

## THE QUANTITATIVE DETERMINATION OF LINOLENIC ACID BY MEANS OF THE LOVELOCK IONISATION DETECTOR\*

GALINA V. NOVITSKAYA

*Institute of Plant Physiology, Academy of Sciences, Moscow (U.S.S.R.)*

(Received August 10th, 1964)

Over the past few years there have been several reports on parallel quantitative determinations of fatty acid mixtures containing linolenic acid by gas-liquid chromatography with a LOVELOCK ionisation detector<sup>1</sup> and by other chromatographic and non-chromatographic methods. It has been shown that in some cases linolenic acid determined by means of an ionisation detector gives abnormally high values<sup>2-4</sup>. It seemed desirable therefore to investigate this technique more closely in order to obtain more accurate results during the study of the fatty acid composition of linseed oil and other fatty acid mixtures rich in linolenic acid.

### EXPERIMENTAL AND RESULTS

Linseed oil triglycerides were separated by reversed-phase partition chromatography as described earlier<sup>5,6</sup>; the system acetone-glacial acetic acid (1:1)/dodecane was used. Total triglycerides and separate triglyceride fractions were converted into fatty acid methyl esters<sup>7</sup>. The ester composition was determined by gas-liquid chromatography<sup>5,\*\*</sup>. Peak area was measured by multiplying peak height by its width at mid-height, and the fatty acid concentration was expressed as weight or moles per cent. Detector voltages used in the various experiments were 750, 1000, 1250 or 1500 V. Detector sensitivity at 750 V decreased considerably; the use of "× 3" amplifier sensitivity at this voltage instead of lower "× 10" sensitivity setting does not affect the accuracy of results.

Fatty acids were separated by reversed-phase chromatography and quantitatively estimated by densitometry<sup>8,9</sup>. The iodine value of the oil was determined by the HANUS method<sup>10</sup>.

The determination of the methyl palmitate:methyl linolenate ratio in a model mixture (1:1 by weight) at various detector voltages gave the results shown in Table I.

It can be seen that the true proportion between the esters was only obtained at 750 V. If higher voltages were applied, the detector response for methyl linolenate became abnormally high. It seems that this ester is more easily ionised by metastable argon atoms than methyl palmitate.

\* Abbreviations and conventions: *P*, *P-O*, *S*, *O*, *L*, *Le* = palmitic, palmitoleic, stearic, oleic, linoleic, linolenic acids respectively, and acyls of these fatty acids in the triglyceride. The sequence of *P*, *S*, ... and other symbols does not represent the actual structure of the triglyceride molecule. [*P*], [*S*], [*OLeLe*], etc. indicate fatty acid and triglyceride concentrations (moles %).

\*\* Argon Chromatograph, W. G. Pye & Co. Ltd., Cambridge, England.

TABLE I

RATIOS OF PALMITATE:LINOLENATE IN A 1:1 MIXTURE FOUND AT VARIOUS DETECTOR VOLTAGES

Detector voltage	Palmitate:linolenate (peak area proportion)
750	1:1.00
1000	1:1.10
1250	1:1.23
1500	1:1.74

TABLE II

DEPENDENCE OF THE RELATIVE DETECTOR RESPONSE FOR FATTY ACID METHYL ESTERS ON THE DETECTOR VOLTAGE\*

Fatty acid	Voltage			
	750	1000	1250	1500
P	1	1	1	1
S	0.61	0.65	0.65	0.63
O	2.31	2.42	2.39	2.31
L	2.93	2.98	2.96	2.96
Le	8.72	11.11	12.55	13.47

\* The response for methyl palmitate is arbitrarily equal to 1 at each voltage.

TABLE III

COMPARISON OF THE DETERMINATION OF LINSEED OIL FATTY ACID COMPOSITION BY GAS-LIQUID CHROMATOGRAPHY AT VARIOUS DETECTOR VOLTAGES AND BY PARTITION CHROMATOGRAPHY (MOLES %)

Fatty acid	PC*	Voltage			
		750	1000	1250	1500
P	7.1	7.6	6.7	6.1	6.0
P-O	—	0.3	0.2	0.2	0.2
S	6.6	5.5	5.2	4.9	4.7
O	20.4	21.6	20.6	19.4	18.7
L	16.3	15.2	13.6	12.6	12.1
Le	49.6	49.7	53.7	56.7	58.3
Iodine value calculated	177.1	176.8	183.4	188.6	191.3
Found	177.2				

\* PC = quantitative determination by reversed-phase partition chromatography and densitometry.

