Comparison of FTIR and Capillary Gas Chromatographic Methods for Quantitation of *trans* Unsaturation in Fatty Acid Methyl Esters[1]

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A computer-assisted method has been developed for estimation of isolated *trans* unsaturation using the peak area of the *trans* absorbance band at 966 cm^{-1} from FTIR spectra of fatty acid methyl esters. Peak areas were used to determine the *trans* content of weighed standards containing from 0 to 100% methyl elaidate and of hydrogenated soybean oil samples containing up to 36% *trans* unsaturation. These data for percent *trans* by FTIR were compared to corresponding data obtained by capillary gas chromatography and the AOCS Official Method 14-61. Determination of isolated *trans* composition in oils using peak areas gave values with the smallest standard deviation for weighed standards and values within 4% of those obtained by capillary gas chromatography and the AOCS Official Method for hydrogenated samples.

Various infrared (IR) spectrophotometric methods for estimation of *trans* unsaturation in lipids have been suggested and routinely used in the fat industry for many years (1-4). These methods are based on measuring peak height at 965-966 cm^{-1} from a manually drawn or zero adjusted (where possible) baseline and comparing this absorbance value to that of standards or to other related absorbances.

Recent advances in equipment technology have incorporated computer capabilities into FTIR instruments allowing manipulations of the IR spectra, i.e., enlargement, isolation of selected regions, and calculation of peak areas, all of which can be outputted to a plotter or a printer. If output is to a printer, an IR absorbance grid suitable for manual measurement of peak height differences in absorbance units is not normally obtained. Standard peak height procedures for estimating isolated *trans* unsaturation, therefore, become more tedious and less accurate.

A simple computer-assisted procedure has been developed for determining isolated *trans* unsaturation in fatty acid methyl esters (FAME) based on integration of the area of the absorbance peak due to *trans* unsaturation. Areas were obtained with a Fourier transform infrared (FTIR) instrument and used to determine percent isolated *trans* in weighed mixtures of methyl elaidate and methyl oleate (0-100% elaidate) and in six partially hydrogenated soybean oil samples. These data were compared to corresponding data determined by the AOCS Official Method (peak height) and by capillary gas chromatography.

MATERIALS AND METHODS

Methyl oleate (*cis*-9-octadecenoate) and methyl elaidate (*trans*-9-octadecenoate) were purchased from Hormel (Hormel Institute, Austin, Minnesota). Purity of both esters was listed as 99+% and confirmed by capillary gas chromatography (cap-GC). Samples of each ester were weighed into a volumetric flask and diluted to volume with carbon disulfide to give a final concentration between 20 and 30 mg/ml. The composition of 11 weighed standard mixtures ranged from 3 to 51% *trans*.

Methyl esters of previously characterized samples of commercial soybean oils, which had been partially hydrogenated at this laboratory using nickel catalyst (unpublished data), were prepared via a modified procedure using 0.5 N $NaOCH_3$ (5). After extraction and drying, the methyl esters were weighed and diluted for analysis by FTIR and cap-GC.

A Perkin-Elmer Infrared Fourier Transform Model 1750 Spectrometer (Perkin-Elmer, Inc., Oak Brook, Illinois) equipped with a Model 7300 professional computer was used for the analyses. Each sample was routinely scanned five times. A computer program was written to calculate areas of the *trans* absorbance peaks between predetermined wave numbers. Percent *trans* was calculated as follows:

$$\%\ trans = \left[\frac{\text{area}}{\text{(cell path, cm) (conc., g/l) (cell constant)}}\right] \times 100$$

Integration of peak areas was performed using baselines drawn between wave numbers as shown in Figure 1. The largest wave number was chosen as the average of several minima generated by samples containing between 5 and 100% elaidate. It intersected the absorbance curve either at a tangent to a peak shoulder or at a flat baseline between two peaks. Several points along the absorption curve which met these

