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Determination of plant triacylglycerols using capillary gas chromatography, high-performance liquid chromatography and mass spectrometry

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ABSTRACT

Molecular species of triglycerides (TG) were determined in plant fats and oils, both qualitatively and quantitatively, by means of capillary gas chromatography (CGC) on a "polarizable" column, reversed-phase C_{18} high-performance liquid chromatography (RP-HPLC) and desorption chemical ionization mass spectrometry (DCI-MS). For the qualitative analysis values of the equivalent carbon number (ECN) and equivalent chain length (ECL) were used for the identification of individual molecular species of TG by means of RP-HPLC or CGC. Plant oils including cocoa butter with smaller numbers of double bonds can be determined without any problems by means of CGC, RP-HPLC and DCI-MS. The determination by CGC and partially also RP-HPLC failed only with oils with either a complex distribution of chain lengths, *i.e.*, rape seed oil (high erucic acid) or a high unsaturation index (linseed oil and blackcurrant oil). However, with complementary results from all three methods it was possible to identify numerous molecular species of triglycerides in various plant oils.

INTRODUCTION

In the last decade, triglyceride (TG) analysis has become almost routine. Its rapid development has mainly been induced by requirements of the food industry, *i.e.*, determination of TG in cocoa butter or dietetic values of polyene TG, *e.g.*, in blackcurrant oil.

The use of reversed-phase high-performance liquid chromatography (RP-HPLC) for the determination of TG has recently been reviewed [1]. The determination of molecular species of TG from plant and animal fats and oils has been performed many times [2–19], mainly by RP-HPLC [2–15] and occasionally by capillary gas chromatography (CGC) [16–19]. Detection of TG is complicated as the ester bond

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absorbs only at low wavelengths, which limits the selection of a suitable mobile phase. All possible detection methods have been used, *i.e.*, refractive index [2–4,6,7], infrared, ultraviolet absorption [4,9,15], light scattering [8,12,14] and mass spectrometry (MS) [10]. Mass spectrometry appears to be the most suitable; however, the method is not commonly available. Therefore, we used UV detection at 222 nm; UV detectors are common and also facilitate the simple collection of individual fractions.

The use of a "polarizable" capillary column that has recently become commercially available is another method for the determination of TG. Determinations of TG on this column have been described several times [16–19], but an exact quantification of TG is not yet available. It has been stated that during CGC [16,19] and GC–MS [21], thermal degradation of polyene TG takes place, so that CGC is not suitable for plant oils such as linseed oil. Unfortunately, also in rape seed oil conclusive results could not be obtained. Therefore, in connection with previous work [22], we tried to quantify the losses. We were also surprised by the fact that data concerning the equivalent chain length (ECL) of individual triglycerides are scarce [23]. Therefore, we tried to determine ECL values at least roughly.

Direct chemical ionization (DCI) MS is a further method that can be used for the identification and quantification of TG. The method has also been applied several times [24,25] primarily to plant oils.

All the above methods have their advantages and shortcomings. However, suitable combinations can be used, with due caution, for the qualitative and quantitative determination of molecular species in any plant fats or oils.

EXPERIMENTAL[a]

Materials

Triglycerides [purified by thin-layer chromatography with silica gel H plates, 20 × 20 cm (Merck, Darmstadt, Germany), using hexane–diethyl ether (90:10)] (MMM, PPP, SSS, AAA, BeBeBe, LgLgLg, PoPoPo, OOO, GaGaGa, ErErEr, LLL, LnLnLn, MMP, PPM, SSM, SSP, OOP, PPO. OOS, SSO, POS) were obtained from Sigma (St. Louis, MO, U.S.A.). Mixed TG (SSA and SAA) were synthesized by interesterification between SSS and AAA using sodium methoxide as catalyst. The reaction was performed at 120°C for 20 min under vacuum with continuous stirring according to Ohshima *et al.* [21]. Analytical-reagent grade solvents were purchased from Lachema (Brno, Czechoslovakia) and Merck.

Plant oils and fats [cocoa butter, olive oil, palm oil, rape oil (high erucic acid), peanut oil, soyabean oil, sunflower oil and linseed oil] were obtained from a local pharmacy (in accordance with the Czechoslovak Pharmacopoeia No. 4) or isolated by cold pressing from plant seeds obtained from special drugstores [grape seeds, cotton seeds, blackcurrant seeds and corn (maize) seeds].

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[a] Nomenclature: M = myristic acid, 14:0; P = palmitic acid, 16:0; Po = palmitoleic acid, 9–16:1; Mg = margaric acid, 17:0; S = stearic acid, 18:0; O = oleic acid, 9–18:1; L = linoleic acid, 9,12–18:2; Ln = linolenic acid, 9,12,15–18:3; A = arachidic acid, 20:0; Ga = gadoleic acid, 11–20:1; Be = behenic acid, 22:0; Er = erucic acid, 13–22:0; Lg = lignoceric acid, 24:0; SSS = triglyceride tristearin; PSO = triglyceride palmitostearoolein.

