the temperature range investigated (170–200 °C), the K110 GC peaks were symmetrical and base line resolved. At 170 °C the separation between the peaks was greatest, but the peak shapes were relatively broad; specifically, the values for full width at half-maximum (fwhm) were 5.4 and 8.4 s for peaks of the 16:0 and the cis isomer *c*9,*c*12-18:2, respectively, which are the first and last eluting components in this FAME mixture. As the temperature was systematically increased to 200 °C, the fwhm values decreased to 2.7 and 3.9 s for peaks of the same components, respectively. When the column was connected to the GC/MI interface, the corresponding fwhm values observed at 200 °C, namely 3.3 and 4.2 s, respectively, were still acceptable. MI/FT-IR spectra were collected for GC runs with T_f = 200 °C, since under such conditions the sharpness of the individual GC peaks was preserved. With isooctane as solvent (bp 99.2 °C), the initial temperature could be raised from 45 to 75 °C, thus limiting the GC analysis to about 18 min when T_f was set at 200 °C.

Analysis of Soybean Oil and Margarine FAMEs. The gas chromatogram observed for refined soybean oil FAMEs shows five peaks for 16:0, 18:0, and three C_{18} cis FAMEs (18:1, 18:2, 18:3). Upon hydrogenation, other isomers were found in varying amounts, depending on the extent of hydrogenation and the nature of the catalyst used. Parts A and B of Figure 1 show chromatograms for soybean oil that had been hydrogenated with a sulfur-containing nickel catalyst for 4 h (iodine value 96) and for margarine, respectively. GC peaks of the detected C_{18} FAMEs were sequentially referenced numerically starting with 18:0, as shown in Figure 1. Observed envelopes and overlapping shoulders suggest that several components correspond to GC peaks (e.g., isomers 2, 3, and 4) and that the true number of individual isomers is probably larger than the number of assigned peaks.

The MI/FT-IR spectra of the different FAME isomers were similar, except for a few subtle, yet characteristic, differences due to the number, position, and configuration of double bonds. The partial overlap of GC peaks was generally not detrimental to these measurements. Parts A–C of Figure 2 show the spectra of 18:0, *t*9,*t*12-18:2, and *c*9,*c*12-18:2 FAME standards, respectively, each of which exhibits unique features, for example, at 1123, 972, and 730 cm^{-1}, respectively. The common FT-IR bands observed for long hydrocarbon chain FAMEs, such as 18:0 (Table I), are due primarily to the intense CH_2 asymmetric (2935 cm^{-1}) and symmetric (2863 cm^{-1}) stretching vibrations, the CH_2 in-plane bend (1463 cm^{-1}) and CH_2 rock (727 cm^{-1}) deformation vibrations, and the strongly absorbing methyl ester carbonyl band at 1754 cm^{-1}.

The intensity of the strong CH_2 asymmetric stretch band appeared to decrease relative to that of the ester carbonyl stretch, as the number of double bonds increased (Table II); this intensity characteristic was used as a simple identification criterion based on the degree of unsaturation.

Some of the highly characteristic bands that allowed differentiation of the C_{18} FAMEs are listed in Table III. The unsaturated group =C—H stretch proved to be useful and occurred at four specific positions: 3035, 3018, 3010, and 3005 cm^{-1}. Figure 3 shows the expansion of this region where band position, intensity, and shape were clearly different for the different FAMEs shown. Also, the CH out-of-plane deformation vibrations in *trans*- or *cis*-$R_1HC{=}CHR_2$ groups exhibited unique resonances at 972 or 730 cm^{-1}, respectively (Table III; Figure 4). On the basis of the presence or absence of these characteristic features, the configuration of double bonds in the new isomers formed during hydrogenation could be readily established. For instance, isomer 8 exhibited an FT-IR spectrum similar to that of *c*9-18:1 (isomer 6) with a band at 3009 cm^{-1} (Figure 5A), which suggests that it is a cis monoene FAME, with the double bond at a carbon other than C9, since the retention time at which it eluted was slightly longer than that of the *c*9-18:1 isomer.

