THE ANALYSIS OF OILS AND FATS BY GAS CHROMATOGRAPHY

II. THE ALKALINE ISOMERISATION OF LINOLEIC ACID

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Many methods have been reported for obtaining the methyl esters of the fatty acids of oils and fats prior to separation by gas–liquid chromatography. These methods can be divided into two main groups: (a) interesterification of the glycerides in the presence of an excess of methanol and an acidic or a basic catalyst, and (b) saponification of the glycerides with alkali, isolation of the free fatty acids and esterification of these acids. Recently[1] six of these methods were compared and it was found that gas chromatography was a suitable method for determining the major fatty acid components of oils and fats.

It has been pointed out[2–4] that mild saponification conditions should be employed since there is evidence that polyunsaturated acids may be partially isomerised during a saponification with concentrated alkali. This isomerisation has been the subject of various recent discussions[5,6], and the complex nature of the mixtures of isomerisation products obtained has been illustrated[7].

HILDITCH *et al.*[8] have shown that, when linoleic acid is heated with alkali, isomerisation takes place only slowly at 170°, but AST[4], using mild saponification conditions for safflower oil, found that a considerable amount of linoleic acid had been isomerised.

LANDOWNE AND LIPSKY[9] have shown that certain unconjugated positional isomers of methyl linoleate can be separated on a polar capillary column, and LITCHFIELD, ISBELL AND REISER[10] have separated three of the four possible geometric isomers of methyl linoleate on a similar type of column. An examination of the retention times of these isomeric esters indicates that it is unlikely that they could be separated from each other on our butanediol succinate (BDS) packed column; they would all be included in the methyl linoleate peak.

CARTONI, LIBERTI AND RUGGIERI[11] have examined the products obtained by the alkali isomerisation of linoleic acid, linolenic acid and dehydrated ricinoleic acid on polar and non-polar capillary columns. These workers obtained, from linoleic acid, four peaks which they assign to conjugated isomers. The results which they obtain for the relative proportions of the isomerisation products are very similar to those reported in Table V. With 6.6 % potassium hydroxide–ethylene glycol as the isomerisation reagent they found little change in the relative proportions of the isomerisation products for reaction times of up to 45 min; with a reaction time of 90 min they found a large increase in the relative proportion of the conjugated *trans,trans* isomers.

The purpose of the present work is to investigate the effect of saponification

temperature on the linoleic acid content of oils which contain only very small amounts of linolenic acid and also to investigate, by means of gas–liquid chromatography, the nature of the linoleic acid isomerisation products formed.

EXPERIMENTAL

Saponification procedures

(a) The oils were saponified with methanolic potassium hydroxide using the "general procedure" described by AST[4]. The isolated free fatty acids were converted to their methyl esters by heating under reflux with methanol containing 1 % by volume sulphuric acid. This procedure will be referred to subsequently as Method 1.

(b) The oils (200 mg) were heated with 1 ml of 6 % potassium hydroxide in diethylene glycol for one hour in either a constant temperature air or oil bath. After this heating period the mixture was cooled, acidified with 4 *N* hydrochloric acid and the liberated fatty acids were extracted with hexane. After evaporation of the hexane on a steam bath the acids were converted to their methyl esters as described in (a) above.

(c) Safflower oil was saponified by heating with 6 % potassium hydroxide in ethylene glycol following the procedure (b) above.

Gas–liquid chromatography

The methyl esters of the fatty acids were separated on a Perkin Elmer 800 chromatograph with butanediol succinate (BDS) as the stationary liquid as described previously[1] except that most of the chromatographic runs were carried out isothermally at a column temperature of 200°.

Equivalent chain lengths were determined by the conventional graphic procedure[12].

RESULTS AND DISCUSSION

Four oils with a low linolenic acid content were saponified with methanolic potassium hydroxide and also with potassium hydroxide–diethylene glycol at four different temperatures. The acids were separated and chromatographed as their methyl esters. The amounts of each ester were calculated relative to the amount of methyl palmitate present. The results are shown in Table I.

It is found that there is no significant change, with an increase in the temperature of saponification, in the relative amounts of either stearic acid or oleic acid. There is a decrease, however, in the relative amounts of linoleic acid with an increase in the temperature of saponification. There are no significant losses of linoleic acid at 120°; at 150° about 87 % of the linoleic acid is unchanged, at 180° about 44 % is unchanged and even at the reflux temperature of diethylene glycol about 5 % remains unchanged. Safflower oil was also saponified with potassium hydroxide–ethylene glycol at 180° and at the reflux temperature of ethylene glycol and the losses in linoleic acid are consistent with those obtained with the diethylene glycol reagent.