Capillary gas chromatography of intact triglycerides

Total TG were analysed on a fused-silica capillary column (25 m × 0.25 mm I.D.) with a 0.1-μm layer of triacylglycerol phase (TAP) (Chrompack, Middelburg, The Netherlands) with a flame ionization detector at 375°C. The carrier gas was hydrogen at 100 cm/s. The column temperature was programmed from 320 to 350°C at 1.5°C/min. An OCI-3 injector (SGE, Kensington, Australia) adapted to the "moveable on-column" injection technique [22] was used. A Model PU 4900 chromatographic system (Pye Unicam, Cambridge, U.K.) was used for the analysis.

High-performance liquid chromatography

HPLC was performed using a G-I gradient LC system (Shimadzu, Kyoto, Japan) with two LC-6A pumps (0.5 ml/min), an SCL-6A system controller, an SPD ultraviolet detector (222 nm), a SIL-1A sample injector and a C-R3A data processor. A 250 mm × 4 mm I.D. analytical column packed with SGX C_{18} (5-μm particles) was used (Tessek, Prague, Czechoslovakia). After injection of 1 μl of TG solution (5 mg/ml), a convex propionitrile (PCN)–methyl *tert.*-butyl ether (MTBE) gradient from 75:25 to 25:75 for 30 min was applied.

Mass spectrometry

A Finnigan MAT 90 mass spectrometer was used with an ion-source temperature of 200°C and a heating rate of 1°C/s. Ammonia (3×10^{-4} bar) was the most suitable reaction gas of three gases tested (methane, isobutane and ammonia). The equivalent carbon number (ECN) and ECL values were obtained using an IBM PC-compatible computer; ECL values were calculated by means of the Statgraphics program (Statistical Graphics, Rockville, MD, U.S.A.).

RESULTS AND DISCUSSION

Qualitative analysis

In principle, there are two possible methods for the identification of TG. The first consists in correlation between the chromatographic behaviour and structure of a compound, and the second in a direct identification of TG, either intact (*e.g.*, by means of GC–MS [21] or LC-MS [10]) or after transesterification by means of fatty acids [2,3,6,15]. TG in individual plant oils have so far been predominantly identified on the basis of data for TG content presented earlier [16] and on the basis of acid analysis obtained by transesterification of fractions resulting from RP-HPLC [2,3,6,9], and also according to values for quasi-molecular ions (QM^+) in DCI-MS [24,25].

Values of ECL, commonly used for the identification of fatty acids [26] and diglycerides [27], are useful for the identification of TG by CGC. However, for TG, ECL values were applied more than 10 years ago and only packed column was used [23]. Table I presents ECL values for more than 60 molecular species of TG, both synthetic (see Experimental) or contained in plant oils. The reproducibility was better than 0.03 unit. The ECL values of saturated TG were determined in agreement with fatty acids. For the determination of ECL values in other TG, the relationship between log (retention time) and number of carbon atoms was used. The values were calculated (and illustrated) using the Statgraphics program (see Experimental). As shown in Table I, TG differing by a single double bond can be separated, and in some instances

TABLE I

EQUIVALENT CHAIN LENGTH VALUES OF SOME TRIGLYCERIDES

TG	ECL[a]	TG	ECL[a]
MMM	42.00	OLL	55.26
MMP	44.00	OOLn	55.40
MPP	46.00	LLL	55.85
MMO	46.15	LLLn[b]	56.02[c]
PPP + MPS	48.00	LLnLn[b]	56.48[c]
MPO	48.15	Γ-LnLnLn	57.03[c]
MPL	48.45	α-LnLnLn	57.12[c]
PoPoPo	48.51	SSA	56.00
PPS	50.00	SOA + POBe	56.17
PPO	50.16	OOA + SOGa + POEr	56.24
MOO	50.21	OLA + SLGa + PLEr	56.61
PPoO	50.22	OLGa	56.85
PPL	50.47	LLA	57.11
PPoL	50.61	LLGa	57.27
MOL	50.64	SAA	58.00
MLL	51.10	PoLGa + SOBe + SSEr	58.17
PSS	52.00	PGaEr + SOEr + OOBe	58.24
PSO	52.17	PLLg + OOEr	58.51
POO	52.23	OLBe + SLEr	58.61
PSL	52.48	OLEr	58.86
PoOO	52.51	LLBe	59.11
POL	52.63	LLEr	59.28
PSLn	52.67	AAA	60.00
PoOL	52.87	SOLg	60.17
PLL	53.12	OOLg + PErEr + SGaEr	60.24
PoLL	53.25	OGaEr + GaGaGa	60.51
PLLn	53.50	LAEr + LGaBe + OLLg	60.61
SSS	54.00	LGaEr	60.86
SSO	54.17	LLLg	61.11
SOO	54.23	LnGaEr	61.40
SSL + OOO	54.50	SErEr	62.24
SOL	54.60	OErEr	62.51
OOL	54.85	LErEr	62.86
SLL	55.12	LnErEr	63.40

[a] Each value represents the mean ± S.D. (no greater than ±0.02) from five chromatograms.
[b] Triglycerides with α-Ln and Γ-Ln are not separated.
[c] This TG_{54} are eluted after SSA (TG_{56}).

even TG having identical numbers of carbon atoms and double bonds, *e.g.*, POO and PSL or OLL and OOLn. This is demonstrated in Fig. 1, in which the gas chromatogram of sunflower oil is shown.

