Analysis of Triacylglycerols by Silver-Ion High-Performance Liquid Chromatography–Atmospheric Pressure Chemical Ionization Mass Spectrometry

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ABSTRACT: Triacylglycerols of the seed oils rich in α- and/or γ-linolenic acid moieties were separated by silver-ion high-performance liquid chromatography (HPLC) followed by on-line atmospheric pressure chemical ionization–mass spectrometric (APCI–MS) detection. Mass spectra of most triacylglycerols exhibited abundant $[M + H]^+$ and $[M - RCO_2]^+$ ions, which defined the molecular weight and the molecular association of fatty acyl residues of a triacylglycerol, respectively. Silver ions formed weaker complexes with triacylglycerols containing γ-linolenic acid than with those containing α-linolenic acid, i.e., the elution order of molecules was $XYT_\gamma > XYT_\alpha$, $XT_\gamma T_\gamma > XT_\gamma T_\alpha > XT_\alpha T_\alpha$, and $T_\gamma T_\gamma T_\gamma > T_\gamma T_\gamma T_\alpha > T_\gamma T_\alpha T_\alpha > T_\alpha T_\alpha T_\alpha$, where T_α = α-linolenic acid, T_γ = γ-linolenic acid, and X, Y = fatty acids different from linolenic acid. Furthermore, silver-ion HPLC resulted in partial separation within equally unsaturated triacylglycerols according to differences in the combined number of acyl carbons. Regioisomeric forms of triacylglycerols were not determined from the seed oil samples, although differences were measured with reference compounds in the relative abundances of $[M - RCO_2]^+$ ions formed by a loss of a fatty acyl residue from the *sn*-2 position and the *sn*-1/3 positions. Silver-ion HPLC/APCI–MS provided valuable information for structure elucidation of seed oil triacylglycerols: 43 molecular species were identified from cloudberry seed oil, 39 from evening primrose oil, 79 from borage oil, 44 from alpine currant, and 56 from black currant seed oils. The quantitation requires to be studied further, especially in those cases where several molecular weight species of triacylglycerols eluted in a single chromatographic peak.

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Silver-ion chromatography is a practical technique for the separation of triacylglycerols according to differences in unsaturation (reviewed in Refs. 1–4). Previous studies have been performed by thin-layer chromatography (TLC) on silica gel plates impregnated with silver ions. More recent studies are mostly based on high-performance liquid chromatographic (HPLC) separation either on a reversed-phase column (silver ions in the mobile phase) (5) or on a silver-loaded cation-exchange column (6), or on a silver-loaded silica column (7). In general, the separation of triacylglycerols on silver-ion chromatography is based on (i) the number of double bonds in the acyl chains, (ii) the distribution of double bonds between the fatty acyl residues within a single molecule, (iii) the configuration and position of double bonds within a fatty acyl residue, and (iv) the position at which the fatty acid is esterified to the glycerol backbone.

The method introduced by Christie (8), based on separation on a silver-loaded cation-exchange column, has been most widely used and applied to the analysis of various samples, such as seed and vegetable oils (8–11), milk fat (12–14), and fish oils (15–17). The technique has not yet been used for the analysis of samples containing both α-linolenic acid (18:3n-3) and γ-linolenic acid (18:3n-6) moieties in their triacylglycerols, although resolution of ester derivatives of α- and γ-linolenic acids has been reported (18). Few studies of oils rich in γ-linolenic acid have been conducted by silver-ion chromatography, for example, evening primrose oil by silver-ion HPLC (19) and silver-ion TLC (20), and borage oil by silver-ion supercritical fluid chromatography (21). Triacylglycerols of the seeds of the plants belonging to the genus *Ribes* are relatively abundant both in γ- and α-linolenic acids. The composition of black currant seed oil has been studied, for example, by reversed-phase HPLC (22–24), high-temperature gas chromatography (GC) (24), and direct inlet chemical ionization-mass spectrometry (MS) (24,25). Recently, a separation of α- and γ-linolenic acid containing triacylglycerols with an identical number of acyl carbons and degree of unsaturation has been obtained by capillary supercritical fluid chromatography (25). However, the differentiation of co-eluting components was not possible with a flame-ionization detector (FID).

Components separated by silver-ion HPLC are most often detected with an evaporative light-scattering detector (ELSD) or with an FID, which, unfortunately, do not produce structural information on molecular compositions. Therefore, identification of the components has been done either by collection of the separated HPLC fractions and analysis of their

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Abbreviations: APCI, atmospheric pressure chemical ionization; APCI–MS, atmospheric pressure chemical ionization–mass spectrometry; ELSD, evaporative light-scattering detector; FID, flame-ionization detector; HPLC, high-performance liquid chromatography; GC, gas chromatography; MS, mass spectrometry; TLC, thin-layer chromatography; RIC, reconstructed ion chromatogram.

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fatty acid compositions as methyl esters by GC, or by comparison of the retention times of unknown peaks with those of reference components. An application of an on-line combination of argentation HPLC, on a silver-loaded silica column, with MS utilizing electrospray ionization has been reported (26). Mass spectra of triacylglycerols exhibited only abundant $[M + Na]^+$ ions without information on fatty acid moieties. Information on both the molecular weights and the fatty acyl residues of triacylglycerols has been achieved by combining reversed-phase HPLC separation with atmospheric pressure chemical ionization–mass spectrometry (APCI–MS) (27,28). Mass spectra of most triacylglycerols consisted of abundant $[M + H]^+$ and $[M - RCO_2]^+$ ions.

The objectives of this study were to apply silver-ion HPLC for the separation of α- and γ-linolenic acid containing triacylglycerols in seed oils and APCI–MS for identification purposes. Chromatographic separation of triacylglycerols containing α- and γ-linolenic acid moieties is essential, because double-bond positional isomers cannot be distinguished as such by MS.

EXPERIMENTAL PROCEDURES

Materials. Oils from the seeds of black currant (*Ribes nigrum*), alpine currant (*R. alpinum*), and cloudberry (*Rubus chamaemorus*) were extracted with supercritical carbon dioxide at Flavex (Rehlingen, Germany). Evening primrose oil (*Oenothera biennis*) and borage oil (*Borago officinalis*) were commercial products purchased from a local health food shop. The oils were dissolved in *n*-hexane, after which the triacylglycerols were purified by elution through a short column of Florisil™ (Fluka Chemie AG, Buchs, Switzerland) with 10 mL *n*-hexane/diethyl ether (4:1, vol/vol). After evaporating the solvent with a stream of nitrogen, the triacylglycerols were dissolved in 1,2-dichloroethane to a concentration of ~10 mg/mL and stored under nitrogen at –20°C. All solvents used were HPLC-grade supplied by Rathburn (Walkerburn, Scotland) or Merck (Darmstadt, Germany). The triacylglycerol standards *rac*-1,2-distearoyl-3-palmitoyl-*sn*-glycerol (*rac*-18:0-18:0-16:0), 1,3-distearoyl-2-palmitoyl-*sn*-glycerol (*sn*-18:0-16:0-18:0), *rac*-1,2-dipalmitoyl-3-oleoyl-*sn*-glycerol (*rac*-16:0-16:0-18:1), 1,3-dipalmitoyl-2-oleoyl-*sn*-glycerol (*sn*-16:0-18:1-16:0), *rac*-1,2-dioleoyl-3-palmitoyl-*sn*-glycerol (*rac*-18:1-18:1-16:0), 1,3-dioleoyl-2-palmitoyl-*sn*-glycerol (*sn*-18:1-16:0-18:1), *rac*-1,2-dipalmitoyl-3-linoleoyl-*sn*-glycerol (*rac*-16:0-16:0-18:2), 1,3-distearoyl-2-linoleoyl-*sn*-glycerol (*sn*-18:0-18:2-18:0), *rac*-1,2-dioleoyl-3-γ-linolenoyl-*sn*-glycerol (*rac*-18:1-18:1-18:3n-6), 1,3-dioleoyl-2-γ-linolenoyl-*sn*-glycerol (*sn*-18:1-18:3n-6-18:1), 1,3-dioleoyl-2-α-linolenoyl-*sn*-glycerol (*sn*-18:1-18:3n-3-18:1), trilinoleoylglycerol (18:2-18:2-18:2), and trilinolenoylglycerol (18:3n-3-18:3n-3-18:3n-3) were purchased from Larodan (Malmö, Sweden).

GC. Fatty acid methyl esters were prepared by sodium methoxide-catalyzed transesterification of the purified triacylglycerol fractions of the seed oils (29). Separations of methyl esters on an NB-351 fused silica capillary column (25 m × 0.32 mm i.d. with 0.20 μm film; HNU-Nordion Ltd., Helsinki, Finland) were conducted with a Varian 3300 gas chromatograph (Limerick, Ireland) equipped with a split injector (split ratio 40:1) and an FID. The temperature program was from 120°C (2 min) with a rate of 3°C/min to 230°C (10 min). The injector temperature was held at 225°C and the detector at 240°C. Helium was used as a carrier gas.

