Method for Characterization of Triacylglycerols and Fat-Soluble Vitamins in Edible Oils and Fats by Supercritical Fluid Chromatography

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ABSTRACT: This study demonstrates the usefulness of capillary supercritical fluid chromatography (SFC) for the characterization of triacylglycerols of edible oils and fats. Triacylglycerols were separated according to the acyl carbon number and the degree of unsaturation on a 25% cyanopropyl/25% phenyl/50% methylpolysiloxane stationary phase. Valuable information concerning the triacylglycerol composition of berry oils was obtained, despite the overlapping of certain triacylglycerol peaks. Simultaneous analysis of fat-soluble vitamins and triacylglycerols is not practical by capillary SFC with flame-ionization detection because of the low concentration of naturally-occurring fat-soluble vitamins in edible oils. Therefore, higher loading of the sample, which led to overloading of triacylglycerols, was required to get reasonable peaks for fat-soluble vitamins. The method was applied to the characterization of triacylglycerols and tocopherols in sea buckthorn pulp and seed oil, and cloudberry seed oil without any sample purification prior to SFC. In addition, the stationary phase proved useful for separating the more complex mixtures of triacylglycerols found in milk fat and in fish oil.

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KEY WORDS: Berry pulp oil, capillary supercritical fluid chromatography, cloudberry, fish oil, mass spectrometry, milk fat, sea buckthorn, seed oil, tocopherols, triacylglycerols.

The analysis of various edible oils typically involves separation by high-performance liquid chromatography (HPLC) or gas chromatography (GC). In capillary-column GC, triacylglycerols are separated either according to the combined number of acyl carbons on nonpolar stationary phases or according to both the number of acyl carbons and double bonds on polar stationary phases (1). HPLC analysis on reversed-phase columns results in the elution of triacylglycerols in ascending order of the combined chainlengths of the fatty acyl residues, with each of the double bonds reducing the retention time of the solutes by an equivalent of approximately two methylene groups (2). HPLC analysis of triacylglycerols on silver ion columns offers the possibility of separating molecules mainly according to the degree of unsaturation (3,4). However, neither GC nor HPLC has been applied to the simultaneous analysis of triacylglycerols and trace levels of fat-soluble vitamins.

The analysis of triacylglycerols by capillary supercritical fluid chromatography (SFC) is most commonly demonstrated as a separation based on the acyl carbon number of the individual triacylglycerol, resulting in quite a highly resolved chromatogram. This kind of separation has been demonstrated with palm kernel oil (5), soybean oil (6), seed oils (7,8), butterfat (7,9,10), and fish oils (7,8,11). In some situations, as in fish oil process control, simultaneous separation according to lipid classes and the acyl carbon number of triacylglycerols together with tocopherols, cholesterol, and free fatty acids by SFC is advantageous when compared with HPLC and high-temperature capillary GC (12). Borch-Jensen *et al.* (13) also have applied a 50% cyanopropylphenyl/50% methylpolysiloxane stationary phase (DB-225, 20 m × 100 μm i.d. with 0.1 μm film; J&W Scientific, Folsom, CA) to SFC analysis of fish oil. The separation was clearly affected, not only by the acyl carbon number, but also by the interaction between the double bonds of the triacylglycerols and the cyano groups of the stationary phase. However, peak resolution and analysis time were unsatisfactory.

In this study, triacylglycerols were separated according to both the number of acyl carbons and the number of double bonds by using capillary SFC with a polar stationary phase. The method was applied to screening triacylglycerol compositions of pulp and seed oils with high repeatability. In addition, the screening of tocopherols in these oils without sample purification is demonstrated. The potential of the used stationary phase to separate more complex mixtures of triacylglycerols, such as milk fat and fish oil, is also briefly discussed.

EXPERIMENTAL PROCEDURES

Materials. Reference compounds of α-, β-, γ-, and δ-tocopherols (Merck, Darmstadt, Germany) were diluted in *n*-hexane (Rathburn Chemicals Ltd., Walkerburn, Scotland), and reference compounds of triacylglycerols (Sigma Chemical Co., St. Louis, MO) and vitamin D_3 (Fluka Chemie AG, Buchs, Switzerland) in dichloromethane (Merck). Sea buckthorn (*Hippophaë rhamnoides*) seed and pulp oil and cloudberry (*Rubus chamaemorus*) seed oil were extracted with supercritical carbon dioxide at Flavex (Rehlingen, Germany).

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Lipids were isolated by centrifugation and phase exchange with sodium sulfate from a bovine milk sample collected after 144 h of delivery. Rainbow trout (*Salmo gairdneri*) lipids were extracted with a modified procedure of Bligh and Dyer as described by Christie (14). All samples were stored under nitrogen at below 0°C to avoid oxidation, and were diluted (1:35, vol/vol) in dichloromethane before chromatography.

