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Comparative study of the determination of triacylglycerol in vegetable oils using chromatographic techniques

Amalia A. Carelli and Arturo Cert

Instituto de la Grasa y sus Derivados, CSIC, Avda. Padre Garcia Tejero 4. Apdo. 1078, 41012 Seville (Spain)

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ABSTRACT

The triacylglycerols of some vegetable oil samples were determined using isocratic HPLC with refractive index (RI) detection, gradient solvent HPLC with evaporative light scattering detection (ELSD), capillary GC and theoretical calculations from FAME analysis in order to establish the suitability of these techniques. The response factors and the repeatability were investigated. Generally, the HPLC-RI detection technique can be used without application of response factors. HPLC-ELSD yields inaccurate results for low concentrations. Calculations assuming a 1,3-random 2-random distribution of fatty acids gave good results for olive oil and acceptable results for sunflower oil. The GC analysis requires the use of response factors.

INTRODUCTION

Vegetable oils possess a characteristic and more or less unique pattern of triacylglycerols (TAGs) that can be used to determine origin and to detect adulteration. Thus in olive oil, a criterion of purity is based on the trilinolein content [1].

The triacylglycerol composition of oils is usually obtained by means of the IUPAC method, which uses isocratic non-aqueous reversed-phase high-performance liquid chromatography (HPLC) with refractive index (RI) detection [2], rendering separations based on the equivalent carbon number (ECN) of triacylglycerols.

While the poor solubility and long retention times of the higher saturated TAGs makes gradient elution desirable, this is not possible with RI detection. Therefore, a number of other detection methods have been tried. Of these, evaporative light scattering or "mass" detection (ELSD), is not affected by changes in mobile phase composition or small variations in room temperature, provides a better signal-to-noise ratio and is easy to use [3,4]. However, the detector response depends on the physical properties and concentration of each eluting material, giving sigmoidal response curves, only a small portion of which is linear [4–6].

On the other hand, capillary gas chromatography (GC) offers high efficiency and high speed for the analysis of complex mixtures of acylglycerols with a broad range of relative molecular masses. On phenylmethylsilicone stationary phases the triacylglycerols are separated by carbon number (CN), and each carbon number peak is split up giving a fine structure governed by the number of unsaturations in order of increasing retention time [7]. Several applications to the analysis of fats and oils using a laboratory-made movable cold on-column injector and flame ionization detection (FID) have been described [7,8], but information is scarce about other injection systems and relative response factors [9].

Otherwise, the triacylglycerol composition can be calculated utilizing fatty acid methyl ester (FAME) determinations and computer programs, by applying the 1,3-random 2-random distribution theory either to the results of total and 2-glycerol fatty acid

Correspondence to: A. Cert, Instituto de la Grasa y sus Derivados, CSIC, Avda. Padre Garcia Tejero 4, Apdo. 1078, 41012 Seville, Spain.

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analysis [10] or only to the total fatty acid composition [11].

The purpose of this work was to establish the suitability of methods other than the IUPAC standard method to determine the triacylglycerol composition in vegetable oils, comparing the results obtained using the following techniques: isocratic HPLC with RI detection, gradient solvent HPLC with ELSD, capillary GC on a phenylmethylsilicone phase using a standard split injector and theoretical computer calculations from FAME analysis. For this, relative response factors were calculated from triacylglycerol standards and analyses of some vegetable oils were accomplished.

The following abbreviations for fatty acids are used: A = arachidic acid, eicosanoic acid, C20:0; B = behenic acid, docosanoic acid, C22:0; G = gadoleic acid, *cis*-11-eicosenoic acid, C20: 1; L = linoleic acid, *cis*,*cis*-9,12-octadecadienoic acid, Cl 8:2; Ln = linolenic acid, *cis*,*cis*,*cis*-9,12,15-octadecatrienoic acid, C18:3; M = myristic acid, tetradecanoic acid, C14:0; N = nonadecanoic acid, C19:0; 0 = oleic acid, *cis*-9-octadecenoic acid, C18:1; P = palmitic acid, hexadecanoic acid, C16:0; Po = palmitoleic acid, *cis*-9-hexadecenoic acid, C16:1; S = stearic acid, octadecanoic acid, C18:0.

EXPERIMENTAL

Materials

All reagents were of analytical-reagent grade, except acetone and acetonitrile, which were of HPLC grade from Merck.