Silver-ion HPLC. Two different HPLC systems were used: (i) a Merck Hitachi L-6200A Intelligent pump (Hitachi, Tokyo, Japan), equipped with an SSI Model LP-21 lo-pulse unit (Scientific Systems Inc., State College, PA), combined with mass spectrometric detection, and (ii) a Shimadzu LC-9A pump (Kyoto, Japan), equipped with a Shimadzu FCV-9AL low-pressure gradient elution unit, combined with an ELSD (Cunow DDL21; Cergy-Saint-Christophe, France).

Silver-ion HPLC was performed according to the procedure introduced by Christie (6,8). A cation-exchange column containing sulfonic acid moieties (ET 250/4 Nucleosil 100-5 SA, 250 mm × 4 mm i.d., 5 μm particle size; Macherey-Nagel, Düren, Germany) was loaded with silver ions. Triacylglycerols in 1,2-dichloroethane (~5 μL) were injected onto the column and separated at ambient temperature using a ternary solvent gradient consisting of A) dichloromethane/1,2/dichloroethane (1:1, vol/vol), B) acetone, and C) acetone/acetonitrile (4:1, vol/vol). The program steps of the linear gradient were: 0 min 100% A; 10 min 50% A–50% B; 30 min 70% B–30% C; 60 min 100% C; 70 min 100% C. The flow rate was 0.8 mL/min. The column was equilibrated for 20 min at the initial conditions before each analysis.

MS. The MS determinations were conducted with a Finnigan MAT TSQ-700 instrument (San Jose, CA) equipped with an APCI source and an ICIS II data system. The MS parameters were optimized for the ionization of triacylglycerols using loop injection technique. During the HPLC–MS analyses, the vaporizer temperature was 400°C, capillary heater temperature 200°C, corona current 5 μA, sheath gas (nitrogen) pressure 50 psi, and auxiliary gas (nitrogen) flow 5 mL/min. Positively-charged ions with *m/z* values of 400–1100 were scanned with a scan time of 0.7 s. The HPLC flow of 0.8 mL/min was introduced into the APCI source without any splitting.

RESULTS

Fatty acid compositions. The samples studied were selected to represent seed oils containing (i) only α-linolenic acid, (ii) only γ-linolenic acid, and (iii) both α- and γ-linolenic acids (Table 1). Cloudberry seed oil was the example of an oil rich in α-linolenic acid (28 mol%). Evening primrose oil and borage oil were the examples of γ-linolenic acid oils. Borage oil contained 22 mol% γ-linolenic acid and evening primrose oil 10 mol%. The proportion of α-linolenic acid in these oils was negligible. The seed oils of black currant and alpine currant contained significant proportions of both linolenic acid isomers. Black currant seed oil contained 14 mol% both

TABLE 1
Fatty Acid Compositions (mol%) of the Triacylglycerols of Cloudberry, Evening Primrose, Borage, Alpine Currant, and Black Currant Seed Oils

	Cloudberry		Evening primrose		Borage		Alpine currant		Black currant	
Fatty acid	Mean[a]	SD	Mean[a]	SD	Mean[a]	SD	Mean[a]	SD	Mean[a]	SD
14:0	0.41	0.02								
16:0	3.15	0.07	6.17	0.08	10.76	0.28	5.55	0.07	5.72	0.10
16:1n-7	0.41	0.02		0.16	0.00					
16:1n-5	0.46	0.01		0.16	0.01					
18:0	1.36	0.00	1.38	0.02	3.61	0.03	1.40	0.02	1.59	0.02
18:1n-9	14.94	0.05	9.09	0.03	16.51	0.02	19.25	0.06	13.49	0.05
18:1n-7	0.64	0.02	0.67	0.01	0.65	0.01	0.94	0.03	0.75	0.01
18:2n-6	40.23	0.16	72.02	0.07	37.19	0.05	41.10	0.08	45.96	0.03
18:3n-6			9.62	0.02	22.41	0.12	9.18	0.03	14.24	0.03
18:3n-3	28.24	0.07	0.21	0.00	0.13	0.00	17.79	0.01	13.81	0.02
18:4n-3					0.13	0.01	3.41	0.01	2.92	0.01
20:0	1.11	0.02	0.25	0.01	0.22	0.02			0.29	0.01
20:1n-9	0.74	0.03	0.17	0.01	3.92	0.13			0.87	0.01
20:2n-6	4.42	0.12			0.18	0.01				
22:0	0.51	0.00			0.14	0.02				
22:1n-9					2.37	0.13				
22:2	2.02	0.06								
24:1n-9					1.34	0.09				
Others	1.38	0.29	0.42	0.03	0.11	0.04	1.39	0.05	0.36	0.02

[a]Average of three gas chromatographic analyses of fatty acid methyl esters.

γ-linolenic and α-linolenic acid, whereas the corresponding proportions for alpine currant oil were 9 and 18 mol%, respectively. An additional feature of the seed oils of plants belonging to the genus *Ribes* was the presence of stearidonic acid (18:4n-3). All the oils studied were abundant in oleic acid (9–19 mol%) and linoleic acid (37–72 mol%).

APCI-MS of triacylglycerols. APCI of most triacylglycerols yielded very simple mass spectra exhibiting abundant $[M + H]^+$ and $[M - RCO_2]^+$ ions, which defined the molecular weight and the molecular association of fatty acyl residues, respectively. As reported earlier by Byrdwell and Emken (27) and Neff and Byrdwell (28), the relative abundances of $[M + H]^+$ and $[M - RCO_2]^+$ ions were strongly affected by the degree of unsaturation of a triacylglycerol, e.g., saturated molecules did not produce any $[M + H]^+$ ion, whereas $[M + H]^+$ ion was the base peak in the mass spectra of more unsaturated triacylglycerols (typically the combined number of double bonds in the acyl chains is ≥4). During the optimization of the MS parameters, it was observed that the ionization efficiency of triacylglycerols decreased with increasing capillary temperature. However, the capillary temperature had to be high enough to produce a relatively low and stable signal background. Therefore, the practical capillary temperature, with the instrumentation and eluent composition used, was in the range of 170–200°C. The vaporizer temperature had to be high enough to produce good sensitivity, the optimum being around 400°C.

Silver-ion HPLC/APCI-MS of reference components. It was shown by Nikolova-Damyanova *et al.* (18) that methyl and phenacyl esters of γ-linolenic acid were less retained on a silver-loaded cation-exchange column than those of α-linolenic acid. This let us to suggest that it may also be possible to separate triacylglycerols containing α- and γ-linolenic acid moieties with the technique. Figure 1 shows the separation of 1,3-dioleoyl-2-γ-linolenoyl-*sn*-glycerol and 1,3-dioleoyl-2-α-linolenoyl-*sn*-glycerol by HPLC on a silver-loaded Nucleosil-5 SA column. The interaction of γ-linolenic acid containing isomer with silver ion was weaker than that of α-linolenic acid containing isomer. The components were separated to the baseline with a resolution of 2.7.

The separation of triacylglycerols on silver-ion HPLC was also affected by the regiospecific distribution of fatty acids (*sn*-2 vs. *sn*-1/3) in a triacylglycerol molecule (Fig. 2, Table 2). Isomeric triacylglycerols differing in the position of unsaturated fatty acids were partially separated, whereas sat-

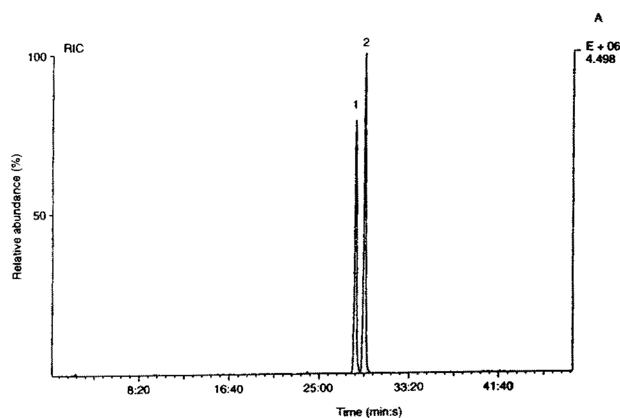

FIG. 1. Separation of 1,3-dioleoyl-2-γ-linolenoyl-*sn*-glycerol (peak number 1) and 1,3-dioleoyl-2-α-linolenoyl-*sn*-glycerol (peak number 2) by silver-ion high-performance liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry. The analytical conditions have been described in the Experimental Procedures section. RIC, reconstructed ion chromatogram.

