trans Fatty Acid Content of Commercial Margarine Samples Determined by Gas Liquid Chromatography on OV-275

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ABSTRACT AND SUMMARY

Gas liquid chromatography (GLC) of margarine methyl esters on a 20 ft x 1/8 in. column containing 15% OV-275 on 100/120 mesh Chromosorb P AW-DMCS at 220 C provided practical separations of *trans* octadecamonoenoates from the corresponding *cis*-isomers and of *trans, trans-* and *cis,trans*-octadecadienoates from the corresponding *cis,cis*-isomers. *Trans* content by GLC was in reasonable agreement (±1-2%) with total *trans* content by infrared analysis in six of seven commercial margarine samples.

INTRODUCTION

Fatty acids containing *trans* double bonds are normal constituents of the human diet being formed by rumen microorganisms and appearing in the milk and tissues of ruminants (1,2). Additional *trans* fatty acids are produced during the commercial hydrogenation of liquid vegetable oils to produce oleomargarines, cooking oils, salad oils, and shortenings. On the basis of USDA research statistics fats available for consumption in the United States contain 5-8% *trans* fatty acids. Long-term, multigeneration studies in rats in Deuel's laboratory (3-5) are usually the basis for statements that the *trans* fatty acids are a harmless addition to the diet. Aaes-Jørgenseen (6,7) and Thomasson (8) have also made significant contributions in this area. A variety of factors—continuing public concern regarding the composition of dietary fats, recent innovations in labeling regulations (9), and occasional misunderstandings engendered by publication of pseudo-research data in the popular media—have led to increased interest in this area.

Hydrogenated fats contain a complex mixture of fatty acids since double bond migration occurs in addition to inversion of geometric configuration. While the residual double bond is at or near the location of the original double bond, all of the *cis* and *trans* monoenoic acids with double bonds between C_8 and C_{12} are usually present (10). As noted by Ackman (11) the gas liquid chromatographic relative retention times of all or most of the *cis* and *trans* octadecenoate isomers have been reported for Apiezon-L, neopentyl glycol succinate polyester, diethylene glycol succinate polyester, XE-60, polyphenyl ether, and β-cyanoethyl methyl-siloxane phases. While interesting analyses have been reported on 150-200 ft capillary columns, definitive analyses of margarine samples require a relatively complicated analytical procedure as reviewed by Dutton (12) and Connacher (13). Ottenstein et al. (14) have recently shown that oleate and elaidate could be separated on packed columns using either SP-2340 or OV-275. It was of interest to determine if OV-275 could be used constructively for analysis of the mixture of *cis* and *trans* positional isomers of octadecenoate and octadecadienoate found in commercial oleomargarines.

MATERIALS AND METHODS

Margarine samples were obtained at local retail grocery stores, extracted with petroleum ether, saponified, and methylated with BF_3-methanol. Fatty acid composition was determined by gas liquid chromatography on a 6 ft x 2

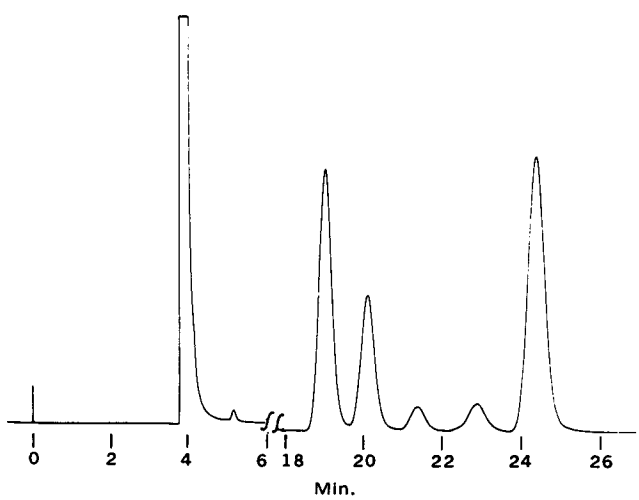

FIG. 1. Fatty acid methyl ester standards. From left to right *trans*-18:1ω9, *cis*-18:1ω9, *trans,trans*-18:2ω6, *cis,trans*-18:2ω6, and *cis,cis*-18:2ω6. 20 ft x 1/8 in. column of 15% OV-275 on Chromosorb P AW-DMCS at 230 C.