Unfortunately, several critical pairs were identified, first SSL and OOO, as also has been reported elsewhere [16–18]. During the analysis of blackcurrant oil, TG with one or two positional isomers of 18:3 acid (*i.e.*, α-18:3 and Γ-18:3 acids) could not be separated. The third type of critical pairs was found in TG differing in the length of critical acyl groups bound to individual glycerol carbons, *e.g.*, $TG_{56:2}$ (type 011; AOO and/or SOGa and/or POEr). In these TG a similar phenomenon to that occurring in

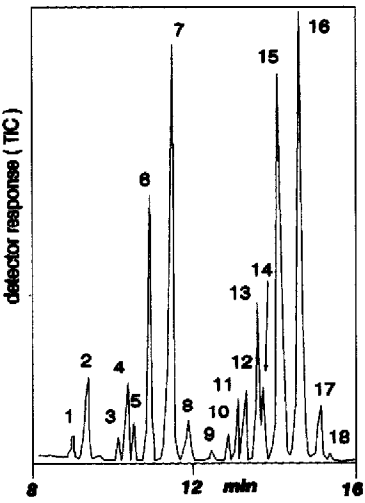


Fig. 1. Triacylglycerol profile (CGC) of soyabean oil. Peaks: 1 = POP, 2 = PLP, 3 = POS, 4 = POO, 5 = PLS, 6 = PLO, 7 = PLL, 8 = SOS, 10 = SOO, 11 = OOO + SLS, 12 = SLO, 13 = OOL, 14 = SLL, 15 = OLL, 16 = LLL, 17 = LLLn, 18 = probably LLnLn. TIC = total ion current.

wax esters [28] could be observed. The results given in Table I are summarized in Table II. The ECL values presented here thus make the identification of common plant oils possible, but unfortunately peak identification is only tentative.

For the analysis of TG by means of RP-HPLC, reproducible values of ECN, thanks mainly to studies by Podlaha and Töregård [29,30], make the identification of TG on the basis of their retention characteristics possible. In this work we utilized their program and equations [29,30] in order to calculate ECN values from our experimental data. The results are presented in Table III.

TABLE II

DIFFERENCES IN EQUIVALENT CHAIN LENGTH WITH CHANGES IN DOUBLE BOND(S) THROUGH THE CHANGE FROM LOWER TO HIGHER UNSATURATED FATTY ACIDS IN TRIGLYCERIDES

TG	Change in ECL[a]	TG	Change in ECL[a]
001	+0.16 ± 0.01 (6)	122	+1.27 ± 0.01 (2)
011	+0.22 ± 0.02 (6)	113	+1.40 ± 0.00 (3)
002	+0.47 ± 0.02 (4)	023	+1.50
111	+0.51 ± 0.00 (5)	222	+1.85
012	+0.62 ± 0.02 (5)	223	+2.02
003	+0.67	233	+2.48
112	+0.86 ± 0.01 (4)	333	+3.03[b]
022	+1.11 ± 0.01 (5)	333	+3.12[c]

[a] Value ± S.D. (number of determinations in parentheses).
[b] Γ-LnLnLn.
[c] α-LnLnLn.

TABLE III

EQUIVALENT CARBON NUMBER VALUES OF SOME TRIGLYCERIDES

TG	ECN[a]	TG	ECN[a]	TG	ECN[a]
α-LnLnLn	31.21	OOL	43.88	LLLg	49.21
β-LnLnLn	31.52	PoOO	44.38	PSO	49.27
LLnLn	33.59	POL	44.40	LGaEr	49.37
LLLn	35.96	MOO	44.84	OOEr	49.93
OLnLn	37.56	PPL	45.00	PPS	50.00
PLnLn	38.09	MPO	45.38	OLBe	50.09
LLL	38.52	LLA	45.59	POEr	50.51
LnLnGa	39.38	OLGa	45.70	SOGa	50.60
PoLL	39.52	MPP	46.00	OOA	50.71
OLLn	39.53	OOO	46.26	SLA + PLBe	50.84
SLnLn	39.91	SOL	46.32	LErEr	51.22
MLL	40.08	POO	46.80	SSO + POA	51.23
PLLn	40.29	PSL	46.95	GaGaGa	51.72
LnLnEr	41.23	SSLn	47.07	OGaEr	51.75
OLL	41.40	PPO	47.33	PSS	52.00
PLL	41.91	LLBe	47.40	OOBe	52.68
OOLn	41.91	LnGaEr	47.40	SLBe + PLLg	52.78
PoOL	42.06	OLEr	47.55	OErEr	53.60
SLLn	42.11	PPP	48.00	SSS	54.00
MOL	42.46	OOGa	48.08	OOLg	54.63
PPoL	42.48	PLEr	48.10	SLLg	54.72
POLn	42.49	OLA	48.21	OAA + POLg	55.13
MPL	43.04	SLGa	48.29	SSA	56.00
LLGa	43.32	POGa	48.66	SOLg	57.09
MMO	43.42	SOO	48.75	SAA	58.00
SLL	43.74	SSL + PLA	48.90	AAA	60.00

[a] Each value represents the mean ± S.D. (no greater than ±0.01) from six chromatograms.