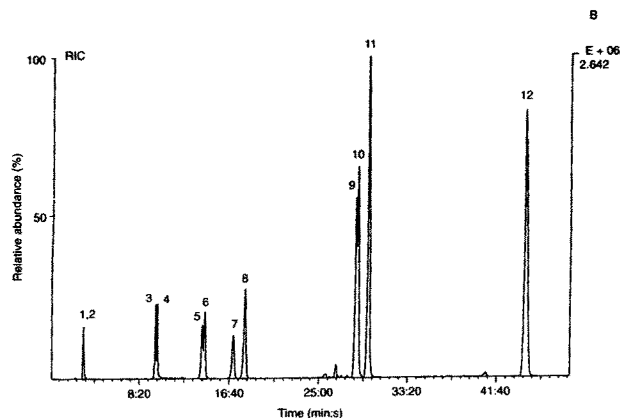

FIG. 2. Separation of triacylglycerols by silver-ion high-performance liquid chromatography according to the regiospecific distribution of fatty acids in the *sn*-2 and *sn*-1/3 positions. Components were detected by atmospheric pressure chemical ionization–mass spectrometry. Analytical conditions have been described in the Experimental Procedures section. Peak identifications: 1 = *rac*-1,2-distearoyl-3-palmitoyl-*sn*-glycerol, 2 = 1,3-distearoyl-2-palmitoyl-*sn*-glycerol, 3 = 1,3-dipalmitoyl-2-oleoyl-*sn*-glycerol, 4 = *rac*-1,2-dipalmitoyl-3-oleoyl-*sn*-glycerol, 5 = 1,3-dioleoyl-2-palmitoyl-*sn*-glycerol, 6 = *rac*-1,2-dioleoyl-3-palmitoyl-*sn*-glycerol, 7 = 1,3-distearoyl-2-linoleoyl-*sn*-glycerol, 8 = *rac*-1,2-dipalmitoyl-3-linoleoyl-*sn*-glycerol, 9 = 1,3-dioleoyl-2-γ-linolenoyl-*sn*-glycerol, 10 = *rac*-1,2-dioleoyl-3-γ-linolenoyl-*sn*-glycerol, 11 = trilinoleoylglycerol, 12 = trilinolenoylglycerol. See Figure 1 for abbreviation.

urated isomers were not separated at all due to the lack of interaction with silver ions. In general, the most unsaturated fatty acid moiety of a triacylglycerol esterified to the *sn*-1/3 position formed stronger interaction with silver ion than that esterified to the *sn*-2 position; for example, 1,3-dioleoyl-2-γ-linolenoyl-*sn*-glycerol eluted slightly earlier than *rac*-1,2-dioleoyl-3-γ-linolenoyl-*sn*-glycerol. With the chromatographic system used in the present study, *rac*-1,2-dioleoyl-3-palmitoyl-*sn*-glycerol had slightly stronger retention to the stationary phase than 1,3-dioleoyl-2-palmitoyl-*sn*-glycerol, thus, being an exception of the general rule. A reverse elution order of these two components or closely related ones has been achieved both by silver-ion TLC (30) and silver-ion HPLC (7,13,31). However, exceptions to the general elution order of isomeric triacylglycerols analyzed by silver-ion TLC has also been reported (30). Various eluent compositions used in separate studies may modify the stationary phase differently, thus, affecting the sample/stationary phase interaction and elution order of molecules. In addition to the regiospecific distribution of fatty acids, the separation also may be affected by differences in the chain length of the esterified fatty acyl residues, as was assumed from the good baseline separation (resolution 3.1) of 1,3-dioleoyl-2-linoleoyl-*sn*-glycerol and *rac*-1,2-dipalmitoyl-3-linoleoyl-*sn*-glycerol. Adlof (32) has recently reported the effect of chain length on the separation of triacylglycerols, diacylglycerol-monoacetates, monoacylglycerol-diacetates, and triacetin.

The regiospecific distribution of fatty acids also had an effect on the mass spectra of triacylglycerols obtained by APCI–MS (Table 2). The abundance of $[M - RCO_2]^+$ ion formed by a loss of a fatty acyl residue from the *sn*-2 position was less than that formed by a loss of a fatty acyl residue from the primary glycerol positions. As an example, the relative abundance of $[M - 16{:}0]^+$ ion in the APCI mass spectrum of 1,3-dioleoyl-2-palmitoyl-*sn*-glycerol was 8.5% of the abundance of the base peak, and in the mass spectrum of *rac*-1,2-dioleoyl-3-palmitoyl-*sn*-glycerol, it was 69.9%. This confirms the elution order of these regioisomeric triacylglycerols discussed above.

Silver-ion HPLC/APCI–MS of seed oils. Triacylglycerols of the seed oils studied were efficiently separated by silver-ion HPLC/APCI–MS as can be seen from the reconstructed ion chromatograms (RIC) (same as total ion chromatograms) shown in Figures 3 and 4. The analyses of triacylglycerols by silver-ion HPLC combined with ELSD produced very similar separations, differing mainly in the proportions of the separated fractions and, therefore, the ELSD chromatograms are not presented here. The identification of the eluted compo-

TABLE 2
Retention Properties and Mass Spectrometric Fragmentation of Triacylglycerols Analyzed by Silver-Ion HPLC/APCI–MS

Compound number	Triacylglycerol	t_r (min:s)	$[M + H]^+$ *m/z* (abundance)	$[M - R_1COO]^+$ *m/z* (abundance) [ion]	$[M - R_2COO]^+$ *m/z* (abundance) [ion]	Resolution (*R*)
1	*rac*-18:0-18:0-16:0	3:05	863.8 (n.d.)	579.5 (100.0) = $[M - 18{:}0]^+$	607.6 (56.4) = $[M - 16{:}0]^+$	$R_{1,2}{}^a = 0.00$
2	*sn*-18:0-16:0-18:0[b]	3:05				
3	*sn*-16:0-18:1-16:0	9:49	833.8 (8.9)	551.5 (33.5) = $[M - 18{:}1]^+$	577.6 (100.0) = $[M - 16{:}0]^+$	$R_{3,4} = 0.99$
4	*rac*-16:0-16:0-18:1	9:58	833.8 (16.2)	551.5 (88.7) = $[M - 18{:}1]^+$	577.6 (100.0) = $[M - 16{:}0]^+$	
5	*sn*-18:1-16:0-18:1	14:11	859.8 (20.7)	577.5 (100.0) = $[M - 18{:}1]^+$	603.6 (8.5) = $[M - 16{:}0]^+$	$R_{5,6} = 0.74$
6	*rac*-18:1-18:1-16:0	14:24	859.8 (14.9)	577.5 (100.0) = $[M - 18{:}1]^+$	603.6 (69.9) = $[M - 16{:}0]^+$	
7	*sn*-18:0-18:2-18:0	17:01	887.8 (3.8)	603.5 (100.0) = $[M - 18{:}0]^+$	607.5 (37.5) = $[M - 18{:}2]^+$	$R_{7,8} = 3.09$
8	*rac*-16:0-16:0-18:2	18:06	831.7 (5.4)	551.4 (100.0) = $[M - 18{:}2]^+$	575.5 (75.6) = $[M - 16{:}0]^+$	
9	*sn*-18:1-18:3n-6-18:1	28:26	881.8 (100.0)	599.5 (65.3) = $[M - 18{:}1]^+$	603.4 (59.0) = $[M - 18{:}3]^+$	$R_{9,10} = 0.57$
10	*rac*-18:1-18:1-18:3n-6	28:39	881.8 (90.5)	599.5 (50.8) = $[M - 18{:}1]^+$	603.4 (100.0) = $[M - 18{:}3]^+$	
11	18:2-18:2-18:2	29:39	879.7 (100.0)	599.4 (51.5) = $[M - 18{:}2]^+$		
12	18:3n-3-18:3n-3-18:3n-3	44:20	873.7 (100.0)	595.3 (44.9) = $[M - 18{:}3]^+$		

[a]Subscripts refer to the compound numbers. [b]Co-eluted with the compound number 1.HPLC/APCI–MS, high-performance liquid chromatography/atmospheric pressure chemical ionization–mass spectrometry.