TABLE IV  
DETERMINATION OF THE FATTY ACID COMPOSITION OF SEPARATE TRIGLYCERIDE FRACTIONS AT VARIOUS DETECTOR VOLTAGES (MOLES %)

Fatty acid	P			S			O			L			Le								
	750	1500	750	750	1500	750	750	1500	750	750	1500	750	750	1500							
Detector voltage																					
$K_2$	$R_2$	$c^*$	$f$	$c$	$f$	$c$	$f$	$c$	$f$	$c$	$f$	$c$	$f$	$c$							
55	0.32	7.7	7.5	—	7.1	9.5	8.8	—	9.0	17.1	16.9	—	14.6	24.4	23.5	—	21.0	42.0	43.3	39.7	48.3
57	0.42	7.7	8.8	—	7.5	—	—	—	—	19.3	20.4	—	17.2	5.2	9.7	—	8.2	63.3	61.0	53.5	67.0
59	0.63	—	—	—	—	—	—	—	—	—	—	—	—	33.3	33.4	—	27.5	66.8	66.6	55.0	72.5

\*  $c$  = Calculated from other fatty acids concentration according to equations (1)-(4);  $f$  = found at this voltage. The data obtained at 1500 volts were only used for calculations of the linolenic acid concentration.

It was therefore of interest to determine whether methyl esters of other fatty acids changed their ionisation pattern depending on detector voltage. The data of Table II show that the relative detector response for stearic, oleic, and linoleic acid esters remains practically unchanged at all voltages studied. In the same range the response for methyl linolenate grows abnormally.

In order to investigate the practical implications of the relationship found we carried out a parallel determination of the fatty acid composition of linseed oil with a known iodine value by reversed-phase partition chromatography and by gas-liquid chromatography at 750–1500 V.

As shown in Table III reliable quantitative data can be obtained by densitometry and by gas-liquid chromatography at 750 V. At higher voltages the detector response for methyl linolenate becomes too high.

After determining the fatty acid composition of the total linseed oil triglycerides we analyzed separate fractions of glycerides of the same polarity. Each fraction was characterized by the polarity constant

$$K_2 = 100 - m + 2e,$$

where  $m$  is the number of carbon atoms and  $e$  the number of double bonds. Each polarity constant value  $K_2$  corresponds to a different  $R_2$  value (ratio of  $R_F$  of triglyceride to the  $R_F$  value of butyl hexabromostearate<sup>6</sup>). The  $K_2 = 55$  fraction is a mixture of *PLLe*, *SLeLe*, and *OLLe* and the  $K_2 = 57$  fraction is a mixture of *PLeLe*, *LLLe*, and *OLeLe*. The  $K_2 = 59$  fraction contains only linoleodilinolenin. Thus, the molar relationships between the fatty acids in different fractions can be expressed as follows:

$$[Le] = 2[S] + [O] + [P] \quad (1)$$

and

$$[L] = [O] + [P] \quad (2)$$

for the  $K_2 = 55$  fraction;

$$[Le] = 2[O] + 2[P] + \frac{1}{2}[L] \quad (3)$$

for the  $K_2 = 57$  fraction, and

$$[Le] = 2[L] \quad (4)$$

for the  $K_2 = 59$  fraction.

The fatty acid composition of separate triglyceride fractions determined by gas-liquid chromatography at 750 V and 1500 V, as well as the composition of these fractions calculated from equations (1)–(4), are shown in Table IV. These data demonstrate that the values found agree with the calculated ones only at 750 V.

#### ACKNOWLEDGEMENTS

The author wishes to thank Dr. A. G. VERESHCHAGIN for his advice and encouragement during this investigation and A. V. KAVERINA for her excellent assistance.

## SUMMARY

The fatty acid composition of total linseed oil triglycerides and of different triglyceride fractions separated in a reversed-phase system have been determined by gas-liquid chromatography with a LOVELOCK argon-ionisation detector. At all detector voltages in the range of 750-1500 V the detector response was directly proportional to a given mass of substance for methyl esters of all fatty acids, with the exception of linolenic acid. The mass-response proportionality for methyl linolenate was observed only at 750 V.

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*J. Chromatog.*, 18 (1965) 20-24