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GAS CHROMATOGRAPHY–MASS SPECTROMETRY AND DESORPTION CHEMICAL IONIZATION MASS SPECTROMETRY OF TRIACYLGLYCEROLS FROM THE GREEN ALGA *CHLORELLA KESSLERI*

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SUMMARY

The distributions of triacylglycerols in the green alga *Chlorella kessleri* cultivated heterotrophically were determined by means of gas chromatography–mass spectrometry and desorption chemical ionization mass spectrometry. Quantitative and qualitative results obtained by the two methods are correlated.

INTRODUCTION

Most organisms, ranging from bacteria to mammals or angiosperm plants, contain complex mixtures of neutral lipids, primarily triacylglycerols, as important energy sources. A detailed analysis of triacylglycerol mixtures is generally difficult[1]. Hydrolysis of the mixture followed by derivatization and gas–liquid chromatography (GLC) or gas chromatography–mass spectrometry (GC–MS) of the degradation products provides detailed information concerning the distribution of acids in the whole mixture, however, it does not yield data about the structures of the individual components. A limited amount of information can be deduced from ion fragments and molecular ions if the sample is directly measured in a mass spectrometer[2]. GLC of intact triacylglycerols on a packed column usually yields qualitative and quantitative information about the chain lengths of the triacylglycerols present[3], or, occasionally, about their unsaturation as determined by GC–CI-MS[4] (gas chromatography–chemical ionization mass spectrometry). The recent progress in high-temperature capillary column chromatography, on-column injection, GC–MS and the use of short capillary columns (about 10 m) has yielded certain structural information about complex mixtures of these high-molecular-weight, poorly volatile lipids[5].

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Hites[2] analyzed a mixture of triacylglycerols by means of the electron impact (EI)–MS method using a direct sample introduction. CI-MS results in an increased proportion of the quasimolecular ion (from several tenths % of the base peak in EI-MS to tens % of the base peak in CI-MS)[6]. Desorption chemical ionization (DCI)-MS permits direct introduction of sample into the mass spectrometer, intensity of the quasimolecular ion is increased and fragmentation is usually greatly reduced[7].

The presence of triacylglycerols in green alga has been reported by several authors[8–11]. However, the composition of the fatty acids or individual compounds was not studied. We have described[12,13] the qualitative and quantitative distribution of fatty acids present in *Chlorella kessleri* after hydrolysis of total lipids. In the present study we examined the composition of fatty acids in the triacylglycerol group of lipids. For the analysis of intact triacylglycerols in the alga we used capillary GC, GC–MS and simultaneous DI-MS. The method can also be applied to a wide range of other common, primarily plant triacylglycerols.

EXPERIMENTAL

Triacylglycerols standards

Tripalmitin, tristearin, triolein, trilinolein and trilinolenin were obtained from Serva Feinbiochemica (F.R.G.) and 1-palmito-2-oleo-3-stearin from Carl Roth (F.R.G.).

Preparation of natural triacylglycerols

A lyophilized biomass (10 g) of the green fresh-water alga *Chlorella kessleri* cultivated under heterotrophic conditions was extracted with chloroform–methanol (2:1) for 24 h at 20°C. The extract was then subjected to preparative thin-layer chromatography (TLC) on silica gel using hexane–diethyl ether–acetic acid (88:11:1) as solvent system. The fraction having R_F 0.6 corresponds to the mixture of triacylglycerols (120 mg).

Methyl esters of fatty acids were prepared by transesterification of triacylglycerols (5 mg) with sodium methanolate[14]. They were then separated and identified by GC–MS using an HP-5995 B apparatus (Hewlett-Packard, Avondale, PA, U.S.A.) with a fused-silica capillary column (60 m × 0.2 mm I.D.) containing SP 2330 (Supelco, Bellefonte, PA, U.S.A.) under the following conditions: injector temperature 250°C, splitting ratio 1:50, helium linear velocity 25 cm/s, temperature programmed from 150 to 230°C at 2°C/min, ionization voltage 70 eV and scan range 40–600 a.m.u. Double bond positions were determined by means of GC–MS pyrrolidides [*i.e.* R–CO–N$(CH_2)_4$][13].

