TABLE V

Summary of Statistical Calculations

	Methods			
	No. 1 modified official	No. 2 Oklahoma State filter screen	No. 3 Buchner funnel	No. 4 Purdue University Shimer filter
Average results				
Cottonseed meal	8.16	8.32	8.35	8.43
Soybean oil meal	2.65	2.68	2.72	2.74
Dairy feed	9.02	9.13	9.00	9.26
Alfalfa meal	23.93	24.00	23.85	23.77
Standard deviation within-labs.				
Cottonseed meal	0.37	0.24	0.33	0.26
Soybean oil meal	0.20	0.18	0.18	0.15
Dairy feed	0.35	0.25	0.24	0.39
Alfalfa meal	0.58	0.52	0.43	0.42
All samples	0.40	0.33	0.31	0.32
Excluding alfalfa meal	0.31	0.22	0.26	0.29
Standard deviation between-labs.				
Cottonseed meal	0.57	0.42	0.55	0.48
Soybean oil meal	0.27	0.21	0.25	0.27
Dairy feed	0.49	0.36	0.29	0.52
Alfalfa meal	0.80	0.84	0.62	0.51
All samples	0.57	0.51	0.46	0.46
Excluding alfalfa meal	0.46	0.34	0.39	0.44

method, the Oklahoma State Filter Screen method, and the Modified Official method. However these differences, while statistically significant in some cases, are actually quite small and do not provide a clear-cut basis for deciding which method is best.

Conclusions

The results obtained in this collaborative study show that none of the methods tested offer sufficient advantages in precision and accuracy to warrant selection as an official method to the exclusion of other methods studied.

Also the precision of all methods tested show these methods to be inadequate for checking crude fiber specification limits set up by the N.S.P.A. on soybean oil meal. This statement would also apply to any other product where specification limits on crude fiber are narrower than the precision shown in Table VI.

Ease and speed for manipulation are also an important criterion for choosing an official method. A poll of committee members showed no definite preference for any one method studied; every method received the support of two or more of the committee members.

TABLE VI

A.O.C.S. Precision Calculations

Agreement within labs.
(95% confidence limits)
(Two single determinations in one lab. should not differ by more than)

Method	No. 1	No. 2	No. 3	No. 4
All samples	1.11	0.91	0.86	0.89
Excluding alfalfa	0.86	0.62	0.71	0.79

Agreement between labs.
(95% confidence limits)
(Single determinations in two labs. should not differ by more than)

Method	No. 1	No. 2	No. 3	No. 4
All samples	1.58	1.41	1.27	1.27
Excluding alfalfa	1.28	0.95	1.08	1.26

The Liaison Committee is still hopeful that a method can be devised that will give a precision in line with trade specifications and practices. The committee will continue to work toward the development of such a method.

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Quantitative Fatty Acid Analysis of Vegetable Oils by Gas-Liquid Chromatography[1,2]

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THE DEVELOPMENT of gas-liquid chromatography (G.L.P.C.) by James and Martin (6, 7) and subsequent applications of other workers (3, 4, 5, 8, 9, 16, 18) made possible the rapid separation of micro quantities of fatty acid esters. The separations were based mainly on chain length, using liquid phases such as silicone and Apiezon greases. The technique was utilized successfully in this laboratory for the quantitative determination of C_{16}, C_{18}, C_{20}, C_{22}, and C_{24} fatty acid esters in rapeseed oil (2). The quantitative aspects were checked by using known mixtures of pure fatty acid esters which showed that the G.L.P.C. results were accurate to within one unit percentage.

The successful separation of saturated and unsaturated fatty acid esters by Orr and Callen (14) with polyester liquid phases and the further developments by other workers (1, 10, 11, 12, 13, 17) led to the complete analysis of fats and oils on a micro scale. Investigations in this laboratory on fatty acid compositions of vegetable oils, animal fats, and partially hydrogenated oils showed good agreement between measured iodine values and those calculated from G.L.P.C. data and led to the work presented herein. The fatty acid composition of six commercial vegetable oils and two