The triacylglycerols trilinolein (LLL), trimyristin (MMM),trinonadecanoin (NNN), triolein (OOO), 1,2-dioleoyl-3-palmitoyl-*rac*-glycerol (POO), 1,3-dipalmitoyl-2-oleoylglycerol (POP), tripalmitin (PPP), tripalmitolein (PoPoPo) and tristearin (SSS), of purity greater than 98% (GC), were obtained from Fluka (Buchs, Switzerland). Standard solutions for HPLC analysis were prepared mixing 20–300 mg of each triacylglycerol in 20 ml of chloroform. For GC analysis the solutions were diluted tenfold with hexane.

For the assays, virgin olive oil and refined sunflower oil were chosen because both contain the same fatty acids but in different proportions. On the other hand, the genetic variety of sunflower oil with a high oleic acid content was used, as it shows a fatty acid composition similar to that of olive oil.

Solutions of oils of 5% in acetone and 0.5% in hexane were used for HPLC and GC analysis, respectively.

HPLC analysis

The HPLC separations were done on a LiChrospher 100 RP-18 (5 μm) column (25 cm × 4 mm I.D.) using an HP 1050 gradient pumping unit (Hewlett-Packard, Avondale, PA, USA). Using RI detection, an HP 1047A detector and a mobile phase of acetone-acetonitrile (1: 1) at a flow-rate of 1.15 ml/min were used. Using ELSD, a Model 750/14 detector (ACS, Macclesfield, UK) was used with the following chromatographic conditions: flow-rate, 1 ml/min; elution using a two-step linear binary gradient from acetone-acetonitrile (30:70) to acetone-acetonitrile (65:35) at 20 min and then increasing to 100% acetone at 40 min; evaporator temperature, 45°C; air pressure, 2 bar; and photomultiplier sensitivity, 3. Between 5 and 10 μl of a solution of oil in acetone (500 mg in 10 ml) were injected.

For the analysis with RI detection, response factors relative to 000 were used. For LLL, POO and POP the factors were experimentally determined using standards, and for the remaining mixed TAGs the factors (F_{xyz}) were calculated from the values for homogeneous TAGs through the equation

$$\frac{1}{F_{xyz}} \quad \frac{1}{3F_{xxx}} + \frac{1}{3F_{yyy}} + \frac{1}{3F_{zzz}} \tag{1}$$

This expression is deduced from the following two equations:

$$F_{xyz} = \frac{n_{ooo} - n_s}{n_{xyz} - n_s} \tag{2}$$

gives the factor as a function of refractive indices of the TAG (n_{xyz}), 000 (n_{ooo}) and the chromatographic solvent (n_s);

$$n_{xyz} = \frac{1}{3} n_{xxx} + \frac{1}{3} n_{yyy} + \frac{1}{3} n_{zzz} \tag{3}$$

assumes [12] that the refractive index of a mixed TAG can be calculated from those of the homogeneous TAGs.

GC analysis

Chromatographic analysis of triacylglycerols was performed using a Chrompack (Middelburg, Neth-

erlands) CP9000 gas chromatograph fitted with a flame ionization detector and a split injection system (splitting ratio 1:30). Separations were carried out on a high-temperature aluminium-clad fused-silica capillary column (25 m × 0.25 mm I.D.) coated with methyl-65% phenylsilicone of thickness 0.1 μm (Quadrex, New Haven CT, USA). The operating conditions were oven temperature 350°C for 1 min, then increased at 0.5°C/min to 360°C and remaining at 360°C for 8 min, injector temperature 360°C, detector temperature 365°C and carrier gas helium at 130 kPa.

FAMEs from triacylglycerols

Standard solutions of triacylglycerols and oil samples were transmethylated by alkaline methanolysis followed by esterification of the fatty acids in acidic medium according to the IUPAC method [13]. GC analysis was carried out on a Supelcowax-10 fused-silica capillary column (30 m × 0.32 mm I.D.) of film thickness 0.25 μm, maintained at a temperature of 220°C for 3 min and then increased at 3°C/min to 255°C (held for 5 min), using helium as the carrier gas.

Fatty acids in the 2-position in the triacylglycerols of oils

The triacylglycerols of oils were partially hydrolysed by pancreatic lipase and then separated by silica gel thin-layer chromatography [14]. The monoacylglycerol band was scraped off and treated as indicated for FAME analysis.

Theoretical calculation of TAGs from FAME analysis

As a prior step for the TAG calculation from the fatty acid composition, the efficiency of the transmethylation method was tested by processing a standard of homogeneous TAGs. The calculations for the determination of the TAG composition from the total and 2-glycerol fatty acids were carried out with the mean values from five FAME analyses.