Even here critical pairs are formed, but different pairs to those in CGC are involved, *e.g.*, LLBe and LnGaEr have identical ECN, whereas their ECL differ by 2.29. Unfortunately the overlap of critical pairs and position isomers is more serious in RP-HPLC than in GC. The results are summarized in Tables IV and V, and a characteristic RP-HPLC record for soyabean oil is also shown in Fig. 2, indicating an excellent separation of individual TG.

TABLE IV

DIFFERENCES IN EQUIVALENT CARBON NUMBER WITH CHANGES OF TWO METHYLENE UNITS IN DIFFERENT GROUPS OF TRIGLYCERIDES

TG group	Change in ECN[a]
000 (by definition)	2.00
001 or 011 or 002	1.95 ± 0.01 (24)
012	1.91 ± 0.03 (8)
111 or 112 or 122 or 022 or 133 or 023 or 033	1.84 ± 0.04 (22)

[a] Value + S.D. (number of determinations in parentheses).

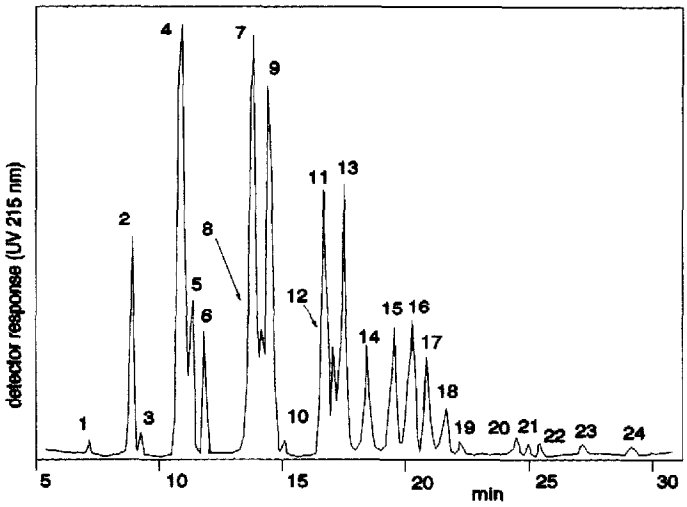


Fig. 2. RP-HPLC analysis of soyabean oil TGs using the gradient mode. Peaks: 1 = LLnLn 2 = LLLn, 3 = OLnLn, 4 = LLL, 5 = OLLn, 6 = PLLn, 7 = OLL, 8 = OOLn, 9 = PLL, 10 = POLn, 11 = OOL, 12 = SLL, 13 = POL, 14 = PPL, 15 = OOO, 16 = SOL, 17 = POO, 18 = PSL, 19 = PPO, 20 = SOO, 21 = SSL, 22 = PSO, 23 = PPS, 24 = SSO.

Podlaha and Töregård [29] gave a value of δ-ECN = 1.94 for an increase in carbon number of 2 in unsaturated TG, where as in this present study (see Table IV) an agreement with this value was only found in TG groups 001 or 011 or 002. For more unsaturated TG a lower δ-ECN value was found. However, Podlaha and Töregård [29] also obtained lower values, *e.g.*, for a change from PoPoPo to OOO a δ-ECN value of 1.89 for a single acid was reported and for a change from OOO to GaGaGa a δ-ECN value of 1.78 for a single acid. However, they did not include these results in their final table and did not even discuss them. In spite of this, we know that minor differences can be influenced by, *e.g.*, different columns, operating conditions, instruments and operators. The reproducibility of ECL values was better than 0.03 unit; for statistical values, see Tables III, IV and V.