TABLE I

Fatty Acid Composition of Margarine Samples[a]

Sample no.	Fatty acids (%)						
	16:0	18:0	18:1[b]	*t*-18:1	*c*-18:1	18:2	18:3
1	12.2	7.8	48.1	24.0	23.1	29.7	3.2
2	10.5	7.2	64.2	30.5	34.6	15.5	tr
3	17.6	7.8	41.9	17.6	24.5	30.6	1.9
4	13.1	8.1	64.2	33.6	33.5	11.7	tr
5	8.5	8.0	32.2	18.0	17.8	47.7	–
6	10.4	6.3	25.2	6.3	18.7	58.3	–
7	12.3	6.1	35.8	13.1	23.3	45.1	–

[a]Single analyses on each sample on each column.

[b]Total 18:1 determined on DEGS-PS, all other values from OV-275

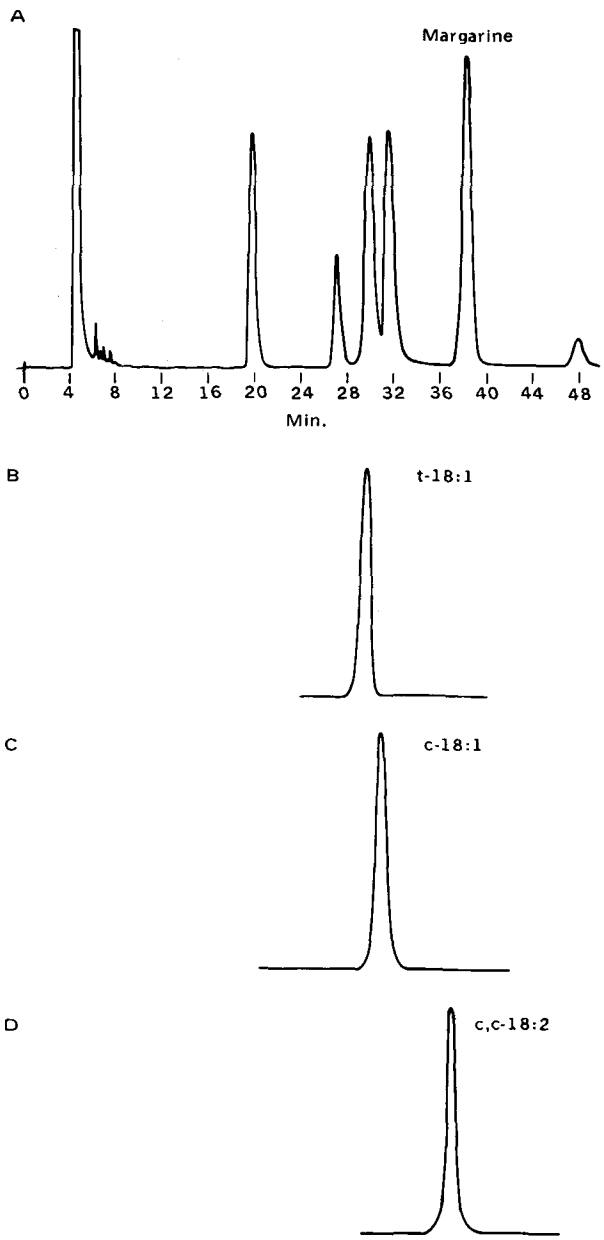

FIG. 2. Gas liquid chromatographic (GLC) analysis of a margarine sample (methyl esters): (A) prior to argentation-thin layer chromatography and eluted fractions; (B) *trans*-monoenoates; (C) *cis*-monoenoates; and (D) *cis,cis*-dienoates. 20 ft x 1/8 in. column of 15% OV-275 on Chromosorb P AW-DMCS at 220 C.