For each of the oils studied the decrease in linoleic acid content at saponification temperatures of 150° and 180° is accompanied by the formation of conjugated octadecadienoic acids and the total amounts of these conjugated acids formed account

TABLE I

AMOUNTS OF METHYL ESTERS RELATIVE TO METHYL PALMITATE (= 1.00)

Acid	*18:0*	*18:1*[9]	*18:2*[9,12]	*18:3*[9,12,15]	*18:2*[conj]	*18:2*[conj]	*20:0*	*18:2*[conj]	*20:1*[11]	*18:2*[conj]	*18:2*[conj]	*Total***
ECL of methyl ester (BDS: 200°)*	*18.00*	*18.38*	*18.87*	*19.59*	*19.84*	*19.96*	*20.00*	*20.17*	*20.32*	*20.45*	*20.62*	
Groundnut oil												
Method 1	0.39	7.02	2.27	0.01	—	—	0.14	—	0.12	—	—	2.54
KOH–DEG: 120°	0.42	6.92	2.24	—	—	—	0.18	—	0.13	—	—	2.55
150°	0.45	6.98	1.98	—	0.10	← 0.26 →		← 0.15 →		0.01	0.02	2.51
180°	0.42	6.95	1.21	—	0.42	← 0.52 →		← 0.15 →		0.13	0.05	2.48
234°	0.40	6.92	0.11	—	0.31	← 0.36 →		← 0.36 →		1.09	0.10	2.33
Corn oil												
Method 1	0.21	2.78	4.48	0.14	—	—	0.10	—	0.08	—	—	4.80
KOH–DEG: 120°	0.22	2.80	4.50	0.09	—	—	0.10	—	0.08	—	—	4.77
150°	0.21	2.80	4.18	0.06	0.25	← 0.26 →		← 0.10 →		0.01	—	4.85
180°	0.21	2.80	1.36	—	← 3.14 →			← 0.18 →		0.09	0.10	4.87
234°	0.22	2.76	0.18	—	← 1.33 →			← 0.44 →		2.18	0.18	4.31
Soyabean oil												
Method 1	0.33	1.94	4.62	0.82	—	—	0.01	—	0.01	—	—	5.44
KOH–DEG: 120°	0.34	1.98	4.63	0.80	—	—	0.01	—	0.01	—	—	5.43
150°	0.34	2.00	4.12	0.51	← 0.41 →			← 0.11 →		0.22	0.08	5.45
180°	0.34	1.98	2.14	0.09	← 0.24 →			← 0.23 →		0.38	0.15	5.33
234°	0.33	2.02	0.25	—	← 1.98 →			← 0.57 →		1.83	0.43	5.06
Safflower oil												
Method 1	0.33	1.73	13.82	0.02	—	—	0.04	—	0.02	—	—	13.90
KOH–DEG: 120°	0.31	1.67	13.56	0.01	0.05	← 0.09 →		—	0.02	—	—	13.73
150°	0.32	1.70	10.80	—	1.23	← 1.26 →		← 0.12 →		0.16	0.08	13.65
180°	0.30	1.70	6.38	—	2.88	← 3.44 →		← 0.33 →		0.26	0.11	13.40
234°	0.32	1.68	0.59	—	3.40	← 3.80 →		← 1.36 →		3.51	0.65	13.25
KOH–EG: 180°	0.32	1.70	5.10	—	3.54	← 4.10 →		← 0.30 →		0.32	0.31	13.67
197°	0.33	1.70	1.76	—	4.83	← 5.85 →		← 0.63 →		0.30	0.31	13.68

* ECL = equivalent chain length.

** Total amounts of methyl esters with ECL of 18.87 and greater.

TABLE II

UNISOMERISED LINOLEIC ACID (PER CENT)

	Method 1	*Potassium hydroxide–diethylene glycol*				*Potassium hydroxide–ethylene glycol*	
		*120°**	*150°*	*180°*	*234°*	*180°*	*197°*
Groundnut oil	100	99	87	53	5	—	—
Corn oil	100	100	93	30	4	—	—
Soyabean oil	100	100	89	46	5	—	—
Safflower oil	100	98	78	46	4	37	13

* Saponification temperature.

for the loss of linoleic acid. At a saponification temperature of 234° the same conjugated acids are formed but about 8 % of the C_{18} acids is not accounted for (Table II).