For comparison of the results with those from HPLC experiments, the TAGs were arranged by their ECN, calculated from the equation

$$ECN = CN - 2.52\ b_O - 2.43\ b_{Po} - 2.27\ b_L - 2.09\ b_{Ln} \quad (4)$$

where CN is the carbon number and b_O, b_{Po}, b_L and b_{Ln} are the number of double bonds attributable to oleic, palmitoleic, linoleic and linolenic acid, respectively. The coefficients were calculated by means of the reference triacylglycerols, taking into account that the logarithm of the relative retention time shows a linear relationship with ECN.

When comparison with GC data was required, the TAGs were put in order according to their CN and unsaturation number.

RESULTS AND DISCUSSION

A standard solution of TAGs analysed by HPLC with RI detection, using isocratic conditions, gave the relative response factors indicated in Table I. The factor for SSS could not be calculated as this compound gave a broad chromatographic peak at very long retention time. The experimental factors for homogeneous and mixed TAG were in agreement with those reported in the literature [12] and calculated from eqn. 1, respectively.

The TAG compositions found for olive, sunflower and high oleic sunflower oil samples applying the HPLC-RI method are given in Tables II, III and IV, respectively, the identities of the chromatographic peaks being established assuming the 1,3-random 2-random fatty acid distribution and discarding the TAGs with a level lower than 0.1%. It

TABLE I

RESPONSE FACTORS RELATIVE TO TRIOLEYLGLYCEROL USING HPLC WITH RI DETECTION

Results are means of five determinations with confidence interval at a significance level a = 5%.

TAG	Concentration (mg/ml)	Response factor	
		Experimental	Literature [12]
MMM	5.21	1.08 ± 0.06	1.106
PPP	5.33	1.05 ± 0.09	1.080
PoPoPo	5.82	1.02 ± 0.06	–
SSS	5.10	–	1.050
OOO	5.51	1	1
LLL	5.51	0.89 ± 0.05	0.924
POO	2.06	0.99 ± 0.05	1.025[a]
POP	4.21	1.02 ± 0.06	1.052[a]

[a] Calculated by eqn. 1 from literature data [12].

TABLE II

TAG COMPOSITIONS (%) OF AN OLIVE OIL DETERMINED BY HPLC-RI AND HPLC-ELSD AND BY CALCULATION FROM TOTAL FAME ANALYSIS

The values are given as means of five determinations with confidence interval at a significance level α = 5%.

TAG	RI detection		From total FAME analysis	ELSD (uncorrected)
	Uncorrected	Corrected		
OLnL	0.24 ± 0.06	0.22 ± 0.05	0.18 ± 0.01	ND[a]
OLL + POLO	0.95 ± 0.24	0.89 ± 0.22	0.91 ± 0.03	0.39 ± 0.05
LnOO + PLL	1.76 ± 0.05	1.63 ± 0.05	1.40 ± 0.03	0.89 ± 0.06
POLn	0.64 ± 0.06	0.60 ± 0.06	0.42 ± 0.01	0.22 ± 0.09
OLO + PO00	10.01 ± 0.16	9.78 ± 0.16	11.18 ± 0.15	10.92 ± 0.72
PLO + PPoO	4.30 ± 0.25	4.33 ± 0.26	3.65 ± 0.05	4.20 ± 0.20
PLP	ND	ND	0.22 ± 0.01	ND
0 0 0	44.93 ± 0.50	44.82 ± 0.49	44.29 ± 0.17	49.19 ± 0.57
PO0 + SOL	22.76 ± 0.21	23.16 ± 0.22	22.99 ± 0.18	21.79 ± 0.56
POP + PLS	2.63 ± 0.09	2.70 ± 0.09	2.84 ± 0.05	2.06 ± 0.29
GOO	0.53 ± 0.22	0.53 ± 0.22	0.41 ± 0.05	0.11 ± 0.02
SOO + AOL	8.18 ± 0.16	8.24 ± 0.16	8.22 ± 0.07	8.65 ± 0.55
POS	1.75 ± 0.09	1.76 ± 0.09	2.00 ± 0.02	1.14 ± 0.12
AOO	0.70 ± 0.08	0.71 ± 0.08	0.73 ± 0.03	0.27 ± 0.03
SOS + AOP	0.62 ± 0.07	0.62 ± 0.07	0.55 ± 0.01	0.21 ± 0.03

[a] ND = Not detected.

can be seen that the precision (R.S.D.) is good (less than 5%) for TAG concentrations higher than 8%, average (less than 15%) forTAG concentrations between 2 and 8% and bad (up to 43%) for concentrations lower than 2%. The introduction of the response factors calculated as indicated under Experimental results in slightly different compositions, most of which lie within the confidence intervals. LLL is the compound most affected by the correction owing to its low response factor (0.89). There-

TABLE III

TAG COMPOSITION (%) OF A SUNFLOWER OIL DETERMINED BY HPLC–RI AND HPLC-ELSD AND BY CALCULATION FROM TOTAL FAME ANALYSIS

The values are given as means of five determinations with confidence interval at a significance level a = 5%.

