Journal of Chromatography, 366 (1986) 261–282
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 793

QUALITATIVE AND QUANTITATIVE ANALYSIS OF PEANUT OIL TRIACYLGLYCEROLS BY REVERSED-PHASE LIQUID CHROMATOGRAPHY

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(Received April 1st, 1986)

SUMMARY

Triacylglycerols (TGs) from an african peanut oil were analysed and fractionated by reversed-phase liquid chromatography (RPLC) using a differential refractometer as a detector. The fatty acids of the 33 collected fractions were analysed by gas chromatography after pentadecanoic acid (15:0) was added as an internal standard to quantitate the TG fractions. The three major fatty acids in the oil were octadecenoic acid (oleic acid, 44.5%), octadecadienoic acid (linoleic acid, 32.3%) and hexadecanoic acid (palmitic acid, 13.9%) together amounting to *ca.* 90% of the total fatty acids. Very long-chain saturated fatty acids (20:0–26:0) were also found. Thirty TGs could be easily identified from the fatty acid composition of the fractions alone. Dioleoyllinoleoylglycerol (18:1, 18:1, 18:2) was the main TG, amounting to nearly 17% of the oil, followed by palmitoyloleoyllinoleoylglycerol (16:0, 18:1, 18:2) (13%), oleoyldilinoleoylglycerol (18:2, 18:2, 18:1) (12%) trioleoylglycerol (18:1, 18:1, 18:1) (10%) and palmitoyldioleoylglycerol (16:0, 18:1, 18:1) (8%). These five TGs, which were virtually the only TGs in their respective fractions, together represented *ca.* 60% of the peanut oil TGs. Straight parallel lines were found for different series of TGs on plotting the logarithm of the relative retention time of the identified TGs (and those further identified) *versus* the number of double bonds. Other straight parallel lines were also observed on plotting the carbon numbers *versus* the equivalent carbon numbers of the oil TGs. These linear relationships were used to predict the different TGs present in the complex fractions. Their proportions in most instances were easily determined from the fatty acid composition of the fractions. In a very few instances a mathematical method had to be applied to solve the problem. Using the above-mentioned methods, 84 TGs could be identified and their percentages determined in the studied peanut oil. The very long-chain saturated fatty acids were always found associated with unsaturated fatty acids, preferentially with two molecules of linoleic acid. On the other hand, correction factors, determined from commercial simple TGs, were applied to peak areas before calculating the percentages of the 33 eluted TG fractions. For the major fractions of the oil, the series of values thus obtained was comparable to the series determined by the internal standardization procedure. Data reported here for peanut oil TGs are likely to be useful in identifying and quantifying the component TGs of other oils analysed by RPLC under the reported conditions.

0021-9673/86/$03.50

INTRODUCTION

Oils are complex mixtures of triacylglycerols (TGs) and the study of their TG composition was undertaken in the past using different methods of separation[1]. Reversed-phase liquid chromatography (RPLC) is now virtually the only method used in the analysis of TG mixtures. With columns of low efficiency, the number of peaks on the chromatogram is also low. Each peak corresponds to several TGs, the identification of which can be achieved by analysing the component fatty acids of each TG fraction that has been previously collected[2,3]. This method yields accurate results but is time consuming and not suitable for routine analysis. The present utilization of more efficient columns has increased the quality of separations[4–6] and for the less complex oils one peak on the chromatogram generally corresponds to one individual TG.

With these very efficient columns the first problem is the identification of the individual TGs. The notion of partition number[1] is no longer suitable, as two TGs with the same partition number (critical pairs) are now well separated[7]. Podlaha and Töregård[8], using commercial TGs, have demonstrated a linear relationship between the equivalent carbon number and the carbon number of TGs which showed the same unsaturation characteristics. The straight lines corresponding to the different unsaturation characteristics were shown to be parallel. Tracing of these parallel lines from a few known TGs theoretically permits the identification of unknown TGs on the chromatogram. On the other hand, Goiffon and co-workers[9,10] have established several linear relationships between the number of double bonds of TGs and the logarithm of the retention time with respect to that of trioctadecenoin (triolein), espressed as log α. In particular this relationship was observed with the series of TGs obtained by substituting a given fatty acid successively for the three component fatty acids of a simple TG. The authors concluded that log α of a given TG was the sum of log α of the three constituent fatty acids, the latter values being equal to 1/3 log α of the corresponding simple TG. The retention time of any TG or the identification of a TG whose retention time is known can thus be either determined graphically or calculated from log α of each fatty acid.

Such linear relationships were later verified by Takahashi *et al.*[11] by means of TGs identified in several oils from the fatty acids of the collected peaks. Stolyhwo *et al.*[12] extended these linear relationships to higher molecular weight TGs identified in natural oils, probably from Goiffon and co-workers' diagram. Several workers have used these properties in the qualitative analysis of several oils by RPLC[13–15].

In previous work[3] we analysed peanut oil by RPLC on a column of relatively low efficiency but the fatty acid analysis of the collected peaks did not lead to a complete identification of the constituent TGs. In this work we studied the RPLC analysis of peanut oil with a more efficient column. Starting from the fatty acid composition of the 33 collected peaks, 30 TGs could immediately be identified. The linear relationships demonstrated by Podlaha and Töregård[8] and Goiffon *et al.*[10] were experimentally verified from this wide range of natural TGs and then used to identify the other TGs present in the oil.

The second problem in the RPLC analysis of oil TGs is the quantitation of the individual TGs present in the mixture. The most accurate method is to collect the TG fractions corresponding to the TG peaks on the chromatogram at the outlet

of the detector. Addition of a known amount of a fatty acid absent from the oil (generally an odd-carbon-numbered fatty acid) permits the amount of TGs to be calculated after gas chromatographic (GC) analysis of fatty acids[3]. However, the method is time consuming and with highly efficient columns that practically separate individual TGs it is desirable to be able to determine the TG composition of the oil directly from the chromatogram. With the most commonly used detectors, *i.e.*, differential refractometer and UV absorption detectors, the peak areas depend on the nature of the TG[16,17]. The new light-scattering detector would not suffer from this drawback[12,18–20]. In this work we used a differential refractometer, which is still widely used in the RPLC analysis of oil TGs. On the one hand the exact percentages of the individual TGs were determined after collection of the TG fractions[3] and on the other hand the response factors of the detector with respect to a variety of saturated and unsaturated simple TGs were measured and the response factors corresponding to the TGs identified on the chromatogram were calculated and used to calculate the TG composition of peanut oil from the peak areas. The two series of data were compared in order to test whether the direct determination of the oil TG composition from the chromatogram would be possible.

EXPERIMENTAL

Samples

The peanut oil used was from Upper Volta. The TG fraction was isolated by silicic acid column chromatography[21] and its purity was checked by thin-layer chromatography[22].