Analysis of triacylglycerols

GLC was performed in an HP 5130A appratus (Hewlett-Packard) with an OCI-3 on-column injector (SGE, Australia) and a column (11 m × 0.31 mm I.D.) containing 0.17-μm cross-linked methylsilicone (Hewlett-Packard). Injection were made at 180°C and the temperature programmed at 1°C/min up to 320°C. A flame ionization detector (350°C), sensitivity 1 × 4, was employed.

GC–MS was performed in a Finnigan Mat 1020 B apparatus with a column (12 m × 0.2 mm I.D.) containing 0.11-μm cross-linked Ultra 1 (Hewlett-Packard).

On-column injection at 100°C, a carrier gas (helium) linear velocity of 110 cm/s, temperature programming from 250 to 350°C at 1°C/min and an ionization voltage of 70 eV were used. Scans over the range 200–650 a.m.u. were made in 0.5 s.

DCI-MS was performed in a MAT 411 apparatus (Varian, F.R.G.) under the following conditions: temperature of ion source, 220°C; reaction gas, methane (0.63 millibar); electron energy, 160 eV; emission current, 0.4 mA; acceleration voltage, 9.2 kV.

RESULTS AND DISCUSSION

High-temperature GC–MS of native triacylglycerols yields direct information about their compositions. However, discrimination of higher fractions with increasing carbon number[15,16] has been described in GLC, and is accompanied by problems in MS[15].

Our GC–MS studies also showed that the intensity of the total ion flow decreases even when an identical molecular weight distribution is maintained. For instance, the relative abundance of the RCO^+ fragment (where R is the hydrocarbon chain of the particular fatty acid) always decreases to 1/2 with increasing double bond number (100% for OOO, 50% for LLL and 25% for LnLnLn). Therefore, the composition of the individual triacylglycerols can be characterized only semiquantitatively (Table I).

Triacylglycerols isolated from the green alga comprise fractions having 51–57 carbon atoms. Ions representing RCO^+, $(RCO + 74)$, $(RCO + 128 + 14\,n)$ (where n is an integer number from 1 to 36) and $(M - RCOO)^+$ have already been described[15,17,18]. For unsaturated acyls, the presence of $(RCO - 1)^+$ and $(M - RCOO - 1)^+$ could also be demonstrated[15,17]. Ions within the molecular ion region, *i.e.*, M^+ and $M - 18$, were not obtained as the practical limit of the quadrupole mass spectrometer used is 800 a.m.u. However, it should be mentioned that the intensity of M^+ and $(M - 18)^+$ is usually up to two orders of magnitude lower than that of $(M - RCOO)^+$.

Wakeham and Frew[15] showed that by means of MS it is possible to determine those triacylglycerols that are not separated by GLC but which have different numbers of double bonds. On non-polar columns[19,20] only triacylglycerols of the UUU, UUS, USS and SSS type (S = stearic acid, U = unsaturated, C_{18} acid) were separated according to their unsaturation. Under optimum conditions on a non-polar column, we separated triacylglycerols differing only by a single double bond (Fig. 1). A similarly separation, but in an different order, has been achieved on a polar capillary column[5].

In this way, we could differentiate not only the total distributions of combinations of individual acyls, *e.g.*, C_{18}–C_{18}–C_{18} from C_{16}–C_{18}–C_{20}, but also combinations differing only in the unsaturation, *i.e.*, $C_{18:0}$–$C_{18:1}$–$C_{18:2}$ from $C_{18:1}$–$C_{18:1}$–$C_{18:1}$, SSL from OOO. Fig. 2 illustrates the MS of two peaks, POO + PLS and LnOS + LOO + LLS. It follows from the m/z values that a combination of S,O,L and Ln acids is involved (for RCO^+, m/z is 267, *i.e.* S, 265 and 264, *i.e.* O, 263 and 262, *i.e.* L and 261 and 260, *i.e.* Ln). On the basis the intensitives of the ions RCO^+ and $(RCO + 74)^+$ for any acyl, together with a correlation of the unsaturation of each acyl, it is possible to deduce a semiquantitative distribution of the individual triacylglycerols in the peak (Table I).

TABLE I

PERCENTAGE DISTRIBUTION OF TRIACYLGLYCEROLS IN THE GREEN ALGA *C. kessleri* DETERMINED BY MEANS OF GLC, GC–MS AND DCI-MS AND THE COMPOSITION OF TRIACYLGLYCEROLS DETERMINED BY GC–MS

P = Palmitic acid, Po = palmitooleic acid, S = stearic acid, O = oleic acid, L = linoleic acid, Ln = linolenic acid, A = arachidic acid; w = weak, m = medium, l = large.