DANIELS AND RICHMOND[2] found that, when oil extracted from flour was heated with potassium hydroxide–ethylene glycol at 180°, 2.9 % of the linoleic acid remained unisomerised. They found that the main isomerisation product was an acid whose methyl ester had a retention time relative to methyl stearate on a polyethylene glycol adipate column of 1.79 and an equivalent chain length (ECL) of 19.95, and also that the peak obtained on the chromatogram for this methyl ester was excessively broad indicating that more than one component might be present. AST[4], using milder saponification conditions with safflower oil, found two linoleic acid isomerisation products, the main product on a diethylene glycol succinate column giving an ECL of 20.6 and the minor product an ECL of 21.3. The above authors found that the amounts of isomerisation products formed accounted for the loss of linoleic acid.

In the present investigation five isomerisation products were detected and retention data and ECL values for these products are shown in Table III. It was found that in certain chromatograms two peaks were obtained with ECL values of 19.84 and 19.96 respectively but in others these two peaks were obtained as one broad misshapen peak. This misshapen peak could usually be split into two incompletely

TABLE III

RETENTION DATA AND EQUIVALENT CHAIN LENGTHS

Column: BDS.

Methyl ester	*Relative retention time*			*Equivalent chain length*		
	180°	*190°*	*200°*	*180°*	*190°*	*200°*
18:0	1.00	1.00	1.00	18.00	18.00	18.00
$18:2^{9,12}$	1.31	1.30	1.30	18.78	18.82	18.87
$18:2^{conj.cis,trans}$	1.84	1.80	1.75	19.78	19.83	19.84
$18:2^{conj.cis,trans}$	1.88	1.86	1.82	19.85	19.93	19.96
$18:2^{conj.cis,cis}$	2.01	1.97	1.94	20.04	20.11	20.17
20:0	1.98	1.90	1.84	20.00	20.00	20.00
$20:1^{11}$	2.16	2.10	2.03	20.25	20.31	20.32
$18:2^{9,11\,trans,trans}$	2.26	2.16	2.11	20.39	20.40	20.45
$18:2^{conj.?}$	2.40	2.32	2.22	20.56	20.62	20.62

separated peaks by running chromatograms at lower column temperatures. These five isomerisation products could also be obtained by heating linoleic acid with the potassium hydroxide–diethylene glycol reagent and four of these products could be obtained by the dehydration of methyl ricinoleate by vacuum distillation in the presence of potassium hydrogen sulphate (Table IV). It is interesting to note that at a reaction temperature of 150° there is essentially no isomerisation of linoleic acid but when the reaction temperature is increased to 180° 98 % of the linoleic acid is isomerised. This is in agreement with the results of HILDITCH[8], who found that linoleic acid isomerised only slowly at 170°.

TABLE IV

PERCENTAGE AMOUNTS OF METHYL ESTERS

Ester	*ECL*	*Linoleic acid–KOH–DEG*				*Dehydration of methyl ricinoleate*
		120°	*150°*	*180°*	*234°*	
16:0	16.00	—	—	—	—	1.3
18:0	18.00	0.1	0.1	0.1	0.1	1.0
18:1[9]	18.38	0.8	0.8	0.8	0.8	5.0
18:2[9,12]	18.87	99.1	99.1	2.2	1.8	51.4
18:2[conj.*cis,trans*]	19.84	—	—	40.2	55.4	20.2
18:2[conj.*cis,trans*]	19.96	—	—	41.2		
18:2[conj.*cis,cis*]	20.17	—	—	5.9	14.7	6.7
18:2[conj.*trans,trans*]	20.45	—	—	5.1	21.3	13.8
18:2[conj.?]	20.62	—	—	4.4	5.9	—

Certain of these isomerisation products could be identified by comparing the chromatograms obtained on the BDS column with those obtained on an Apiezon L column. Retention times obtained on this latter column were similar to those obtained by BODY AND SHORLAND[13]. The retention times obtained on both columns for a pure sample of methyl octadeca-9(*trans*)-11(*trans*)-dienoate[14] agreed with those obtained for the isomerisation product having an ECL of 20.45 (BDS; 200°).

The relative amounts of each of the isomerisation products are shown in Table V and it can be seen that, with saponification temperatures of 150° and 180°, the main products are the *cis,trans* isomers with smaller amounts of the other isomers. Soyabean oil gives a higher proportion of the *trans,trans* isomer than the other oils at these saponification temperatures. This oil contains a greater proportion of linolenic acid than the other oils and since linolenic acid is also isomerised under these conditions it is possible that an isomerisation product of linolenic acid has a retention time similar to the above *trans,trans*-octadecadienoate. This would also explain the results of DANIELS AND RICHMOND[2] who assumed that their ester, ECL 20.42, was obtained solely by the isomerisation of linolenic acid although it was obtained in an amount greater by 30 % than the linolenic acid originally present. In order to clarify this point linolenic acid was heated with the potassium hydroxide–diethylene glycol reagent at 150° and 180°. At the lower temperature a small amount of the linolenic acid was isomerised with the formation of a product having an ECL of 20.42 (BDS; 200°); at the higher temperature most of the linolenic acid isomerised, the major product having an ECL of 20.42 and the minor product 20.15. The formation of these products