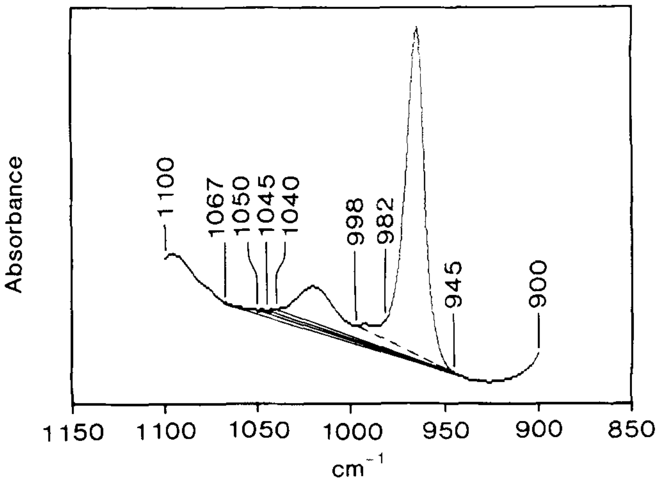

FIG. 1. FTIR absorbance spectrum from 1100 to 900 cm^{-1} of methyl elaidate in CS_2. Baselines for calculation of area of *trans* absorbance band start at 1040, 1050, 1067 and 1045 cm^{-1} for FTIR methods 1, 2, 3 and 4, respectively, and end near 945 cm^{-1}. The AOCS Official Method 14-61 recommends a baseline between 998 and 944 cm^{-1} for methyl esters.

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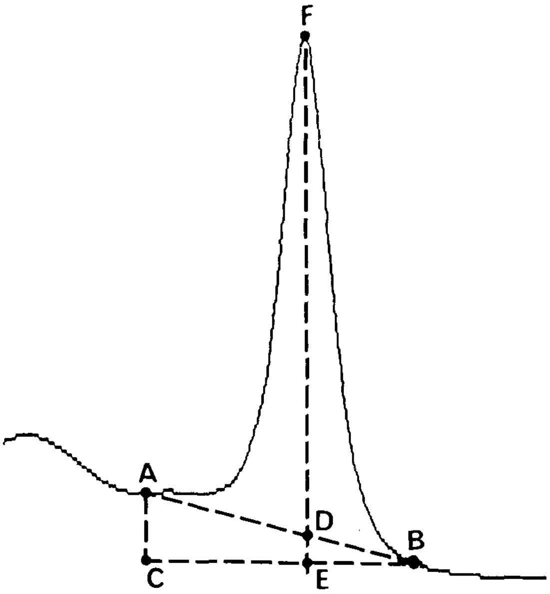

FIG. 2. FTIR absorbance band at 966 cm^{-1} used to calculate percent *trans* unsaturation by the AOCS Official Method 14-61. Wave numbers and absorbance values at A, B and F are obtained from the computer-displayed spectrum and allow calculation of the absorbance DF.

criteria were chosen. The lower terminus of the baseline was approximately that used in the AOCS Official Method (3). Four separate programs were written to calculate percent *trans* using different baselines (Fig. 1).

Differential absorbance at the maximum of the *trans* absorbance band at 966 cm^{-1} was also used to estimate *trans* according to the AOCS Official Method Cd 14-61 (3). A baseline was drawn between 10.02 μ (Point A, 998 cm^{-1}) and 10.59 μ (Point B, 944 cm^{-1}) (Fig. 2). The absorbance at designated wave numbers for points A, B and F were obtained from the computer display. From these values, absorbance at D (A_D) was calculated using plane geometry equations for proportional right triangles as $A_B + [(A_A - A_B) \times \frac{EB}{CB}]$. The difference in absorbances at F and D replaced the area value in the above equation used for calculating percent *trans*.

Capillary gas chromatography was performed on a Varian Model 3400 chromatograph (Varian, Sugar Land, Texas). Samples were run on a 100-m × 0.25-mm SP 2560 capillary column (split ratio 100:1) (Supelco, Inc., Bellefonte, Pennsylvania) temperature programmed from an initial 15 min hold at 165°C to 220°C at 3°C/min. Analog data were collected with a ModComp computer (Modular Computer System, Inc., Ft. Lauderdale, Florida) which calculated peak areas. Areas for *trans* monoenes and *trans* dienes were combined for comparison with IR data.

RESULTS AND DISCUSSION

The availability of modern FTIR instruments equipped with small computers allows percent *trans* to be calculated by either peak area or maximum absorbance value. Because peak height, the basis of the AOCS method, is only one variable used to calculate the area of a triangle (which a Gaussian peak closely approximates), areas of *trans* absorption bands should also yield an accurate estimate of isolated *trans* unsaturation. The area under a peak depends, of course, on the baseline chosen. The baseline recommended by the AOCS Official Method used starting points at 10.02 μ (998 cm^{-1}) and 10.59 μ (944 cm^{-1}). As seen in Figure 1, these wave numbers determine a line drawn between two absorbance minima. Workers at our laboratory have found that the higher wave number varies with the proportion of *trans* concentration and, therefore, have used different minima to determine the baseline. Therefore, for comparison, baselines were chosen at various ab-

TABLE 1

Percent Methyl Elaidate in Weighed Mixtures Determined by Capillary GC and Infrared Methods