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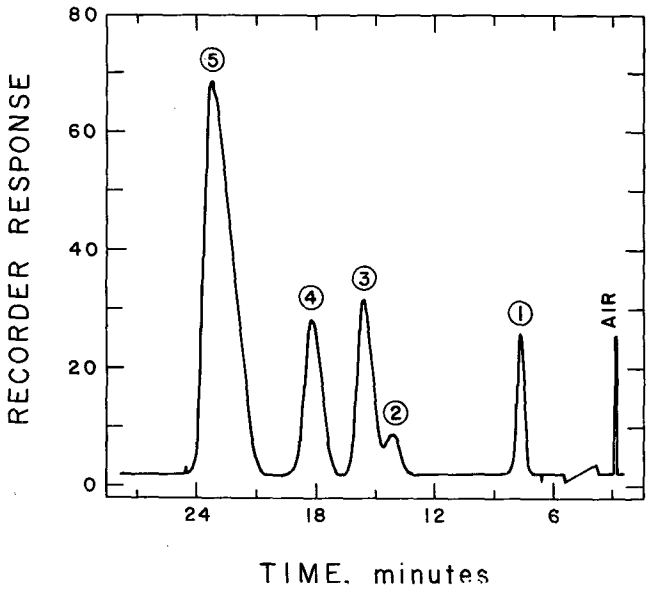

FIG. 1. Separation of the methyl esters of palmitic (1), stearic (2), oleic (3), linoleic (4), and linolenic (5) acids by using adipic acid-diethylene glycol polyester. Column 6 ft. by ¼ in. packed with C22 firebrick 40–60 mesh coated with adipic acid-diethylene glycol polyester (4:1 w/w), column temperature 205°C., bridge current 200 ma, flow rate of helium gas 60 ml. per minute, recorder 2.5 mv range, chart speed 20 in. per hour, injector temperature 250°C., sample size 1 microliter.

synthetic mixtures are compared by G.L.P.C. and those calculated from iodine values and spectral data.

A major problem with polyester liquid phases has been the thermal instability of the polymer at operating temperatures above 200°C. Further investigations in this laboratory have shown that the thermal stability of the polyester polymer can be improved without loss of resolution.

Materials and Methods

The synthetic mixtures were made up from the purified samples of the methyl esters of palmitic, stearic, oleic, linoleic, and linolenic acids which were prepared in the laboratory. Methyl palmitate and methyl stearate were purified by fractional distillation through a Podbielniak Heli-Grid column. Methyl oleate was prepared by fractional distillation and fractional crystallization. Linoleic and linolenic acids were obtained by debromination of the corresponding purified tetra- and hexabromostearic acids. The acids were converted to esters with diazomethane in ether. Nordihydroguaiaretic acid was added to the synthetic mixture which was stored under purified nitrogen at −25°C. when not in use.

The samples of soybean, linseed, corn, sunflowerseed, olive, and peanut oils were obtained from commercial sources. Two methods were used to convert the glyceride esters of the commercial oils to methyl esters. In the first method the oil was saponified and acidified, and the free fatty acids were taken up in solvent and washed free from acid. The solvent was removed, and diazomethane in ether was added to form the methyl esters. In the second method, which is more rapid with neutral oils, the glyceride esters were changed to methyl esters by interesterification, using sodium methoxide as a catalyst. The procedure was as follows: 10 ml. of methanol and 1 ml. of a solution containing 0.5 g. of sodium in one liter of methanol were added to 100 mg. of oil in a 100-ml. round-bottom flask. The mixture was refluxed for one-half hour and cooled to room temperature; 0.1 ml. of glacial acetic acid was added to inactivate the catalyst. The solvent was removed by evaporation under reduced pressure on a rotary laboratory evaporator in a water bath at 60–80°C.

The gas-liquid chromatographic units of conventional design were built in the laboratory. Temperature of the oven was controlled by an electronic control unit. The injection block was maintained at 30–50° above the column temperature by means of a separate Variac control. The detection system employed two Gow-Mac thermal detectors in the reference channel and two in the sample channel. The bridge was operated at 160–200 ma. from a 12-volt storage battery. The recorders were operated on the 2.5 or 5.0 mv range, depending on the sample size. Helium gas used as the carrier was passed through a preheater column before entering the system. Flow rates were measured with a soap-bubble meter connected to the outlet tube. The polyester columns were used at 205°C. with flow rates of 40–60 ml. of helium per minute and the silicone column at 225°C. and a flow rate of 50 cc. per minute.

The polyesters were synthesized according to the procedure of Koroly and Beavers (9), using succinic or adipic acid and diethylene glycol, 1,4-butanediol or ethylene glycol. The general method of preparation and treatment, outlined for the succinate-diethylene glycol polyester, is as follows. A mixture of 118 g. of succinic acid, 126 g. of diethylene glycol, 2.5 g. of diglycerol,[4] and 250 mg. of zinc chloride was placed in a round-bottom flask equipped with a stirrer, thermometer, and air condenser. The mixture was heated for 4 hrs. at 160°C. while a stream of nitrogen was passed through the flask. The air condenser was re-

[4] Diglycerol was not used in preparations which used 1,4-butanediol or ethylene glycol.