Several standard mixtures of TGs were used. A standard mixture of simple saturated TGs (trihexanoin, trioctanoin, tridecanoin, tridodecanoin and tritetradecanoin) from Nu-Chek-Prep (Elysian, MN, U.S.A.) was used to determine the parameters of the TG peaks on the chromatogram. It was chromatographed first alone, then with added trihexadecenoin and trioctadecenoin (Nu-Chek-Prep). For the quantitation of the TG fractions of peanut oil, three standard mixtures of TGs were used successively: (i) a mixture of simple saturated TGs from trioctanoin to trihexadecanoin in equal proportions (Sigma, St. Louis, MO, U.S.A.) with an equal proportion of trioctadecenoin added; (ii) a mixture of simple trimonounsaturated TGs (Sigma) in various proportions from tritetradecenoin to trieicosenoin; and (iii) a mixture of simple TGs of various degrees of unsaturation (G2 HPLC Standard from Nu-Chek-Prep), *viz.*, trihexadecanoin, trihexadecenoin, trioctadecanoin, trioctadecenoin and trioctadecatrienoin. The proportions of the different TGs in these mixtures were controlled by GC analysis of the constituent fatty acids.

Liquid chromatography

A Model 6000A solvent delivery system and an R401 differential refractometer (Waters Assoc., Milford, MA, U.S.A.) were used. The 250 × 4.0 mm I.D. Hibar LiChrospher 100 CH-18 (3 μm particles) column was purchased from Merck (Darmstadt, F.R.G.). The analyses were carried out isocratically at a constant temperature of 30°C, using acetone–acetonitrile (70:30, v/v) as the mobile phase at a flow-rate of 1.2 ml min^{-1}. The solvents of analytical-reagent grade (Merck) were vacuum degassed for 5 min before use. Different solubilizing solvents and different concentra-

tions were chosen according to the TG mixtures being analysed. The samples, containing simple trisaturated TGs, were dissolved in chloroform–acetone (50:50, v/v) at a concentration of 60 mg ml^{-1} and 300 μg (5 μl) were injected in each run, that is, 50 μg of each individual TG. The simple trimonounsaturated TGs were dissolved in pure acetone at a concentration of 38 mg ml^{-1} and 10 μl were injected, that is, 49–130 μg depending on the individual TG in the mixture. The peanut oil TGs were also dissolved in acetone (100 mg ml^{-1}) and 2 mg (20 μl) were fractionated at each time as described previously[3]. Under the conditions used the detector response was found to be linearly related to the amount of individual TG detected. Peak areas were measured by means of an ICAP 10 integrator–calculator (LTT, Paris, France).

Gas chromatography

The fatty acid composition of the collected TG fractions was determined by gas chromatography (GC) of the methyl esters prepared from methanol–boron trifluoride[23]. The analyses were performed on a Becker–Packard Model 417 gas chromatograph, equipped with a 30 m × 0.4 mm I.D. glass capillary column coated with Carbowax 20M (Applied Science Labs., State College, PA, U.S.A.) at a constant temperature of 195°C and a nitrogen flow-rate of 3 ml min^{-1}. The column was equipped with a ROS injector[24] (SPIRAL, Dijon, France) and the apparatus with a flame ionization detector. The peak areas were measured with an ICAP 10 integrator. Calibration factors for quantitative determinations were calculated using standard mixtures of fatty acids (Nu-Chek-Prep).

The presence of hexacosanoic acid (26:0) in certain TG fractions of peanut oil was demonstrated by GC on a 10 m × 0.4 mm I.D. glass capillary column coated with silicone SE-30 (Applied Science Labs.) at a temperature programmed from 165 to 280°C at 6°C min^{-1} and a hydrogen flow-rate of 3 ml min^{-1}.

Calculations

The quantitation of the different TG fractions of peanut oil was performed by internal standardization. To each fraction of TGs collected was added a known amount of pentadecanoic acid (15:0) (Nu-Chek-Prep) before preparation of the methyl esters. The amount of the TG fatty acids was calculated by comparison with the amount of internal standard (15:0) and the amount of the TG fraction was calculated from that of the component fatty acids.

The partition number (PN) of the TG peaks on the chromatogram was calculated from the total acyl carbon number (C) of the TG and the total number of double bonds (N) according to the equation[1]

$$PN = C - 2N$$

C and N were calculated from the number of carbon atoms and the number of double bonds, respectively, taking into account the percentage of the constituent fatty acids in the TG fraction.

For each TG fraction of peanut oil a reduced retention time ($t_r - t_0$) relative to that of triolein (= selectivity factor) was determined graphically and expressed as "experimental" log α (ref. 25).

In the complex TG fractions containing more than one TG, the individual TGs

were identified by comparing the experimental log α of the TG fractions with log α "calculated" for all the possible TGs in peanut oil. The calculation of log α of a given TG involved the previous determination of log α of several simple TGs and log α of the corresponding fatty acids[9,10]. The log α values of the simple saturated TGs shown in Table I were obtained by extrapolation of the linear relationship of log α *versus* the carbon number of the TGs of carbon numbers 18 (trioctanoin) to 42 (tritetradecanoin). The log α values of the following unsaturated simple TGs were directly determined by RPLC. The log α values of fatty acids also presented in Table I were taken to be equal to one third of the log α values of the corresponding simple TGs[10].

TABLE I

LOGARITHM OF THE REDUCED RETENTION TIME OF SIMPLE TRIACYLGLYCEROLS RELATIVE TO THAT OF TRIOCTADECENOIN (TRIOLEIN) AND THOSE OF THE CORRESPONDING FATTY ACIDS RELATIVE TO OCTADECENOIC (OLEIC) ACID

Triacylglycerols		*Fatty acids*	
Structure	*Log α**	*Structure*	*Log α***
16:0, 16:0, 16:0	+0.144	16:0	+0.048
18:0, 18:0, 18:0	+0.483	18:0	+0.161
20:0, 20:0, 20:0	+0.822	20:0	+0.274
22:0, 22:0, 22:0	+1.161	22:0	+0.387
24:0, 24:0, 24:0	+1.509	24:0	+0.503
26:0, 26:0, 26:0	+1.855	26:0	+0.618
16:1, 16:1, 16:1	−0.318	16:1	−0.106
18:1, 18:1, 18:1	0.000	18:1	0.000
20:1, 20:1, 20:1	+0.345	20:1	+0.115
22:1, 22:1, 22:1	+0.675	22:1	+0.225
18:2, 18:2, 18:2	−0.354	18:2	−0.118

* Log α of the saturated simple triacylglycerols (TGs) were determined graphically after extrapolation of the linear relationship between log α and carbon number of the four simple TGs from trioctanoin (C_{24}) to tritetradecanoin (C_{42}).

** Log α of fatty acids were taken as one third of the corresponding TGs log α.

The log α value of one TG (ABC) composed of the three fatty acids (A, B and C) was calculated using the equation[10]

$$\log \alpha\,(ABC) = \log \alpha\,(A) + \log \alpha\,(B) + \log \alpha\,(C)$$

PN and log α were calculated for all the 286 possible individual TGs in peanut oil (eleven different fatty acids present in the oil) and used to identify the TGs present in the complex TG fractions of the oil, by comparison with the experimentally determined values.