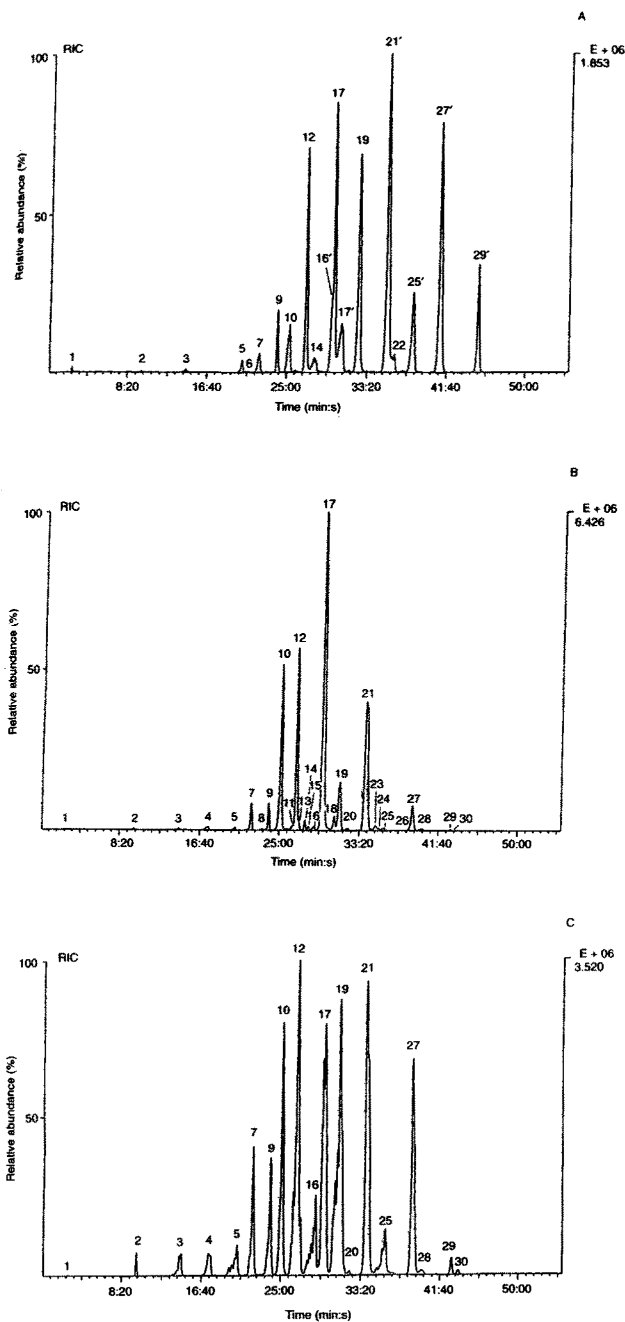

FIG. 3. RIC of the triacylglycerols of A) cloudberry seed oil, B) evening primrose oil, and C) borage oil achieved by silver-ion high-performance liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry. A seven-point smoothing has been applied to the chromatograms. The analytical details have been described in the Experimental Procedures section. Peak numbers refer to Table 3. See Figure 1 for abbreviation.

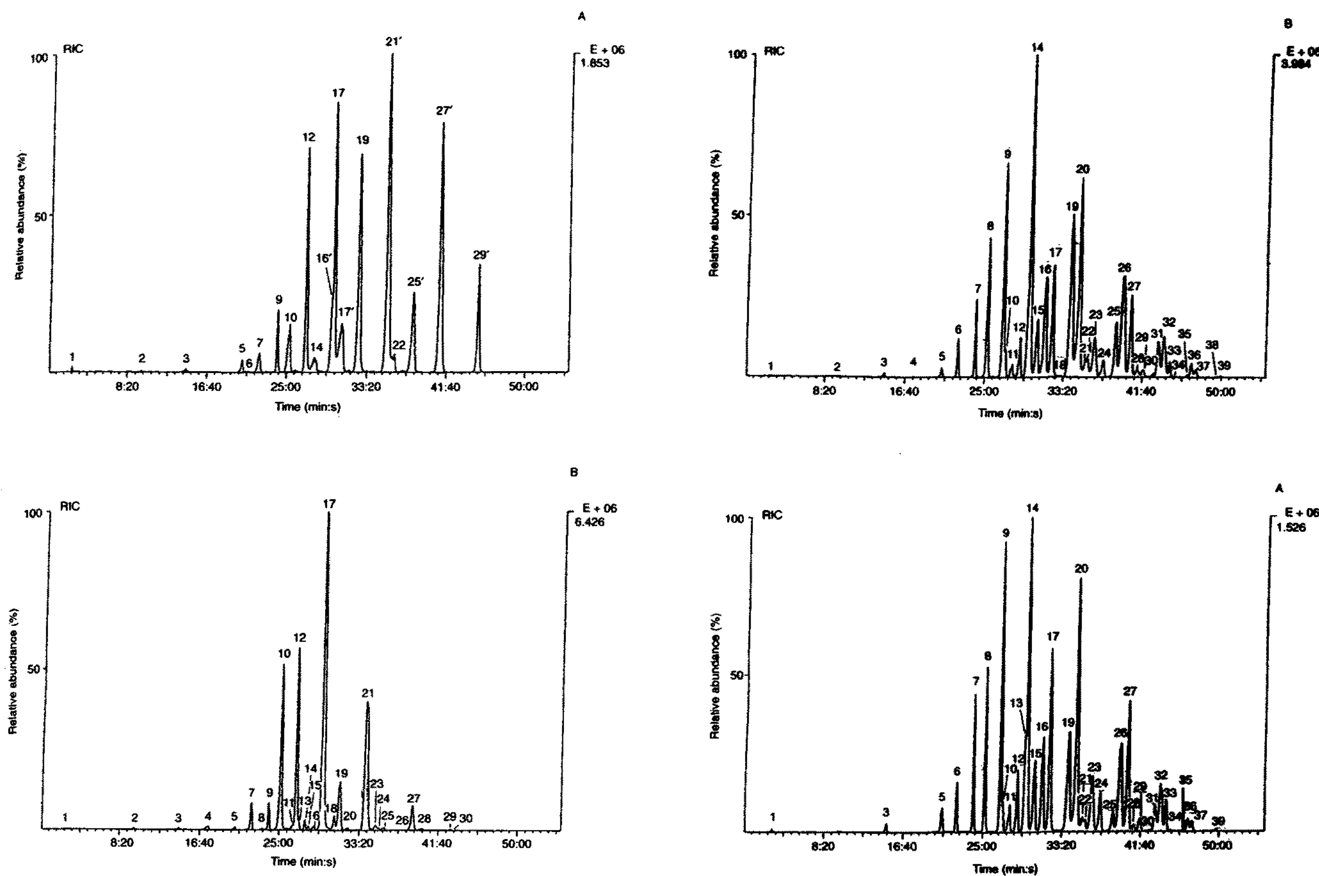

FIG. 4. RIC of the triacylglycerols of A) alpine currant seed oil, and B) black currant seed oil achieved by silver-ion high-performance liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry. A seven-point smoothing has been applied to the chromatograms. The analytical details have been described in the Experimental Procedures section. Peak numbers refer to Table 4. See Figure 1 for abbreviation.

nents and their proportions are presented in Table 3 and 4. Almost all the molecular species listed in the tables exhibited both $[M + H]^+$ and $[M - RCO_2]^+$ ions in the mass spectra. Triacylglycerols having together two or fewer double bonds in their acyl chains formed no or only a weak molecular ion and, thus, they were identified mainly according to the ions formed by the loss of one fatty acid moiety. None of the samples analyzed contained significant amounts of saturated triacylglycerols; thus, the first eluted peak without retention to the silver-ion column was possibly due to the presence of carotenoids, especially in cloudberry seed oil. The quantitative estimates of the separated triacylglycerol fractions were based on the peak areas in the RIC (APCI–MS) and in the ELSD chromatograms without corrections according to molecular response factors. In the present study, no attempt was made to estimate the relative amounts of different molecular species eluting in a single chromatographic peak. The proportions of triacylglycerols obtained by the two detection systems were not always in accordance, which was due to variable detector response of different triacylglycerol species. In the following, the analysis of each sample by silver-ion HPLC/APCI–MS will be discussed in more detail.