Methods. The analyses were carried out with a Lee Scientific Series 600 supercritical fluid chromatograph (Dionex, Salt Lake City, UT) equipped with a flame-ionization detector (FID). The temperature of the FID was held at 340°C, and nitrogen was used as a make-up gas. A Valco switching valve (Valco Instruments, Inc., Houston, TX) with an internal sample loop volume of 1.0 µL was used for timed split/dynamic split injections. The timed split loop–open time was 0.7 s for berry pulp and seed oils and 0.3 s for others, unless otherwise mentioned. Frit restrictors (30 cm × 50 µm i.d.; Dionex) were installed both at the dynamic split outlet and at the detector end of the analytical column. SFC-grade CO_2 (Scott Specialty Gases, Plumsteadville, PA) was used as a carrier fluid at a column flow rate of 0.37 mL min^{-1} as measured with propane at the initial conditions of the chromatographic program. Columns used for SFC were SB-Octyl-50 (Dionex), 10 m × 50 µm i.d. with 0.25 µm film, and SB-Cyanopropyl-25 (Dionex), 10 m × 50 µm i.d. with 0.25 µm film. A linear density gradient from 0.140 g mL^{-1} with 0.010 g mL^{-1} min^{-1} was used for elution at a constant temperature of 140°C for both columns. Mass spectrometric data were obtained on a Finnigan MAT (San Jose, CA) TSQ-700 triple quadrupole mass spectrometer with ammonia negative ion chemical ionization at ammonia pressure of 8500 mtorr at ion source temperature of 200°C, as reported elsewhere (15). Sample introduction was performed with a direct probe.

RESULTS AND DISCUSSION

Separation of triacylglycerols according to the combined number of acyl carbons is relatively easy on a nonpolar liquid phase. The saturated triacylglycerols, differing by one methylene group, were almost baseline-separated in an SB-Octyl-50 (50% octyl/50% methylpolysiloxane) stationary phase. In addition to the acyl carbon number, the stationary phase effects a partial separation of triacylglycerols according to the degree of unsaturation as a function of polarity (Fig. 1A). In comparison with saturated triacylglycerols, the unsaturated triacylglycerols have reduced retention on the stationary phase, resulting in overlapping of, e.g., 52:0 and some unsaturated triacylglycerols with 54 acyl carbons. Although such overlapping occurs, this stationary phase allows adequate group separation based on acyl carbon numbers for most purposes, especially for pulp and seed oils that do not contain significant amounts of saturated triacylglycerols. The chromatograms of sea buckthorn pulp and seed oil represent the separation of relatively simple natural mixtures of triacylglycerols (Fig. 1B and 1C). The separation of cloudberry seed oil was similar to that of sea buckthorn seed oil and, therefore, is not presented here.

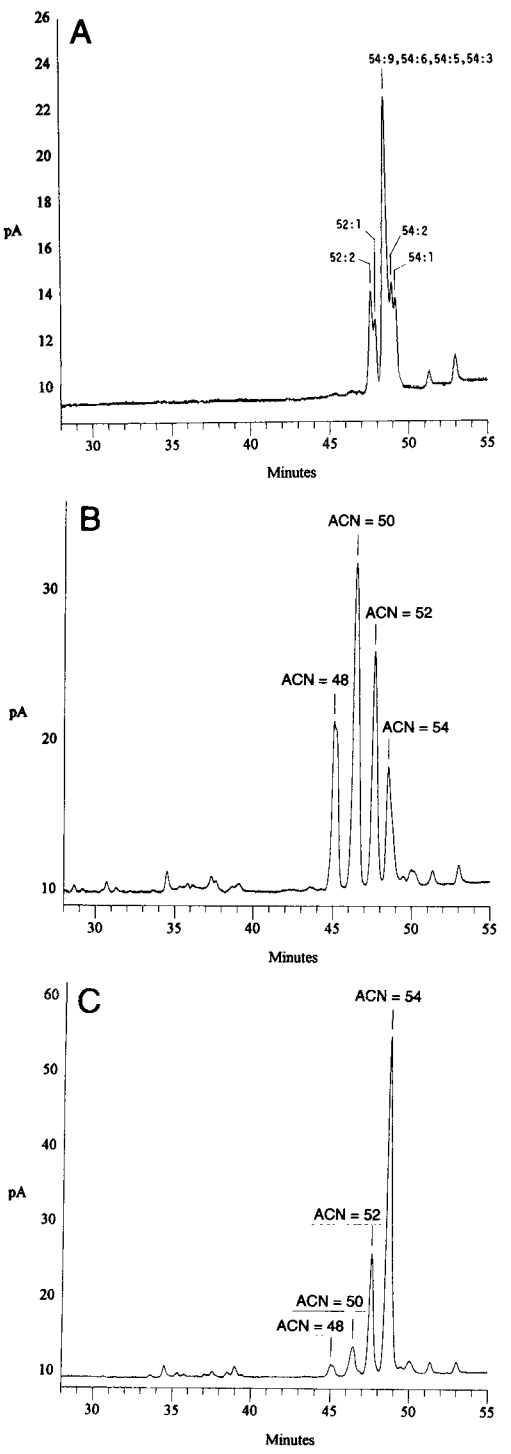

FIG. 1. The separation of triacylglycerols on an SB-Octyl-50 (Dionex, Salt Lake City, UT) column: A) a mixture of unsaturated triacylglycerols, B) sea buckthorn pulp oil, and C) sea buckthorn seed oil. ACN = acyl carbon number.