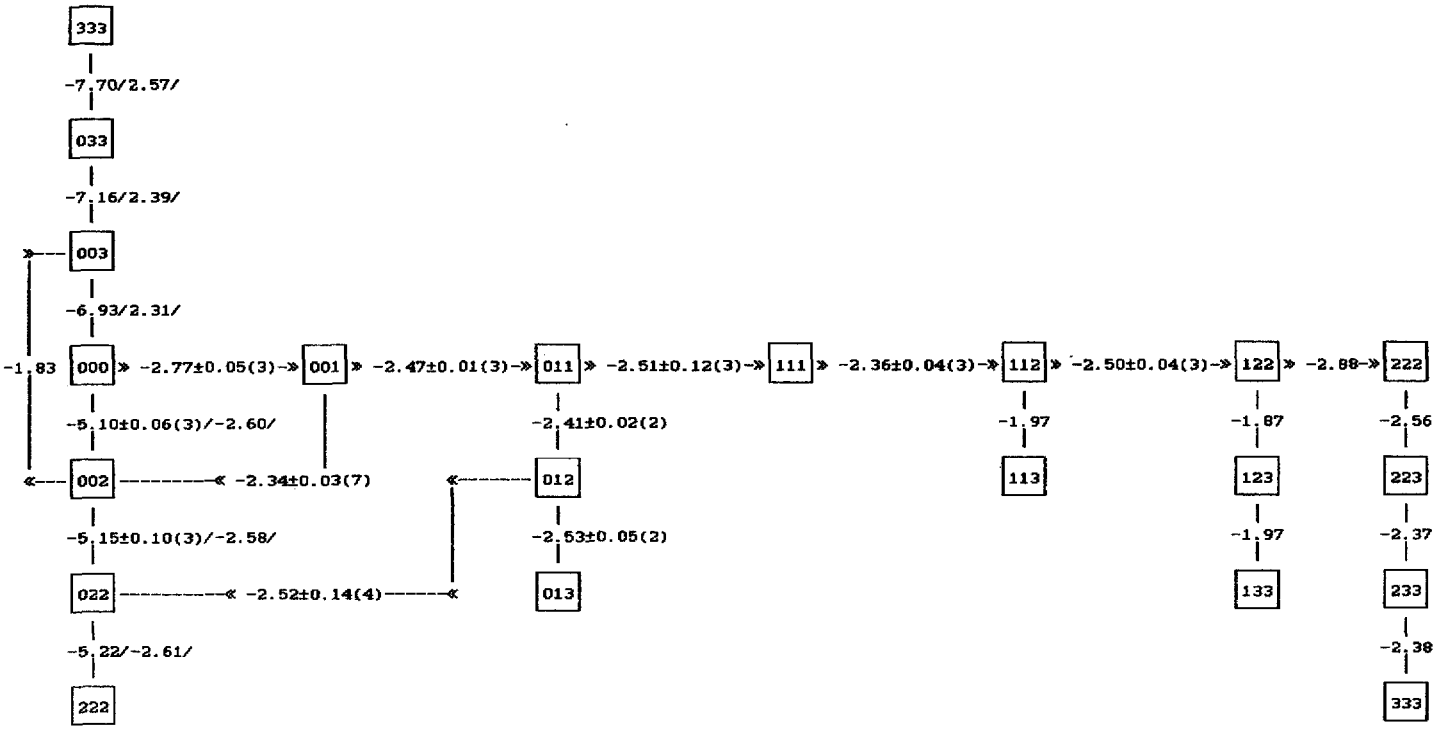
The δ-ECN values presented in Tables IV and V are scattered. Even in this instance we are partially in agreement with literature data [29,30] where values higher by a maximum of 0.3 δ-ECN were presented, probably owing to the use of different chromatographic conditions. For instance, for a change of groups from 000 to 001 a difference of ECN between 2.99 and 3.32 was reported, compared with a value of 2.77 $\pm$ 0.05 in this work (see Table V). Almost the same holds for a change of groups from 111 to 112, where a value of δ-ECN of 2.38 was reported compared with a value of 2.36 $\pm$ 0.04 in this work. On the basis of Tables III–V and from a knowledge of the total fatty acid composition and the assumption of a random distribution, it is possible to predict relatively accurately the structure of individual peaks, without their time-consuming collection and without using the expensive LC–MS method.

However, for an accurate and error-free qualitative determination of molecular species, we also used the method DCI-MS (see also Fig. 3, in which a partial record of the QM^+ region of soyabean oil TG is presented) which makes it possible to determine

TABLE V

DIFFERENCES IN EQUIVALENT CHAIN LENGTH WITH CHANGES IN DOUBLE BOND(S) THROUGH THE CHANGE FROM LOWER TO HIGHER UNSATURATION (CARBON NUMBER CONSTANT)

Value ± S.D. (number of determinations in parentheses) (values only for one double bond in square brackets).



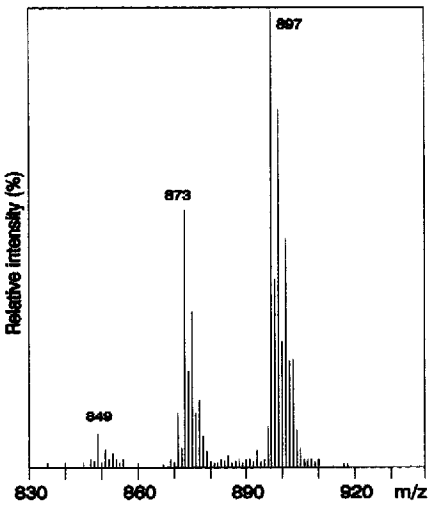


Fig. 3. DCI-MS of soyabean triacylglycerols. Only the range QM^+ is shown.

molecular weight of individual TG directly. This method was used for total mixture(s) of triglycerides. Therefore, this method does not confirm the stucture of TG in peak(s) but in the total mixture. Ammonia appeared to be the most suitable ionization gas, mainly owing to the formation of very intensive QM^+. The structures of TG presented in Tables VII–XI were thus verified by three independent methods.

TABLE VI

QUANTITATIVE YIELDS (RESPONSES) FROM A STANDARD MIXTURE OF TRIGLYCERIDES BY MEANS OF DIFFERENT TECHNIQUES (DETECTORS)

TG[a]	Relative response		
	CGC	RP-HPLC	DCI-MS
PPP	1.00	1.00	1.00
SSS	1.00	1.00	1.00
AAA	0.98	0.99	0.99
BeBeBe	0.90	0.99	0.99
LgLgLg	0.73	0.98	0.95
PoPoPo	1.00	1.00	1.00
OOO	0.98	0.99	0.99
GaGaGa	0.93	0.98	0.98
ErErEr	0.85	0.97	0.96
SSS	1.00	1.00	1.00
SSO	1.00	1.01	0.99
SOO	1.00	1.04	0.97
OOO	0.98	1.08	0.95
LLL	0.83	1.17	0.92
LnLnLn	0.14	1.74	0.86

[a] Intact TG were mixed in equal amounts (by weight).

