THE ANALYSIS OF OILS AND FATS BY GAS CHROMATOGRAPHY

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(Received May 22nd, 1964)

Gas–liquid chromatography has become a most important method for the determination of the fatty acid composition of natural oils and fats and the acidic part of complex lipids. The importance of the method has increased with the use of polyester stationary liquids, which enables a separation of the fatty acid methyl esters to be achieved on a basis of unsaturation[1,2].

In most of the published work on the determination of fatty acids, the acids were first converted to the corresponding methyl esters before separation by gas–liquid chromatography. Various investigators have studied the quantitative aspects of this conversion using methanol–hydrochloric acid[3], boron trifluoride–methanol[4], methylation of the silver salts of the acids by methyl iodide[5], and recently the results of a collaborative test have been published[6].

There have been many methods reported for obtaining the methyl esters of the fatty acids of oils and fats and these may be divided into two main groups, (a) transesterification of the glycerides in the presence of an excess of methanol, and (b) saponification of the glycerides with alkali, isolation of the free fatty acids and esterifying these acids. There are also many variations in procedure within these main groups. The usual catalyst for the transesterification is an alkali metal methoxide[2,7–11], but potassium hydroxide[12] and mineral acid[13] have also been used. Many variations, both in the reaction time and the strength of the alkali, in the saponification procedures have been reported[14–18].

It has been pointed out[18,19] that it is important to use mild conditions for the saponification of lipids since there is evidence that polyunsaturated acids may be partially isomerised during a saponification with concentrated alkali. Transesterification methods are therefore to be preferred. These latter methods are also less time consuming.

The purpose of the present investigation is to compare two different types of ionisation detectors with respect to their response to long-chain methyl esters and also to compare the following procedures for obtaining these methyl esters from oils and fats:

(a) Transesterification:

1. sodium methoxide catalyst, 40 min reaction time[10];
2. potassium hydroxide catalyst[12];
3. sodium methoxide catalyst, 4 h reaction time[8].

(b) Saponification-esterification:

4. saponification with 7.8 % ethanolic potassium hydroxide[19];
5. saponification with 0.5 *N* ethanolic potassium hydroxide[15];
6. cold saponification with 50 % aqueous potassium hydroxide[17].

EXPERIMENTAL

Preparation of methyl esters

(*a*) *Transesterification procedures*

1. A mixture of 5 g of the oil and 16 ml of 0.2 *N* sodium methoxide in methanol was heated under reflux for 40 min. The mixture was cooled, acidified with 2 *N* sulphuric acid and the resulting mixture was poured into an equal volume of cold, saturated sodium chloride solution. The methyl esters were extracted with 3 × 10 ml portions of light petrol (b.p. 40–60°) and the combined extracts were washed with small portions of ice-cold water until the washings were neutral. The light petrol extract was dried for 30 min over anhydrous sodium sulphate and, after filtration, the bulk of the light petrol was removed by distillation.

2. A mixture of 5 g of the oil and 25 ml of 0.2% potassium hydroxide in methanol was heated under reflux until the mixture was homogeneous, and then for a further 10 min. The mixture was cooled, poured into an equal volume of cold water and the methyl esters extracted with 3 × 20 ml portions of diethyl ether. The combined extracts were washed with small portions of water until the washings were neutral. The ether extract was dried over anhydrous sodium sulphate and, after filtration, the bulk of the ether was removed by distillation.

3. A mixture of 2 g of the oil and 35 ml of methanol was heated under reflux for a few minutes, 3.5 ml of 1 % sodium methoxide in methanol were added and the resulting solution heated under reflux for 4 h. The mixture was cooled, poured into an equal volume of water and the methyl esters obtained by extraction into ether as in procedure 2 above.

(*b*) *Saponification–esterification procedures*

4. A mixture of 10 g of the oil and 50 ml of 7.8 % potassium hydroxide in 95 % ethanol was heated under reflux for 3 h. The resulting solution was poured into an equal volume of cold water and the unsaponifiable material removed by extraction with ether. The aqueous layer, containing the potassium salts of the fatty acids was acidified with 2 *N* sulphuric acid and the free fatty acids were extracted with 3 × 10 ml portions of diethyl ether. The combined ether extracts were dried for 30 min over anhydrous sodium sulphate, and, after filtration, the ether was removed by distillation.

The fatty acids obtained were esterified by heating under reflux for 4 h with 50 ml methanol containing 1 % by volume sulphuric acid. The resulting solution was poured into an equal volume of water and the methyl esters were extracted with 3 × 10 ml portions of diethyl ether. The combined ether extracts were washed with an aqueous sodium bicarbonate solution and finally with water. The ether extract was dried for 30 min over anhydrous sodium sulphate and, after filtration, the bulk of the ether was removed by distillation.