TAG	RI detection		From total FAME analysis	ELSD (uncorrected)
	Uncorrected	Corrected		
LLL + POLL	20.06 ± 0.53	19.01 ± 0.52	18.12 ± 0.16	18.91 ± 1.62
OLL + POLO	28.57 ± 0.66	27.99 ± 0.68	29.91 ± 0.07	28.96 ± 3.38
PLL	9.05 ± 0.16	9.06 ± 0.18	7.83 ± 0.05	9.53 ± 0.33
OLO + GLL	15.30 ± 0.42	15.64 ± 0.45	16.55 ± 0.07	15.44 ± 1.05
PLO + SLL	12.68 ± 0.15	12.96 ± 0.17	12.76 ± 0.06	14.00 ± 0.54
PLP	0.83 ± 0.23	0.87 ± 0.24	0.86 ± 0.01	0.45 ± 0.02
000 + GOL	4.79 ± 0.62	5.10 ± 0.65	3.19 ± 0.04	5.34 ± 0.22
SOL + POO + ALL	5.91 ± 0.91	6.42 ± 0.98	7.19 ± 0.05	5.58 ± 0.23
PLS + POP	1.03 ± 0.46	1.10 ± 0.49	1.37 ± 0.02	0.66 ± 0.11
BLL	0.59 ± 0.31	0.58 ± 0.30	0.69 ± 0.02	0.33 ± 0.14
SOO + AOL	1.18 ± 0.54	1.26 ± 0.58	1.55 ± 0.01	0.80 ± 0.12

TABLE IV

TAG COMPOSITION (%) OF A HIGH OLEIC SUNFLOWER OIL DETERMINED BY HPLC-RI AND HPLC-ELSD AND BY CALCULATION FROM TOTAL FAME ANALYSIS

The values arc given as means of five determinations with confidence interval at a significance level $a = 5\%$.

TAG	RI detection		From total FAME analysis	ELSD (uncorrected)
	Uncorrected	Corrected		
LLL	1.63 ± 0.06	1.45 ± 0.05	10.1	0.92 ± 0.17
OLL	2.44 ± 0.25	2.25 ± 0.24	1.53 ± 0.02	1.80 ± 0.17
PLL	1.10 ± 0.28	1.04 ± 0.26	<0.1	0.49 ± 0.06
OLO + PO00	6.99 ± 0.59	6.74 ± 0.57	16.04 ± 0.12	7.93 ± 0.26
PLO	1.61 ± 0.69	1.58 ± 0.68	1.86 ± 0.02	1.32 ± 0.12
0 0 0	61.93 ± 1.56	62.21 ± 1.56	54.28 ± 0.08	65.07 ± 2.00
PO0 + SOL	9.63 ± 0.45	9.87 ± 0.45	11.38 ± 0.14	9.72 ± 0.58
POP	0.23 ± 0.13	0.23 ± 0.13	0.42 ± 0.01	0.10 ± 0.03
GOO	0.57 ± 0.15	0.57 ± 0.15	0.61 ± 0.01	0.14 ± 0.04
SOO + AOL	10.91 ± 0.50	11.07 ± 0.51	9.34 ± 0.19	10.34 ± 0.50
POS	0.45 ± 0.17	0.46 ± 0.18	0.81 ± 0.02	0.15 ± 0.04
AOO + BLO	0.70 ± 0.22	0.70 ± 0.22	1.16 ± 0.04	0.37 ± 0.08
SOS	ND[a]	ND	0.39 ± 0.02	ND
BOO + BOP	1.82 ± 0.41	1.82 ± 0.41	2.17 ± 0.13	1.65 ± 0.16

[a] ND = Not detected.

fore, the application of correction factors is unnecessary if they are very close to unity. In this work, the HPLC-RI method is taken as the reference method, as the oils analysed do not contain TAGs with long retention times and the response factors are known.