SFC of milk fat and fish oil samples was complex due to the composition of these samples (Fig. 2). Nevertheless, triacylglycerols of milk fat were separated into groups that represent different acyl carbon numbers. The separation was sim-

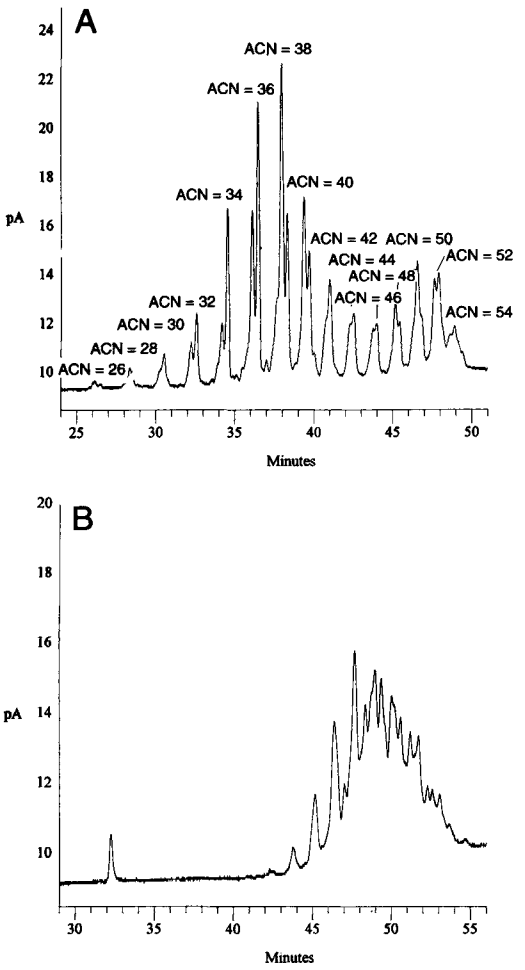

FIG. 2. The separation of triacylglycerols on an SB-Octyl-50 column: A) milk fat, and B) rainbow trout oil. Abbreviation and company source as in Figure 1.

ilar to that obtained by GC on a nonpolar stationary phase reported by Kuksis (16). The SB-Octyl-50 column was not able to separate rainbow trout oil triacylglycerols because of the presence of highly unsaturated triacylglycerols with more than 10 double bonds. In general, the nonpolar stationary phase yields valuable information of the acyl carbon distribution. However, often more detailed data about the triacylglycerol composition are needed.

The main effort of this study concentrated on the development of a reliable method for the screening of triacylglycerols and tocopherols in edible oils by capillary SFC with a 25% cyanopropyl/25% phenyl/50% methylpolysiloxane stationary phase and FID. The separation of triacylglycerols on the stationary phase used is based on the combined number of acyl carbons and the degree of unsaturation. Screening of triacylglycerols and tocopherols in the same oil matrix is difficult to obtain with a single chromatographic run because the low concentration of tocopherols requires higher loading of the sample, which definitely ruins the chromatography of triacylglycerols due to the low capacity of 50-μm i.d. capillary columns.

The chromatograms of sea buckthorn pulp and seed oil, determined on the cyanopropyl phase, are shown in Figures 3A and 3B, respectively. The separation of cloudberry seed oil was similar to that of sea buckthorn seed oil. The triacylglycerol composition of sea buckthorn pulp oil is clearly different from the seed oil, as previously observed in the chromatograms obtained on the nonpolar column (Fig. 1). The main components of the pulp oil were triacylglycerols with 48, 50, and 52 acyl carbons, whereas 54-acyl carbon triacylglycerols were the main components in the sea buckthorn and the cloudberry seed oil. The peaks were nearly separated on the baseline, but the number of peaks in the chromatograms was less than the number of molecular species recorded by mass spectrometric analysis (Table 1). This was due to the fact that certain triacylglycerols were overlapping each other in the chromatograms. The broadness and deformed shape of some peaks were indications of nonuniform retention of different compounds within a single peak.