5. A mixture of 5 g of the oil and 40 ml of 0.5 *N* potassium hydroxide in 95 % ethanol was heated under reflux for 2 h. The resulting solution was poured into an equal volume of cold water. The fatty acids were obtained and converted into their methyl esters by the same method as in procedure 4.

6. 10 g of the oil was added, with vigorous stirring, to a solution of 2.6 g potassium hydroxide in 2.5 ml of water. The reaction was exothermic, and, after cooling the reaction vessel, 0.1 ml of ethanol was added. The resulting mixture was allowed

to stand overnight at room temperature. The solid mass obtained was dissolved in 150 ml of cold water and this solution was processed to give the free fatty acids and then their methyl esters by the method used in procedure 4.

Gas–liquid chromatography

The methyl esters of the fatty acids were separated on two different gas chromatographs, a Perkin Elmer 800 (PE 800) instrument with a dual flame ionisation detector, and a Pye Argon Chromatograph (PAC) with a strontium-90 ionisation detector. The operating parameters were:

PE 800. 6 ft. × $^1/_8$ in. O.D. stainless steel column packed with butanediol succinate (BDS) on HMDS Chromosorb W (80–100 mesh) (8:92, w/w); for the linseed and corn oil esters the column temperature was programmed from 180° to 210° at a rate of 3.3 degs/min; for the coconut oil esters the column temperature was programmed from 150° to 210° at a rate of 6.7 degs/min. The flash heater temperature was 300°; flow-rate of nitrogen was 30 ml/min. Samples were injected onto the chromatographic column by means of a Hamilton micro-syringe.

PAC. 4 ft. × $^1/_4$ in. O.D. glass column packed with PEGA on acid-washed Celite 545 (100–120 mesh) (10:90, w/w); for the linseed and corn oil esters the column temperature was 180°. It was not possible to estimate all the methyl esters from coconut oil at one column temperature; the lower molecular weight esters were separated at 140° and the higher molecular weight esters were separated at 180° and a quantitative relationship between the chromatograms obtained at the two temperatures was established using the area of the methyl palmitate peak[3,20]. Argon was the carrier gas used at a pressure of 10 lb./sq.in., the applied voltage was 1250 V. Samples were placed on the chromatographic column with a micro-dipper pipette.

The areas under the peaks were obtained by multiplying the peak height by the width at half peak height[21], and the percentage areas obtained by the internal normalisation technique.

RESULT AND DISCUSSION

It has been shown[22] that, for accurate quantitative results using gas chromatographic methods, calibration of the detector with pure compounds is essential. ETTRE AND KABOT[23] have recently published results of the response of the PE 800 dual flame ionisation detector to fatty acid methyl esters. These workers used BDS-coated open tubular columns and found that the areas per cent were proportional to the weight per cent of the esters. In the present work this has been confirmed when conventional packed columns were used (Table I).

The response of a β-ray ionisation detector appears to depend on the geometry of the cell and the operating conditions employed. The variations in response to fatty acid methyl esters have been discussed by ACKMANN AND BURGHER[24]. The response factors obtained in the present work are shown in Table II, and it was found that the response factors for the lower molecular weight fatty esters are dependent on the applied voltage. It is therefore necessary to calibrate the detector for each applied voltage setting.

Pure samples of linoleic and linolenic methyl esters were not available to the present authors for calibration purpose but since the relative amounts of these two

TABLE I

RESPONSE OF A FLAME IONISATION DETECTOR TO FATTY ACID METHYL ESTERS

Ester	*Weight response relative to methyl palmitate* (= 100)	
	a	*b*
Methyl octoate	99	97
Methyl decoate	100	101
Methyl laurate	100	100
Methyl myristate	99	98
Methyl palmitate	100	100
Methyl stearate	101	101

a = present work.
b = results of ETTRE AND KABOT[23].

esters are similar on the two different instruments, it may be concluded that the responses of the two different ionisation detectors to these esters were similar.

The results obtained for the relative amounts of the major constituent fatty acids of linseed oil, corn oil and coconut oil are shown in Table III. It can be seen that, for linseed oil and corn oil, good agreement is obtained for all six procedures used for obtaining the methyl esters. Also, there is good agreement of the results obtained on the two different instruments. For the coconut oil there is good agreement of the results obtained on the two different instruments. There is also good agreement of the results obtained by five of the procedures for obtaining the methyl esters but the relative proportion of lauric acid is much lower in the methyl esters obtained by transesterification procedure 3.

