Analysis of Fats and Oils by Gas-Liquid Chromatography and by Ultraviolet Spectrophotometry[1]

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SINCE THE ORIGINAL description and application of gas-liquid chromatography published in 1952 by James and Martin (1), developments in instrumentation and in techniques and diversified applications have proceeded at a phenomenal pace. Principal improvements in instrumentation have been concerned with better control of temperature of column, sample injection and collection sites, and with more practical and sensitive detectors. There have also been numerous advances in techniques, such as sample introduction, trapping of emerging components, and column packing. The discovery of new stationary liquid phases which permit complete separation of saturated and unsaturated esters of the same chain-length (2, 3, 4) gave impetus to a new surge of interest for application to fats as a quantitative method of analysis.

In this country thermal conductivity detectors have become most widely used in high temperature gas-liquid chromatography (GLC). Improved types are available which can detect amounts of a substance of the order of 10^{-6} g. More sensitive ionization detectors have been developed within the past several years which are capable of detecting about 10^{-9} g. of a component. However it has not been feasible to utilize this extreme sensitivity with some stationary phases, such as succinate polyester of diethylene glycol, one of the most effective for separating fatty acid esters, because of instability of the polyester at the high column temperatures required for achieving practical retention times. Indeed, there is some question as to whether the increased order of sensitivity is necessary for routine analysis of fats and oils.

There are numerous publications dealing with instrumentation, techniques, new stationary phases, discussions of factors involved in GLC fractionations, and with experimental demonstration of the effective separation of various fatty acids as their methyl esters. However there are surprisingly few publications on the use of this technique for quantitative expression of fatty acid composition of our common fats and oils. Similarly there is a dearth of information on the comparison of analyses by GLC with the known composition of complex mixtures of fatty acids or with analyses of fats by other methods. Published analyses by GLC have been limited largely to a specific tissue lipid or to one particular fat or oil (5, 6, 7, 8, 9, 10, 11). Comparative analyses of known mixtures or of fats analyzed by other means have been reported on only a few samples (4, 11, 12, 13).

The present study was undertaken to obtain more complete information on the quantitative aspects of GLC as a practical analytical technique for determining fatty acid composition of natural fats and oils. For this purpose, the more common type of apparatus with thermal conductivity detector was employed. Analyses by GLC of known mixtures of pure fatty acid esters and of a series of fats and oils were compared with known values or with analyses by an alkali-isomerization ultraviolet spectrophotometric method. Analyses of the fats and oils by an independent laboratory with a similar type of equipment but with different means of computing percentage composition are included.

Experimental

Apparatus. Conventional type of GLC equipment was employed. The air bath was heated electrically, controlled to ±0.5°C. by a thermoregulator, and was uniform in temperature to within ±1°C. It housed a detector and coiled stainless steel columns. A four-filament thermal conductivity cell, and 2-millivolt, 11-in. strip chart recorder requiring 2 seconds for full-scale pen deflection were employed. Means for attenuating the detector signal to the recorder was provided. The chart speed was 30 in. per hour. The sample injection site was heated and controlled independently up to 350°C. Samples for analysis were introduced by microsyringe through a silicone rubber disc into the heated injection site. Provision was also made to heat the exit tube from the detector to avoid condensation. The emerging components could be collected by means of a cold trap.

Column Packing and Operation. Two coiled stainless steel columns, 8 ft. long by ¼ in. O. D., with different packing and operating conditions were employed. Column I was packed with 6.6 g. of succinate polyester of diethylene glycol (LAC-3-R-728, Cambridge Industries Company, Cambridge, Mass.) on 20 g. of 42–60 mesh Chromosorb and was operated at 235°C., 30 psig. of helium, and flow rate of 75 ml. per minute at column exit.

Column II was packed with 3.5 g. of the succinate polyester on 10.6 g. of Celite 545 and was operated at 240°C., 40 psig. of helium, and flow rate of 45 ml. per minute at column exit.

The operating conditions for each of the columns were adjusted so as to complete a chromatogram in about 30 min. Hence the retention times for the individual components were not widely different for either column. The columns were used interchangeably for the mixtures of pure esters and for some of the esters of the fats. There appeared to be no significant difference in the performance of the columns for the samples investigated. Reasonably sharp, symmetrical, separated peaks were obtained for the major components. In general, attempts were made to operate the columns at a sensitivity which would permit nearly maximum use of chart width for the peaks of major components. For example, when 0.5 μl samples were used, the sensitivity was about 6 times as great as when 3 μl samples were employed, resulting in approximately equal areas for a given component peak. Several days' conditioning of the columns at operat-

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ing temperatures were required before the rate of decomposition or "bleed" of the stationary phase was reduced sufficiently to maintain a steady base-line. It was found desirable to condition freshly prepared columns in a separate air bath or oven in order to avoid excessive contamination of the detector.

Estimation of Percentage of Components. The percentage composition by GLC was obtained by measuring the area under each peak with a planimeter and calculating the ratio of these areas to the sum of the area under all of the component peaks.