(i) Cloudberry seed oil. The elution order of the triacylglycerols of cloudberry seed oil, representing an oil rich in α-linolenic acid, was $SSM > SMM > MMM > SMD > MMD > SDD > MDD > SMT_\alpha > MMT_\alpha = DDD > SDT_\alpha > MDT_\alpha >$

TABLE 3
Identification of the Triacylglycerols of Cloudberry Seed Oil, Evening Primrose Oil, and Borage Oil by Silver-Ion HPLC/APCI–MS

		Cloudberry seed oil				Evening primrose oil				Borage oil			
Peak number	Abbreviation[a]	ACN:n[b]	Triacylglycerol[c]	MS[d] %	ELSD[e] %	ACN:n	Triacylglycerol	MS %	ELSD %	ACN:n	Triacylglycerol	MS %	ELSD %
1		Not triacylglycerols		0.1	[f]	Not triacylglycerols		<0.1	0.5	Not triacylglycerols		<0.1	n.d.
2	*SSM*	50:1	**16:0/16:0/18:1**[g]	<0.1	0.3	50:1	**16:0/16:0/18:1**	0.1	n.d.[h]	50:1	**16:0/16:0/18:1**	0.3	0.3
		52:1	16:0/18:0/18:1			52:1	16:0/18:0/18:1			52:1	16:0/18:0/18:1		
		54:1	18:0/18:0/18:1			54:1	18:0/18:0/18:1			54:1	18:0/18:0/18:1		
		56:1	18:0/20:0/18:1							56:1	18:0/20:0/18:1		
		58:1	18:0/22:0/18:1							58:1	18:0/22:0/18:1		
3	*SMM*	50:2	16:0/16:1/18:1	0.2	0.5	52:2	**16:0/18:1/18:1**	0.1	n.d.	52:2	**16:0/18:1/18:1**	1.0	0.7
		52:2	**16:0/18:1/18:1**			54:2	18:0/18:1/18:1			54:2	18:0/18:1/18:1 + 16:0/18:1/20:1		
		54:2	18:0/18:1/18:1							56:2	18:0/18:1/20:1 + 16:0/18:1/22:1		
		56:2	20:0/18:1/18:1							58:2	18:0/18:1/22:1 + 16:0/18:1/24:1		
		58:2	22:0/18:1/18:1										
4	SSD					50:2	**16:0/16:0/18:2**	0.5	n.d.	50:2	**16:0/16:0/18:2**	1.3	0.4
						52:2	16:0/18:0/18:2			52:2	16:0/18:0/18:2		
						54:2	18:0/18:0/18:2			54:2	18:0/18:0/18:2 + 16:0/20:0/18:2		
										56:2	18:0/20:0/18:2 + 16:0/22:0/18:2		
										58:2	18:0/22:0/18:2 + 16:0/24:0/18:2		
5	*MMM*	54:3	18:1/18:1/18:1	0.5	3.2	54:3	18:1/18:1/18:1	0.3	0.3	54:3	**18:1/18:1/18:1**	1.8	0.6
										56:3	18:1/18:1/20:1		
										58:3	18:1/18:1/22:1		
										60:3	18:1/18:1/24:1		
6	*MMM*	50:3	16:1/16:1/18:1	<0.1	n.d.								
7	*SMD*	52:3	**16:0/18:1/18:2**	1.1	0.8	52:3	**16:0/18:1/18:2**	2.1	1.3	52:3	**16:0/18:1/18:2**	4.2	8.1
		54:3	18:0/18:1/18:2			54:3	18:0/18:1/18:2			54:3	18:0/18:1/18:2 + 16:0/20:1/18:2		
		56:3	20:0/18:1/18:2			56:3	20:0/18:1/18:2			56:3	18:0/20:1/18:2 + 16:0/22:1/18:2		
		58:3	22:0/18:1/18:2							58:3	18:0/22:1/18:2 + 16:0/24:1/18:2		
										60:3	18:0/24:1/18:2		
8	*SDD*[i]					52:4	16:0/18:2/18:2[i]	0.1	n.d.				
9	*MMD*	54:4	**18:1/18:1/18:2**	2.2	4.5	54:4	**18:1/18:1/18:2**	1.5	0.7	54:4	**18:1/18:1/18:2**	3.5	5.5
		56:4	18:1/20:1/18:2			56:4	18:1/20:1/18:2			56:4	18:1/20:1/18:2		
										58:4	18:1/22:1/18:2 + 20:1/20:1/18:2		
										60:4	18:1/24:1/18:2 + 20:1/22:1/18:2		
10	*SDD*	52:4	**16:0/18:2/18:2**	3.3	1.4	52:4	**16:0/18:2/18:2**	13.7	10.5	50:4	16:0/16:2/18:2	8.6	8.2
		54:4	18:0/18:2/18:2			54:4	18:0/18:2/18:2			52:4	**16:0/18:2/18:2**		
		56:4	20:0/18:2/18:2			56:4	20:0/18:2/18:2			54:4	18:0/18:2/18:2		
		58:4	22:0/18:2/18:2							56:4	20:0/18:2/18:2		
		60:4	24:0/18:2/18:2							58:4	22:0/18:2/18:2		
11	*SDT*$_\gamma$[i]					52:5	16:0/18:2/18:3[i]	0.3	n.d.				
12	*MDD + SMT*$_\gamma$	54:5	**18:1/18:2/18:2**	8.8	14.3	54:5	18:1/18:2/18:2	13.7	15.3	52:4	16:0/18:1/18:3	16.1	23.7
		56:5	20:1/18:2/18:2							54:4	16:0/20:1/18:3		
										56:4	16:0/22:1/18:3		
										58:4	16:0/24:1/18:3		
										54:5	**18:1/18:2/18:2**		
										56:5	20:1/18:2/18:2		
										58:5	22:1/18:2/18:2		
										60:5	24:1/18:2/18:2		
13	*SMT*$_\gamma$					52:4	16:0/18:1/18:3	0.2	n.d.				
14	*SMT*$_\alpha$ *+ DDD*[i]	52:4	**16:0/18:1/18:3**	1.1	1.9	54:6	18:2/18:2/18:2[i]	0.6	n.d.				
		54:4	18:0/18:1/18:3										
		56:4	20:0/18:1/18:3										
15	*MDT*$_\gamma$[i]					54:6	18:1/18:2/18:3[i]	0.2	n.d.				

(continued on next page)

Peak number	Abbreviation[a]	Cloudberry seed oil ACN:n[b]	Triacylglycerol[c]	MS[d] %	ELSD[e] %	Evening primrose oil ACN:n	Triacylglycerol	MS %	ELSD %	Borage oil ACN:n	Triacylglycerol	MS %	ELSD %
16	MMT_γ					54:5	18:1/18:1/18:3	0.3	n.d.	54:5	**18:1/18:1/18:3**	3.5	1.5
										56:5	18:1/20:1/18:3		
										58:5	18:1/22:1/18:3 + 20:1/20:1/18:3		
										60:5	18:1/24:1/18:3 + 20:1/22:1/18:3		
										62:5	20:1/24:1/18:3		
16′	MMT_α	54:5	**18:1/18:1/18:3**	3.3	n.d.								
		56:5	18:1/20:1/18:3										
17	$DDD + SDT_\gamma$	54:6	18:2/18:2/18:2	11.4	14.9	52:5	16:0/18:2/18:3	34.7	59.9	52:5	**16:0/18:2/18:3**	14.0	14.5
						54:6	**18:2/18:2/18:2**			54:6	18:2/18:2/18:2		
										54:5	18:0/18:2/18:3		
										56:5	20:0/18:2/18:3		
17′	SDT_α	52:5	**16:0/18:2/18:3**	3.6	1.5								
		54:5	18:0/18:2/18:3										
		56:5	20:0/18:2/18:3										
18	DDT_γ[i]					54:7	18:2/18:2/18:3[i]	1.0	n.d.				
19	MDT_γ					54:6	18:1/18:2/18:3	5.5	1.2	52:6	16:1/18:2/18:3	16.9	16.4
										54:6	**18:1/18:2/18:3**		
										56:6	20:1/18:2/18:3		
										58:6	22:1/18:2/18:3		
										60:6	24:1/18:2/18:3		
19′	MDT_α	54:6	**18:1/18:2/18:3**	13.7	17.4								
		56:6	20:1/18:2/18:3										
20	$DDT_\gamma + MDT_\alpha$[i]					54:7	**18:2/18:2/18:3**	0.2	n.d.	54:7	18:2/18:2/18:3	<0.1	n.d.
						54:6	18:1/18:2/18:3[i]			54:6	**18:1/18:2/18:3**		
21	$DDT_\gamma + ST_\gamma T_\gamma$					54:7	18:2/18:2/18:3	20.8	10.2	52:6	16:0/18:3/18:3	16.4	16.0
										54:7	**18:2/18:2/18:3**		
21′	DDT_α	54:7	**18:2/18:2/18:3**	21.1	27.7								
		56:7	18:2/20:2/18:3										
22	$ST_\alpha T_\alpha$	52:6	16:0/18:3/18:3	1.0	n.d.								
23	DDT_α[i]					54:7	18:2/18:2/18:3[i]	0.4	n.d.				
24	$DT_\gamma T_\gamma$					54:8	18:2/18:3/18:3	0.1	n.d.				
25	$MT_\gamma T_\gamma$					54:7	18:1/18:3/18:3	0.3	n.d.	52:7	16:1/18:3/18:3	2.3	0.4
										54:7	**18:1/18:3/18:3**		
										56:7	20:1/18:3/18:3		
										58:7	22:1/18:3/18:3		
										60:7	24:1/18:3/18:3		
25′	$MT_\alpha T_\alpha$	54:7	**18:1/18:3/18:3**	5.8	1.8								
		56:7	20:1/18:3/18:3										
26	$MDTe$					54:7	18:1/18:2/18:4						
27	$DT_\gamma T_\gamma$					54:8	18:2/18:3/18:3	2.9	0.2	54:8	18:2/18:3/18:3	9.4	3.7
27′	$DT_\alpha T_\alpha$	54:8	18:2/18:3/18:3	16.9	8.2								
28	$DDTe$					54:8	18:2/18:2/18:4	0.2	n.d.	54:8	18:2/18:2/18:4	0.1	n.d.
29	$T_\gamma T_\gamma T_\gamma$					54:9	18:3/18:3/18:3	0.1	n.d.	54:9	18:3/18:3/18:3	0.5	0.1
29′	$T_\alpha T_\alpha T_\alpha$	54:9	18:3/18:3/18:3	5.7	1.2								
30	$DT_\gamma Te$					54:9	18:2/18:3/18:4	<0.1	n.d.	54:9	18:2/18:3/18:4	0.1	n.d.