HPLC analysis using elution gradient and ELSD gives a sharp SSS peak, in contrast to the HPLC-RI method where this peak is very broad. For the calculation of the response factors a standard solution with a high content of reference TAG (000) was used, as the detector response might be expected to be non-linear at a low concentration range [4–6]. The results in Table V indicate that there is a rapid increase in the response factors for amounts of TAGs less than 10 μg, in agreement with the decrease in detector response cited in the literature [4,5]. Consequently, the results of the oil analysis (Tables II-IV) show very low values for the low-concentration (less than 2%) TAGs, in comparison with those obtained by HPLC-RI. The values for the remaining TAGs differ by up to 15% from the corresponding results obtained by the HPLC-RI method. On the other hand, the precision of the measurements is average (R.S.D. 510%) for medium and low concentrations and bad (R.S.D. up to 40%) for concentrations less than 1%. Although the time of analysis is short and the precision is acceptable, the use of HPLC-ELSD does not seem advisable for TAG analysis because of the wide range of TAG concentrations in the samples.

The TAG compositions of the oils calculated from the total FAME analysis assuming a 1,3-random 2-random distribution (Tables II-IV) show a very good precision (R.S.D. 2 and 10% for concentrations higher and lower than 2%, respectively), in accordance with the very good precision of the

TABLE V

RESPONSE FACTORS RELATIVE TO TRIOLEYLGLYCEROL USING HPLC-ELSD

TAG	Concentration (mg/ml)	Injection volume (μl)			
		2.5	5	10	15[a]
LLL	3.285	2.68	1.5	1.38	1.39 ± 0.03
0 0 0	13.75	1	1	1	1
PPP	3.105	1.69	1.14	1.13	1.16 ± 0.02
s s s	2.175	2.08	1.30	1.24	1.17 ± 0.06

[a] Means of five determinations with confidence interval at a significance level a = 5%.

FAME analysis method. In addition, these results are very close to those obtained by calculation from the total and 2-glycerol fatty acid compositions (difference less than 5%), except for POO in olive and high oleic sunflower oils (difference 10%), and are nearer to the HPLC-RI data than those obtained by calculation from the total and 2-glycerol fatty acid compositions. Therefore, the 2-glycerol fatty acid data seem unnecessary for calculation of the theoretical TAG composition.

Comparing the computer and HPLC-RI data, similar values are obtained for olive and sunflower oils, except OLO, PLO and POS in the former (difference 7-14%) and OLL, PLL and 000 in the latter (difference 7-38%). This corroborates the 1,3-random 2-random distribution for the olive oil and indicates an acceptable approximation for the sunflower oil. In contrast, the values for the high oleic sunflower oil are very discordant, indicating that the theoretical distribution is not applicable to this oil. This is in accord with the drastic changes in TAG composition observed throughout the development of this mutant seed [15]. Results from olive and sunflower oils confirm the TAG assignment for the chromatographic peaks of these oils, although the attribution of some minor TAGs might be uncertain.

Analysis of a standard solution by GC with FID, using split injection, gave very diverse relative response factors (Table VI), in accordance with the results reported using a movable cold on-column injector and a phenylmethylsilicone stationary phase [9]. Except for LLL, the factors exhibited a linear relationship with the retention times of the TAGs (see Fig. 1), with a correlation coefficient of 0.987. This decrease in response with elution time has been attributed to quenching by the bleeding level of the methylphenylsilicone stationary phase [9], but in our experiments a contribution of the mass discrimination effect due to the split injection mode could be possible. The low response of LLL is in agreement with the losses of highly unsaturated TAGs reported in the literature [7] and suggests an alteration of the compound during analysis. The compositions of the vegetable oils, obtained applying the experimental factor for the LLL and those calculated by means of the regression curve for the remaining TAGs, are given in Tables VII, VIII and IX, where the peak identities (as in HPLC) were established assuming the 1,3-random 2-random fatty acid distribution. With respect to precision, the R.S.D. is less than 3% for peaks greater than 2%, rises to 15% for small peaks of short retention times and reaches up to 30% for small peaks with long retention times.