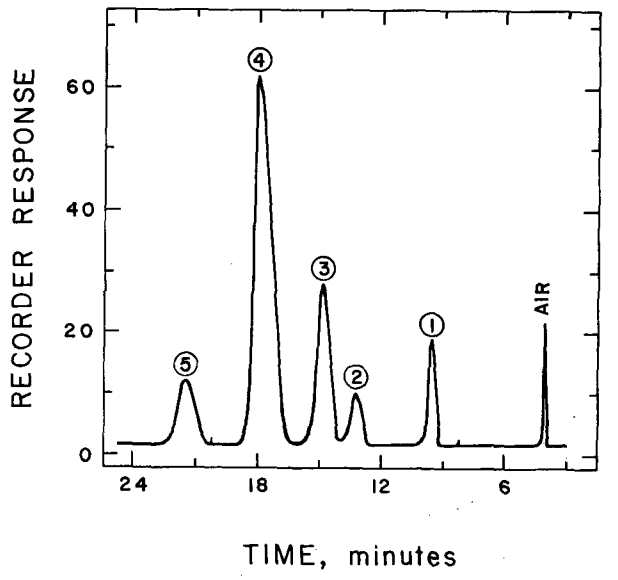

FIG. 2. Separation of the methyl esters of palmitic (1), stearic (2), oleic (3), linoleic (4), and linolenic (5) acids by using succinic acid-diethylene glycol polyester. Column 6 ft. by ¼ in. packed with C22 firebrick 40–60 mesh coated with succinate acid-diethylene glycol polyester (4:1 w/w), column temperature 205°C., bridge current 200 ma, flow rate of helium gas 60 ml. per minute, recorder 2.5 mv range, chart speed 20 in. per hour, injector temperature 250°C., sample size 1 microliter.

placed by a short condenser and the round-bottom flask, which was connected to a vacuum pump. The clear solution was heated with stirring for another 4 hrs. at 160°C. to 180°C. under 2-mm. of pressure. During this time excess diethylene glycol was removed, and the polymer became increasingly viscous. At the end of the reaction time the polymer was cooled to room temperature, dissolved in acetone, and passed through a short column of Amberlite IR-120 to remove the zinc chloride catalyst.

The polyester (30 g.) was dissolved in 300 ml. of acetone, and a fraction was precipitated by addition of 110 ml. of Skellysolve "F" (petroleum ether b.p. 30–50°C.). The solvent was removed from the viscous precipitated fraction under reduced pressure on a rotary laboratory evaporator. The fractionation of the polyesters reduced the amount of "bleeding." A similar fractionation used on commercial polyesters, such as the LAC-IR-296 and LAC-IR-496, also reduced "bleeding."

Celite (60–100 mesh) was used as a solid support for the polyester liquid phases. The polyester was dissolved in acetone and was added to the solid support in the ratio of 1:4 (w/w); the solvent then was evaporated under reduced pressure in a rotary evaporator, and the solid (13 g.) was packed in a copper tube 6 ft. long by ¼ in. in diameter.

Washed silicone grease, prepared according to procedure outlined by Cropper and Heywood (3, 4), was applied to crushed C_{22} firebrick (40–60 mesh) in the ratio of 1:4 (w/w) in the same manner, using ethyl acetate as the solvent. The solid was packed into a 36-in. by ¼-in. copper column.

Sample sizes of esters varying from 1 to 3 microliters, depending on the oil under consideration, were injected with a micro syringe.

Iodine values were measured by the Wijs method, using a 1-hr. reaction time and 0.1 *N* sodium thiosulfate (19). Spectral analyses for conjugated diene and triene acids were made on the free fatty acids of the commercial oils and on the methyl esters of the synthetic mixtures by the standard A.O.C.S. procedure (19).

Results and Discussion

The separation of the esters of palmitic, stearic, oleic, linoleic, and linolenic acids by the adipate polyester of diethylene glycol (Figure 1) shows an incomplete separation of stearic and oleic esters under the conditions used. A plot of logarithm of emergence time against carbon number shows that methyl linolenate emerges before the methyl arachidate. The succinate-diethylene glycol polyester completely separated stearic and oleic esters (Figure 2). However a log plot shows that the methyl linolenate has an emergence time coincident with methyl eicosenoate, which would complicate the analysis of oils containing both of these acids, *e.g.*, rapeseed oil.