mm 10% DEGS-PS on 80/100 mesh Supelcoport column at 200 C (Supelco, Inc., Bellefonte, PA). *trans* Isomers were analyzed on a 20 ft x 1/8 in. 15% OV-275 on Chromosorb P AW-DMCS column at 220 C (Supelco, Inc.). Margarine samples (10% in $CHCl_3$) were also analyzed by the infrared procedure of Allen (15). This procedure was standardized with trielaidin-triolein mixtures using a Beckman AccuLab 4 infrared spectrophotometer. Fatty acid methyl esters were separated by degree of unsaturation and geometric configuration on Redi-Coat-AG thin layer chromatographic plates (Supelco, Inc.) using the solvent system petroleum ether-ethyl ether (90:10, v/v). Fractions were visualized with fluorescein.

RESULTS

The seven margarine samples were found (Table I) to contain 25-64% monoenoic acids, of which 6-33% was in

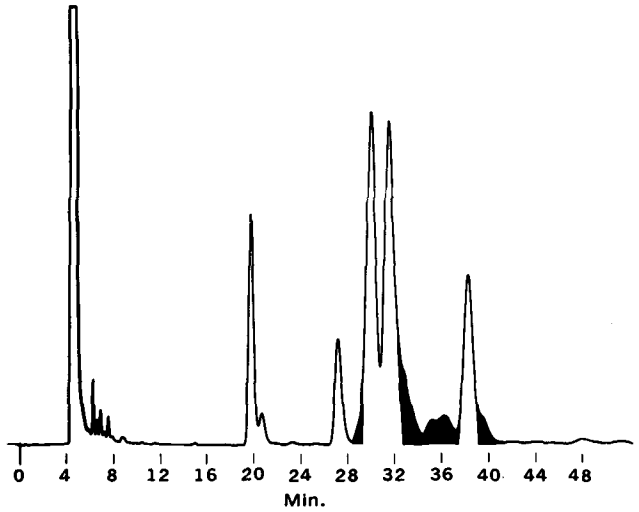

FIG. 3. Comparison of *trans* fatty acid content of seven commercial margarine samples determined by infrared analysis and packed column gas liquid chromatography (△) oleate-elaidate standards (X) margarine samples.

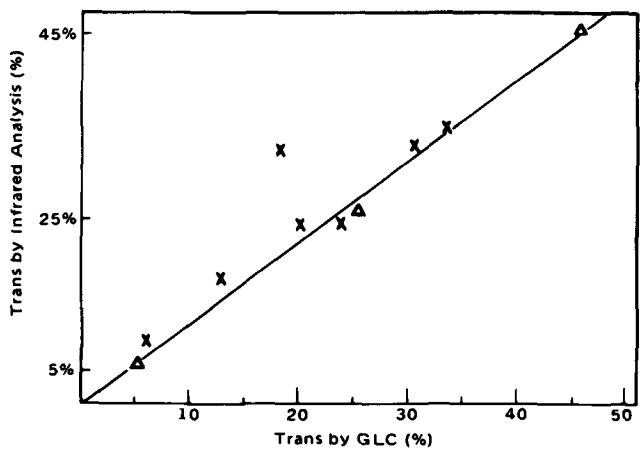

FIG. 4. Margarine sample showing maximum amount of material encountered not clearly contained (shaded areas) within the "elaidate," "oleate," or "linoleate" peaks. Conditions as in Figure 3.

the *trans* configuration, and 11-58% dienoic acids. Good agreement was obtained between the fatty acid analyses on OV-275 and DEGS-PS. For single analyses the sum of *c*-18:1 and *t*-18:1 on OV-275 corresponded reasonably well to the total 18:1 determined on DEGS-PS.