The differences between the GC data and the compositions calculated from FAME analysis are, in olive oil (Table VII) and sunflower oil (Table VIII), greater than with the HPLC-RI technique,

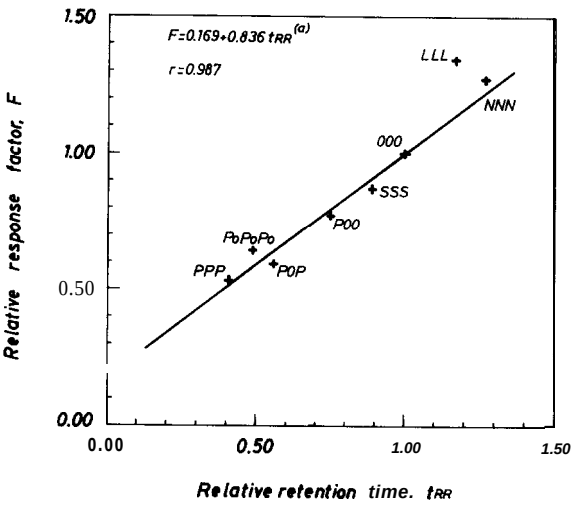

Fig. 1. Response factors versus retention times, both relative to 000, for GC analysis using FID and split injection. [a] The data for LLL were not taken into account in the calculation.

TABLE VI

RESPONSE FACTORS (*F*) AND RETENTION TIMES (t_{RR}), BOTH RELATIVE TO 000, USING GC-FID AND SPLIT INJECTION

The values are means of five determinations with confidence interval at a significance level α = 5%.

TAG	Concentration (μg/ml)	t_{RR}	*F*
PPP	533	0.41	0.53 ± 0.02
PoPoPo	582	0.49	0.64 ± 0.02
POP	421	0.56	0.59 ± 0.02
POO	206	0.75	0.77 ± 0.04
SSS	510	0.89	0.87 ± 0.02
000	557	1	1
LLL	551	1.17	1.34 ± 0.07
NNN	495	1.27	1.27 ± 0.09

TABLE VII

COMPARISON OF TAG COMPOSITIONS (%) OF AN OLIVE OIL DETERMINED BY GC-FID ANALYSIS WITH THOSE OBTAINED BY HPLC-RI AND TOTAL FAME ANALYSIS

The values are means of five determinations with confidence interval at a significance level a = 5%.

TAG	GC-FID		From total FAME analysis	HPLC-RI
	F	*%*		
POP	0.64	2.37 ± 0.04	2.69 ± 0.06	2.60
PPoO + PLP	0.66	0.65 ± 0.05	0.82 ± 0.02	0.94
POS	0.76	1.59 ± 0.03	2.00 ± 0.02	1.76 ± 0.04
PO0 + PLS	0.80	23.11 ± 0.04	21.97 ± 0.20	21.94
PLO + PO00	0.83	4.36 ± 0.13	4.96 ± 0.05	5.28
POLn + PoOL + PLL	0.86	0.47 ± 0.05	0.80 ± 0.01	1.02
SOS + AOP	0.92	0.60 ± 0.02	0.55 ± 0.01	0.62 ± 0.07
SOO	0.95	8.42 ± 0.07	8.11 ± 0.08	8.14
000 + SOL	1.00	49.35 ± 0.35	45.35 ± 0.17	45.93
OLO + LnOS	1.04	6.85 ± 0.29	9.41 ± 0.16	8.21
OOLn + OLL	1.09	1.22 ± 0.32	1.93 ± 0.04	2.11
OLnL	1.13	0.21 ± 0.08	0.18 ± 0.01	0.22 ± 0.05
AOO	1.16	0.56 ± 0.11	0.73 ± 0.03	0.71 ± 0.08
GOO + AOL	1.20	0.26 ± 0.06	0.52 ± 0.03	0.53 ± 0.22

indicating a greater deviation of the GC results, but for the high oleic sunflower oil (Table IX) considerable deviations, such as those found between HPLC-RI results and theoretical calculations, are observed.

In the comparison between GC and HPLC-RI measurements, only a few chromatographic peaks have the same identity. In order to extend the number of comparable data, theoretical compositions deduced from FAME analysis were applied to the

TABLE VIII

COMPARISON OF TAG COMPOSITION (%) OF A SUNFLOWER OIL DETERMINED BY GC-FID ANALYSIS WITH THOSE OBTAINED BY HPLC-RI AND TOTAL FAME ANALYSIS

The values are means of five determinations with confidence intervals at a significance level a = 5%.