The equivalent carbon number (*ECN*)[8] was determined graphically from the linear relationship[10]: log α = f(*C*) of the simple saturated TGs of carbon number 18–42, extrapolated for the TGs of higher carbon number.

It must be noted that the equation

$$\log \alpha = 0.057\ C - 2.60$$

derived from the plot of $\log \alpha = f(C)$ under our experimental conditions leads to the equation

$$ECN = 17.54 \log \alpha + 45.60$$

which permits the direct calculation of *ECN* from the "experimental" $\log \alpha$ of the TG fraction.

The response factors of the detector to TGs were calculated by the internal normalization method, starting from the ratio of the weight percent of the TG in the standard mixture to the corresponding percentage peak area on the chromatogram. They were referred to the response factor to trioctadecenoin (triolein) taken as unity. In the first standard mixture of the trisaturated TGs, trioctanoin was not taken into account because its peak on the chromatogram was incompletely separated from the solvent peak; nor was trioctadecanoin (tristearin) in the third mixture because it partly precipitated at the injection point owing to its poor solubility in the solvent system used and was therefore not quantitatively detected.

The response factors of the simple saturated TGs from trioctadecanoin to trihexacosanoin were determined graphically from the linear relationship established for the four simple TGs of the first standard mixture, tridecanoin to trihexadecanoin. The response factors to the saturated and unsaturated fatty acids were calculated as one third of those of the corresponding simple TGs. The response factors applied to the mixed TGs of peanut oil were calculated by adding those of the constituent fatty acids[10].

For the long-chain saturated fatty acids, the response factors of which were determined graphically, an alternative method was used to obtain experimental data, starting from the peanut oil TGs containing these fatty acids associated with unsaturated fatty acids. By comparing the experimental weight percent of these TGs in the oil and the corresponding peak areas on the chromatogram, an "experimental" response factor could be calculated for each of them. Taking into account the response factors to unsaturated fatty acids determined experimentally from the standard mixtures, a response factor for the component saturated fatty acid could be calculated assuming as above[10] that the response factors of fatty acids were additive. Only those TGs exhibiting a not too low weight percentage in the oil were used in the calculations.

RESULTS

Chromatographic parameters of the TG fractions

Fig. 1 shows the chromatogram recorded in the analysis by RPLC of the peanut oil used in this work. The profile is comparable to that obtained by Dong and Dicesare[4] with the same type of column and with the same solvent mixture. In total 33 TG peaks can be identified, most of which are well individualized without any overlapping; those in high concentration are eluted within 20 min.

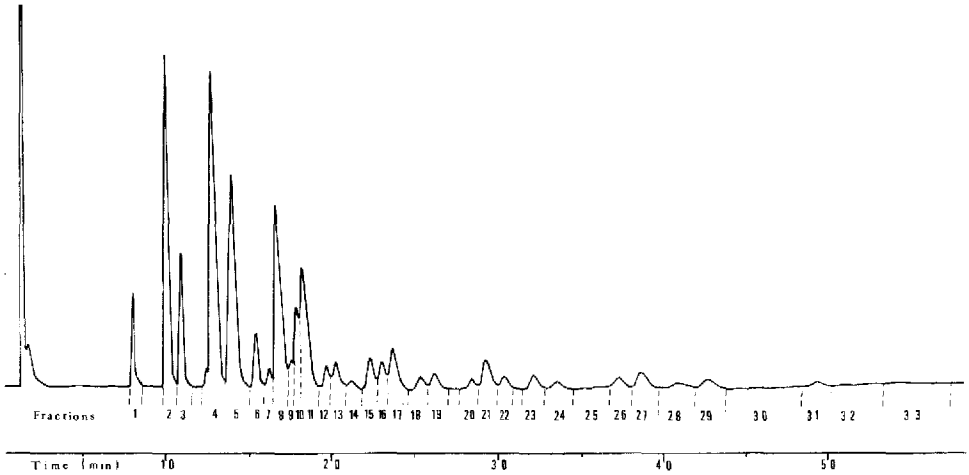

Fig. 1. Chromatogram recorded during the collection of the 33 fractions of peanut oil triacylglycerols separated by reversed-phase liquid chromatography on a 250 × 4 mm I.D. Hibar RP 18 (3 μm) column, eluted with acetone–acetonitrile (70:30, v/v) at 1.2 ml min^{-1}.

The 33 fractions were collected at the outlet of the detector. Three frationations of 2 mg of peanut oil TGs each time were necessary in order to collect enough material in the lowest fractions for further analysis. Table II shows the results obtained in the fatty acid analysis of these 33 fractions.

The relative proportions of the 33 TG fractions in peanut oil, derived from the amount of internal standard (15:0) added to each fraction, are reported in the first line (mol-% values). It can be seen that five fractions each represent more than 10% and together amount to nearly two thirds of the total TGs (62.4%). Fraction 4 represents 16.5%.

The next two sections in Table II report the fatty acid composition of the TG fractions from which the relative percentage of total saturated, monounsaturated and diunsaturated fatty acids can be calculated. These results permitted, in most instances, not only the class of the TG fractions (TGs showing the same unsaturation pattern) to be identified, but also the main individual triacylglycerol(s) in the fractions. This is the case for 28 fractions in which 30 individual TGs could be identified (fractions 28 and 33 contained two easily identifiable main TGs, the other fractions only one). Only in fractions 9, 18, 25, 30 and 32 was it not possible to characterize the main individual TGs accurately.

The next section in Table II shows the partition numbers of the 33 TG fractions calculated from the total carbon number and the total number of double bonds of the contituent fatty acids of each fraction. These numbers were generally very close to those expected, namely the even numbers from 42 to 56. A large difference was observed for fraction 7 (47.39 instead of 48 expected). This could be explained by the very small amount of this fraction, which moreover is poorly separated from the following fraction present in high proportion and thus highly contaminated with 18:1, decreasing the proportion of 20:1 present in this fraction. Such a large deviation is also observed for the very minor fractions 32 and 33.