TABLE VII

RELATIVE COMPOSITION (%) OF TRIGLYCERIDES FROM COCOA BUTTER, CORN, COTTON AND GRAPE OIL

For experimental conditions, see Experimental.

TG	Cocoa			Corn			Cotton			Grape		
	GC	LC	MS	GC	LC	MS	GC	LC	MS	GC	LC	MS
MPL	0	0	0	0	0	0	1.7	0.6	1.1	0	0	0
MPO	0.6	0	0.5	0	0	0	0.6	0	0.5	0	0	0
PPP	0.6	0	0.6	0	0	0	0.5	0	0.4	0	0	0
MLL	0	0	0	0	0	0	1.1	0	0.7	0	0	0
PPoL + MOL	0	0	0	0	0	0	0.8	0	0.4	0	0	0
PPL	1.7	3.3	2.1	0	0	0	8.1	7.7	7.9	0.8	0.3	0.6
PPO	23.3	18.0	19.7	3.9	1.5	2.7	6.2	1.5	3.1	0.5	0	0.3
PPS	0.8	0	0.7	1.9	0.4	1.2	0.1	0	0.1	0	0	0
PMgO	0.6	0	0.5	0	0.2	0.1	0	0	0	0	0	0
PPLn	0	0	0	0	0.1	0.1	0	1.4	0.5	0	0	0
PoLL	0	0	0	0	0	0	0.1	0.3	0.1	0	0	0
PLL + PoOL	0	0	0	17.1	16.4	16.8	20.8	24.5	21.3	15.7	17.6	16.1
POL	0.3	1.0	0.9	13.8	10.4	12.1	9.6	10.9	10.0	16.1	15.5	15.2
POO	2.5	6.3	"[a]	4.0	2.5	"	4.4	2.0	4.9	1.8	1.4	1.9
PSL	3.0	5.1	7.1	1.9	2.8	5.6	2.3	1.1	"	0.4	0.1	"
PSO	36.0	30.8	33.6	0.5	0.1	0.3	1.2	0.5	0.8	0.3	0.3	0.2
PSS	1.4	0	1.3	0	0	0	0	0	0	0	0	0
MgSO	0.5	0	0.5	0	0	0	0	0	0	0	0	0
LLLn	0	0	0	0.3	1.2	0.8	0	1.5	0.4	0	0	0
LLL	0	0	0	17.7	25.6	22.3	15.9	23.2	17.1	30.1	32.6	30.8
OLLn	0	0	0	0	0.6	"	0	0	0	0	0	0
OLL	0	0	0	18.8	22.5	21.4	14.1	15.4	14.8	18.9	19.1	18.7
OOLn	0	0	0	0	0.3	"	0	0	0	0	0	0
OOL	0	0	0	11.2	10.9	13.0	6.1	5.1	6.4	4.2	3.4	12.3
SLL	0	0	0	2.1	1.9	"	1.7	1.1	"	7.1	6.9	"
OOO[b]	1.3	1.7	3.5	4.1	3.1	4.9	2.6	1.6	3.1	0	0	0
SOL	0.3	1.3	"	1.7	1.2	"	1.3	0.9	"	2.4	1.9	3.5
SSL[b]	?	1.6	?	0	0	0	0	0	0	0.9	0.7	"
SOO	2.6	7.9	5.1	0.7	0.3	0.6	0.6	0.7	0.6	0.6	0.2	0.3
SSO	22.4	22.0	22.0	0.3	0	0.1	0.2	0	0.1	0.2	0	0
SSS	0.7	0	0.6	0	0	0	0	0	0	0	0	0
SLA	0.1	0	0.3	0	0	0	0	0	0	0	0	0
OOA	0.3	1.0	"	0	0	0	0	0	0	0	0	0
SOA	0.9	0	0.8	0	0	0	0	0	0	0	0	0
OAA	0.2	0	0.2	0	0	0	0	0	0	0	0	0

[a] The symbol " expresses the sum of the value above and in this line because the TG have the equal M.W. (*e.g.*, PLL and POLn have the same M.W. and in DCI-MS they show QM^+ with the same value).
[b] Peaks are not separated by CGC.

Quantitative analysis

As during the analysis of standards the responses of the detectors in all three methods were very similar up to triglyceride 222 (LLL), in a further analysis the response of the detectors was used directly without any calibration (Table VI). It

TABLE VIII

RELATIVE COMPOSITION (%) OF TRIGLYCERIDES FROM OLIVE, PALM, SOYABEAN AND SUNFLOWER OIL

For experimental conditions, see Experimental.