Samples. The individual fatty acid methyl esters used in known mixtures ranged from 98 to 99+% pure, as judged by independent analysis. The methyl esters of the fats and oils were prepared and freed of unsaponifiables by a method described elsewhere (14).

Other Method of Analysis. An alkali-isomerization spectrophotometric method (15) was used in conjunction with iodine values for analysis of known mixtures of methyl esters and methyl esters of the fats and oils.

Results and Discussion

Known pure fatty acid methyl esters and mixtures of these were employed to establish satisfactory operating conditions and to obtain reference data. Examples of chromatograms of two such mixtures are shown in Figures 1 and 2, and comparisons of calculated with known compositions in Table I. Substantially complete resolution of the components was achieved, as judged by visual inspection of the chromatograms. The calculated compositions show a good order of agreement with the known percentages and with those determined by the spectrophotometric method. There is a tendency toward somewhat low values for the slower-moving polyunsaturated acid esters and correspondingly high values for the faster-moving saturated components. Whether this is an inherent fault of the method for calculating percentage, an accumulative effect of similar minor impurities, incomplete resolution, or because of transesterification with stationary phase is not known. The same tendency is evident in the few examples published (4, 12, 16).

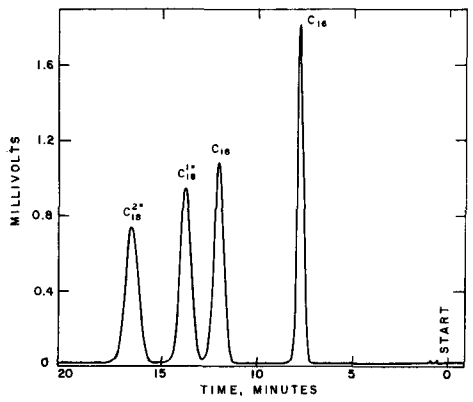

FIG. 1. Separation of components of mixture I by GLC.

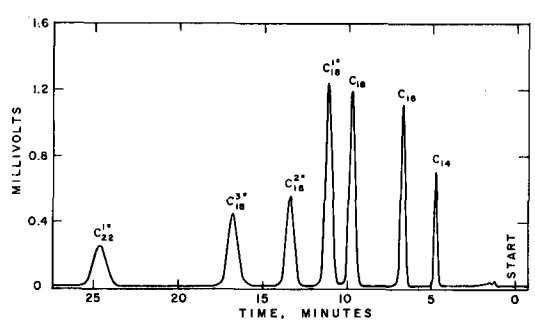

FIG. 2. Separation of components of mixture II by GLC.

TABLE I

Composition of Known Mixtures by GLC and Spectrophotometric Analyses

Component (%)	Mixture I			Mixture II		
	Known	Found		Known	Found	
		GLC	U.V. spectro.		GLC	U.V. spectro.
Myristate	.....	.....	.....	5.9	6.4	.....
Palmitate	24.9	27.0	.....	11.8	13.8	.....
Stearate	25.0	25.3	.....	19.1	20.9	.....
Oleate	25.0	24.8	24.1	23.3	23.8	.....
Linoleate	25.1	22.9	24.8	13.3	12.5	12.3
Linolenate	.....	.....	.....	15.3	12.9	15.7
Erucate	.....	.....	.....	11.2	9.6	.....

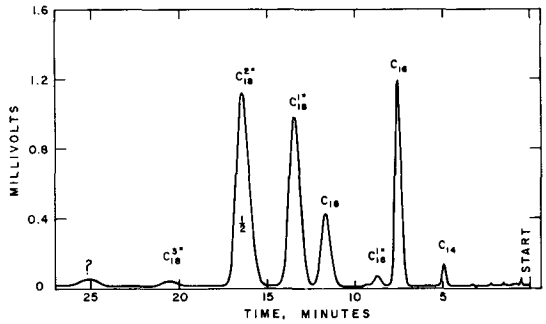

FIG. 3. Separation of components of lard methyl esters by GLC. Lard obtained from pigs fed high level of safflower oil. Area of peak for linoleate to be multiplied by 2.

A typical chromatogram is shown for a sample of lard methyl esters in Figure 3. This lard was produced from pigs fed a high level of safflower oil. On account of the large proportion of methyl linoleate in this sample it was necessary to attenuate the detector signal for this peak to one-half. The component represented by the small peak at 25 min. was not identified. Collections were made of material at this point and at lesser "suggested" peaks beyond 25 min. Examination of collected fractions led to inconclusive results because of contamination by artifacts from the stationary phase. The presence of some tetraenoic acid ester in the material collected beyond 25 minutes of retention time was detected by the alkali-isomerization spectrophotometric method.

Figure 4 is a chromatogram of methyl esters of sesame oil, one of the vegetable oils examined. The concentration of oleate and linoleate are about 38 and 45%, respectively, while the concentration of stearate is only about 5.4%. Yet their peaks appear well separated.