Mixtures of pure compounds (see Fig. 3C and 3D) were used to confirm the elution order of the triacylglycerols, as well as for spiking them into the berry oils. The resolution between the saturated triacylglycerols differing by one methylene group was typically 1.1. Similar resolution was achieved with unsaturated triacylglycerols having an identical number of acyl carbons but differing by one double bond. The resolution tended to increase with an increasing number of double bonds, e.g., the resolution between 54:5 and 54:6 was already up to 2.0. The retention times of individual compounds in a mixture of pure triacylglycerols were somewhat lower than those of the corresponding compounds in the mixtures to be analyzed. This gives a clue as to how triacylglycerols may affect the stationary phase and act as a part of the stationary phase, resulting in slightly slower elution of individual triacylglycerols. Therefore, great care must be taken if peaks are identified only by using the retention times of reference compounds. Comparison of the difference in retention times between saturated and unsaturated triacylglycerols in Table 2 led to the assumption that the used stationary phase interacted slightly stronger with double bonds *via* the free electron pair of the nitrogen of the cyano group rather than a dominating polar interaction with unsaturated triacylglycerols, for example $\Delta t_{R(54:0,52:0)} = 0.72$ min and $\Delta t_{R(54:1,52:1)} = 0.84$ min. The mass spectrometric data of the respective edible oils overviewed in Table 1 were also used to assist peak identification.

Table 3 presents the data of the retention times and the proportions of triacylglycerols of pulp and seed oils studied. The data showed good retention time repeatability for each berry oil. In addition, the proportions of triacylglycerols based on the peak areas had good repeatability with the exception of peaks with a relative peak area less than 3.0% of the total.

Some of the triacylglycerols in berry oils that differed in their acyl carbon number and in the degree of unsaturation overlapped each other, for example, 52:4, 54:3, and 56:2. However, the overlapping was incomplete with triacylglycerols having from 48 to 52 acyl carbons and less than four double bonds, which are seen as slightly broadened peaks in