The percentage composition of each oil examined (Tables I and II) was determined from areas obtained by drawing tangents to the curves on the recorder chart and using the height of the triangle and width of the base. G.L.P.C. results are the means of duplicate determinations. Two synthetic mixtures of fatty acids were made up to resemble soybean and linseed oils, and the compositions of these are given in Table III as individual determinations.

TABLE I

Fatty Acid Compositions of Corn, Sunflowerseed, Soybean, and Linseed Oils by G.L.P.C. and Standard Analysis

		Palmitic	Stearic	Oleic	Linoleic	Linolenic	Iodine value
Corn	(1)	12.2	2.0	28.9	56.3	0.8	123.8[b]
	(2)	12.0	2.3	28.3	56.6	0.8	123.8[b]
	(3)	12.1	2.3	28.7	56.2	0.7	123.2[b]
	(4)	....	18.2[a]	17.7	64.1	....	125.6[c]
Sunflowerseed	(1)	6.6	3.7	15.5	74.2	....	141.1[b]
	(2)	6.6	4.0	15.5	73.9	....	140.6[b]
	(3)	7.2	4.1	16.2	72.5	....	138.8[b]
	(4)	....	16.2[a]	5.5	78.3	....	139.6[c]
Soybean	(1)	11.5	4.5	25.0	49.4	9.6	131.5[b]
	(2)	11.5	3.9	24.6	52.0	8.0	131.5[b]
	(3)	11.9	4.4	24.0	51.9	7.8	130.3[b]
	(4)	....	19.8[a]	14.7	58.8	6.7	131.3[c]
Linseed	(1)	6.7	3.5	16.2	17.4	56.2	190.2[b]
	(2)	6.9	3.6	16.0	15.0	58.5	191.9[b]
	(3)	6.1	3.8	15.5	15.3	59.3	194.0[b]
	(4)	....	4.1[a]	19.6	25.3	51.0	193.2[c]

(1) Succinate G.L.P.C.—esters by diazomethane. (2) Succinate G.L.P.C.—esters by interesterification. (3) Adipate G.L.P.C.—esters by interesterification. (4) Standard analyses (19).

[a] Total saturated acids from standard analysis.
[b] Calculated from G.L.P.C. results.
[c] Measured by Wijs method.

Both methods for preparing the methyl esters give closely similar data with the succinate polyester column (Tables I and II) except for linoleic acid in soybean oil, linolenic acid in linseed oil, and oleic and linoleic acids in peanut oil. Standard deviations from the mean for all G.L.P.C. results were as follows: palmitic 0.23, stearic 0.08, oleic 0.88, linoleic 0.22, and linolenic 0.16 units percentage. The interesterification method is preferred for neutral oils because the procedure is simple and requires less time to prepare the samples for G.L.P.C. analysis.

The two types of polyester columns gave analytical results in close agreement when the esters were formed by the interesterification method. The succinate poly-

TABLE II

Fatty Acid Composition of Olive and Peanut Oils by G.L.P.C. and Standard Analyses

	Olive oil				Peanut oil			
	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)
Palmitic	13.6	13.6	13.4	....	9.9	11.4	10.1	....
Palmitoleic	1.2	1.3	1.2	....	....	....	....	....
Stearic	3.3	3.2	3.1	14.3[a]	3.2	3.3	3.2	21.9[a]
Oleic	73.5	74.0	76.2	76.2	57.5	54.7	53.9	51.2
Linoleic	7.4	7.0	5.5	9.0	23.2	25.7	24.2	26.9
Linolenic	1.0	0.9	0.6	0.5	....	....	....	....
Arachidic	....	....	....	....	1.3	1.2	1.7	....
Eicosenoic	....	....	....	....	1.6	1.1	1.6	....
Behenic	....	....	....	....	2.3	1.8	3.2	....
Lignoceric	....	....	....	....	1.0	0.8	2.1	....
Iodine value	79.4[b]	79.0[b]		82.1[c]	90.4[b]	92.0[b]	89.1[b]	90.1[c]

(1) Succinate G.L.P.C.—esters by *diazomethane*. (2) Succinate G.L.P.C.—esters by interesterification. (3) Adipate G.L.P.C.—esters by interesterification. (4) Standard analyses (19).

[a] Total saturated acids. [b] Calculated from G.L.P.C. results. [c] Measured by Wijs method.