On the OV-275 column with margarine samples a broadening of both the *trans* 18:1 peak and the *cis* 18:1 peak was observed with loss of baseline separation (compare Figure 1 and Figure 2). Linoleaidate, *trans,trans*-18:2ω6 and *cis*-9,*trans*-12-18:2ω6 standards are well separated from the corresponding *cis,cis*-isomer. Presence of *cis,trans*-18:2, *trans,cis*-18:2 and *trans,trans*-18:2 positional isomers in the margarine samples, however, produced a pair of relatively broad, partially resolved peaks.

Argentation-TLC followed by elution and GLC on OV-275 on a typical margarine showed that the "elaidate" peak contained only *trans*-18:1, the "oleate" peak contained only *cis*-18:1, and the "linoleate" peak contained only *cis,cis*-18:2 (Figure 2).

Reasonable agreement (±1-2%) between GLC analysis and infrared analysis on single samples (Figure 3) can be obtained if a few precautions are observed. The infrared abosrption band for *trans* unsaturation at 10.4 microns is rather weak. A rather concentrated sample of tub margarines is required to produce a peak distinguishable

from baseline absorption. The band at 9.6 microns must therefore be adjusted to the highest practical absorbancy (≅0.9). When this was not done, a value for *trans* twice the GLC value was obtained by infrared analysis.

For 70% of the margarines analyzed, the "elaidate" peak corresponded well to total *trans* by infrared. Two stick margarines produced by the same manufacturer utilizing a "slow" hydrogenation process contained significant quantities of *cis,trans,* and *trans,trans* positional isomers of 18:2. Inclusion of these GLC peaks in the total *trans* calculation reduced the difference between methods from 4.2% to 1.7% for one sample. The other sample contained additional partially resolved peaks on the leading edge of "elaidate" and the trailing edge of "linoleate" (Figure 4). As experience is gained with samples of this last type it may be possible to reconcile the data from the two analyses.

DISCUSSION

The infrared analysis procedure is simple, rapid, and accurate and requires minimal time in sample preparation. GLC analysis requires derivative formation and a somewhat longer instrument run, ca. 40 min. Most laboratories performing fat analyses, however, routinely prepare methyl esters. The extra time involved is therefore only the somewhat slower running time on OV-275 as compared to DEGS-PS. Since GLC provides data beyond the total *trans* content afforded by infrared analysis, the potential usefulness of the chromatographic method is related to the interest or lack of interest in this information.

Several points should be noted with regard to the use of the OV-275 column. A reasonably well-packed 20 ft column is required to attain adequate peak resolution. Some laboratories may find it somewhat awkward to prepare a column of this length. At operating temperature (220C) the phase is sensitive to trace impurities of oxygen which rapidly degrades the separation. Use of a device to remove O_2 and H_2O from the carrier gas will prolong column life. An occasional particularly well-packed column may be run at 230 C with baseline separation of elaidate-oleate standards complete in 19.5 min.

Inclusion of all of the *trans* 18:1 positional isomers encountered in six of seven margarine samples within a single, albeit somewhat asymmetric, peak is considered a decided advantage. Spreading of the *trans* and *cis* octadecamonoenoates into a series of peaks usually results in overlap between the two fatty acid types. For practical, routine purposes it may be preferable to have a complete separation between *cis* and *trans* isomers with minimal separation of positional isomers. Partial resolution such as noted on a Silar column (13) severely impedes sample quantitation. Limited asymmetry of the "oleate" and "linoleate" peaks noted herein, indicative of the presence of *cis* positional isomers, does not interfere with quantitation by elementary techniques. The OV-275 column appears to be useful for routine analytical work except perhaps in the case of fats containing an unusually broad spectrum of positional and geometric isomers. It will be of interest to see what range of positional isomers falls within the range of the major peaks.

ACKNOWLEDGMENTS

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