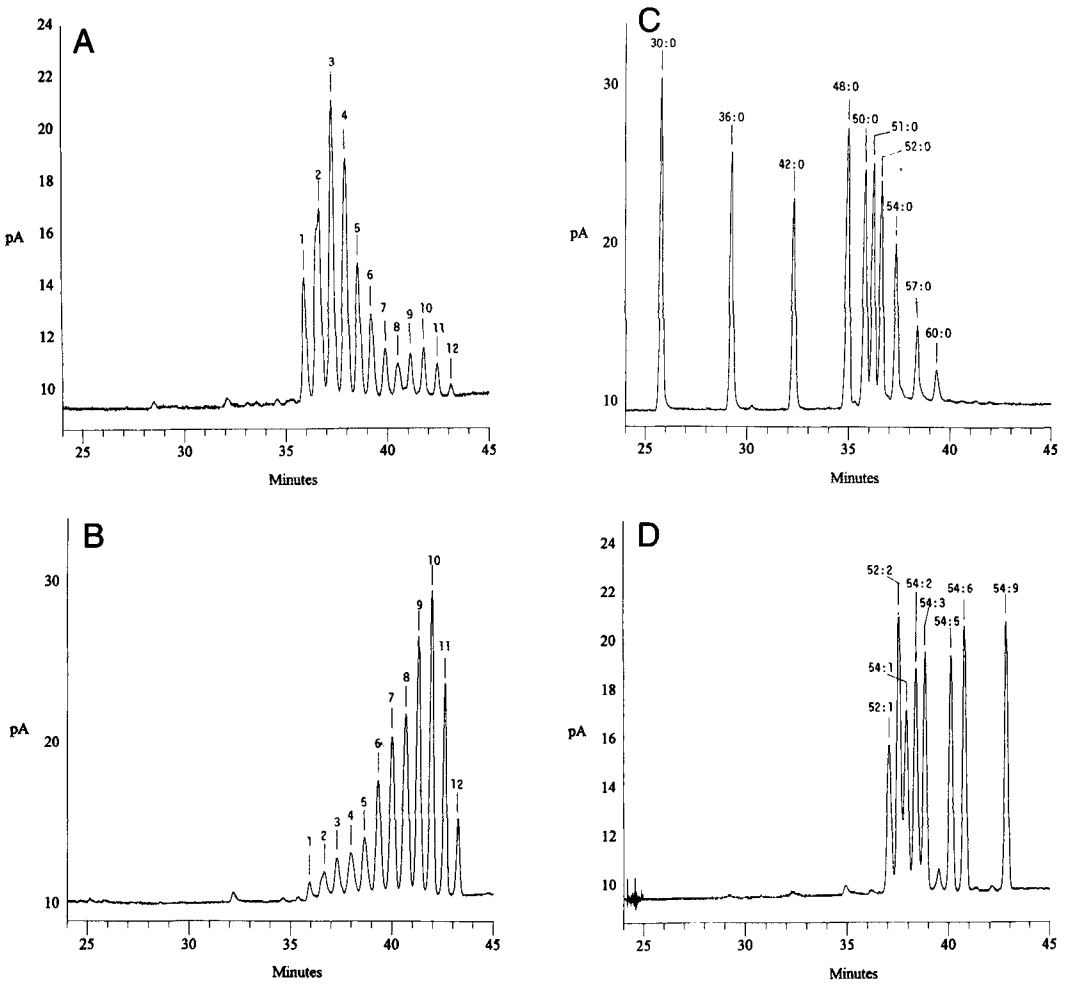

FIG. 3. The separation of triacylglycerols on an SB-Cyanopropyl-25 (Dionex, Salt Lake City, UT) column: A) sea buckthorn pulp oil, B) sea buckthorn seed oil, C) a mixture of saturated triacylglycerols, and D) a mixture of unsaturated triacylglycerols. For peak identification, see Table 3.

the chromatograms of sea buckthorn pulp and seed oil in Figure 3. The peak identifications were partially tentative for the 48 and 50 acyl carbon-containing triacylglycerols because the retention time difference of triacylglycerols decreased along the linear density ramp, with overlapping becoming more complete.

The elution temperature and the density program were optimized for separating triacylglycerols of berry oils in reasonable time with good resolution, i.e., relatively narrow peak shapes. The use of a slower ramp in the linear density program or lower temperature does not necessarily result in better resolution due to peak broadening. Higher elution temperatures, even with the minimum linear density ramp, will result in great loss of resolution because of the strongly increased diffusion in the separation medium.

Figure 4 shows, as an example, the similarity of the profiles for the proportions of triacylglycerols in cloudberry seed oil according to SFC–FID and mass spectrometry (MS) data. The proportions based on SFC–FID analysis were calculated according to the peak areas, presuming that the response of unsaturated triacylglycerols containing 48, 50, 52, or 54 acyl carbons on FID did not differ much from each other. The proportions of triacylglycerols obtained from MS analysis were calculated according to the ^{13}C corrected intensities of the deprotonated molecular ions of triacylglycerols in the averaged spectra. Profiles for the proportions of triacylglycerols according to the SFC–FID and MS data were also similar for sea buckthorn pulp and seed oil, which supported the assumption of the elution order, although the data were not directly comparable.

The 25% cyanopropyl/25% phenyl/50% methylpolysiloxane stationary phase also permitted efficient separation of triacylglycerols of milk fat (Fig. 5A). The profile of milk fat obtained on the SB-Cyanopropyl-25 column was similar to that obtained on the SB-Octyl-50 column (Fig. 2), except for better resolution, especially among the later-eluting peaks. The similarity of the chromatograms is explained by the high content of saturated triacylglycerols in milk fat. Although the

TABLE 1
Mass Spectrometric Data of the Proportions of Triacylglycerols of Sea Buckthorn Pulp and Seed Oil, and Cloudberry Seed Oil

	Sea buckthorn pulp oil		Sea buckthorn seed oil		Cloudberry seed oil	
Triacylglycerol	Proportion[a] (%)	SD	Proportion[a] (%)	SD	Proportion[a] (%)	SD
48:3	2.15	0.03	0.31	0.02	—	—
48:2	12.65	0.32	1.80	0.07	—	—
48:1	9.45	0.14	1.40	0.04	—	—
48:0	0.45	0.04	—	—	—	—
50:4	1.19	0.03	0.39	0.00	—	—
50:3	8.05	0.23	1.61	0.03	0.39	0.03
50:2	20.70	0.37	3.86	0.28	0.41	0.02
50:1	10.93	0.31	1.95	0.09	—	—
52:6	0.36	0.04	2.46	0.08	1.25	0.05
52:5	0.95	0.01	5.32	0.17	2.51	0.04
52:4	2.40	0.06	6.48	0.17	2.73	0.07
52:3	6.94	0.15	4.54	0.07	1.41	0.05
52:2	10.24	0.25	3.13	0.31	0.72	0.03
52:1	0.73	0.08	0.36	0.05	—	—
54:9	0.40	0.02	3.10	0.16	5.14	0.11
54:8	1.03	0.04	9.06	0.14	14.37	0.34
54:7	1.61	0.09	14.80	0.55	22.56	0.33
54:6	1.62	0.11	14.37	0.69	21.52	0.48
54:5	1.31	0.07	10.36	0.33	12.64	0.30
54:4	1.11	0.04	5.55	0.25	4.90	0.13
54:3	1.98	0.04	2.41	0.05	1.80	0.12
54:2	0.63	0.04	0.79	0.03	0.33	0.09
56:7	—	—	—	—	0.57	0.03
56:6	—	—	—	—	1.20	0.04
56:5	—	—	0.44	0.05	1.44	0.04
56:4	—	—	0.69	0.04	1.10	0.04
56:3	—	—	0.79	0.08	0.56	0.05
56:2	—	—	0.68	0.12	—	—
56:1	—	—	0.47	0.08	—	—
58:4	—	—	—	—	0.31	0.10
trace[b]	3.29	0.56	2.92	0.39	2.07	0.30

[a]Four replicates.
[b]The combined proportion of components corresponding to less than 0.3% of the total is reported as trace.