Carbon number	*Composition (GC–MS)*	*Age distribution*	
		GLC	*DCI-MS*
48	PPP	0.4	0.1
50	PoPoLn	0.0	0.6
	PoPoL	0.2	1.1
	PPLn	0.6	1.2
	PPL	1.2	1.1
	PPO	0.9	0.5
52	PoLL(l) + PLnL(l)	1.7	3.4
	PPL(l) + PLnO(m)	8.2	10.2
	PLO	3.7	5.5
	POO(l) + PLS(m)	5.8	1.4
	POS	0.9	0.8
54	LnLnL	0.0	0.7
	LnLnO(m) + LnLL(l)	2.4	4.3
	LnLnS(w) + LnLO(m) + LLL(l)	19.2	25.4
	LnLS(w) + LnOO(m)	20.1	21.6
	LnOS(w) + LOO(m) + LLS(l)	16.2	12.4
	LnSS(w) + LOS(m) + OOO(l)	11.78	5.4
	LSS(m) + OOS(l)	4.5	0.9
	OSS	1.4	0.6
56	LnLnA	0.0	1.0
	LnLA	0.2	1.1
	LLA	0.3	0.7
58	LAA	0.3	0.0

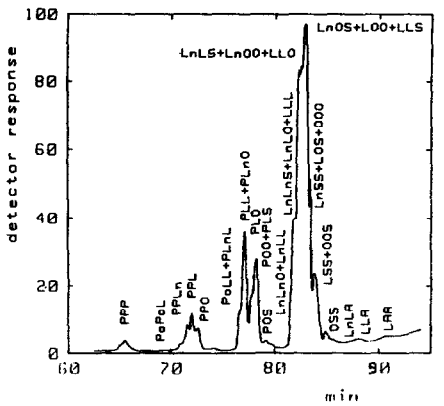

Fig. 1. GLC of triacylglycerols.

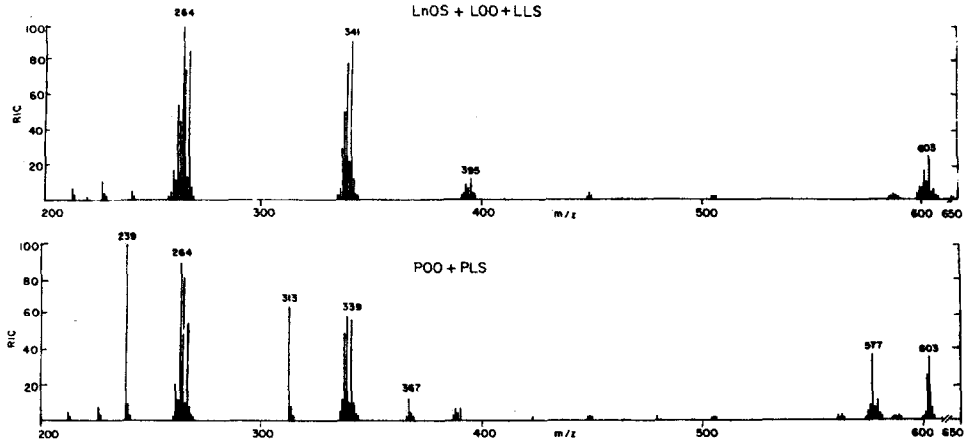

Fig. 2. EI-MS of LnOS + LOO + LLS and POO + PLS.

In the peak LnOS + LOO + LLS, LOO (l), LLS (m) and LnOS (w) are large, medium and weak constituents, respectively. Another theoretically possible component, PoLnA, was not detected. We assume that, due to the absence of the ion at m/z 295 (RCO^+ for A), this component comprises less than 1% of the peak. In exceptional cases it is also possible to detect even acyl substitution on individual glycerol atoms. For instance, in the peak POO-PLS, palmitoyl can be detected through the presence of the ion at m/z 589 $(M - RCOO - CH_2)^+$ in positions 1 or 3 of both triacylglycerols. Unfortunately, in most cases the intensity of this ion is low and it was not possible to determine the stereospecificity of individual acyls. Also, in the peak POO + PLS, two other theoretically possible combinations, *viz.*, PoOS and PoPoA, were not detected (absence of the ions at m/z 237 and 236 for Po and at m/z 295 for A, respectively).