			FTIR method[a]									
Weighed	GC		1		2		3		4		AOCS	
0	.15		.17		.29		.81		0		0	
2.76	3.00	(8.7)[b]	2.79	(1.1)	3.01	(9.1)	3.44	(24.6)	2.74	(-.7)	1.11	(-59.8)
9.70	10.25	(5.7)	9.71	(.1)	9.90	(2.1)	10.29	(6.1)	9.63	(-.7)	11.35	(17.0)
11.67	11.86	(1.6)	11.80	(1.1)	12.05	(3.3)	13.12	(12.4)	11.72	(.4)	9.92	(-15.0)
13.50	13.43	(-.5)	13.53	(.2)	13.87	(2.7)	14.97	(10.9)	13.71	(1.6)	12.96	(-4.0)
18.40	17.68	(-3.9)	16.55	(-10.1)	17.77	(-3.4)	18.90	(2.7)	17.87	(-2.9)	18.43	(.2)
19.80	19.15	(-3.3)	20.49	(3.5)	20.51	(3.6)	21.23	(7.2)	20.20	(2.0)	19.27	(-2.7)
26.85	26.09	(-2.8)	26.27	(-2.2)	26.33	(-1.9)	27.54	(2.6)	26.06	(-2.9)	25.07	(-6.6)
28.20	28.32	(.4)	26.49	(-6.1)	27.35	(-3.0)	28.16	(-.1)	27.61	(-2.1)	27.07	(-4.0)
33.30	34.28	(2.9)	33.73	(1.3)	33.66	(1.1)	34.04	(2.2)	33.25	(-.2)	33.09	(-.6)
34.10	34.04	(-.2)	32.94	(-3.4)	33.32	(-2.3)	33.75	(-1.2)	33.21	(-2.6)	35.49	(4.1)
51.00	51.83	(1.6)	51.35	(.7)	50.94	(-.1)	51.25	(.5)	51.00	(0)	51.63	(1.2)
100.00	99+		98.24	(-1.8)	97.43	(-2.6)	98.32	(-1.7)	99.17	(-.8)	—	

[a] Baselines were determined by a line drawn between the smallest and largest wave numbers given for each FTIR method. Integrated areas were those under the absorbance peak between the middle and lowest wave numbers. Method 1, 1040-981-947 cm^{-1}; 2, 1050-981-945 cm^{-1}; 3, 1067-982-945 cm^{-1}; 4, 1045-982-945 cm^{-1}. The middle wave number (upper integration limit) was lowered to 977 cm^{-1} for samples containing 5-12% *trans* and to 973 cm^{-1} for samples with 0-5% *trans*.

[b] Numbers in parentheses are percent error from weighed values.

sorbance minima, and areas of the isolated *trans* absorbance peaks were calculated between the smallest wave number and a perpendicular line dropped from the peak minima to the baseline.

A series of standards containing weighed amounts of methyl elaidate and methyl oleate (from 3 to 51% elaidate) was prepared and analyzed with the FTIR under four different baseline options. Values for percent isolated *trans* determined by GC (area percent) and by FTIR for weighed standards are presented in Table 1. IR data for percent *trans* were determined from peak height (AOCS method, column 7) and from peak areas calculated using baselines indicated in the table.

Repetitive determinations of percent *trans* with the 28.20% elaidate standard resulted in standard deviations ($P<.05$) of ± 0.42 for FTIR (21 runs, 5 scans each) and ± 0.22 for GC (6 runs). Linear regression analysis of the elaidate-oleate standards gave the best correlation coefficient (0.9999) and standard deviation (0.39) with FTIR method 4 (Fig. 3). Data obtained by the capillary gas chromatographic and AOCS methods for the weighed standards had correlation coefficients and standard deviations of 0.9993 and 0.58 and of 0.9972 and 1.17, respectively. Sleeter (6) has reported similar results with FTIR using a second order polynomial fit of *trans* area data.

Areas determined by cap-GC for elaidate ranged from -3.9 to 8.7% of the weighed values. Corresponding values determined by the four FTIR programs deviated from the weighed standards by -10.1 to 3.5 (Method 1), -3.4 to 9.1 (Method 2), -1.7 to 24.6 (Method 3), and from -2.9 to 2.0 (Method 4) percent. The AOCS peak height method had percent differences of from -59.8 to 17.0.

Standards containing less than 10% isolated *trans* required a different integration end-point for accurate estimation of *trans* due to the smaller width of the *trans* absorption band. Pure methyl elaidate produced an absorption band with minima at 944 and 985 cm^{-1}. As the *trans* concentration decreased to less than 10%, the peak minima were 944 and 977 cm^{-1}. Below 5% *trans*, peak minima were 944 and 973 cm^{-1}.