TG	Olive			Palm			Soyabean			Sunflower		
	GC	LC	MS	GC	LC	MS	GC	LC	MS	GC	LC	MS
MMO	0	0	0	1.0	0	0.5	0	0	0	0	0	0
MPP	0	0	0	0.6	0	0.3	0	0	0	0	0	0
MLO	0	0	0	0.3	0.6	0.4	0	0	0	0	0	0
MPL	0	0	0	0.4	0.6	0.5	0	0	0	0	0	0
MPO	0	0	0	2.8	0.5	1.4	0	0	0	0	0	0
PPP	0	0.4	0.4	11.6	7.1	9.0	0	0	0	0	0	0
MOO	0	0	0	0.6	0	0.3	0	0	0	0	0	0
PPL	1.4	2.6	1.4	10.3	10.7	10.5	3.9	1.4	1.2	3.8	1.2	3.3
PPO	7.3	4.2	5.3	27.0	24.0	26.3	1.2	0.2	0.4	1.0	0	0.7
PPS	0	0	0	0.5	0.2	0.4	0	0.2	0.2	0	0	0
PLLn	0	0	0	0	0	0	1.6	2.6	2.0	0	0	0
PLL + PoO	0.4	2.4	1.8	1.6	4.1	3.1	17.1	13.8	14.6	15.8	16.8	15.9
POLn	0	0	0	0	0	0	0	0.1	"	0	0	0
PoOO	7.8	2.1	9.0	0	0	0	0	0	0	0	0	0
POL	"[a]	8.2	"	8.1	10.8	9.8	12.1	10.7	8.8	6.9	6.0	6.1
POO	28.3	20.1	24.4	19.2	22.1	20.9	4.2	1.9	2.8	3.2	0.6	4.0
PSL	0	0	0	2.5	2.4	2.4	2.8	1.0	"	3.9	1.0	"
PSO	1.9	1.3	1.6	5.7	4.9	5.2	1.0	0.4	0.2	1.1	0.6	0.8
PSS	0	0	0	0.3	0	0.2	0	0	0	0	0	0
LLnLn	0	0	0	0	0	0	0	0.4	0.4	0	0	0
LLLn	0	0	0	0	0	0	2.5	8.4	7.4	0	0	0
OLnLn	0	0	0	0	0	0	0	0.4	"	0	0	0
LLL	0	1.0	0.6	0	0	0	16.5	16.8	24.5	22.8	28.0	26.1
OLLn	0	2.8	1.3	0	0	0	0	4.6	"	0	0	0
OLL	1.5	3.7	2.9	0.4	1.0	0.7	15.4	15.0	19.4	22.2	23.1	22.3
OOLn	0.6	0	"	0	0	0	0	1.9	"	0	0	0
OOL	0	0	0	1.0	2.6	1.9	8.7	10.1	12.4	5.3	7.5	15.0
SLL	0	0	0	0	0.6	0.3	3.9	4.0	"	7.9	10.2	"
OOO[b]	32.5	31.6	32.0	2.4	4.7	3.0	4.5	2.3	4.8	0.9	1.4	4.1
SOL	0	0	0	0.6	0.5	0.5	2.3	2.9	"	3.2	2.4	"
SSL[b]	0	0	0	0	0	0	?	0.2	"	0.6	0.4	1.7
SOO	4.9	4.9	4.9	2.0	1.7	1.8	0	0.5	0.6	1.4	0.8	"
SSO	0.4	0.7	0.6	0.7	0	0.4	1.3	0.2	0.3	0	0	0
OOGa	0.4	0	0.3	0	0	0	0	0	0	0	0	0
OOA	0.5	0	0.2	0.2	0	0.1	0	0	0	0	0	0
SOA	0	0	0	0.2	0	0.1	0	0	0	0	0	0

[a] See Table VII.
[b] Peaks are not separated by CGC.

follows from Tables VII–XI that the values obtained by means of the three independent methods are often different; for an explanation, see below.

Quantification by CGC, RP-HPLC and DCI-MS failed with three of the above twelve oils, especially linseed oil and blackcurrant oil. In these two oils problems with quantification of individual molecular species were clearly apparent. It follows from

TABLE IX

RELATIVE COMPOSITION (%) OF TRIGLYCERIDES FROM BLACKCURRANT OIL

TG	CGC	RP-HPLC	DCI-MS
PLLn	0.2	4.3	4.4
PLL	12.1	2.7	6.5
POLn	13.2	3.4	"[a]
POL	37.1	1.7	1.3
LnLn?[b]	0	0.9	0.8
LnLnLn	0	3.0	11.7
LnLnLn[c]	0	9.8	"
LLnLn[c]	0	20.2	20.5
LLLn[c]	0	21.8	25.5
OLnLn[c]	0	3.3	"
LLL	0.1	11.9	20.1
OLLn[b]	0	6.1	"
SLnLn[b]	0	1.6	"
OLL	1.3	2.2	5.3
OOLn[b]	2.1	3.0	"
OOL	13.8	1.8	3.9
SLL	20.1	2.3	"

[a] See Table VII.
[b] Probably TG with 18:4w3 acid.
[c] Indistinguishable between 18:3w3 and 18:3w6 acids in TG.

Table IX and even better from Table XI that the results obtained by determination by CGC and by RP-HPLC are contradictory, sometimes differing by two orders of magnitude. Only the DCI-MS method is reasonably satisfactory. As reported by Geeraert [20], in polyene TG discrimination occurs. However, in contrast to his study, we were able to analyse commonly available plant oils under optimum conditions

TABLE X

RELATIVE COMPOSITION (%) OF TRIGLYCERIDES FROM LINSEED OIL

TG	CGC	RP-HPLC	DCI-MS
PLnLn	5.2	4.2	4.3
PLLn	4.6	3.1	3.2
PLL	5.6	0.6	0.7
POL	10.7	0.8	1.0
LnLnLn	8.6	50.7	49.3
LLnLn	5.8	19.2	18.9
LLLn	5.9	3.6	13.0
OLnLn	12.9	9.2	"[a]
LLL	4.8	2.4	5.6
OLLn	7.6	3.2	"
OLL	8.1	1.4	2.9
SLLn	4.2	0.8	"
OOL	5.6	0.5	0.8
OOO	10.4	0.3	0.3

[a] See Table VII.