The 33 TG fractions of peanut oil separated on this type of column are dis-

TABLE II

PERCENTAGE AND FATTY ACID COMPOSITION OF THE 33 FRACTIONS OF PEANUT OIL TRIACYLGLYCEROLS FRACTIONATED BY REVERSED-PHASE LIQUID CHROMATOGRAPHY

Parameter	*Fraction*								
	*1**	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Mol-%**	2.08	11.66	4.95	16.54	13.57	2.53	0.67	10.30	0.99
16:0	0.08	0.02	30.92	0.23	28.20	62.41	9.01	0.23	5.49
18:0	0.24	–	0.16	0.02	3.42	0.27	0.77	–	1.14
20:0	–	–	–	–	–	–	–	0.12	10.61
22:0	–	–	–	–	–	–	–	–	–
24:0	–	–	–	–	–	–	–	–	–
26:0	–	–	–	–	–	–	–	–	–
16:1	0.66	0.17	0.58	0.04	0.27	0.47	0.85	–	–
18:1	0.33	32.59	1.68	63.72	30.80	2.61	31.04	97.32	50.98
20:1	–	–	–	0.78	–	–	23.36	0.96	4.70
22:1	–	–	–	–	–	–	0.53	–	–
18:2	98.48	67.22	66.66	35.21	37.31	34.24	34.44	1.37	27.08
*CN***	53.96	53.99	52.11	54.03	52.29	50.23	54.87	54.05	54.59
*DB****	5.96	5.02	4.07	4.05	3.17	2.15	3.74	3.03	3.29
*PN****	42.04	43.95	43.97	45.93	45.95	45.93	47.39	47.99	48.01
Log α***	−0.353	−0.244	−0.201	−0.127	−0.085	−0.035	−0.011	0.000	+0.026
*ECN****	39.60	41.40	42.20	43.40	44.10	44.95	45.40	45.55	45.95

* A trace of 18:3 (0.21%) was also found in this fraction.
** Calculated from the amount of pentadecanoic acid (15:0) added as an internal standard.
*** For definitions, calculations and graphical determinations, see Experimental.

tributed in eight groups corresponding to the eight even numbers from 42 to 56. These eight groups correspond to the eight TG fractions of an African peanut oil of similar composition previously analysed by RPLC on a much less efficient column[3]. This shows the efficiency of the type of column used in this work in the separation of critical pairs of TGs, that is, those exhibiting the same partition number[7].

In Fig. 2, 48 individual TGs identified in the peanut oil studied are defined by their number of double bonds (abscissa) and their experimental log α (ordinate). Twenty seven were immediately identified from the fatty acid composition of the corresponding TG fractions (Table II); 21 were identified later (see below). The values lie on several series of straight, parallel lines. One series of parallel lines corresponds to triacylglycerols obtained by substituting each time one linoleic acid for palmitic acid starting from a saturated TG containing two or three molecules of palmitic acid. Another series of parallel lines is obtained by substituting oleic acid for palmitic acid. Other series of parallel lines can be obtained by substituting linoleic acid or oleic acid for the other saturated fatty acids. This diagram constitutes an experimental verifi-

10	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*
2.59	10.35	1.20	2.09	0.48	1.45	1.18	2.99	0.86
2.99	26.30	32.89	59.38	5.50	3.97	4.54	0.94	23.30
23.23	6.29	30.04	5.12	1.03	1.75	1.77	29.08	17.84
5.34	0.19	–	–	–	0.74	23.49	3.41	23.43
–	–	–	–	–	29.33	4.42	0.18	0.13
–	–	–	–	–	0.07	–	–	–
–	–	–	–	–	–	–	–	–
0.12	0.09	0.27	0.28	0.19	1.07	0.37	0.18	0.75
30.60	60.03	6.59	28.75	65.05	3.42	29.00	61.93	3.42
1.80	–	–	0.21	28.04	1.83	3.61	0.85	–
–	–	–	0.46	0.19	0.60	–	–	–
35.92	7.10	30.21	5.80	–	57.22	32.80	3.43	31.13
54.24	52.43	52.01	50.49	55.36	57.46	55.86	54.21	53.98
3.13	2.23	2.02	1.24	2.80	3.64	2.96	2.09	1.99
47.98	47.97	47.97	48.01	49.76	50.18	49.94	50.03	50.00
+0.034	+0.043	+0.081	+0.094	+0.115	+0.138	+0.151	+0.164	+0.194
46.15	46.25	46.90	47.15	47.50	47.90	48.10	48.35	48.90

(Continued on pp. 270 and 271)

cation and an extrapolation, by means of a wide variety of natural mixed and simple TGs from peanut oil, of the relationships established by Goiffon *et al.*[10].

These straight and parallel lines can be traced out from a reduced number of known TGs, particularly from commercially available simple TGs or from TGs easily identifiable in a natural mixture as in this work. They can then be utilized to identify the unknown TGs, as was done for the present peanut oil. The data obtained here can also serve as references in the analysis of other natural mixtures of TGs when using the same conditions, in particular the same eluting solvent.

In Fig. 3, 52 individual TGs identified in peanut oil are defined by their equivalent carbon number (*ECN*) determined graphically (abscissa) and their exact carbon number calculated from that of the constituent fatty acids (ordinate). Twenty eight TGs were immediately identified and 24 were identified later as in Fig. 2. All the TGs with the same unsaturation characteristics (class) lie on a straight line and the different lines corresponding to nine classes are parallel. These results obtained from a large number of natural TGs confirm and extend the linear relationships established

TABLE II (*continued*)

Parameter	*Fraction*								
	19	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*
Mol-%**	1.29	0.65	2.20	1.07	1.18	0.86	0.16	0.86	1.44
16:0	31.95	4.20	0.86	1.52	27.94	22.43	13.62	2.51	0.97
18:0	32.67	2.64	0.96	1.71	4.20	19.23	6.68	1.57	0.51
20:0	1.58	0.55	0.57	28.83	4.19	23.74	1.63	0.42	0.13
22:0	0.04	0.98	31.15	3.57	29.62	1.15	2.67	1.43	32.97
24:0	–	28.25	1.25	–	–	–	1.85	30.89	0.94
26:0	–	–	–	–	–	–	15.87	–	–
16:1	0.34	0.52	0.14	0.37	0.45	0.24	3.40	0.39	0.37
18:1	30.52	6.05	31.55	59.54	2.22	31.44	14.88	31.05	62.95
20:1	0.12	0.43	0.58	0.48	–	–	0.96	0.84	–
22:1	0.10	0.87	0.25	–	0.19	–	–	–	–
18:2	2.68	55.51	32.69	3.98	31.19	1.77	38.44	30.90	1.16
*CN***	52.18	59.08	58.00	56.07	56.13	54.20	57.60	59.63	58.05
*DB***	1.09	3.57	2.94	2.05	1.96	1.06	2.88	2.82	1.97
*PN***	50.00	51.94	52.12	51.97	52.21	52.08	51.83	53.99	54.11
Log α***	+0.209	+0.246	+0.260	+0.277	+0.302	+0.322	+0.352	+0.369	+0.385
*ECN****	49.10	49.75	49.95	50.25	50.70	51.00	51.60	51.85	52.10

by Podlaha and Töregård[8] from 18 commercial model TGs. As before, the straight and parallel lines can be traced out from a very limited number of model or natural TGs and then utilized to characterize the unsaturation and chain length of unknown TGs in a natural mixture, as was done in this work for peanut oil.