[a]Fatty acyl residues: S = saturated, M = monoenoic, D = dienoic, T_α = α-linolenic acid, T_γ = γ-linolenic acid, Te = tetraenoic fatty acid moiety. [b]ACN is the combined number of acyl carbons and n the combined number of double bonds in the acyl chains of a triacylglycerol. [c]The distribution of fatty acyl residues between *sn*-1, *sn*-2, and *sn*-3 positions has not been differentiated. [d]Proportions of triacylglycerols based on mass spectrometric (MS) detection. [e]Proportions of triacylgycerols based on evaporative light-scattering detection (ELSD). [f]The proportion was 23.4% of all peaks in ELSD chromatogram, %ELSD proportions presented here are calculated excluding the proportion of peak 1. [g]The triacylgycerol exhibiting the most abundant $[M + H]^+$ ion in the spectrum has been typed in bold font. [h]n.d. = Not detected or separated. [i]Tentative identification.

$DDT_\alpha \geq ST_\alpha T_\alpha > MT_\alpha T_\alpha > DT_\alpha T_\alpha > T_\alpha T_\alpha T_\alpha$, where S = saturated, M = monoenoic, and D = dienoic fatty acyl residue, and T_α = α-linolenoyl residue (Fig. 3A; Table 3). The elution order was similar to that reported earlier for linseed oil (6) with few exceptions. In the present study, one α-linolenoyl residue formed stronger interaction with silver ion than two linoleoyl residues, resulting in nearly baseline separations of MDD and SMT_α fractions, as well as DDD and SDT_α frac-

TABLE 4
Identification of the Triacylglycerols of Alpine Currant Seed Oil and Black Currant Seed Oil by Silver-Ion HPLC/APCI–MS

		Alpine currant seed oil				Black currant seed oil			
Peak number	Abbreviation[a]	ACN:n[b]	Triacylglycerol[c]	MS[d] (%)	ELSD[e] (%)	ACN:n	Triacylglycerol	MS (%)	ELSD (%)
1		Something other than triacylglycerols		0.1	4.2	Something other than triacylglycerols		<0.1	0.6
2	*SSM*					52:1 54:1	16:0/18:0/18:1 18:0/18:0/18:1	<0.1	0.1
3	*SMM*	52:2 54:2	**16:0/18:1/18:1**[f] 18:0/18:1/18:1	0.3	<0.1	52:2 54:2	**16:0/18:1/18:1** 18:0/18:1/18:1	0.1	0.1
4	*SSD*					52:2	16:0/18:0/18:2	<0.1	n.d.[g]
5	*MMM*	54:3	18:1/18:1/18:1	1.0	0.2	54:3	18:1/18:1/18:1	0.4	0.1
6	*SMD*	52:3 54:3	**16:0/18:1/18:2** 18:0/18:1/18:2	1.6	2.2	52:3 54:3	**16:0/18:1/18:2** 18:0/18:1/18:2	1.3	1.7
7	*MMD*	54:4	18:1/18:1/18:2	3.2	7.1	54:4 56:4	**18:1/18:1/18:2** 18:1/20:1/18:2	2.1	3.7
8	*SDD*	52:4 54:4	**16:0/18:2/18:2** 18:0/18:2/18:2	5.1	4.0	52:4 54:4	**16:0/18:2/18:2** 18:0/18:2/18:2	5.1	5.3
9	*MDD*	54:5	18:1/18:2/18:2	9.2	17.5	54:5 56:5	**18:1/18:2/18:2** 20:1/18:2/18:2	8.3	15.0
10	SMT_{γ}	52:4	16:0/18:1/18:3	0.6	n.d.	52:4	16:0/18:1/18:3	0.6	nd
11	SMT_{α}	52:4 54:4	**16:0/18:1/18:3** 18:0/18:1/18:3	1.0	0.4	52:4 54:4	**16:0/18:1/18:3** 18:0/18:1/18:3	0.5	0.2
12	MMT_{γ}	54:5	18:1/18:1/18:3	2.0	0.9	54:5 56:5	**18:1/18:1/18:3** 18:1/20:1/18:3	1.6	0.6
13	MMT_{α}	54:5	18:1/18:1/18:3	2.7	n.d.				
14	$SDT_{\gamma} + DDD + MMT_{\alpha}$	52:5 54:6	16:0/18:2/18:3 **18:2/18:2/18:2**	11.9	17.2	52:5 54:5 54:6	16:0/18:2/18:3 18:1/18:1/18:3 **18:2/18:2/18:2**	15.9	25.4
15	SDT_{α}	52:5 54:5	**16:0/18:2/18:3** 18:0/18:2/18:3	3.4	2.2	52:5 54:5	**16:0/18:2/18:3** 18:0/18:2/18:3	2.8	1.3
16	MDT_{γ}	54:6	18:1/18:2/18:3	4.6	3.8	54:6 56:6	**18:1/18:2/18:3** 20:1/18:2/18:3	6.1	5.6
17	MDT_{α}	54:6	18:1/18:2/18:3	7.8	12.1	54:6 56:6	**18:1/18:2/18:3** 20:1/18:2/18:3	5.2	4.7
18	DDT_{γ}					54:7	18:2/18:2/18:3	<0.1	n.d.
19	$DDT_{\gamma} + ST_{\gamma}T_{\gamma}$	52:6 54:7	16:0/18:3/18:3 **18:2/18:2/18:3**	6.1	4.6	52:6 54:7	16:0/18:3/18:3 **18:2/18:2/18:3**	11.6	13.6
20	DDT_{α}	54:7	18:2/18:2/18:3	12.3	14.7	54:7	18:2/18:2/18:3	11.1	12.9
21	$ST_{\alpha}T_{\alpha} + MT_{\gamma}T_{\gamma}$	52:6	16:0/18:3/18:3	0.3	n.d.	52:6 56:7	**16:0/18:3/18:3** 20:1/18:3/18:3	0.4	0.1
22	$MT_{\gamma}T_{\gamma}$	54:7	18:1/18:3/18:3	0.3	0.4	54:7	18:1/18:3/18:3	0.5	0.2
23	$MT_{\gamma}T_{\alpha} + MDTe$	54:7	18:1/18:3/18:3 + 18:1/18:2/18:4	3.3	1.5	54:7	18:1/18:2/18:4 + 18:1/18:3/18:3	2.6	0.9
24	$MT_{\alpha}T_{\alpha}$	54:7	18:1/18:3/18:3	2.0	0.6	54:7	18:1/18:3/18:3	0.8	0.1
25	$DT_{\gamma}T_{\gamma}$	54:8	18:2/18:3/18:3	1.0	n.d.	54:8	18:2/18:3/18:3	3.1	1.0
26	$DT_{\gamma}T_{\alpha} + DDTe$	54:8	18:2/18:3/18:3 + 18:2/18:2/18:4	6.3	2.4	54:8	18:2/18:3/18:3 + 18:2/18:2/18:4	8.3	4.0
27	$DT_{\alpha}T_{\alpha}$	54:8	18:2/18:3/18:3	6.1	2.5	54:8	18:2/18:3/18:3	4.1	1.2
28	$MT_{\gamma}Te$	54:8	18:1/18:3/18:4	0.2	0.1	54:8	18:1/18:3/18:4	0.2	<0.1
29	$MT_{\alpha}Te$	54:8	18:1/18:3/18:4	0.6	0.2	54:8	18:1/18:3/18:4	0.3	<0.1
30	$T_{\gamma}T_{\gamma}T_{\gamma}$	54:9	18:3/18:3/18:3	<0.1	n.d.	54:9	18:3/18:3/18:3	0.1	n.d.
31	$T_{\gamma}T_{\gamma}T_{\alpha} + DT_{\gamma}Te$	54:9	18:3/18:3/18:3 + 18:2/18:3/18:4	1.5	0.3	54:9	18:2/18:3/18:4 + 18:3/18:3/18:3	2.2	0.6
32	$T_{\gamma}T_{\alpha}T_{\alpha} + DT_{\alpha}Te$	54:9	18:2/18:3/18:4	3.2	0.9	54:9	18:2/18:3/18:4 + 18:3/18:3/18:3	2.7	0.6
33	$T_{\alpha}T_{\alpha}T_{\alpha}$	54:9	18:3/18:3/18:3	1.3	0.2	54:9	18:3/18:3/18:3	0.6	<0.1
34	*MTeTe*	54:9	18:1/18:4/18:4	<0.1	n.d.	54:9	18:1/18:4/18:4	<0.1	n.d.