TAG	GC-FID		From total FAME analysis	HPLC-RI
	F	*%*		
POP	0.64	0.28 ± 0.02	0.48 ± 0.09	0.39
PLP	0.66	0.86 ± 0.04	0.86 ± 0.01	0.87 ± 0.24
POS	0.76	0.29 ± 0.03	0.50 ± 0.01	–
PO0 + PLS	0.80	3.80 ± 0.14	3.31 ± 0.04	2.77
PLO	0.83	7.83 ± 0.07	8.71 ± 0.07	8.50
PLL + POOL	0.86	8.28 ± 0.13	8.10 ± 0.04	9.25
POLL		ND[a]	0.19 ± 0.01	0.20
SOS	0.92	ND	0.13 ± 0.00	–
SOO + SLS	0.95	1.61 ± 0.12	1.50 ± 0.02	0.98
000 + SOL	1	9.58 ± 0.50	7.61 ± 0.05	9.19
OLO + SLL	1.04	21.03 ± 0.13	20.65 ± 0.07	19.8
OLL	1.09	27.10 ± 0.23	29.91 ± 0.08	27.97
LLL	1.34	19.32 ± 0.33	18.06 ± 0.16	18.81

[a] ND = Not detected.

TABLE IX

COMPARISON OF TAG COMPOSITION (%) OF HIGH OLEIC SUNFLOWER OIL DETERMINED BY GC-FID ANALYSIS WITH THOSE OBTAINED BY HPLC-RI AND TOTAL FAME ANALYSIS

The values are means of five determinations with confidence interval at a significance level a = 5%.

TAG	GC-FID		From total FAME analysis	HPLC-RI
	F	%		
POP	0.64	0.31 ± 0.02	0.42 ± 0.01	0.23 ± 0.13
PLP	0.66	0.16 ± 0.01	< 0.1	N D
POS	0.76	0.51 ± 0.04	0.82 ± 0.02	0.46 ± 0.18
PO0	0.80	8.58 ± 0.09	9.66 ± 0.14	–
PLO + PO00	0.83	1.36 ± 0.11	2.14 ± 0.03	
PLL + POOL	0.86	0.70 ± 0.06	< 0.1	
SOS	0.92	0.35 ± 0.06	0.39 ± 0.01	ND
SOO	0.95	10.99 ± 0.39	9.26 ± 0.20	–
000 + SOL	1	64.68 ± 0.53	56.48 ± 0.07	
OLO	1.04	5.79 ± 0.20	15.90 ± 0.12	
OLL	1.09	1.90 ± 0.08	1.55 ± 0.02	2.25 ± 0.24
LLL	1.34	1.43 ± 0.07	< 0.1	1.45 ± 0.05
AOO	1.16	0.82 ± 0.11	0.78 ± 0.02	
GOO	1.20	0.54 ± 0.07	0.61 ± 0.01	0.57 ± 0.15
BOO	1.41	1.88 ± 0.24	2.00 ± 0.12	
SOO + AOL		10.99 ± 0.39		11.07 ± 0.51
000 + SOL + PO0		13.26		72.09
OLO + PO00 + PLO		7.15		8.32
AOO + BLO		0.82 ± 0.11		0.70 ± 0.22
BOO + BOP		1.88 ± 0.24		1.82 ± 0.41

[a] ND = Not detected.

HPLC-RI peaks of olive and sunflower oils, giving approximate values whose errors were not evaluated. In olive oil (Table VII), great variations (255 50%) occur for the minor peaks PPoO + PLP, POLn + PoOL + PLL, OOLn + OLL, AOO and COO + AOL, and considerable variations (10–20%) for the medium peaks POP, PLO + PoOO and OLO + LnOS. In sunflower oil (Table VIII), better results are obtained; variations from 25 to 50% are found for the small peaks POP, POO + PLS and SOO + SLS and less than 10% for the remainder. Consequently, HPLC-RI seems a more appropriate technique than GC for TAG determination, in spite of its lower precision. For high oleic sunflower oil (Table IX), comparisons were made between the sums of several peaks, as the percentages derived from theoretical calculations are not applicable. The results show a variability similar to those for the other oils. The similarity of results for olive and sunflower oils reinforces the applicability of the theoretical calculations for the identification of the main components of the chromatographic peaks in oils complying with a 1,3-random 2-random distribution. For high oleic sunflower oil, the proposed peak identities are a tentative approach that renders acceptable results.

As an example, Figs. 2, 3 and 4 show the chromatograms of high oleic sunflower oil obtained by HPLC-RI, HPLC-ELSD and GC-FID, respectively. These illustrate the different elution orders of triacylglycerol species according to the method used and the long time of analysis required when the isocratic HPLC method with RI detection is employed.