TG type composition of the TG fractions

Identification of the TG types. A TG type is defined as a TG in which the three fatty acids are known but not their positioning. Identification of the TG types present in each of the 33 fractions was carried out as follows. The 286 possible TG types of the analysed peanut oil[1] obtained by combinating three fatty acids out of the eleven present in the oil were listed. The partition number (*PN*) and the logarithm of the reduced retention time relative to triolein (log α) were determined for each of them. The TG types were then distributed into groups of the same partition number. Only the eight groups of *PN* 42–56, those displayed in the oil (Table II), were retained. In each group the possible TG types were classified according to increasing log α, which

28	*29*	*30*	*31*	*32*	*33*
0.67	0.97	0.25	0.89	0.21	0.52
22.07	28.16	12.50	1.63	16.73	21.48
9.90	5.06	5.95	1.23	16.64	12.42
0.37	4.03	1.44	—	1.14	0.58
10.86	29.14	4.21	0.85	1.23	11.13
21.89	0.20	2.61	33.22	16.59	20.47
—	—	17.50	—	10.86	—
0.47	0.30	0.35	—	1.10	0.56
4.01	31.51	31.29	61.42	8.73	31.64
—	—	1.63	0.48	—	—
—	—	—	—	—	—
30.43	1.60	22.52	1.17	26.98	1.72
57.91	56.07	58.59	60.01	58.74	57.73
1.96	1.05	2.35	1.93	1.91	1.07
53.99	53.97	53.89	56.15	54.91	55.59
+0.410	+0.428	+0.475	+0.493	+0.523	+0.536
52.55	52.85	53.65	54.00	54.55	54.75

represents the elution order[9,10]. Of the numerous possible TG types in each group, the only ones retained were those presenting (i) a calculated log α close to the experimental value and (ii) a degree of unsaturation (class) and a chain length (*CN*) characteristic of the fraction, as deduced from Fig. 3 (carbon numbers *versus* equivalent chain length). It is noteworthy that this diagram has permitted the existence of TGs containing hexacosanoic acid (26:0) to be postulated. The existence in trace amounts of this very long-chain fatty acid was then confirmed in the oil by GC using a silicone stationary phase.

Taking into account conditions (i) and (ii), it was observed that six TG fractions consisted of only one TG type, thirteen fractions of two TG types, thirteen fractions of three TG types and only one fraction showed four constituent TG types. If we examine the elution order of the different TG types according to the partition number, we can observe that in most instances, especially for the major TG in each fraction, the elution order followed the order of increasing partition number. However, some TGs did not follow this rule, and thus was always so with the trisaturated

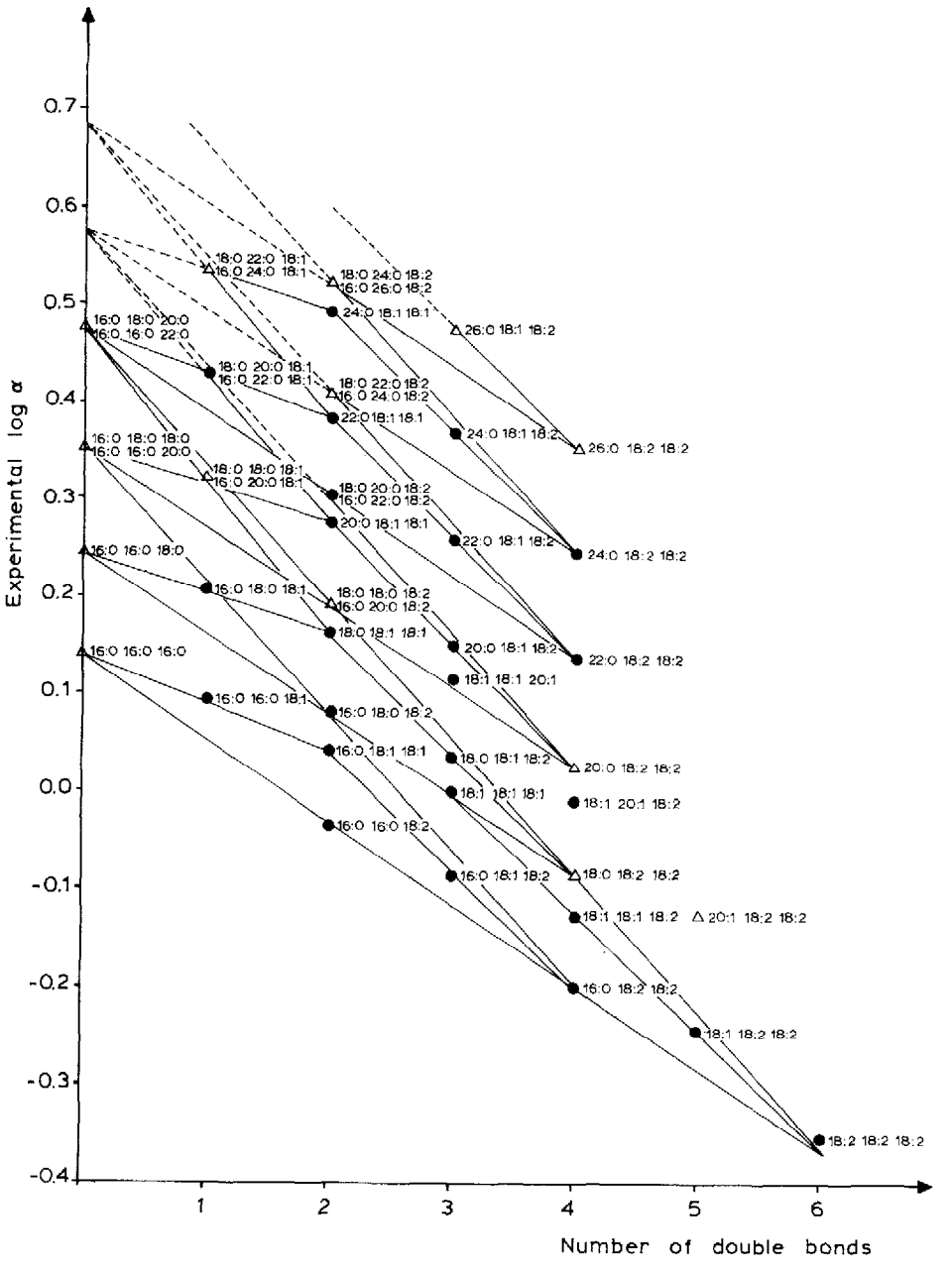

Fig. 2. Plot of log α *versus* number of double bonds for 48 peanut oil triacylglycerols. Log α is the logarithm of the reduced retention time of the TGs relative to that of trioctadecenoin (triolein) and was determined from the chromatogram in Fig. 1. The number of double bonds was calculated from that of the three component fatty acids of the TGs after identification. Twenty seven TGs (●) were immediately identified from the fatty acid composition of the fractions collected (Table II). The other 21 TGs (△) were identified later (Table III).