TABLE 2
Retention Times of Pure Triacylglycerols on an SB-Cyanopropyl[a] Column

Saturated triacylglycerols			Unsaturated triacylglycerols		
Compound	t_R (min)[b]	SD	Compound	t_R (min)[b]	SD
30:0	25.78	0.04	52:1	36.99	0.05
36:0	29.24	0.02	52:2	37.47	0.05
42:0	32.27	0.02	54:1	37.83	0.10
48:0	34.94	0.04	54:2	38.28	0.09
50:0	35.82	0.04	54:3	38.74	0.07
51:0	36.18	0.06	54:5	40.00	0.06
52:0	36.57	0.06	54:6	40.66	0.06
54:0	37.29	0.05	54:9	42.70	0.05
57:0	38.31	0.03			
60:0	39.28	0.01			

[a]From Dionex (Salt Lake City, UT).
[b]Five replicates.

TABLE 3
Retention Times and Proportions of Triacylglycerols in Sea Buckthorn Pulp and Seed Oil and in Cloudberry Seed Oil

Peak number	Compound[a] major/minor(s)	t_R[b] (min)	SD	Proportion (%)	SD
		Sea buckthorn pulp oil			
1	48:1	35.93	0.03	8.03	0.05
2	48:2/50:1[c]	36.71	0.03	19.77	0.10
3	50:2/48:3, 52:1	37.34	0.03	21.63	0.06
4	52:2/50:3	37.99	0.03	19.15	0.37
5	52:3/50:4, 54:2	38.61	0.03	9.77	0.05
6	52:4/54:3	39.24	0.03	6.22	0.09
7	54:4/52:5	39.89	0.03	3.85	0.06
8	54:5/52:6	40.49	0.03	3.06	0.19
9	54:6	41.11	0.03	3.04	0.03
10	54:7	41.77	0.03	3.24	0.03
11	54:8	42.44	0.03	1.79	0.14
12	54:9	43.13	0.02	0.66	0.04
		Sea buckthorn seed oil			
1	48:1	35.92	0.02	1.29	0.03
2	50:1/48:2	36.66	0.03	2.99	0.19
3	50:2/52:1, 48:3	37.28	0.03	3.55	0.16
4	52:2/50:3	37.96	0.03	4.55	0.11
5	52:3/54:2, 50:4, 56:1	38.63	0.03	5.19	0.09
6	52:4/54:3, 56:2	39.30	0.04	8.78	0.06
7	54:4/52:5, 56:3	39.98	0.04	11.70	0.10
8	54:5/52:6, 56:4	40.64	0.05	14.30	0.11
9	54:6/56:5	41.27	0.04	16.36	0.13
10	54:7	41.94	0.05	16.84	0.15
11	54:8	42.58	0.04	10.57	0.10
12	54:9	43.22	0.03	3.72	0.06
		Cloudberry seed oil			
4	52:2/50:3	37.71	0.12	1.37	0.12
5	52:3/54:2	38.45	0.11	2.05	0.40
6	52:4/54:3	39.07	0.09	4.59	0.23
7	54:4/52:5, 56:3	39.77	0.08	8.56	0.12
8	54:5/52:6, 56:4	40.42	0.06	14.79	0.25
9	54:6/56:5, 58:4	41.11	0.06	22.52	0.24
10	54:7/56:6	41.80	0.05	24.88	0.20
11	54:8/56:7	42.46	0.04	15.74	0.15
12	54:9	43.08	0.05	5.50	0.13

[a]Based on the use of spiked reference compounds and mass spectrometry data in Table 1.
[b]Five replicates.
[c]Partial separation, integrated as one peak.

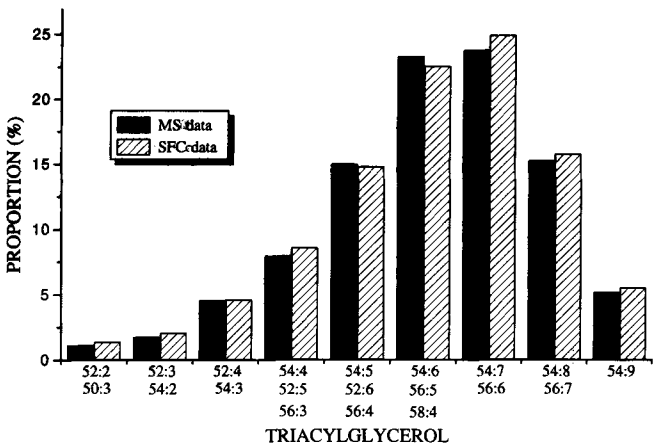

FIG. 4. The proportions of triacylglycerols in cloudberry oil measured by capillary supercritical fluid chromatography (SFC) and negative-ion chemical ionization mass spectrometry (MS).