For the hydrogenated soybean oil mixture 7H, each of the four isomers 10–13 (Figure 1A) showed a spectrum similar to that of the *t*9,*t*12-18:2 standard (same retention time as isomer 13) with bands at 3035 and 3005 cm^{-1} as well as the unique and sharp feature at 972 cm^{-1} (Figure 5B), although they eluted at different times. These characteristics indicated that isomers 10–12 in mixture 7H were also *trans,trans*-dienes like isomer 13. For the hydrogenated soybean oil mixture 6A and the two margarines investigated, FAMEs 10 and 11 produced FT-IR spectra characteristic of cis isomers. Only FAMEs 12 and 13 in the margarines were *trans,trans*-dienes, but these species were not found in mixture 6A. Careful inspection of the chromatograms obtained at 170 °C (Figure 1) revealed that, for the margarines (Figure 1B) and mixture 6A, FAMEs 10 and 11 eluted after those in mixture 7H (Figure 1A) by 4 and 5 s, respectively. These data

from the narrow retention time window where compounds 10 and 11 elute indicated that the various test materials investigated contained different species. Thus, these cis isomers were labeled 10′ and 11′ for the margarines and mixture 6A.

Isomers 16 and 17, which were identified as *c*9,*t*12-18:2 and *t*9,*c*12-18:2, respectively, on the basis of their relative retention times (Kobayashi, 1980), showed identical FT-IR spectra with features characteristic of both cis (3018 cm^{-1}) and trans (971 cm^{-1}) double bonds (Table III). On the other hand, isomer 14 showed a spectrum that was quite similar to those of isomers 16 and 17 (Figure 6A,B) except that the =C—H stretch band was narrower than those of isomers 16 and 17 and was shifted to 3010 cm^{-1}, as expected for a *cis*-monoene FAME, suggesting that the cis and trans double bonds in isomer 14 are separated by more than a single methylene group, i.e., that it is a non-methylene-interrupted (NMI) *c,t*-18:2 or *t,c*-18:2. Isomer 20 gave rise to a band at 3017 cm^{-1} (Table III) characteristic of a *cis,cis*-diene FAME. FAME 21 (*c*9,*c*12,*c*15-18:3) is a *cis*-triene with relatively intense bands at 3019 and 727 cm^{-1}. In a preliminary search for *trans*-triene FAME isomers, species that eluted before FAME 21 were detected by FT-IR and exhibited bands at 972, 3020, and 730 cm^{-1}, indicating the presence of cis and trans configurations. These data are consistent with the results of Perkins and Smick (1987). The identification of C_{18} triene isomers is the subject of further investigation.

FT-IR Quantitation of Trans Isomers. Determination of *trans*-monoene and -diene FAMEs in argon matrices involved determination of the height of the sharp CH out-of-plane deformation band in *trans*-$R_1HC{=}CHR_2$ groups. Calibration plots of absorbance at 972 and 971 cm^{-1} for *t*9,*t*12-18:2 and *t*9-18:1, respectively, were generated as a function of nanograms injected. The linear regression equations were $y = -0.068 + 0.34x$ for *t*9,*t*12-18:2 and $y = -0.80 + 0.24x$ for *t*9-18:1. The coefficients of determination, r^2, were 0.97 (*t*9,*t*12-18:2) and 0.98 (*t*9-18:1), meaning that 97% (*t*9,*t*12-18:2) and 98% (*t*9-18:1) of the variability in absorbance are accounted for by the independent variable, which is the amount of analyte injected. The 95% confidence bounds of the mean predicted values encompassed the origin, indicating the absence of a bias. Recovery based on determination of the 17:0 FAME internal standard (Table IV) averaged 97.8% with a relative standard deviation (RSD) of 2.4%. Quantitative results for trans species present in the margarines and hydrogenated oils are summarized in Table V. The precision of repeat analyses of the same solution over several days yielded results for isomers 3, 14, and 16 showing that the GC/MI/FT-IR technique leads to reproducible results (Table VI); the precision of determination for replicate injections expressed in terms of RSD was in the range 2.3–5.0%.