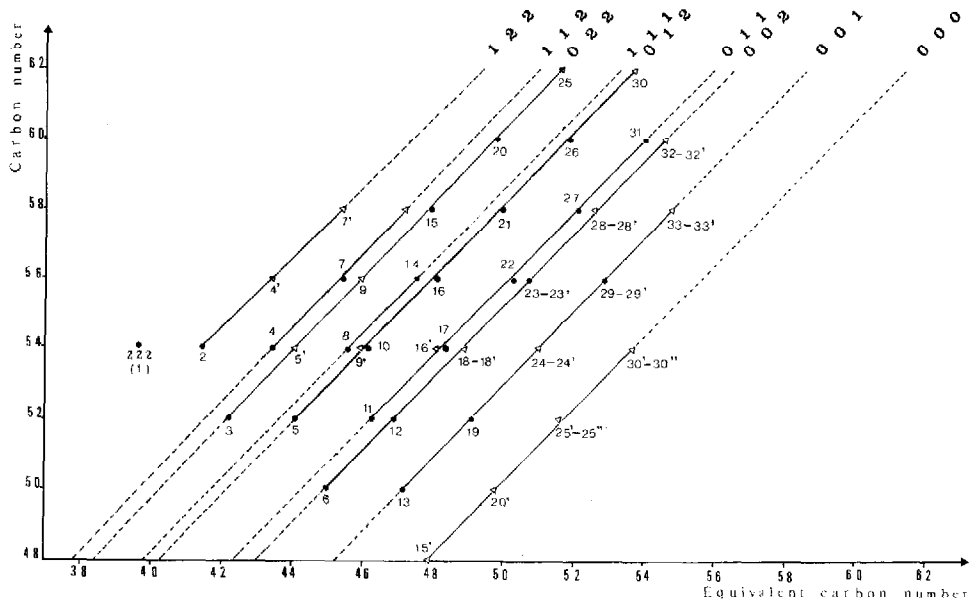

Fig. 3. Plot of carbon number (*CN*) *versus* equivalent carbon number (*ECN*) for 52 peanut oil triacylglycerols (TGs). *ECN*s were determined graphically from extrapolation of the linear relationship of *CN versus* log α (definition in Fig. 2) for the five saturated simple TGs from trihexanoin (C_{18}) to tritetradecanoin (C_{42}). *CN*s were calculated from those of the three component fatty acids of the TGs after identification. The TGs are numbered as the collected fractions in which they were present and according to decreasing percentages in the case of several TGs in the same fraction. Twenty eight TGs (●) were immediately identified from the fatty acid composition of the fractions collected (Table II). Twenty four TGs (△) were identified later (Table III). Each straight line corresponds to one class of unsaturation (0, 1 or 2 double bonds of the three component fatty acids).

TGs. For example, in fraction 15 tripalmitin (16:0, 16:0, 16:0) of *PN* 48 was eluted with TGs of *PN* 50 and in fraction 20 the saturated TG (16:0, 16:0, 18:0) of *PN* 50 was eluted with TGs of *PN* 52. As a general rule, the trisaturated TGs were eluted with unsaturated TGs of higher *PN*. These exceptions have also been found in other studies[10,13].

If we now examine the elution order according to unsaturation, we find that, in general, the more saturated the TGs, the longer is the retention time. Further, with equal numbers of carbon atoms and double bonds, the higher the number of saturated fatty acids in the TG molecule, the longer is the retention time. Thus TGs 112, 111 and 011 were eluted earlier than TGs 022, 012 and 002, respectively. This is also clearly apparent in Fig. 3. However, the carbon number may reverse this order. For example, in fraction 8 triolein of carbon number 54 (class 111) was eluted earlier than TGs of class 022 with carbon numbers of 56 (fraction 9), 58 (fraction 15), 60 (fraction 20) and 62 (fraction 25).

Calculation of the TG type composition. For the TG fractions of the oil containing only one TG type, no calculation was necessary. For the fractions composed of two or even three TG types, the percentages of the different types could be easily calculated in most instances from the fatty acid composition of the fraction (Table

II) on neglecting the contaminant fatty acids present in low percentages. As an example, if we consider fraction 23, the carbon number was found to be 56 and the unsaturation characteristic 001 (two molecules of saturated fatty acids and one of monounsaturated fatty acid). Taking into consideration the fatty acids present in this fraction, the only two possible TG types were 16:0, 22:0, 18:2 and 18:0, 20:0, 18:2. The percentage of the former could be calculated from the percentage of either 16:0 ($27.94 \times 3 = 83.82$) or 22:0 ($29.62 \times 3 = 88.86$) or better from both by taking the mean (86.34). In fact, the percentages of these two fatty acids were corrected for the contaminant fatty acids and the percentage of the TG types was thus calculated to be 88.77 (Table III). The same reasoning was applied to the second TG type (18:0, 20:0, 18:2) to give a percentage of 11.23. Moreover, assuming that the contaminant TG in this fraction is the major TG of the preceding fraction, namely 20:0, 18:1, 18:1, the percentage of this TG (3.33) can be estimated from the percentage of 18:1 in fraction 23 (2.22) and its amount, taking into account the percentage of fraction 23 in the total TGs, can be added to the preceding fraction 22. This mode of calculation was applied to most fractions of the oil. However, for more complex mixtures, a mathematical method was used[26–29]. An example is given in Table IV for the most complex fraction 5, present in high proportion in the oil (13.6%). This fraction contained the maximum possible TG types, namely five, shared in three classes of three different carbon numbers. An additional TG type (18:1, 18:1, 18:2) represented a contaminant originating from fraction 4, almost exclusively constituted of this TG (Table III). The resolution of a system of only five equations (established from the percentage of the five acids present in the fraction) for six unknowns (a, b, c, d, e and f, representing the percentages of the six TG types in the fraction) was rendered possible assuming that the minor palmitoleic acid (0.27%) was present in only one TG type, that in which the other two fatty acids were present in high proportions (palmitic and oleic acids).

As for a certain number of TGs of several fractions, the percentage of the major TG of fraction 5 (16:0, 18:1, 18:2) was taken as the mean of two values, one derived from the proportion of 16:0 (first equation) and the other from the proportion of 18:2 (fifth equation). The contaminant TG was eliminated from fraction 5 (and added to fraction 4) and the new fatty acid percentages were recalculated from the percentages of the TG types effectively present in the pure fraction 5.

The percentages of the 75 TG types present in peanut oil, calculated after separation of 33 fractions, are listed in Table III and expressed as percentages in their own fractions or in the total TGs of the oil. It can be seen that in almost all the fractions one TG type predominates when not unique. Of the 75 TGs present, 5 occurred in high proportions (8.48–16.55%), together representing nearly 60% of the total. The very long-chain saturated (20:0–26:0) and monounsaturated (20:1 and 22:1) were preferentially associated with two molecules of unsaturated fatty acids, especially of linoleic acid (18:2). In Table V the fatty acid composition of the total TGs of peanut oil reconstituted from the composition and the percentages of the 75 TG types (Table III) and that reconstituted from the fatty acid composition and the percentages of the 33 fractions isolated by RPLC (Table II) can be compared with the experimentally determined values. For the eleven fatty acids present in the oil, the corresponding three data were very close to each other, indicating the suitability of the fractionation and analysis methods used in this study.