peaks were not separated at baseline, good repeatability of retention times and acceptable repeatability of relative amounts were obtained with the same chromatographic program optimized for berry oils (Table 4). The chromatogram of rainbow trout oil in Figure 5B does not represent high resolution, but shows the potential of the stationary phase used with further method optimization to produce better separation for screening purposes. The retention time repeatability was still acceptable, but the repeatability of proportions was relatively poor for the majority of the peaks. The complexity of milk fat and fish oil and the lack of pure compounds may lead to incorrect peak identification and, therefore, is not discussed here.

Elution of tocopherols on the cyanopropyl stationary phase was tested by spiking them in sea buckthorn pulp oil. The separation, with the elution order of α-, ß + γ- and δ-tocopherol, was adequate for screening purposes. α-Tocopherol, which

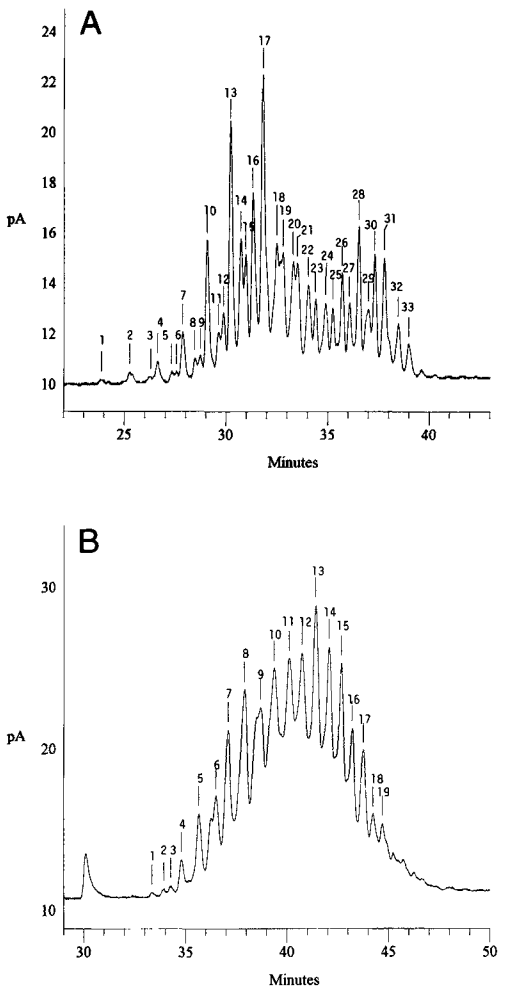

FIG. 5. The separation of triacylglycerols on an SB-Cyanopropyl-25 column: A) milk fat, and B) rainbow trout oil. Peak numbers refer to those in Table 4. Company source as in Figure 3.

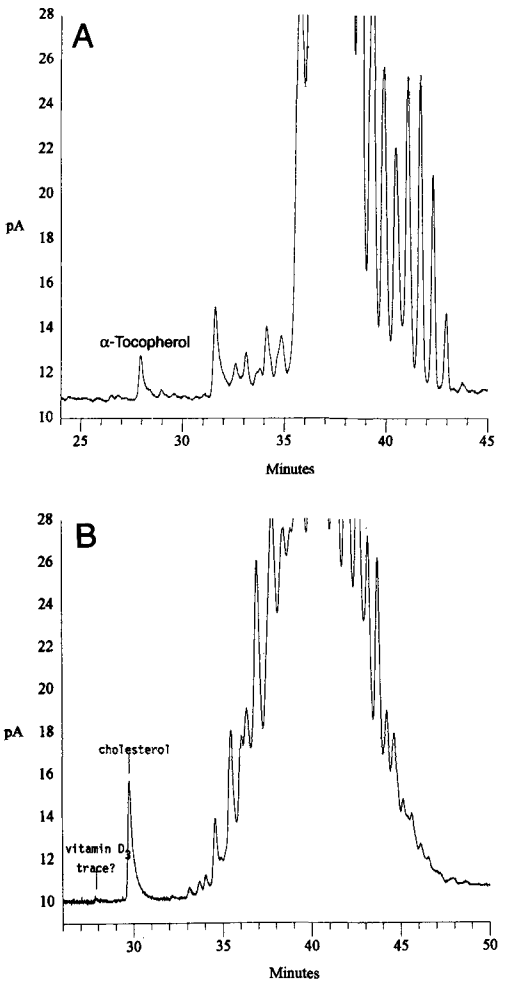

FIG. 6. A) Sea buckthorn pulp oil 4:1 (vol/vol) in CH_2Cl_2, and B) rainbow trout oil 2:1 (vol/vol) in CH_2Cl_2. Timed split loop open time 0.5 s.

