Analysis for Geometrical and Positional Isomers of Fatty Acids in Partially Hydrogenated Fats

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Abstract

A liquid chromatographic procedure for fractionation and analysis of methyl esters from fats is described. First, esters are separated by liquid chromatography on a partially vulcanized rubber column into trienoate, dienoate, monoenoate-palmitate and stearate fractions. The monoenoate-palmitate fraction is separated by liquid chromatography on a silver-saturated cation exchange resin into palmitate, *trans* monoenoate and *cis* monoenoate fractions. Double bond positions in *trans* and *cis* monoenoates are located by ozonization and gas chromatography of fragments. This separation procedure requires less time and uses simpler apparatus with smaller samples than a previously described method based on countercurrent distribution. Data from analyses of two liquid oils and four shortenings are presented.

Introduction

ANALYSIS OF PARTIALLY HYDROGENATED fats and oils for isomeric monoenes and dienes requires a series of separations and subsequent analysis of the fractions for *trans* content and double bond position. Kuemmel (7) described a chromatographic separation of mercury adducts of fatty esters followed by infrared measurement of *trans* content in the recovered ester fractions. Later he used silver nitrate-silicic acid column chromatography for separation of *cis* and *trans* isomers and capillary gas chromatography for measurement of some of the positional isomers (8).

At this laboratory, Jones et al. (6) described the separation of methyl esters into saturated, monoenoic, dienoic and trienoic fractions by countercurrent distribution with an acetonitrile-hexane system. This was followed by countercurrent distribution with an argentation system to separate geometric isomers of monoenoic and dienoic esters. Double bond positions were determined by permanganate-periodate cleavage and gas chromatography of the resulting dibasic esters (5). Although this procedure is capable of giving a complete analysis of monoenes and much information about the dienes, it is laborious and time-consuming. The liquid-liquid chromatographic procedure described in this paper requires 1 day for each chromatographic separation compared to 3–5 days for countercurrent distribution. About 3–5 g of methyl esters is required for the chromatographic method while a countercurrent distribution uses 35–40. Further, double bond positions are determined by an ozonization-pyrolysis technique requiring 1 hr and 5 mg of sample compared to 1 week and a 50–100 mg sample for the permanganate-periodate method (5). The application of the procedure to six commercial partially hydrogenated samples is described in this report.

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Experimental

Materials

Four shortenings from vegetable oils and two liquid oils described as processed soybean oil were analyzed. All were purchased in local markets during the summer of 1965. Direct analyses of methyl esters from the samples are shown in Table I.

Analytical Procedures

The methyl esters were prepared by transesterification with sodium methoxide catalyst. Fatty acid values are area percentages from gas chromatographic curves obtained with an 11% EG-SSX (Applied Science Laboratories, State College, Pa.) column and radium D argon radiation detector. *Trans* double bonds were measured by infrared absorption in carbon disulfide solution with a methyl elaidate standard. Conjugation was measured by ultraviolet absorption according to the AOCS standard method (1).

Capillary gas chromatograms were run at 200C and 20 psi argon on a 200 ft × 0.01 in. column coated with 100% cyanoethylsilicone resin (General Electric Experimental nitrite silicone 238–149–99) (9).

Fractionation

Methyl esters were separated into stearate, monoene, diene, and triene fractions by chromatography on a rubber column as described by Hirsch (4). Samples of 3–4 g were used on a 150 cm × 2 cm I.D. column with a flow rate of 5–6 ml/min of 88% acetone. Separations were monitored by passing the eluant through a recording differential refractometer, and fractions were combined based on information from the recorder chart. Fig. 1 is a drawing of the chart for sample E and illustrates the separation obtained. Analyses of the fractions are shown in Table II. Gas chroma-

TABLE I
Analysis of Methyl Esters from Partially Hydrogenated Fats

Sample	Fatty acid composition (GLC),[a] %					*trans* (IR)	Diene conjugated esters (UV)
	P	S	M	D	T	%	%
Liquid oil A	8.0	3.3	47.6	37.4	3.8	12.0	0.4
Liquid oil B	10.9	4.4	38.0	41.8	4.8	4.9	0.4
Shortening C	17.4	11.2	41.2	27.4	2.7	16.6	0.6
Shortening D	14.5	10.3	62.3	11.8	1.0	29.2	0.5
Shortening E	13.1	10.9	50.5	25.5	....	20.5	0.6
Shortening F[b]	9.9	10.7	62.6	10.4	1.6	23.5	0.3

[a] P = Palmitate, S = Stearate, M = Monoenoate, D = Dienoate, T = Trienoate.
[b] Sample F contained 2.6% palmitoleate and 2.1% of an unidentified component between diene and triene.