The compositions of the fats and oils as determined by GLC are shown in Tables II and III. Samples of the methyl esters of these fats and oils were furnished to an independent laboratory for GLC analysis. Description of their apparatus, conditions of column operation, and results have been published (17). A summary of their GLC analyses is included in the tables.

TABLE II

Fatty Acid Composition of Lard by GLC and by Spectrophotometric Analyses

Component (%)	Lard (I.V. 59.1)				Lard (I.V. 119.9)[a]			
	Gas-liquid chromatography			U.V. spectro.	Gas-liquid chromatography			U.V. spectro.
	0.5 μl	3 μl	1 μl[b]		0.5 μl	3 μl	1 μl[b]	
Myristate	1.7	1.5	1.5	—	1.0	0.9	1.0	—
Palmitate	28.8	26.7	26.9	—	13.5	13.1	14.0	—
Stearate	14.3	14.0	14.1	—	7.3	7.3	7.3	—
Total saturated	44.8	42.2	42.5	42.7	21.9	21.3	22.3	20.8
Palmitoleate	2.7	3.1	4.3	—	0.8	0.7	1.4	—
Oleate	42.9	43.3	43.8	—	20.2	20.1	21.8	—
Total monoenoate	45.6	46.4	48.1	47.3	21.0	20.8	23.2	21.3
Linoleate	8.4	9.8	9.5	8.5	54.6	55.0	54.6	56.3
Linolenate	1.1	1.4	—	0.6	0.9	0.9	trace	1.0
Arachidonate	trace	trace	—	0.3	trace	trace	—	0.5
Pentaenoate	—	—	—	0.2	—	—	—	0.1

[a] From pigs fed high level of safflower oil. [b] Data from Reference (17).

TABLE III

Fatty Acid Composition of Olive, Sesame, and Safflower Oils by GLC and Spectrophotometric Analyses

Component (%)	Olive Oil				Sesame Oil				Safflower Oil[a]			
	Gas-liquid chromatography			U.V. spectro.	Gas-liquid chromatography			U.V. spectro.	Gas-liquid chromatography			U.V. spectro.
	0.5 μl	3 μl	1 μl[b]		0.5 μl	3 μl	1 μl[b]		0.5 μl	3 μl	1 μl[b]	
Palmitate	11.7	10.8	11.7	—	10.0	10.0	11.6	—	7.5	7.1	7.5	—
Stearate	2.9	2.6	2.2	—	5.4	5.4	5.3	—	2.8	2.5	2.1	—
Total saturated	14.6	13.4	13.9	11.8	15.4	15.4	16.9	14.8	10.3	9.6	9.6	8.8
Palmitoleate	1.3	0.9	1.4	—	trace	—	—	—	—	—	—	—
Oleate	77.9	78.4	77.1	—	38.8	38.2	37.6	—	13.6	12.2	13.8	—
Total monoenoate	79.2	79.3	78.5	81.6	38.8	38.2	37.6	39.6	13.6	12.2	13.8	15.0
Linoleate	5.3	6.1	7.5	5.9	44.5	45.0	44.0	44.8	76.1	78.3	76.6	76.2
Linolenate	0.9	1.1	trace	0.7	0.8	0.9	1.5	0.8	—	—	—	—

[a] Trace myristate found by GLC. [b] Data from Reference (17).

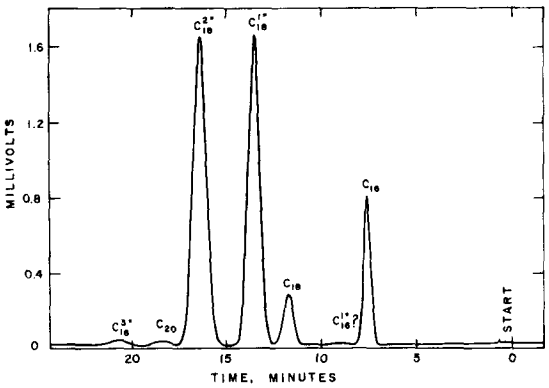

FIG. 4. Separation of components of sesame oil methyl esters by GLC.

In general, the results of GLC analyses by the two laboratories are in good agreement and, where comparisons can be made, are also in good agreement with analysis by the spectrophotometric (and iodine value) method. The advantage of GLC in giving more complete information on the saturated and monoenoic acids is obvious. The determination by GLC of minor amounts of arachidonic and pentaenoic acids in lard was not accomplished. These were more readily determined by the spectrophotometric method. However this method does not distinguish between acids that have the same number of double bonds but different chain-lengths.

Summary

1. Known mixtures of pure fatty acid methyl esters and a number of fats and oils as their methyl esters have been analyzed by conventional GLC with thermal conductivity detectors.

2. Percentage of fatty acid distribution determined by GLC agreed well with known percentages in model mixtures and with analysis by the spectrophotometric method for fats and oils.

3. Determination of very small amounts of arachidonic and pentaenoic acids in lard by GLC was not successful.

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