TABLE XI

RELATIVE COMPOSITION (%) OF TRIGLYCERIDES FROM PEANUT OIL

TG	CGC	RP-HPLC	DCI-MS
PPL	3.9	2.0	3.1
PPO	4.6	1.2	2.8
PLL	5.3	6.1	5.4
POL	9.9	11.6	10.3
POO	10.5	7.6	10.7
PSL	2.5	0.8	"[a]
PSO	1.9	0.9	1.4
LLL	1.7	3.6	3.0
OLL	9.6	17.5	14.5
OLO	10.8	17.3[b]	15.9
SLL	0.9	1.2	"
OOO	12.4[c]	8.1	13.6
PLGa	0	1.1[d]	"
SOL	5.6	2.1	"
POGa	0	1.2[e]	5.1
SOO	3.8	3.3	"
PLA + SSL	?[c]	0.9	"
POA + SSO	1.0	0.8	1.1
LLGa	0.4	?[b]	"
OLGa	1.0	0.6	0.9
LLA	0.5	?[d]	"
OOGa	1.5	0.5	2.1
OLA	1.4[f]	?[e]	"
OOA	1.6	2.5	2.0
SLA + PLBe	?[f]	0.8	"
SOA + POBe	1.6	0.7	0.9
LLBe	0.8	1.5	1.3
OLBe	1.8	1.6	1.6
OOBe	2.3	1.2	1.7
SLBe + PLLg	0.3	0.5	"
SOBe + POLg	0.6	0.5	0.5
LLLg	0.2	0.6	0.4
OLLg	0.5	0.8	0.7
OOLg	0.8	0.7	0.8
SLLg	0	0.2	"
SOLg	0.3	0	0.2

[a] See Table VII.

[b–f] Peaks are not separated by CGC and/or RP-HPLC.

without calibration. The results obtained were within the range of experimental error and within the range of quantitative differentiation caused by cultivation, treatment or storage.

A specific problem occurred with rape seed (high erucic acid) oil. Of the oils investigated, the distribution of chain lengths is most pronounced here (from TG_{50} to TG_{62}). In addition, TG with seven double bonds, *e.g.*, LnLnEr are present. Owing to the large number of fatty acids present (P, S, A, Be, Lg, O, Ga, Er, L and Ln), individual molecular species are not separated, being eluted in a single peak, *e.g.*, in

CGC as POEr + SOGa + OOA (and probably also others) and in RP-HPLC as LGaGa + POO + SSLn + OLEr (and others). Also in DCI-MS there is one QM^+, *e.g.*, of *m/z* 930, in several molecular species of TG. When scanning the region *m/z* 200–300, *i.e.*, for values that are manifested by ions of the types RCO^+ and $(RCO - 1)^+$, we detected ions corresponding to the acids P, S, A, O, Ga, Er and L. By combination of QM^+ (*m/z* 930) and the above-mentioned acids, the presence of, *e.g.*, molecular species PLEr, SLGa, OLA and OOGa can be assumed, positions and other isomers naturally not being considered.

From the above data, it can be clearly concluded that for the identification of such complicated mixtures of TG, a combination of two, better three, methods, must be used. For determination a much more effective column (with a higher number of plates) must be used both in CGC and particularly in RP-HPLC. When using CGC thermal dégradation causes a limitation (polyene TG and TG with more than 63 carbon atoms).

When using RP-HPLC a value of 45 000 plates for SSS was obtained (the manufacturer stated a value of 50 000 plates for toluene as a reference sample), but even this number was not sufficient for a complete separation of some critical pairs.

For the above reasons we do not present a table of quantitative results for rape seed oil as the correlation among values obtained by means of CGC, RP-HPLC and DCI-MS is very poor. Therefore, we also do not present ECN and ECL values.

CONCLUSIONS

It can be concluded that the main contribution to qualitative analysis is represented by the determination of ECL and ECN values (see Tables I and III), and particularly their differences (Tables II, IV and V). These δ-ECN and δ-ECL values make it possible to identify almost any plant fat or oil. The use of a combination of three independent techniques, *i.e.*, CGC, RP-HPLC and DCI-MS, made it possible to identify more than 80 molecular species of triglycerides in a dozen plant oils.

In quantitative analysis, the relative responses of the individual detectors, *i.e.*, of the flame ionization detector in CGC, UV detector in RP-HPLC and mass spectrometer in DCI-MS, were investigated with model mixtures. By optimizing the analytical conditions it was possible to avoid correction factors up to TG of the group 222 (*i.e.*, LLL), so that individual molecular species of TG could be detected in nine common oils without any problems. Certain difficulties occurred during the analysis of linseed, blackcurrant and rape seed oils. It can be concluded that by means of a combination of CGC, RP-HPLC and DCI-MS the qualitative and quantitative analysis of most plant oils and fats is feasible.

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