The necessity to reduce the integration limits for samples low in *trans* unsaturation was not unexpected because calculated areas would include the area between the absorbance trace and the baseline beyond the actual *trans* band. This would result in high values for *trans* and was evident in data that were 7 to 104% higher than in the weighed standards (data not shown).

A similar modification in baseline is not suggested in the AOCS method. With this method, the largest variations from weighed values were also found with samples containing 10% or less methyl elaidate. The percent deviation (-59.8) in the standard containing the smallest proportion of *trans* was improved to -22.5 if peak absorbance was determined from a baseline defined by a tangential line drawn at absorbance minima instead of at predetermined wave numbers. GC data also had its largest percent deviation associated with the samples containing the least *trans* unsaturation.

Hydrogenated oils. Six soybean oils which had been partially hydrogenated were analyzed as methyl esters by the procedures used for the standards. Four of these

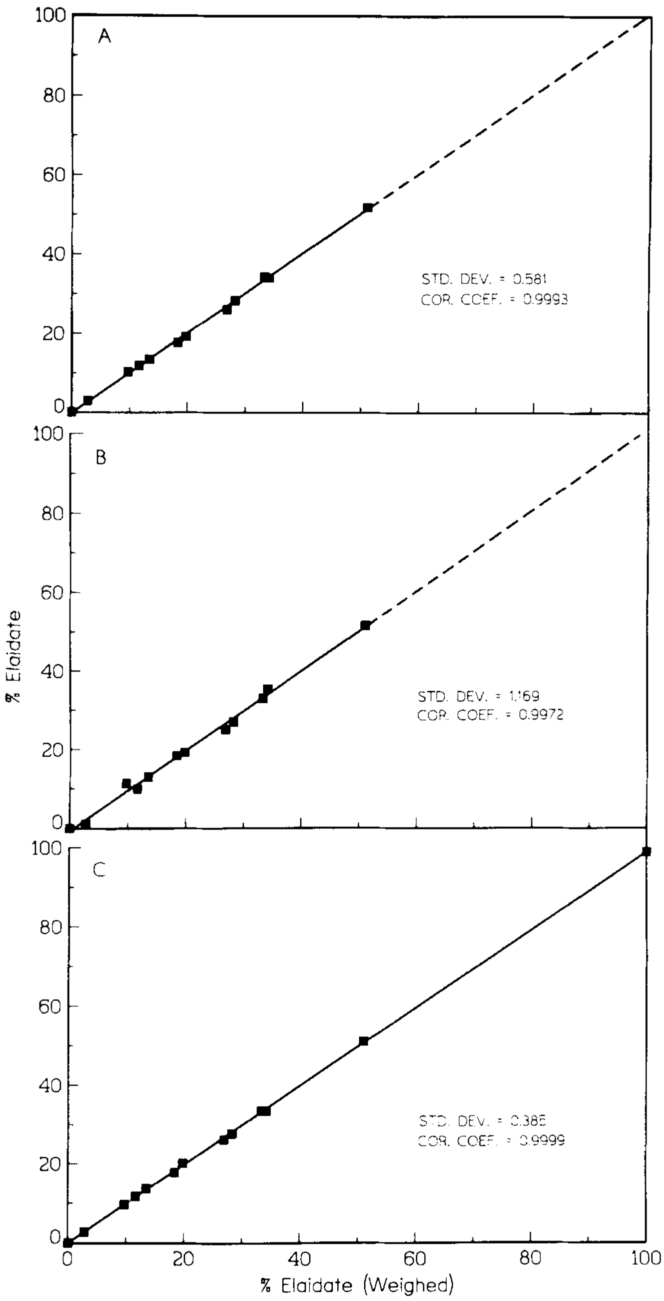

FIG. 3. Linear regression fit of data estimating percent *trans* in weighed standards determined by A, capillary gas chromatography; B, FTIR-AOCS Official Method 14-61, and C, FTIR areas.

oils had been analyzed previously for isolated *trans* unsaturation with a dispersive IR instrument (unpublished data) and contained up to 36% isolated *trans*.