In summary, the TAG composition of vegetable oils can be determined by the isocratic HPLC-RI technique. Correction factors have to be applied if the differences between response factors of major peaks are greater than 10%. Calculation of the composition from FAME analysis is suitable only if

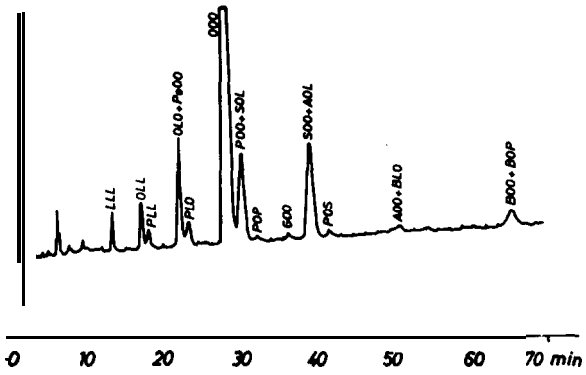

Fig. 2. TAG analysis of high oleic sunflower oil using the isocratic HPLC-RI technique.

it is known for certain that the fatty acids in the sample follow the 1,3-random a-random distribution. In that event, the theoretical composition is useful to establish the main components of the chromatographic peaks resulting from analysis by other techniques. The determination of the fatty acid composition at the 2-position of the glycerol

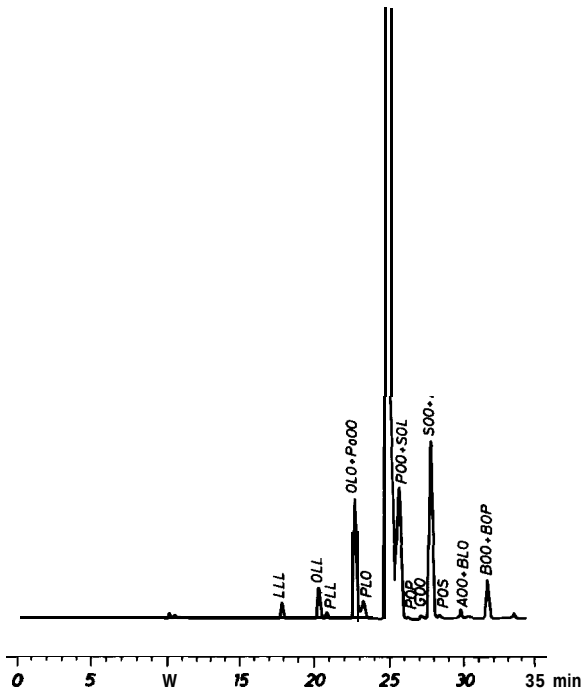

Fig. 3. TAG analysis of high oleic sunflower oil using the gradient solvent HPLC-ELSD technique.

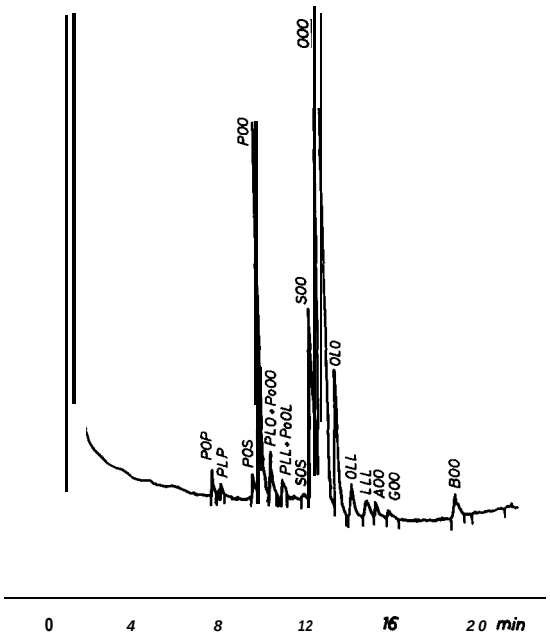

Fig. 4. TAG analysis of high oleic sunflower oil using capillary GC-FID on a phenylmethylsilicone stationary phase.

seems unnecessary for calculation of theoretical TAG composition.

HPLC using a solvent gradient and ELSD is an appropriate technique for the separation of oils containing TAGs with a wide range of ECN, but the quantification of small peaks is very inaccurate.

Finally, capillary GC on a phenylmethylsilicone stationary phase shows great sensitivity and yields a distribution of the chromatographic peaks that, together with that obtained by the HPLC-RI technique, permits, in some instances, determination of the individual TAGs. Nevertheless, the application of response factors is necessary and special care has to be taken in the analysis of oils containing polyunsaturated TAGs.

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