TABLE III

TRIACYLGLYCEROL TYPE COMPOSITION OF PEANUT OIL

Fraction	*Mol-%**	*CN***	*Class****	*TG type*[§]	*Concentration in the fraction (%)*	*Concentration in total TGs (%)*	*Mol-%*[§§]
1	2.08	54	222	18:2, 18:2, 18:2	98.00	2.04	2.40
		52	122	16:1, 18:2, 18:2	2.00	0.04	
2	11.91	54	122	18:1, 18:2, 18:2	99.49	11.85	12.12
		52	112	16:1, 18:1, 18:2	0.51	0.06	
3	4.81	52	022	16:0, 18:2, 18:2	98.19	4.73	4.91
		50	012	16:0, 16:1, 18:2	1.81	0.09	
4	16.95	56	122	20:1, 18:2, 18:2	2.26	0.38	16.68
		54	112	18:1, 18:1, 18:2	97.62	16.55	
		52	111	16:1, 18:1, 18:1	0.12	0.02	
5	13.26	54	022	18:0, 18:2, 18:2	10.59	1.40	13.58
		52	012	16:0, 18:1, 18:2	88.59	11.75	
		50	011	16:0, 16:1, 18:1	0.82	0.11	
6	2.38	50	002	16:0, 16:0, 18:2	100.00	2.38	2.41
7	0.85	58	122	22:1, 18:2, 18:2	1.18	0.01	0.66
		48	001	16:0, 16:0, 16:1	2.01	0.02	
		56	112	18:1, 20:1, 18:2	96.81	0.82	
8	10.62	54	111	18:1, 18:1, 18:1	100.00	10.62	10.61
9	1.11	56	022	20:0, 18:2, 18:2	74.71	0.83	0.95
		54	012	16:0, 20:1, 18:2	25.29	0.28	
10	3.85	54	012	18:0, 18:1, 18:2	100.00	3.85	3.12
11	8.51	52	011	16:0, 18:1, 18:1	99.67	8.48	8.95
		52	011	18:0, 16:1, 18:1	0.33	0.03	
12	1.41	52	002	16:0, 18:0, 18:2	100.00	1.41	1.11

(*Continued on p. 276*)

TABLE III (*continued*)

Fraction	*Mol-%**	*CN***	*Class****	*TG type§*	*Concentration in the fraction (%)*	*Concentration in total TGs (%)*	*Mol-%§§*
13	1.80	50	001	16:0, 16:0, 18:1	97.45	1.76	1.68
		50	001	16:0, 18:0, 16:1	0.94	0.02	
		58	112	18:1, 22:1, 18:2	1.61	0.03	
14	0.45	56	111	18:1, 18:1, 20:1	99.34	0.45	0.29
		56	111	16:1, 18:1, 22:1	0.66	Trace	
15	1.62	48	000	16:0, 16:0, 16:0	2.96	0.05	1.42
		58	022	22:0, 18:2, 18:2	90.67	1.47	
		56	012	16:0, 22:1, 18:2	1.61	0.02	
		56	012	18:0, 20:1, 18:2	4.76	0.08	
16	1.63	56	012	20:0, 18:1, 18:2	84.02	1.37	1.44
		54	011	16:0, 18:1, 20:1	15.98	0.26	
17	2.71	54	011	18:0, 18:1, 18:1	99.41	2.70	2.74
		56	012	22:0, 16:1, 18:2	0.59	0.01	
18	0.91	54	002	16:0, 20:0, 18:2	73.46	0.67	0.80
		54	002	18:0, 18:0, 18:2	26.54	0.24	
19	1.20	52	001	16:0, 18:0, 18:1	98.58	1.18	1.27
		52	001	18:0, 18:0, 16:1	1.09	0.01	
		60	112	20:1, 22:1, 18:2	0.33	Trace	
20	0.71	58	111	18:1, 18:1, 22:1	2.39	0.02	0.68
		50	000	16:0, 16:0, 18:0	6.59	0.04	
		60	022	24:0, 18:2, 18:2	91.02	0.65	
21	2.22	58	012	22:0, 18:1, 18:2	100.00	2.22	2.23
22	1.03	56	011	20:0, 18:1, 18:1	97.38	1.00	1.22
		56	011	18:0, 18:1, 20:1	1.46	0.02	
		56	011	22:0, 16:1, 18:1	1.16	0.01	
23	1.14	56	002	16:0, 22:0, 18:2	88.77	1.01	1.75
		56	002	18:0, 20:0, 18:2	11.23	0.13	

24	0.86	54	001	16:0, 20:0, 18:1	71.21	0.61	1.04
		54	001	18:0, 18:0, 18:1	18.04	0.24	
		54	001	18:0, 20:0, 16:1	0.75	0.01	
25	0.15	52	000	16:0, 16:0, 20:0	7.48	0.01	0.27
		52	000	16:0, 18:0, 18:0	16.33	0.03	
		62	022	26:0, 18:2, 18:2	76.19	0.11	
26	0.90	60	012	24:0, 18:1, 18:2	100.00	0.90	0.78
27	1.44	58	011	22:0, 18:1, 18:1	98.89	1.42	1.41
		58	011	24:0, 16:1, 18:1	1.11	0.02	
28	0.69	58	002	18:0, 22:0, 18:2	33.92	0.23	0.61
		58	002	16:0, 24:0, 18:2	66.08	0.46	
29	0.93	56	001	16:0, 22:0, 18:1	87.25	0.81	0.96
		56	001	18:0, 20:0, 18:1	11.79	0.11	
		56	001	18:0, 22:0, 16:1	0.96	0.01	
30	0.25	54	000	16:0, 16:0, 22:0	18.19	0.04	0.27
		54	000	16:0, 18:0, 20:0	6.22	0.02	
		62	012	26:0, 18:1, 18:2	75.59	0.19	
31	0.89	60	011	22:0, 18:1, 20:1	1.48	0.01	0.72
		60	011	24:0, 18:1, 18:1	98.52	0.88	
32	0.21	60	002	20:0, 22:0, 18:2	4.14	0.01	0.36
		60	002	18:0, 24:0, 18:2	57.97	0.12	
		60	002	26:0, 16:0, 18:2	37.89	0.08	
33	0.52	58	001	18:0, 22:0, 18:1	35.10	0.18	0.56
		58	001	16:0, 24:0, 18:1	63.19	0.33	
		58	001	18:0, 24:0, 16:1	1.71	0.01	

* Calculated from the amount of pentadecanoic acid (15:0) added as an internal standard.

** Carbon number of the TG type calculated from the number of carbon atoms of the three component fatty acids.

*** Class of unsaturation of the TG type (0, 1 or 2 double bonds of the three component fatty acids).

§ TG for which the three component fatty acids are known but not their positioning.

§§ Calculated from peak areas on the chromatogram after correction factors (Table VI) were applied. The series of values has to be compared with the series in column 2.