CONCLUSIONS

The double-bond configuration of hydrogenation products of soybean oil and margarines was established and revealed the presence of as many as four *trans,trans*-diene isomers; *t*9,*t*12-18:2 and other trans FAMEs were quantitated by GC/MI/FT-IR.

The presence of one or two trans double bonds in FAME isomers gave rise to two hydrocarbon stretch vibrations around 3035 and 3005 cm^{-1}, while cis double bonds exhibited only one band at either 3010 cm^{-1} (one cis group) or 3018 cm^{-1} (two or three cis groups). The intensity of the *trans*- or *cis*-=C—H stretch bands was directly proportional to the number of double bonds, while that of the aliphatic chain C—H stretch vibration decreased relative to the C=O stretch band intensity, as the number of double bonds increased in C_{18} FAMEs. Moreover, the intensity of the CH out-of-plane deformation vibration (972 cm^{-1}) in *trans,trans*-diene FAMEs was greater than that in *trans*-monoene FAMEs. Cis,trans, or trans,cis isomers exhibited bands that are characteristic of both configurations.

The *t*9,*t*12-18:2 level was low for margarines, 0.3% (corresponding to 0.9 ng deposited on the Cryolect disk), and was as high as 9.1% for hydrogenated soybean oil mixture 7H, which was hydrogenated with use of a sulfur–nickel catalyst. The highest trans levels (11.1–29.5%) were those of the *t*-18:1 FAME isomers. Since the hydrogenation of soybean oil resulted in a distribution of isomers that led to GC profiles with peaks that were not always base line resolved, indicating that some isomers probably coeluted, further separation of hydrogenated mixtures by thin-layer chromatography and GC prior to MI/FT-IR analysis is planned.

ACKNOWLEDGMENT

We thank C. Kopral for statistical assistance and K. Farren for technical assistance.

LITERATURE CITED

Ackman, R. G. WCOT (Capillary) Gas-Liquid Chromatography. In *Analysis of Oils and Fats*; Hamilton, R. J., Rossell, J. B., Eds.; Elsevier Science: New York, 1987; p 137.

Anderson, R. L.; Fullmer, C. S., Jr.; Hollenback, E. J. Effects of the *trans* Isomers of Linoleic Acid on the Metabolism of Linoleic Acid in Rats. *J. Nutr.* **1975**, *105*, 393–400.

AOAC. *Official Methods of Analyses*, 14th ed.; Association of Official Analytical Chemists: Arlington, VA, 1984; Sections 28.023–28.024, 28.056–28.059.

Bourne, S.; Reedy, G.; Coffey, P.; Mattson, D. Matrix Isolation GC/FTIR. *Am. Lab. (Fairfield, Conn.)* **1984**, *16*, 90–101.

Formo, M. W. Physical Properties of Fats and Fatty Acids. In *Bailey's Industrial Oil and Fat Products*; Swern, D., Ed.; Wiley: New York, 1979; Vol. 1, p 189.

Gildenberg, L.; Firestone, D. Gas Chromatographic Determination of *trans* Unsaturation in Margarine: Collaborative Study. *J. Assoc. Off. Anal. Chem.* **1985**, *68*, 46–51.

Holloway, T. T.; Fairless, B. J.; Freidline, C. E.; Kimball, H. E.; Kloepfer, R. D.; Wurrey, C. J.; Jonooby, L. A.; Palmer, H. G. Performance of a Gas Chromatographic-Matrix Isolation-Fourier Transform Infrared Spectrometer. *Appl. Spectrosc.* **1988**, *42*, 359–369.

Holman, R. T.; Mahfouz, M. M.; Lawson, L. D.; Hill, E. G. In *Dietary Fats and Health*; Parkins, E. G., Visek, W. J., Eds.; American Oil Chemists Society: Champaign, IL, 1983; p 370.