has the highest biological activity of the tocopherols (17), was separated at baseline. The natural concentration of tocopherols was fairly low compared with the total concentration of triacylglycerols in berry oils; therefore, higher loading of the sample was needed to get the tocopherols visualized in the chromatogram. The content of α-tocopherol in sea buckthorn pulp oil was approximately 2.1 mg g^{-1} based on tridecanoin as internal standard (Fig. 6A). The sample had to be at least fifty times more concentrated compared to screening of triacylglycerols to get reasonable quantitation for α-tocopherol in sea buckthorn pulp oil. As expected, the resolution of triacylglycerols was lost. In sea buckthorn seed oil, tocopherols were not present in quantitative amounts with corresponding sample dilution. The problem of low concentration also was faced in the screening for vitamin D_3 in rainbow trout oil (Fig. 6B). In contrast to vitamin D_3, cholesterol was present in higher amounts in rainbow trout oil, thus making quantitation possible.

The method described in this paper gives a useful tool for the screening of triacylglycerols of several pulp and seed oil products. The 25% cyanopropyl/25% phenyl/50% methylpolysiloxane stationary phase provides information on both the number of acyl carbons and the degree of unsaturation of triacylglycerols. Most often, such detailed data on triacylglycerols are required because the acyl carbon number distribution of triacylglycerols is not adequate due to the relatively simple composition of plant-originated oils. For further information fractionation according to the acyl carbon number of triacylglycerols on a nonpolar stationary phase prior to the described method is needed. Although the sensitivity of the FID is clearly less than that of a fluorescence detector for tocopherols, the method is also suitable for the screening of tocopherol content of plant oils without any sample purification step if more concentrated samples are available.

The SFC method, optimized for berry oils, also produced reasonable separation of milk fat. Both nonpolar and polar stationary phase separation can be used to measure changes in the triacylglycerol composition during chemical processes or biological events. The developed method also was applied

TABLE 4
Retention Times and Proportions of Triacylglycerols in Milk Fat and Rainbow Trout Oil

Milk fat					Rainbow trout oil				
Peak no.	t_R[a] (min)	SD	Proportion (%)	SD	Peak no.	t_R[a] (min)	SD	Proportion (%)	SD
1	23.89	0.03	0.17	0.05	1	33.47	0.06	0.11	0.02
2	25.24	0.01	0.49	0.04	2	34.03	0.06	0.13	0.02
3	26.24	0.02	0.18	0.05	3	34.37	0.06	0.20	0.02
4	26.60	0.01	0.80	0.01	4	34.90	0.06	0.93	0.13
5	27.30	0.02	0.26	0.02	5	35.75	0.06	2.42	0.07
6	27.53	0.02	0.27	0.03	6	36.58	0.06	4.42	0.16
7	27.84	0.02	1.72	0.10	7	37.16	0.05	6.12	0.39
8	28.45	0.02	0.62	0.03	8	37.94	0.06	8.07	0.12
9	28.71	0.02	0.74	0.06	9	38.72	0.07	9.60	0.08
10	29.06	0.03	4.60	0.28	10	39.42	0.06	10.81	0.85
11	29.61	0.02	1.32	0.05	11[b]	40.15	0.06	10.33	0.70
12	29.86	0.03	2.04	0.12	12	40.69	0.15	10.31	0.14
13	30.25	0.04	8.27	0.53	13	41.43	0.06	10.56	0.18
14	30.74	0.04	4.30	0.13	14[b]	42.08	0.07	8.94	0.33
15	30.97	0.04	3.39	0.15	15	42.67	0.07	6.68	0.39
16	31.36	0.05	6.55	0.24	16	43.22	0.07	4.45	0.31
17	31.85	0.06	11.62	0.33	17	43.76	0.07	3.44	0.12
18	32.52	0.04	5.15	0.15	18	44.26	0.06	1.45	0.05
19	32.82	0.05	5.15	0.19	19	44.73	0.05	1.04	0.04
20	33.32	0.05	4.10	0.09					
21	33.53	0.05	3.50	0.24					
22	34.06	0.04	3.56	0.13					
23	34.41	0.05	2.21	0.17					
24	34.90	0.05	3.06	0.13					
25	35.26	0.05	2.34	0.15					
26	35.74	0.05	3.09	0.23					
27	36.09	0.06	2.41	0.14					
28	36.27	0.06	4.17	0.23					
29	37.02	0.05	2.74	0.17					
30	37.34	0.06	3.37	0.14					
31	37.81	0.06	4.55	0.22					
32	38.50	0.06	2.00	0.09					
33	39.00	0.06	1.25	0.06					

[a]Five replicates.
[b]Duplicate peak integrated as one peak.

to the separation of rainbow trout oil, but the chromatography was unsatisfactory due to the complex nature of triacylglycerols.

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