In this last mentioned procedure it was found that, when the reaction mixture was poured into water and the methyl esters extracted by ether, the aqueous layer remained cloudy and it was thought probable that some material was not being extracted by the ether. The aqueous layer was acidified, solid sodium chloride was added until the solution was almost saturated, and the resulting solution was ex-

TABLE II

RESPONSE OF A β-RAY IONISATION DETECTOR TO FATTY ACID METHYL ESTERS

Ester	*Weight response relative to methyl palmitate* (= 100) *in dependence on applied voltage* (*V*)			
	1000	*1250*	*1500*	*1750*
Methyl octoate	60	85	98	101
Methyl decoate	66	90	100	101
Methyl laurate	72	93	102	101
Methyl myristate	89	98	99	100
Methyl palmitate	100	100	100	100
Methyl stearate	100	101	103	105

tracted with ether. After this extraction the aqueous layer was clear. This ether solution was dried and chromatographed on the PE 800 instrument. It was found that this solution contained a greater proportion of lauric acid than the original solution of methyl esters obtained by procedure 3.

TABLE III

RELATIVE AMOUNTS OF THE MAJOR CONSTITUENT FATTY ACIDS

Acid	*Flame ionisation detector*						*β-Ray ionisation detector*					
	Transesterification			*Saponification-esterification*			*Transesterification*			*Saponification-esterification*		
	1	*2*	*3*	*4*	*5*	*6*	*1*	*2*	*3*	*4*	*5*	*6*
Linseed oil												
16:0	5.6	5.8	6.2	6.3	5.8	5.8	5.3	5.4	5.4	5.7	5.9	5.8
18:0	3.7	3.6	3.5	3.5	3.6	3.7	3.9	3.8	4.3	3.5	3.8	3.7
18:1	18.6	18.8	18.7	18.3	18.1	18.1	18.4	18.2	18.7	18.4	18.1	18.2
18:2	13.2	13.2	13.5	13.8	13.8	13.7	13.3	13.4	13.7	13.4	13.4	13.7
18:3	58.9	58.6	58.1	58.1	58.7	58.7	59.1	59.2	57.9	58.9	58.8	58.6
Corn oil												
16:0	11.4	11.2	11.7	12.1	11.7	11.5	11.8	11.7	11.6	12.0	12.0	11.5
18:0	2.0	1.8	2.2	2.0	1.9	2.0	2.2	2.0	1.9	2.0	2.1	2.0
18:1	32.0	32.1	32.6	31.7	31.5	32.2	31.8	32.2	32.8	32.1	31.6	32.0
18:2	54.6	54.9	53.5	54.2	54.9	54.3	54.2	54.1	53.7	53.9	54.3	54.3
Coconut oil												
6:0	0.6	0.6	1.1	0.5	0.6	0.6	(0.6)*	(0.6)*	(1.1)*	(0.5)*	(0.6)*	(0.6)*
8:0	8.0	9.6	11.3	9.5	9.2	9.0	8.9	10.9	11.5	9.1	10.4	10.9
10:0	5.8	6.3	5.8	6.8	6.8	6.9	5.5	5.9	5.5	5.5	5.7	5.8
12:0	50.5	50.0	45.7	50.6	49.9	48.7	51.6	49.8	44.8	51.6	50.3	49.5
14:0	19.1	17.8	19.4	18.2	18.4	18.5	19.0	18.5	19.1	18.8	18.6	18.5
16:0	7.2	7.0	8.2	6.9	7.1	7.5	7.3	6.7	8.3	7.0	6.9	7.2
18:0	2.2	2.1	2.3	1.8	1.9	2.3	2.1	2.0	2.5	2.2	2.0	2.0
18:1	5.3	5.1	5.3	4.5	5.0	5.2	4.4	4.5	5.7	4.6	4.6	4.7
18:2	1.3	1.4	1.3	1.2	1.1	1.3	0.6	1.1	1.5	0.7	0.9	0.8

* Results obtained on flame ionisation detector.

These two solutions of methyl esters were combined and it was found that the relative proportions of the methyl esters in this combined solution were similar to those found by the other procedures. A sample of the coconut oil was then transesterified by procedure 3, modified to include the acidification of the reaction mixture and saturation with sodium chloride before ether extraction. The resulting solution of the methyl esters was chromatographed. The results of these experiments are summarised in Table IV. KAUFMANN AND MANKEL[13] have also reported loss of lower molecular weight fatty acids during the extraction stage and they have overcome this loss by extraction with a mixture of docosane and light petrol.