Hwang, D. H.; Kinsella, J. E. The Effects of Trans, Trans Methyl Linoleate on the Concentration of Prostaglandins and Their Precursors in Rat. *Prostaglandins* **1979**, *17*, 543–559.

Jackson, H. W.; Roy, D. J.; Applewhite, T. H. Letter to the Editor. *Can. Inst. Food Sci. Technol. J.* **1980**, *13* (3), A19.

Kobayashi, T. Gas-Liquid Chromatographic Separation of Geometric Isomers of Unsaturated Fatty Acid Methyl Esters Using a Glass Capillary Column. *J. Chromatogr.* **1980**, *194*, 404–409.

Marchand, C. M.; Beare-Rogers, J. L. Complementary Techniques for the Identification of *trans,trans*-18:2 Isomers in Margarines. *Can. Inst. Food Sci. Technol. J.* **1982**, *15* (1), 54–57.

Mossoba, M. M.; Chen, J. T.; Brumley, W. C.; Page, S. W. Application of Gas Chromatography/Matrix Isolation/Fourier Transform Infrared Spectrometry to the Determination of Ethyl Carbamate in Alcoholic Beverages and Foods. *Anal. Chem.* **1988**, *60*, 945–948.

Mossoba, M. M.; Niemann, R. A.; Chen, J.-Y. T. Picogram Level Quantitation of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in Fish Extracts by Capillary Gas Chromatography/Matrix Isolation/

Fourier Transform Infrared Spectrometry. *Anal. Chem.* **1989**, *61*, 1678–1685.
Perkins, E. G.; Smick, C. Octadecatrienoic Fatty Acid Isomers of Partially Hydrogenated Soybean Oil. *JAOCS, J. Am. Oil Chem. Soc.* **1987**, *64*, 1150–1155.
Privett, O. S.; Phillips, F.; Shimasaki, H.; Nozawa, T.; Nickell, E. C. Studies of Effects of Trans Fatty Acids in the Diet on Lipid Metabolism in Essential Fatty Acid Deficient Rats. *Am. J. Clin. Nutr.* **1977**, *30*, 1009–1017.
Reedy, G. T.; Ettinger, D. C.; Schneider, J. F.; Bourne, S. High-Resolution Gas Chromatography/Matrix Isolation Infrared Spectrometry. *Anal. Chem.* **1985**, *57*, 1602–1609.
Sahasrabudhe, M. R.; Kurian, C. J. Fatty Acid Composition of Margarines in Canada. *Can. Inst. Food Sci. Technol. J.* **1979**, *12*, 140–145.
Sebedio, J. L.; Le Quere, J. L.; Semon, E.; Morin, O.; Prevost, J.; Grandgirard, A. Heat Treatment of Vegetable Oils. II. GC-MS and GC-FTIR Spectra of Some Isolated Cyclic Fatty Acid Monomers. *JAOCS, J. Am. Oil. Chem. Soc.* **1987**, *64*, 1324–1333.
Slover, H. T.; Thompson, R. H., Jr.; Davis, C. S.; Merola, G. V. Lipids in Margarines and Margarine-like Foods. *JAOCS, J. Am. Oil. Chem. Soc.* **1985**, *62*, 775–786.

Received for review February 22, 1989. Accepted August 2, 1989.

Isolation and Purification of 7S and 11S Globulins from Broad Beans and Peas

Vladimir V. Suchkov, Irina A. Popello, Valeriy Ya. Grinberg,* and Vladimir B. Tolstogusov

A. N. Nesmeyanov Institut of Organoelement Compounds, USSR Academy of Sciences, Vavilov St. 28, 117312 Moscow, USSR

A simple and effective preparation technique for isolation of 7S and 11S globulins from broad beans and pea seeds has been developed. It is based on differential solubility of 7S and 11S globulins in sodium chloride solution at pH 4.8 and at 5 and 20 °C. The method makes it possible to isolate 6 g of 11S and 1 g of 7S globulins from 100 g of broad bean meal or 1.5 g of 11S and 2 g of 7S globulins from 100 g of pea meal, with only low centrifugation. If necessary, the admixture of 15S component in 11S globulin preparations can be removed by gel chromatography on Sepharose CL-6B.