(continued on next page)

Peak number	Abbreviation[a]	Alpine currant seed oil ACN:n[b]	Triacylglycerol[c]	MS[d] (%)	ELSD[e] (%)	Black currant seed oil ACN:n	Triacylglycerol	MS (%)	ELSD (%)
35	$T_\gamma T_\gamma Te$	54:10	18:3/18:3/18:4	<0.1	n.d.	54:10	18:3/18:3/18:4	0.1	n.d.
36	$T_\gamma T_\alpha Te$	54:10	18:3/18:3/18:4	0.6	0.2	54:10	18:3/18:3/18:4	0.7	0.1
37	$T_\alpha T_\alpha Te$	54:10	18:3/18:3/18:4	0.5	0.1	54:10	18:3/18:3/18:4	0.4	<0.1
38	$T_\gamma TeTe$					54:11	18:3/18:4/18:4	<0.1	n.d.
39	$T_\alpha TeTe$	54:11	18:3/18:4/18:4	0.1	n.d.	54:11	18:3/18:4/18:4	0.1	n.d.

[a]Fatty acyl residues: S = saturated, M = monoenoic, D = dienoic, T_α = α-linolenic acid, T_γ = γ-linolenic acid, Te = tetraenoic fatty acid moiety.
[b]ACN is the combined number of acyl carbons and n the combined number of double bonds in the acyl chains of a triacylglycerol.
[c]The distribution of fatty acyl residues between *sn*-1, *sn*-2, and *sn*-3 positions has not been differentiated.
[d]Proportions of triacylglycerols based on mass spectrometric (MS) detection.
[e]Proportions of triacylgycerols based on evaporative light scattering detection (ELSD).
[f]The triacylgycerol exhibiting the most abundant $[M + H]^+$ ion in the spectrum has been typed in bold font.
[g]n.d. = Not detected or separated.

tions. Furthermore, $ST_\alpha T_\alpha$ eluted slightly after DDT_α as a shoulder peak, and MMT_α and DDD eluted in the same peak. In the case of linseed oil (6), MDD co-eluted with SMT_α, and DDD co-eluted with SDT_α whereas MMT_α and DDD were well separated. Such differences in the elution order of triacylglycerols may be caused by minor differences in the column performance or in the analytical conditions. The most abundant triacylglycerols of cloudberry seed oil, separated by silver-ion HPLC, represented MDD, DDD, MDT_α, DDT_α, and $DT_\alpha T_\alpha$ fractions. The most abundant molecular species of these fractions were 18:1/18:2/18:2, 18:2/18:2/18:2, 18:1/18:2/18:3n-3, 18:2/18:2/18:3n-3, and 18:2/18:3n-3/18:3n-3, respectively (distribution of fatty acids between *sn*-1, *sn*-2, and *sn*-3 positions is not differentiated). Overall, 43 molecular species of triacylglycerols were identified from cloudberry seed oil.

(ii) Evening primrose oil. The general elution order of the triacylglycerols of evening primrose oil (Fig. 3B; Table 3) was much the same as that for cloudberry seed oil. In addition, evening primrose contained a small SSD fraction which eluted between SMM and MMM fractions. Differences were observed in the retention behavior of triacylglycerols containing trienoic fatty acyl residues. The presence of γ-linolenic acid resulted in co-elution of MDD and SMT_γ fractions, and DDD with SDT_γ, where T_γ = γ-linolenoyl residue. Furthermore, the MMT_γ fraction eluted before $DDD + SDT_\gamma$ fraction. Comparison of the retention times of the triacylglycerols of evening primrose oil with those of cloudberry seed oil showed that SMT_γ eluted before SMT_α, MMT_γ before MMT_α, SDT_γ before SDT_α, MDT_γ before MDT_α, DDT_γ before DDT_α, $MT_\gamma T_\gamma$ before $MT_\alpha T_\alpha$, $DT_\gamma T_\gamma$ before $DT_\alpha T_\alpha$, and $T_\gamma T_\gamma T_\gamma$ before $T_\alpha T_\alpha T_\alpha$. Thus, triacylglycerols containing γ-linolenoyl residues formed weaker interactions with silver ions than molecules containing α-linolenoyl residues. The general elution profile of the triacylglycerols of evening primrose oil was very similar to that reported by Christie (19). APCI–MS detection allowed achievement of information on the molecular composition of the minor constituents as well; therefore, more components were resolved and identified in the present study (39 molecular species). Identification of some minor fractions was only tentative, because a fraction yielding a similar mass spectrum was identified elsewhere in the chromatogram. Differences in the regiospecific distribution of fatty acyl residues in triacylglycerols as well as the presence of small amount of α-linolenic acid in evening primrose oil may partially explain this observation. In general, molecular species of triacylglycerols containing dienoic fatty acyl residues were typical constituents of evening primrose oil. The most abundant fractions separated by silver-ion HPLC were SDD, MDD, $DDD + SDT_\gamma$, and DDT_γ consisting of the most abundant triacylglycerols 16:0/18:2/18:2, 18:1/18:2/18:2, 18:2/18:2/18:2, and 18:2/18:2/18:3n-6, respectively.

(iii) Borage oil. The analysis of the triacylglycerols of borage oil by silver-ion HPLC/APCI–MS (Fig. 3C; Table 3) resulted in a more complex separation compared with evening primrose oil. The main elution order of the triacylglycerols of both γ-linolenic acid oils was similar. An additional feature of borage oil was the presence of $ST_\gamma T_\gamma$ which eluted in the same peak as DDT_γ. Both borage oil and evening primrose oil contained small amounts of triacylglycerols with tetraenoic fatty acyl residues (Te), such as $DDTe$ and $DT_\gamma Te$. Several of the peaks in the reconstructed ion chromatogram of borage oil showed a deformed peak shape. This was actually caused by partial separation of molecular species within a single peak representing equally unsaturated molecules. In general, the molecules eluted in the descending order of molecular weight, e.g., the elution order of triacylglycerols within the MMM fraction (peak number 5) was 60:3 ($[M + H]^+$ ion, m/z 969.9) followed by 58:3 (m/z 941.9), 56:3 (m/z 913.8), and finally 54:3 (m/z 885.8). Moreover, the elution order of molecular species was 18:1/18:1/24:1 > 18:1/18:1/22:1 > 18:1/18:1/20:1 > 18:1/18:1/18:1. Similar separations were determined within several other peaks containing saturated and/or monoenoic fatty acid moieties, the chain length of which varied from C_{16} to C_{24} in borage oil. The same phenomenon was also observed with other oils, but to lesser extent. Thus, the separation of triacylglycerols by silver-ion

HPLC is also affected by the chain length of the fatty acyl residues. Adlof (32) has suggested recently that this kind of separation may be due to substrate interaction with the free silanol groups or with the phenylsulphonic acid groups of the stationary phase. The most abundant triacylglycerol fractions of borage oil separated by silver-ion HPLC were $MDD + SMT_\gamma$, $DDD + SDT_\gamma$, MDT_γ, and $ST_\gamma T_\gamma + DDT_\gamma$. The most abundant molecular species of these fractions were 18:1/18:2/18:2, 16:0/18:2/18:3n-6, 18:1/18:2/18:3n-6, and 18:2/18:2/18:3n-6, respectively. In this study, 79 molecular species of triacylglycerols were identified from borage oil, which shows the high complexity of the sample.