The amounts of some of the minor constituent fatty acids of linseed oil and corn oil obtained on the PE 800 instrument are shown in Table V. The amounts of lauric and myristic acid obtained by all six procedures are similar but there are large

TABLE IV

RELATIVE AMOUNTS OF FATTY ACIDS OBTAINED FROM COCONUT OIL

Acid	*a*	*b*	*c*	*d*
6:0	1.0	0.6	0.8	0.9
8:0	12.8	4.9	8.7	10.4
10:0	4.8	7.6	6.3	6.6
12:0	43.5	56.4	50.1	49.6
14:0	19.4	17.4	18.4	18.0
16:0	7.8	6.3	7.0	7.0
18:0	3.0	1.6	2.3	2.0
18:1	6.4	4.1	5.2	4.5
18:2	1.3	1.1	1.2	1.0

a = obtained by procedure 3.
b = extracts of the aqueous layer of procedure 3.
c = extracts a and b combined.
d = obtained by modified procedure 3.

variations in the amounts of the other acids, *e.g.* the amount of the triunsaturated C_{14} acid in corn oil varies from 0.03 % (procedure 1) to 0.95 % (procedure 4). The total amounts of the minor constituent fatty acids of corn oil found by the transesterification procedures are much lower than the amounts obtained by the other procedures.

When the coconut oil methyl esters were chromatographed on the PEGA column a peak was obtained which had a retention time of 0.86 relative to methyl stearate (= 1.00) and an equivalent chain length of 17.50. This peak is probably due

TABLE V

PERCENTAGE AMOUNTS OF THE MINOR FATTY ACID CONSTITUENTS

Acid	*Procedure*					
	1	*2*	*3*	*4*	*5*	*6*
Linseed oil						
12:0	0.08	0.08	0.07	0.08	0.08	0.07
12:unsat.	0.01	0.02	0.01	0.01	0.02	0.04
12:unsat.	0.02	0.04	> 0.01	0.03	0.04	0.05
13:0	0.02	0.04	> 0.01	0.04	0.02	> 0.01
14:0	0.04	0.04	0.05	0.04	0.04	0.02
14:3	0.17	0.11	0.03	0.02	0.04	0.03
Total	0.34	0.33	0.18	0.22	0.24	0.21
Corn oil						
10:0	0.03	0.03	0.02	0.09	0.02	0.02
11:0	0.01	0.03	0.03	0.09	0.09	0.09
12:0	0.33	0.30	0.29	0.37	0.33	0.35
12:unsat.	0.01	0.21	0.15	0.32	0.30	0.35
13:0	0.06	0.43	0.10	0.83	0.81	0.99
14:0	0.12	0.09	0.10	0.09	0.10	0.13
14:3	0.03	0.17	0.10	0.95	0.56	0.77
Total	0.59	1.26	0.79	2.74	2.21	2.70

to the presence of a triunsaturated C_{16} methyl ester. The amounts of this component in the methyl esters obtained by the different procedures varied considerably:

Procedure	1	2	3	4	5	6
Percentage 16:3	0.71	0.01	0.01	0.36	0.07	0.09

When the coconut oil methyl esters obtained by procedures 4 and 5 were chromatographed on the BDS column peaks were obtained for the monounsaturated C_8, C_{10}, C_{12}, C_{14} and C_{16} methyl esters and the amounts of these esters are shown in Table VI.

TABLE VI

PERCENTAGE AMOUNTS OF MONOUNSATURATED ACIDS IN COCONUT OIL

Acid	*Procedure*	
	4	*5*
8:1	0.5	> 0.1
10:1	0.3	0.1
12:1	2.5	1.3
14:1	1.0	0.5
16:1	0.3	0.2

These peaks were barely discernible in the chromatograms of the methyl esters obtained by the other procedures. The amounts of monounsaturated C_{16} fatty acid found by KAUFMANN AND MANKEL[13] in samples of sesame oil and maize oil vary by a factor of 2 depending on the procedure used to obtain the methyl esters from these oils.

CONCLUSIONS

The results given here show that gas chromatography is a convenient method for determining the fatty acid content of oils and fats. When mixtures of fatty acids contain acids with a range of molecular weights then a gas chromatograph with temperature programming simplifies the operations involved. The six procedures for obtaining fatty acid methyl esters from oils and fats give similar results for the fatty acids present in major amounts so long as precautions are taken to obviate the loss of lower molecular weight fatty acids. Further work is obviously necessary to account for the varying results obtained by the different procedures for the minor constituent fatty acids.

SUMMARY

A comparison is made of six procedures of obtaining methyl esters of fatty acids from oils and fats and also of two gas chromatographic instruments for the separation of these esters. The responses of a dual flame ionisation and a β-ray ionisation detector to long-chain fatty acid methyl esters are discussed.

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