TABLE V

RELATIVE PROPORTIONS (PER CENT) OF ISOMERISATION PRODUCTS

ECL	*Temperature of saponification*				
	Potassium hydroxide–diethylene glycol			*Potassium hydroxide–ethylene glycol*	
	150°	*180°*	*234°*	*180°*	*197°*
Groundnut oil					
19.84	36	33	14	—	—
19.96	43	41	16	—	—
20.17	10	12	16	—	—
20.45	4	10	49	—	—
20.62	7	4	5	—	—
Corn oil					
19.84	57	91	32	—	—
19.96	35				
20.17	5	3	9	—	—
20.45	3	3	55	—	—
20.62	—	3	4	—	—
Soyabean oil					
19.84	50	75	41	—	—
19.96					
20.17	13	7	12	—	—
30.45	27	13	38	—	—
20.62	10	5	9	—	—
Safflower oil					
19.84	44	41	27	41	40
19.96	43	49	30	47	49
10.17	4	4	10	4	5
20.45	6	4	28	4	3
20.62	3	2	5	4	3

from linolenic acid would therefore account for the high proportions of *trans,trans* isomer obtained from the saponification of soyabean oil at the lower temperatures.

At a saponification temperature of 234° all the oils give a decrease in the amount of the *cis,trans* isomers and this decrease is accompanied by an increase in the amount of the *cis,cis* isomer and a larger increase in the amount of the *trans,trans* isomer. At this higher saponification temperature some of the *cis,trans* isomers must be converted to the *trans,trans* isomer.

These results obtained by gas–liquid chromatography are supported by results obtained by infrared spectroscopy. With the methyl esters obtained from oils saponified by Method 1 or by potassium hydroxide–diethylene glycol at 120° no absorption was obtained at 985 cm^{-1} and 946 cm^{-1}, the respective characteristic *trans,trans* and *cis,trans* wave numbers. Absorptions at these wave numbers were evident for the methyl esters obtained from oils saponified at 150° and these absorptions became more intense with the methyl esters from oils saponified at the higher temperatures.

Safflower oil was saponified with the potassium hydroxide–diethylene glycol reagent at 180° for various times. There is a decrease in the linoleic acid content with an increase in saponification time. The major isomerisation products are the *cis,trans*

isomers and although the amounts of all isomerisation products increase with an increase in reaction time the relative proportions of these products remain essentially constant (Table VI).

TABLE VI

METHYL ESTERS FROM THE SAPONIFICATION OF SAFFLOWER OIL AT 180°

Ester	*ECL*	*Saponification time (min)*				
		20	*40*	*60*	*90*	*330*
16:0	16.00	1.00	1.00	1.00	1.00	1.00
18:0	18.00	0.34	0.33	0.35	0.33	0.33
$18:1^{9}$	18.38	1.68	1.70	1.70	1.67	1.67
$18:2^{9,12}$	18.87	12.24	10.53	6.38	4.76	0.30
$18:2^{cis,trans}$	19.84	0.51	1.25	2.88	3.54	4.95
$18:2^{cis,trans}$	19.96	0.59	1.55	3.44	4.16	6.05
$18:2^{cis,cis}$	20.17	0.11	0.10	0.33	0.35	0.48
$18:2^{trans,trans}$	20.45	0.10	0.10	0.26	0.51	0.78
$18:2^{conj}$	20.62	0.02	0.05	0.11	0.13	0.68
Unisomerised linoleic acid (per cent)		89	76	46	34	2

CONCLUSIONS

The fatty acid content of oils containing large amounts of linoleic acid and very small amounts of linolenic acid can be determined by gas chromatography after saponification at temperatures not exceeding 120°. When the oils are saponified at temperatures above 120° then isomerisation of the linoleic acid occurs and the amounts of isomerisation products formed increase with both an increase in the saponification temperature and the length of time of saponification. Certain of the isomerisation products can be separated by gas chromatography and it is found that with saponification temperatures up to 197° the main products are conjugated *cis,trans*-octadecadienoic acids. At higher temperatures an increasing amount of the corresponding *trans,trans* isomers are formed.

SUMMARY

Gas chromatography is used to study the nature of the fatty acids obtained by the saponification of oils with a low linolenic acid content. No isomerisation of linoleic acid is found at saponification temperatures not exceeding 120° but increasing amounts of isomerisation products are obtained with an increase of saponification temperature above 120°. The nature of these isomerisation products is discussed.

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