(iv) Alpine currant and black currant seed oils. The RIC of the triacylglycerols of both alpine currant and black currant seed oil (Fig. 4), achieved by silver-ion HPLC/APCI–MS, were more complicated than those discussed above. The triacylglycerols of the currants were separated into 39 peaks, representing molecules from *SSM* (one double bond) to *TTeTe* (11 double bonds). The identification of the molecular species and their proportions are presented in Table 4. Altogether 44 molecular species of triacylglycerols were identified from alpine currant, and 56 from black currant seed oil. The more complex composition of black currant was mainly due to the presence of C_{20} fatty acids in the oil. The elution order of the less unsaturated triacylglycerols of the currants was similar to that achieved with α-linolenic acid and γ-linolenic acid containing oils, i.e., molecules eluted in the order of *SSM* > *SMM* > *SSD* > *MMM* > *SMD* > *MMD* > *SDD* > *MDD*. Both isomers of triacylglycerols containing either one α-linolenoyl or one γ-linolenoyl residue were separated from alpine currant and black currant seed oils. The isomer pairs of SMT_γ–SMT_α, MMT_γ–MMT_α, SDT_γ–SDT_α, MDT_γ–MDT_α, DDT_γ–DDT_α, $MT_\gamma Te$–$MT_\alpha Te$, $DT_\gamma Te$–$DT_\alpha Te$, and $T_\gamma TeTe$–$T_\alpha TeTe$ were well separated so that the molecule containing γ-isomer had a shorter retention time compared with the molecule containing α-isomer. Triacylglycerols with SDT_γ, DDD, and MMT_α structures eluted in the same peak which was in accordance with the results achieved with cloudberry, evening primrose, and borage oils. Molecules containing two linolenoyl residues were separated up to three isomers, which eluted in the order of $XT_\gamma T_\gamma$ followed by $XT_\gamma T_\alpha$ followed by $XT_\alpha T_\alpha$, where X is a fatty acyl residue different from a linolenoyl residue. The elution order of isomers was $ST_\gamma T_\gamma > ST_\alpha T_\alpha$, $MT_\gamma T_\gamma > MT_\gamma T_\alpha > MT_\alpha T_\alpha$, $DT_\gamma T_\gamma > DT_\gamma T_\alpha > DT_\alpha T_\alpha$, and $T_\gamma T_\gamma Te > T_\gamma T_\alpha Te > T_\gamma T_\alpha Te$. Trilinolenoylglycerols were separated into four isomers which eluted in the order of $T_\gamma T_\gamma T_\gamma$ followed by $T_\gamma T_\gamma T_\alpha$, $T_\gamma T_\alpha T_\alpha$, and finally $T_\alpha T_\alpha T_\alpha$.

Triacylglycerols of both currants consisted mainly of molecules with 52 or 54 acyl carbons. In addition, triacylglycerols with 56 acyl carbons were determined in black currant seed oil, which was in accordance with the determined fatty acid composition (approximately 1 mol% C_{20} acids; Table 1) and other studies (25). The elution profiles of triacylglycerols of black currant and alpine currant seed oils achieved by silver-ion HPLC were very similar as were the molecular species compositions of the separated peaks. However, differences were determined in the relative amounts of triacylglycerol fractions (Table 4). The most abundant triacylglycerols of alpine currant eluted in the fractions representing MDD, $SDT_\gamma + DDD$, MDT_α, and DDT_α molecules. The most abundant molecular species of these fractions were 18:1/18:2/18:2, 18:2/18:2/18:2, 18:1/18:2/18:3n-3, 18:2/18:2/18:3n-3, respectively. The main fractions of black currant seed oil were MDD, $SDT_\gamma + DDD + MMT_\alpha$, $DDT_\gamma + ST_\alpha T_\gamma$, and DDT_α, and the most abundant molecular species in these fractions were 18:1/18:2/18:2, 18:2/18:2/18:2, 18:2/18:2/18:3n-6, and 18:2/18:2/18:3n-3, respectively. Differences were observed in the proportions of molecules containing α- and/or γ-linolenoyl residues within equally unsaturated triacylglycerols. Alpine currant contained substantially more 18:1/18:2/18:3n-3 (MDT_α, peak number 17) compared with 18:1/18:2/18:3n-6 (MDT_γ, peak number 16), whereas the proportions of these molecular species in black currant seed oil were nearly equal. Similarly, the relative amount of 18:2/18:2/18:3n-6 + 16:0/18:3n-6/18:3n-6 ($DDT_\gamma + ST_\gamma T_\gamma$, peak number 19) in alpine currant was clearly less than that of 18:2/18:2/18:3n-3 (DDT_α, peak number 20), whereas the corresponding proportions were nearly the same in black currant seed oil. Such differences were expected: the ratio of the proportions of γ-linolenic acid to α-linolenic acid was ~1:1 in black currant and ~1:2 in alpine currant seed oil. Among *DTT* + *DDTe*-molecules, alpine currant contained equal proportions of 18:2/18:3n-6/18:3n-3 + 18:2/18:2/18:4 (peak number 26) and 18:2/18:3n-3/18:3n-3 (peak number 27), whereas the relative amount of 18:2/18:3n-6/18:3n-6 (peak number 25) was substantially low. The corresponding triacylglycerols of black currant seed oil were richer in γ-linolenic acid containing molecules, i.e., 8% $DT_\gamma T_\alpha + DDTe$, 3% $DT_\gamma T_\gamma$ and 4% $DT_\alpha T_\alpha$ according to quantitative estimates produced by APCI–MS.

DISCUSSION

The composition and structure of oils containing γ-linolenic acid are of increasing interest due to their possible positive physiological properties, such as in the treatment of atopic eczema and diabetic neuropathy, and revealing breast pain and premenstrual syndrome (33). The physiological effects of γ-linolenic acid oils have mostly been studied with evening primrose oil and borage oil. The properties of currant seed oils have not been studied intensively, although currants contain significant amounts of γ-linolenic acid and also stearidonic acid, both of which are Δ-6-desaturation products. The medical properties of these oils are due to biochemical molecular reactions, and thus it is important to achieve information on the molecular composition of triacylglycerols. The chromatographic separation of triacylglycerols containing α- and/or γ-linolenic acid moieties is essential, because triacylglycerols containing these fatty acids cannot be distinguished as such by MS detection. In the present study, silver-ion HPLC has been shown to be an efficient method for the sepa-

ration of α- and γ-linolenic acid containing triacylglycerols in complex mixtures. Furthermore, molecular species containing stearidonic acid were determined. The on-line detection of the separated compounds by APCI–MS made it possible to achieve information both on the molecular weight and the molecular association of fatty acids of the triacylglycerol molecular species. The *m/z* values of $[M + H]^+$ ions define unambiguously the number of acyl carbons and double bonds in the molecule. The identification of relatively saturated triacylglycerols may be problematic, because they do not yield any or only a weak $[M + H]^+$ ion. Therefore, the identification of, for example, milk-fat triacylglycerols may be more difficult, and additional information, such as retention time data, will be required. The mass spectra of triacylglycerols achieved by APCI–MS are affected by the structure of the molecule, e.g., the degree of unsaturation and the regiospecific distribution of fatty acids between the *sn*-2 and *sn*-1/3 positions, as well as by the instrumental parameters of the mass spectrometer, such as capillary and vaporizer temperatures. At the present stage of the method, no attempt was made for distinction of regioisomeric triacylglycerols in complex mixtures, although differences were measured in the relative abundances of $[M - RCO_2]^+$ ions formed by a loss of a fatty acyl residue from the *sn*-2 position and the *sn*-1/3 positions of triacylglycerol reference components. The regiospecific analysis of triacylglycerols would require extensive optimization and calibration of the system. The silver-loaded cation-exchange column showed potential to separate, at least partially, unsaturated regioisomeric triacylglycerols by using a ternary solvent gradient. Similar results have been reported earlier using an isocratic eluent system consisting of acetonitrile and hexane (31). It may be possible to separate regioisomers of relatively simple triacylglycerol mixtures with an optimized silver-ion HPLC analysis as reported elsewhere (7). However, the samples of the present study, especially currants, seem to be far too complex for such separation. The quantitative estimates presented in this study were based on uncalibrated systems, i.e., no corrections to the raw data were done according to different response factors of molecular species. The quantitative analysis of triacylglycerols requires to be thoroughly studied. The presence of several molecular species within a single chromatographic peak makes it difficult to quantitate single triacylglycerols. The molecular weights of components eluting in the same peak in silver-ion HPLC may be differentiated according to their $[M + H]^+$; however, a single $[M - RCO_2]^+$ ion may be a fragment formed by a loss of one fatty acyl residue from several molecules. It may be possible to modify the elution system and ionization conditions so that triacylglycerols would yield only abundant ions consisting of the whole molecule without fragmentation. Unfortunately, in such a situation the information on molecular association of fatty acids is lost. Although quantitation may cause difficulties, the on-line detection of triacylglycerols by APCI–MS after separation by silver-ion HPLC provides valuable information for structure elucidation. Mass spectra yield information on both the molecular weight and molecular association of fatty acids of triacylglycerols. Similar data cannot be achieved by GC analysis of fatty acid methyl esters of the collected fractions separated by silver-ion HPLC.

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