DCI-MS made it possible to determine both the qualitative and quantitative distribution of individual triacylglycerols in the mixture. First, the classes of triacylglycerols in samples were determined (Fig. 3). As mentioned above, when using the GC–MS method it is possible to discriminate highly unsaturated triacylglycerols. The agreement between the percentage distribution of triacylglycerols obtained by both methods is good, certainly better than that described by Merritt *et al.*[21] for DCI-MS and reversed-phase high-performance liquid chromatography (RP-HPLC), where several-fold differences were found even for major triacylglycerols.

Discrimination also occurs in DCI-MS[7], however, it does not depend on the number of carbon atoms in the triacylglycerols but rather on the number of double bonds. Therefore, it is possible to utilize the intensities of the pseudomolecular ions for the calculation of the percentage distributions of the individual triacylglycerols.

The content of fatty acids in the triacylglycerol fraction is presented in Table II. It can be seen that seven major acids are involved. However, no acids having more than 22 carbon atoms were not found in total lipids[12,13] or in waxes and ceramides[22].

Metzger *et al.*[11] compared the ^1H NMR spectra of olive, peanut and linseed oil with that of 'algal oil'. It was concluded that both algae and plant oils contain

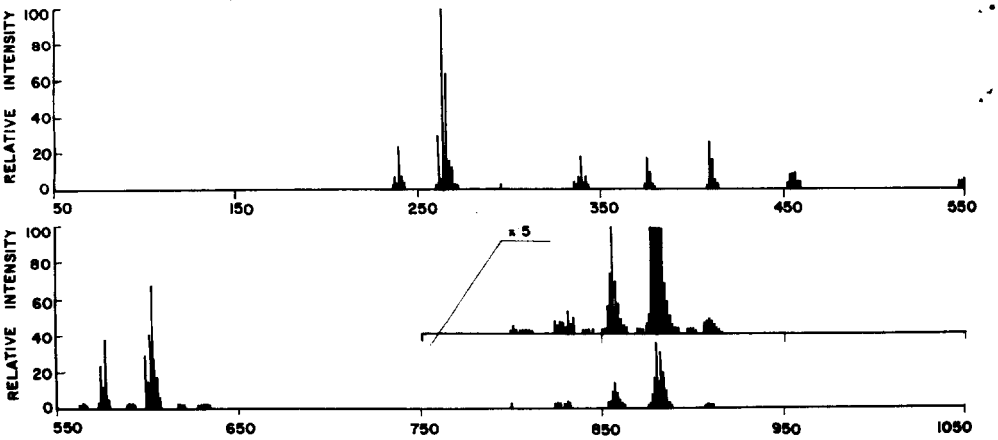

Fig. 3. DCI-MS of triacylglycerols from the green alga *C. kessleri.*

TABLE II

CONTENTS OF FATTY ACIDS IN THE TRIACYLGLYCEROL FRACTION FROM THE ALGA *C. kessleri*

Acid	*%*	*Acid*	*%*
14:0	1.0	18:0	6.4
15:0	0.3	9-18:1	26.8
16:0	9.1	9,12-18:2	39.5
9-16:1	3.7	9,12,15-18:3	10.3
17:0	0.2	20:0	1.1
9-17:1	0.7	13-22:1	0.9

triacylglycerols with identical unsaturation. In other papers[9,10] the presence of triacylglycerols was only briefly mentioned.

The use of DCI–MS in the present study made it possible to determine simultaneously the quality and quantity of individual triacylglycerols. The subsequent analysis by means of GC–MS yielded more accurate data. The separations of the triacylglycerols according to the number of carbon atoms and partially also according to their unsaturation enables individual molecular species to be determined semi-quantitatively by both methods. Naturally, this determination is much more accurate than by GLC, where the structure of each triacylglycerol is deduced from its retention time. As compared with HPLC, the isolation of individual peaks and their subsequent identification by MS are avoided. Thus, GC–MS yields results comparable with those obtained by the LC–MS method preferred by Kuksis[23].

The results obtained can also be applied to other mixtures of triacylglycerols of similar composition but different origin, *e.g.*, plant oils or animal fats.

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