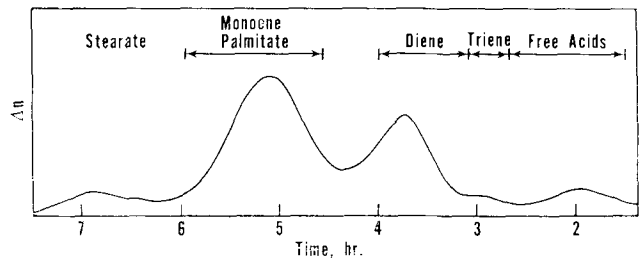

FIG. 1. Differential refractometer tracing for separation of Sample E methyl esters on 150 cm × 2 cm rubber column. Flow rate 5.5 ml per minute of 88% acetone.

tographic analyses of monoene and diene fractions showed only traces of esters from the adjacent liquid chromatogram peaks. Triene positions contained amounts of diene so small that they could only slightly affect the analyses in Table II.

The monoene samples were passed through a silver resin column (3) to give palmitate, *trans* monoene, and *cis* monoene fractions.

Diene and triene fractions were not fractionated further since they contained mainly all *cis* esters which would be eluted only very slowly and incompletely from a silver resin column (3). Capillary gas chromatograms were run, and representative curves are shown in Fig. 2 and 3.

Double Bond Location

Double bond position in the monoenes was determined by the micro-ozonization pyrolysis procedure described by Davison and Dutton (2). Values based on the aldehyde esters from the carboxyl end of the molecule are plotted as bar graphs in Fig. 4 and 5.

Results and Discussion

The scheme of separation and analysis applied to the fats is summarized in Fig. 6. These procedures

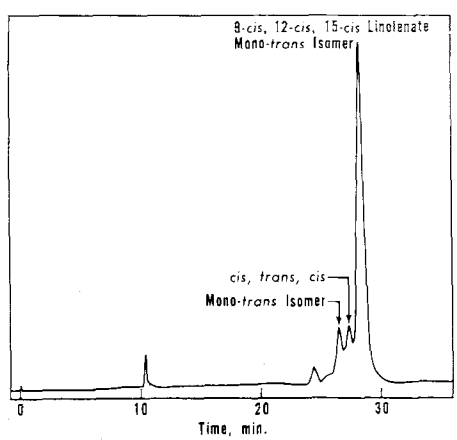

FIG. 2. Capillary gas chromatogram for Sample E triene fraction. 200 ft × 0.01 in. 100% cyanoethylsilicone column 200C 20 psi argon. Unlabeled peaks have not been identified.

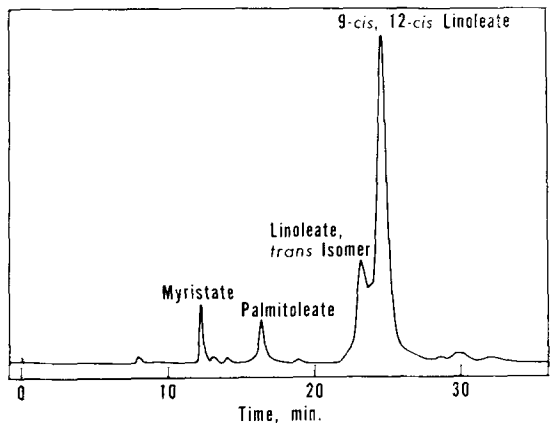

FIG. 3. Capillary gas chromatogram for Sample D diene fraction. 200 ft × 0.01 in. 100% cyanoethylsilicone column 200C 20 psi argon. Unlabeled peaks have not been identified.

provide information for calculating the percentage of all *cis* and *trans* positional monoene isomers in the fat sample. A maximum value—which is believed to be near the correct value for most fat samples—is also calculated for 9-*cis*,12-*cis* linoleate.

The rubber column separation illustrated in Fig. 1 is like the countercurrent distribution used before (6) in that decreasing the carbon chain by two methylene groups has the same effect on the separation as adding a double bond (4,12). Therefore, palmitate is found in the same fraction as the monoenes, and small amounts of myristate and palmitoleate are concentrated in the diene fraction. Subsequent argentation chromatography resolves the palmitate from the *cis* and *trans* monoenes as described in the experimental section.