TABLE IV

DETERMINATION OF THE TRIACYLGLYCEROL TYPE COMPOSITION OF FRACTION 5 OF PEANUT OIL

Fatty acids			*Triacylglycerol types*		
Structure	*Mol-%*		*CN****	*Class****	*Log α****
	*Exptl.**	*Calc.***			
16:0	28.20	29.80	54	022	−0.075
18:0	3.42	3.53	52	012	−0.070
			52	012	−0.063
			50	011	−0.058
16:1	0.27	0.27	50	011	−0.051
18:1	30.80	29.80			
18:2	37.31	36.59			

* Determined experimentally by gas chromatography.

** Calculated from the TG type composition reported in the last column.

*** Carbon number (*CN*), class of unsaturation (class) and retention time relative to trioleoylglycerol (α) of the TG types present in the fraction.

§ The last TG type represents a contaminant from the preceding fraction.

§§ Five equations corresponding to the five fatty acids present in the fraction and derived from the fatty acid composition of the TG types and from that of the fraction.

§§§ Mol-% of the component TG types in the fraction and in the total TGs of the oil.

TABLE V

FATTY ACID COMPOSITION OF TOTAL TRIACYLGLYCEROLS FROM PEANUT OIL

Fatty acid	*Mol-%*		
	*Experimental**	*Reconstituted*** *from fatty acids*	*Reconstituted**** *from TG types*
16:0	13.89	13.60	13.67
18:0	3.91	4.27	4.20
20:0	1.45	1.59	1.58
22:0	2.13	2.51	2.49
24:0	0.91	1.09	1.12
26:0	Trace	0.09	0.13
16:1	0.19	0.23	0.15
18:1	44.49	44.17	44.51
20:1	0.74	0.76	0.77
22:1	Trace	0.04	0.03
18:2	32.29	31.65	31.35

* Determined experimentally by gas chromatography.

** Calculated from the fatty acid composition and the percentage of the 33 collected fractions.

*** Calculated from the TG type composition and the percentage of the 33 collected fractions.

Structure[§]	*Mol-%*	*Equations*[§§]	*Solutions*[§§§]	
			Fraction	*TGs*
18:0, 18:2, 18:2	*a*	$b + d = 28.20 \times 3 = 84.60$	$a = 10.20$	1.38
16:0, 18:1, 18:2	*b*	$a + c + e = 3.42 \times 3 = 10.26$	$b = 85.12$	11.55
18:0, 16:1, 18:2	*c*	$c + d + 2e = 0.27 \times 3 = 0.81$	$c = 0$	0
16:0, 16:1, 18:1	*d*	$b + d + 2f = 30.80 \times 3 = 92.40$	$d = 0.80$	0.11
18:0, 16:1, 16:1	*e*	$2a + b + c + f = 37.31 \times 3 = 111.93$	$e = 0$	0
18:1, 18:1, 18:2	*f*		$f = 3.88$	0.53
				13.57

TG composition from peak areas. In Table VI are reported the response factors to saturated and unsaturated fatty acids derived from the response factors of the differential refractometer (the detector used in this work) to various simple TGs. It also reports a series of data calculated for long-chain saturated fatty acids from a certain number of TGs present in peanut oil.

It can be observed that the response factors relative to the checked saturated fatty acids were higher than that of octadecenoic acid (18:1) to which they were referred. Those corresponding to unsaturated fatty acids were found to be lower.

When compared to the response factors reported elsewhere[13] from literature data of the TG refractive indices, those determined in this work for TGs (three times the values reported in Table VI for the corresponding fatty acids) were observed to be 5–8% higher for saturated TGs and only 1–2% higher for unsaturated TGs. The experimental data reported in this work are therefore fairly comparable to what can be expected from the refractive indices. Could the relatively high figure for octadecenoic acid (18:1) be explained by the nature of the C_{18}-bonded phase used in the analysis?

The data for long-chain saturated fatty acids calculated from certain component TGs of peanut oil and reported in Table VI were found to be higher (except for 26:0) than those determined graphically. However, the different values obtained for each fatty acid were very scattered, especially for 20:0, and under these conditions the mean was not accurate enough to be taken into consideration. Only the experimentally determined response factors were therefore taken into account in calculating

TABLE VI

CORRECTION FACTORS OF FATTY ACIDS RELATIVE TO OLEIC ACID DETERMINED FROM SIMPLE AND PEANUT OIL TRIACYLGLYCEROLS

Values (means ± SEM) represent 1/3 of values found for the corresponding simple triacylglycerols relative to that of triolein taken as unity.

TGs	*Saturated fatty acids (n = 9)*		
	10:0	*12:0*	*14:0*
Simple TGs*	0.409 ± 0.016***	0.407 ± 0.013***	0.387 ± 0.013***
Peanut oil TGs**			

	Unsaturated fatty acids (n=8)
	14:1
Simple TGs*	0.287 ± 0.006

* Correction factors determined from commercial simple triacylglycerol mixtures.

** Calculated from peanut oil triacylglycerols containing one or two molecules of the corresponding saturated fatty acids, using correction factors of the unsaturated fatty acids reported below.

*** Determined from simple saturated triacylglycerol mixtures.

§ Determined graphically from the regression straight line of the preceding values.

the percentages of the 33 TG fractions separated by RPLC from peak areas. The values thus obtained are shown in Table III (last column) and can be compared with those calculated by internal standardization with 15:0 (second column). For the major fractions the difference was found to be low (from 5% for fraction 11 to 0.1% for fraction 8). However, for minor fractions the difference was sometimes relatively high, probably because of an inaccurate determination of the corresponding peak areas.

CONCLUSION

The TG analysis of peanut oil performed in this work was hardly more complete than that reported previously[30], but it was much less time consuming. Fractionation of TGs separated by means of a very efficient column and fatty acid analysis of the fractions thus obtained permitted a large number of individual TGs to be accurately identified and precisely quantitated after an internal standard was added. Comparison of the quantitative data thus obtained with those calculated after experimentally determined response factors of the detector were applied showed that the TG composition of the oil could be precisely calculated directly from peak areas. Under these conditions the analysis of any oil is still more rapid. However, for certain complex fractions of peanut oil, only the fatty acid analysis has permitted what TGs were or were not present to be ascertained.

Further, the detailed analysis of peanut oil carried out in this work has revealed the existence of minor TGs such as those composed of hexacosanoic acid (26:0). It

16:0	*18:0*	*20:0*	*22:0*	*24:0*	*26:0*
0.387 ± 0.018***	0.377§	0.367§	0.358§	0.348§	0.338§
0.417 ± 0.033	0.418 ± 0.051	0.402 ± 0.141	0.408 ± 0.054	0.493 ± 0.069	0.304
(n = 7)	(n = 5)	(n = 3)	(n = 3)	(n = 3)	(n = 1)

16:1	*18:1*	*20:1*	*18:2*	*18:3*
0.283 ± 0.005	0.333	0.302 ± 0.012	0.312 ± 0.011	0.283 ± 0.007

has also shown that a few TGs such as trioctadecadienoin (trilinolein) or trioctadecenoin (triolein) could be fractionated from peanut oil with a high degree of purity, which may lead to commercial applications.

ACKNOWLEDGEMENT

We thank J. Gresti for technical assistance.

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