Preparative isolation of 7S and 11S globulins from legume seeds presents a certain difficulty. A number of techniques are known to be effective only in obtaining enriched globulin fraction: salting out by ammonium sulfate (Danielsson, 1949), isoelectric precipitation (Derbyshire et al., 1976), gel chromatography (Gwiazda et al., 1980). The method of selective thermal denaturating (Varfolomeeva et al., 1985) makes it possible to obtain a precipitate of homogeneous 11S globulins, but with denaturation and loss of considerable amount of 11S and all 7S globulins. Centrifugation in a sucrose gradient (Mori and Utsumi, 1979) yields a homogeneous globulin fraction, but this method cannot be suggested as a preparational one. A successful solution of the problem of preparational isolation of 7S and 11S globulins was achieved by zone precipitation (Scholz et al., 1974; Wright and Boulter, 1974), which combines the principle of isoelectric precipitation with that of gel chromatography. The main drawback of this method is the need to use multiple chromatography techniques to achieve complete separation of components. Thus, none of the methods listed above has proved to be effective in obtaining homogeneous preparations of 7S and 11S globulins in the quantities necessary for the studies of different physicochemical properties of concentrated protein solutions.

7S and 11S globulins are known to have different solubility in solution with low ionic strength (Osborne and Campbell, 1898a–c). 7S globulins are more soluble than 11S globulins. These data formed the basis for developing a simple method of isolating 7S and 11S globulins from broad beans and pea seeds.

MATERIALS AND METHODS

Sorted seeds of broad beans (*Vicia faba* L.) of Fribo variety and those of peas (*Pisum sativum* L.) of Orlovsky-3 variety were ground in a laboratory cyclone mill (UDY Corp., Fort Collins, CO). The flour was sifted with the sieve of 0.3-mm cell size.

Isolation of 11S Globulins. A 100-g portion of meal was dispersed in 900 mL of distilled water and the resultant mixture titrated with 0.5 M solution of NaOH to pH 8.0 and then mixed at 50 °C for 1 h. The suspension was centrifuged at 5000*g* for 30 min (K-70D, MLV, GDR). Dry NaCl was added to the supernatant to 0.5 M. The solution was titrated to pH 4.8 with 0.1 M HCl containing 0.5 M NaCl. The suspension obtained was centrifuged at 5000*g* for 30 min, and supernatant was diluted with distilled water to 0.3 M NaCl concentration. It was centrifuged at 1000*g* for 10 min. After centrifugation the system separated into two liquid phases: 1, lower phase; 2, upper one. The lower liquid phase with a protein concentration of about 50% and 0.3 M NaCl concentration was the fraction enriched with 11S globulin. Dry NaCl was added to this concentrated liquid phase to 0.6 M. The obtained concentrated solution was diluted with 0.6 M NaCl solution to about 5% protein concentration. Then, it was centrifuged at 5000*g* for 30 min. The supernatant was diluted with distilled water to 0.3 M NaCl. The system separated into two phases when kept for 10–15 min. The lower concentrated protein phase contained 11S globulin with negligible admixture of 15S globulin. All the operations were made at 20 °C. Protein yield was 6 g from broad beans and 1 g from pea seeds per 100 g of meal.

Isolation of 7S Globulins. The upper liquid phase was cooled to 5 °C and exposed to this temperature for 1 h. The precipitate obtained (fraction enriched with 7S globulin) was separated by centrifugation at 1000*g* for 15 min at 5 °C. The supernatant with 0.3 M NaCl concentration was diluted with 5 °C cool distilled water to 0.15 M NaCl concentration. The precip-

0021-8561/90/1438-0092$02.50/0