The first peak (to the right) in Fig. 1 represents fatty acids formed during the transesterification of

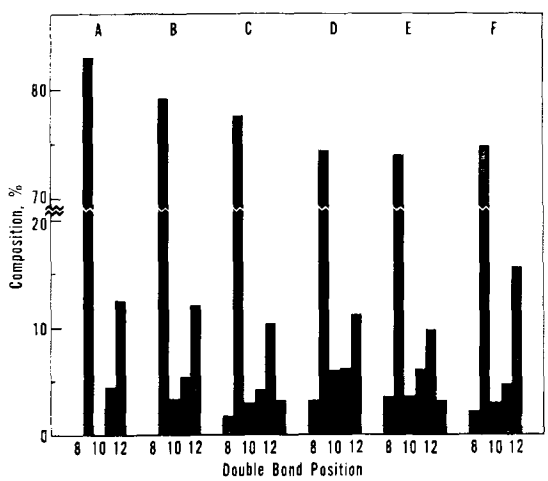

FIG. 4. Double bond position in *cis* monoene fractions from hydrogenated fats.

TABLE II
Analysis of Methyl Ester Fractions from Rubber Column Separations

Sample	Monoene fraction		Diene fraction		Triene fraction		
	trans (IR) %	Palmitate (GLC) %	*trans* (IR) %	a_{230}	*trans* (IR) %	a_{230}	a_{268}
A	19.6	18.8	11.0	1.14	.....	.....	.....
B	7.4	30.9	6.0	0.81	9.7	2.74	1.37
C	25.9	23.9	9.7	0.71	8.1	3.11	0.71
D	34.9	18.4	34.0	4.30	.....	.....	.....
E	26.4	25.9	17.9	2.09	18.7	4.11	2.21
F	26.3	20.2	26.8	1.11	.....	.....	.....

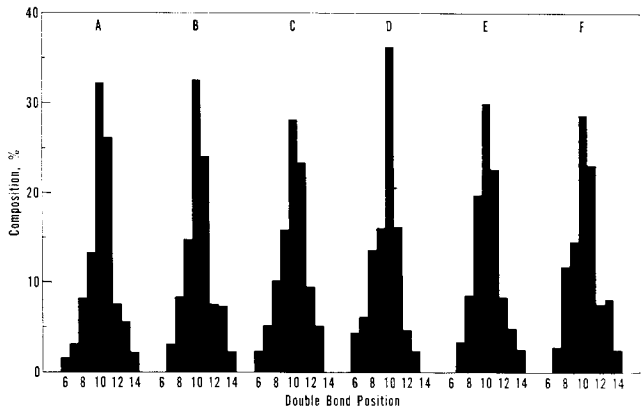

FIG. 5. Double bond position in *trans* monoene fractions from hydrogenated fats.

the triglycerides. This fraction is identified by infrared absorption as mainly free acids. The carbonyl band at 1795 cm^{-1} in methyl esters is shifted to 1712 cm^{-1}. Other ester bands are weak and acid bands are present.

A triene peak was found on the rubber column chromatograms of only three samples, although gas chromatography showed small peaks in the linolenate position for all but one sample. Recovered triene fractions all contained *trans* double bonds as shown in Table II. Absorptions at 268 mμ, although weak, were all part of the three band structure of conjugated triene.

Diene and triene fractions were not fractionated further because *cis,cis* esters are absorbed strongly on the silver resin column (3).

In Fig. 2, the capillary gas chromatogram for the triene fraction from sample E has a large peak at the position for natural all *cis* linolenate. One mono-*trans* isomer of triene would be at the same position (11). Two small peaks correspond to *cis,trans,cis* and to the other unidentified mono-*trans* isomer.

The capillary gas chromatograph of the diene fraction from sample D (Fig. 3) has a large peak in the position for the natural 9-*cis*,12-*cis* linoleate. The small peak could not be identified, but it is in the region where *trans* isomers are found. Other diene fractions showed similar patterns but with smaller and less distinct peaks.

Monoene fractions were analyzed for *trans* ester and palmitate content before fractionation on the silver resin column. Results of double bond location shown in Fig. 4 and 5 are much like previous results on other samples (6). *Cis* monoene fractions all contain large amounts of the 9-isomer. This results from the large amount of oleate in the original oil which has never been isomerized. There is also 12-monoene which is formed from reduction of the 9 double bond in linoleate.