All methods for determining percent *trans* gave similar results and were within two percentage points of the previously determined IR values (Table 2). The percent *trans* calculated from GC data were generally low for the more highly reduced samples when compared to IR data. This difference is due to the complexity of the isomers and the difficulties in obtaining complete separation and identification of all the *trans*-containing ester peaks (Fig. 4). GC area percentages for *trans* dienes have been recalculated to reflect vari-

TABLE 2

Comparison of Methods for Determining Percent Isolated *trans* in Partially Hydrogenated Soybean Oil Methyl Esters

Sample #	Dispersive IR	GC	FTIR Method				AOCS
			1	2	3	4	
1	ND[a]	.15	.27	.94	2.93	.81	.10
			.25[b]	.58[b]	1.61[b]	.27[b]	
2	5.2	4.92	6.24	6.78	8.47	6.16	5.44
			4.93[b]	5.18[b]	6.16[b]	4.89[b]	
3	12.4	11.20	14.02	14.10	15.98	13.90	12.84
			13.15[b]	13.26[b]	14.43[b]	12.92[b]	
4	21.3	22.93	20.70	21.02	22.51	21.21	19.91
5	ND	20.65	24.13	24.53	26.21	24.52	22.93
6	36.0	34.31	36.25	36.62	38.10	36.70	34.01

[a] ND, not determined.

[b] Integration limits for area calculations were from 977 cm^{-1} (#3) and from 973 cm^{-1} (#1 and 2) to the lower value for each method defined in Table 1.

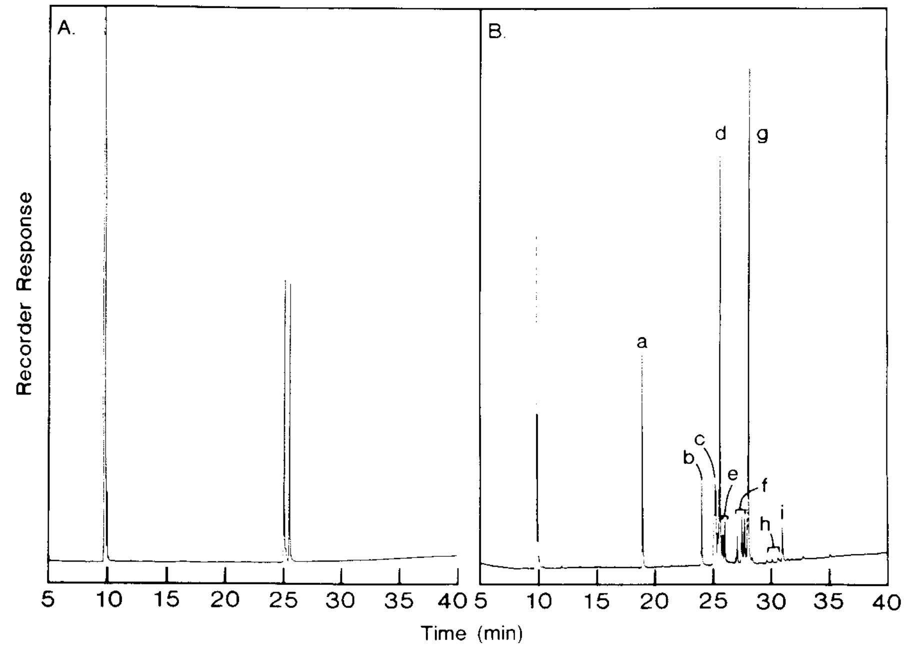

FIG. 4. Capillary gas chromatograms of A) methyl elaidate (51%) and methyl oleate weighed standard, and B) a partially hydrogenated soybean oil (sample 5). Peak identification: a) palmitate; b) stearate; c) *trans*-18:1 isomers; d) oleate; e) unknown (*cis*-18:1 isomers); f) *trans*-18:2 isomers; g) linoleate; h) *trans*-18:3 isomers; i) linolenate.

ations resulting from differing extinction coefficients in mono- and di-*trans* dienoic methyl esters (7,8) and combined with percentages for *trans* monoenes in Table 2. If areas for the tentatively identified *trans* trienes are included, the *trans* values in the highly hydrogenated oils are increased by 0.5 to 1.0% and more closely agree with the FTIR data.

In a study conducted by Madison et al. (9) using partially hydrogenated oils, shortenings and margarines, peak heights of the *trans* IR absorptions were measured from a baseline tangent to peak minima at about 937 and 1015 cm^{-1}. Data obtained with their method differed from corresponding capillary gas chromatographic data by less than two percentage points.

The results in Tables 1 and 2 indicate that calculation of percent *trans* in hydrogenated oils containing less tha ca. 5% *trans* is improved by the use of appropriately selected integration limits. The criteria for selection of these parameters, as described, can be accomplished automatically by appropriate computer programs. Determination of an absolute *trans* value for hydrogenated oils is not easy, but because the agreement between the GC and FTIR data is good, it is reasonable to assume that both methods give accurate results. The best method to use depends on the analytical information required. FTIR is fast and simple but requires more sample than GC and does not provide information on other fatty acid percentages.

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