TABLE III

Fatty Acid Composition of Synthetic Mixtures by G.L.P.C. and Spectral Analyses

	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Iodine value
			Synthetic I			
Actual %	6.0	5.2	19.1	58.7	11.0	146.1[b]
Spectral	....	13.2[a]	16.7	59.2	10.9	144.7[c]
Succinate	6.8	5.6	19.9	57.9	9.8	142.3[d]
G.L.P.C.	7.1	5.1	19.6	58.8	9.4	142.6[d]
Adipate	6.7	4.9	20.4	58.3	9.7	143.2[d]
G.L.P.C.	6.6	4.7	19.9	59.2	9.6	144.0[d]
			Synthetic II			
Actual %	5.5	5.6	19.7	10.5	58.7	187.9[b]
Spectral	....	12.8[a]	18.9	17.7	51.6	181.0[c]
Succinate	6.6	5.6	20.9	9.8	57.1	183.5[d]
G.L.P.C.	6.4	6.2	20.8	10.1	56.5	182.3[d]
Adipate	6.2	6.4	20.8	10.5	56.1	182.0[d]
G.L.P.C.	6.3	5.9	21.6	10.1	56.1	182.0[d]

[a] Total saturated acids.
[b] Calculated from composition of synthetic mixture.
[c] Measured by Wijs method.
[d] Calculated from G.L.P.C. results.

ester column could be used on the present oils since methyl linolenate and methyl eicosenoate were not present in the same sample. A silicone column was used to verify the presence or absence of methyl arachidate in these oils and to confirm the proportions on the basis of chain length. Although the separation of stearic and oleic was incomplete on the adipate polyester column, the calculated amounts of stearic and oleic acids are in good agreement with the results, using the succinate columns where these acids were separated more completely.

Linoleic and linolenic acid contents of the various oils as given by spectral analyses are not in good agreement with the G.L.P.C. data for soybean, corn, sunflowerseed, and linseed oils where one or both of these acids were present in major quantities (Tables I and II). The amount of linolenic acid was lower and of linoleic acid higher by the standard analyses (19) than by the G.L.P.C. method. This discrepancy is reflected in the content of oleic and "saturated" acids which are obtained by difference in the spectral results and is not unexpected since the spectral method is an empirical procedure.

The iodine values calculated from the amounts of unsaturated acids determined by G.L.P.C. are in good agreement with the measured iodine values. An error of 1% in linolenic acid will give rise to an error of 2.7 iodine value units if made in favor of saturated acids, 1.8 for linoleic, and 0.9 for oleic acid. The agreement of calculated and measured iodine values can be used as a measure of the accuracy of the G.L.P.C. method if no conjugated acids are present.

The results on the two synthetic mixtures show good agreement with actual values for both the succinate and adipate columns. Spectral analyses and G.L.P.C. give similar values for linoleic and linolenic acids in mixture I but not in mixture II, where the latter acid is predominant. The measured values and those calculated from the G.L.P.C. results are in agreement. The single values given in this table illustrate the differences that can be expected in duplicate determinations.

The major objection to polyesters based on diethylene glycol is the thermal instability, which limits the life of the column. A study by Pohl (15) on polyester polymers shows that the ether oxygen in diethylene glycol contributes to thermal instability. Substitution of ethylene glycol and 1,4-butanediol as the polyol gave a marked improvement in thermal stability. The succinate-ethylene glycol polyester will give the same separation as the succinate-diethylene glycol (Figure 2). Similarly the succinate-1,4-butanediol polyester (m.p. 85–95°C.) separated the fatty acid esters in the same manner as the adipate-diethylene glycol (Figure 1) with an improved resolution of stearic and oleic esters. Solvent fractionation of these polyesters was not required in the preparative procedure, and a further improvement in column life was obtained by drying the packing under vacuum at 55°C. for 16 hrs. prior to filling the column.

It is concluded from the present data that the analytical results obtained by G.L.P.C., using polyester columns, are reliable. The method is rapid and ideally suited to small quantities of fats and oils. It should find many applications in research problems.

Summary

The fatty acid composition of a number of vegetable oils and of two synthetic mixtures of methyl esters are compared by gas-liquid chromatography and by standard methods. The calculated iodine values from G.L.P.C. results are in good agreement with measured iodine values and are indicative of the reliability of the G.L.P.C. values. Standard methods gave lower values for linoleic acid and higher values for linolenic acid than did G.L.P.C. This deviation was particularly evident in oils with a high proportion of linolenic acid, *e.g.*, linseed oil. The results of G.L.P.C. are considered to be accurate to within one unit percentage. Thermal stability of the polyester polymers can be improved by using 1,4-butanediol or ethylene glycol as the polyols instead of diethylene glycol.

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