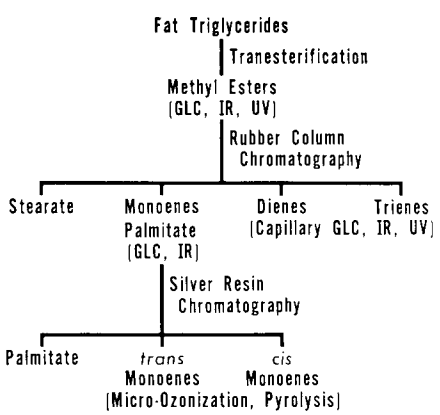

FIG. 6. Flow diagram for separation and analysis of fat samples.

TABLE III

Calculated Value for Oleate and Maximum Value for Linoleate in Original Fats

Sample	Oleate %	Linoleate, maximum value %
A	29.9	32.2
B	26.9	38.5
C	21.1	24.1
D	26.1	6.6
E	24.1	19.7
F	31.3	7.0

In the *trans* monoene fractions, all double bonds are widely scattered since all *trans* bonds are in esters that have been isomerized during the hydrogenation. The 10- and 11-monoenes, which are present in the greatest amount, could be formed by adsorption of linoleate on the catalyst followed by movement of a double bond to form a conjugated system which is rapidly hydrogenated. Such hydrogenation would be expected to yield equal amounts of 10- and 11-monoenes. However, there is more 10- than 11-monoene and the excess 10-monoene may be formed by isomerization of the large amount of oleate present; this isomerization would be expected to yield about as much 10- as 8-monoene.

The percentage of any monoenoic isomer in the fat may be calculated. This has been done for the 9-*cis* isomer (oleate) and values are given in Table III. The method of calculation is outlined for sample A as follows. When the *trans* content of the monoene is corrected for palmitate, we have 19.6/(1.000–0.188) = 24.1% *trans*. There is 47.6% monoene in the fat, so *cis* monoene in the fat is 47.6(1.000–0.241) = 36.1%. The *cis* monoene has 82.9% 9-isomer so 36.1 × 0.829 = 29.9% 9-*cis* monoene in the original sample.

Maximum values for natural 9-*cis*,12-*cis* linoleate are also given in Table III. The calculation for sample A is as follows. The diene fraction contains 11.0% *trans* compared with a methyl elaidate standard. Since dienes with one methylene group between the double bonds have approximately 85% as much infrared absorption as monoenes (10) the *trans* content is corrected to 11.0/0.85 = 12.9%. Argentation countercurrent distribution of dienes in previous work (11) did not provide evidence of *trans,trans* dienes. At a low level of *trans* content, it seems reasonable to assume that *trans* double bonds are nearly all present as mono-*trans* esters. Kuemmel (7) made this assumption and we do also for this calculation. There are also 1.0% conjugated esters calculated by AOCS method (1) from ultraviolet absorption. Then a maximum value for 9-*cis*,12-*cis* linoleate is 37.4(100.0–12.9–1.0) = 32.2%. This value will be high by the amount of the all *cis* isomer other than 9,12 present (such as 12,15 from linolenate) but should be close to the correct value.

ACKNOWLEDGMENTS

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REFERENCES

1. AOCS Official Methods, cd. 7–58.
2. Davison, V. L., and H. J. Dutton, Anal. Chem. *38*, 1302–1305 (1966).

3. Emken, E. A., C. R. Scholfield and H. J. Dutton, JAOCS *41*, 388 (1964).
4. Hirsch, J., Coloq. Intern. Centre Natl. Rech. Sci. (Paris) *99*, 11 (1961).
5. Jones, E. P., and J. A. Stolp, JAOCS *35*, 71–76 (1958).
6. Jones, E. P., C. R. Scholfield, V. L. Davison and H. J. Dutton, Ibid. *42*, 727–730 (1965).
7. Kuemmel, D. F., Anal. Chem. *34*, 1003–1007 (1962).
8. Kuemmel, D. F., Ibid. *38*, 1611–1614 (1966).
9. Litchfield, C., R. Reiser, A. F. Isbell and G. L. Feldman, JAOCS *41*, 52–55 (1964).
10. Scholfield, C. R., E. P. Jones, R. O. Butterfield and H. J. Dutton, Anal. Chem. *35*, 1588 (1963).
11. Scholfield, C. R., R. O. Butterfield and H. J. Dutton, Anal. Chem. *38*, 1694–1697 (1966).
12. Scholfield, C. R., J. Nowakowska and H. J. Dutton, JAOCS *37*, 27–30 (1960).

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