ELSEVIER

Journal of Chromatography A, 787 (1997) 276–282

JOURNAL OF CHROMATOGRAPHY A

Short communication

Supercritical fluid chromatography–gas chromatography of volatiles in cloudberry (*Rubus chamaemorus*) oil extracted with supercritical carbon dioxide

P. Manninen[a,*], H. Kallio[b]

[a]*Aromtech Oy, Valtakatu 27 A, FIN-94100 Kemi, Finland*

[b]*Department of Biochemistry and Food Chemistry, University of Turku, FIN-20014 Turku, Finland*

Received 14 March 1997; received in revised form 27 May 1997; accepted 9 June 1997

Abstract

A supercritical fluid chromatographic–gas chromatographic method was developed and applied to the analysis of volatiles of cloudberry oil extracted with supercritical carbon dioxide. Capillary supercritical fluid chromatography was used for the pre-separation of the oil and for the introduction of the volatile fraction into gas chromatography. Altogether 69 components could be reliably identified using chemical and electron impact ionization mass spectrometry. The major groups of compounds were aryl compounds and esters of fatty acids. The most abundant compounds were benzoic acid and benzyl alcohol with the relative amounts of approximately 58 and 6%, respectively. © 1997 Elsevier Science B.V.

Keywords: *Rubus chamaemorus*; Oils; Cloudberry; Sample preparation

1. Introduction

Volatile compounds in edible oils are typically analyzed by utilizing different headspace techniques and gas chromatography (GC) [1–5]. The headspace methods typically involve either trapping of volatiles on Tenax or taking a sample from an equilibrated gas phase of a sample vial. The use of Tenax traps narrows the range of the volatiles available for further analysis. On the other hand, the proportions of sample components in the gas-phase equilibrium in the headspace sample vial is dependent on the vapor pressures and diffusion rates of the sample components. Therefore, the proportions of the components in the gas-phase equilibrium are not necessarily the same as in the solid or liquid phase.

Supercritical fluid extraction (SFE) combined with GC has been used for the analysis of volatiles of spices and herbs [6–9]. The volatiles have typically been extracted with supercritical CO_2 at pressures of 10–30 MPa. Triacylglycerols start to dissolve in supercritical CO_2 already at 9 MPa [10], which leads to problems in SFE–GC of volatiles in lipid-containing matrices. Therefore, for example, a volatile fraction of raw beef obtained with SFE contained lipids and had to be analyzed with headspace technique [11]. Capillary supercritical fluid chromatography (SFC) with flame ionization detection (FID) has been used for the separation of essential oils [12]. The wide solvent peak obtained in SFC–FID chromatograms overlaps the early eluting compo-

*Corresponding author.

0021-9673/97/$17.00
PII S0021-9673(97)00651-1

nents restricting the analysis of most volatile and low-molecular-mass compounds. In addition, a capillary column is easily overloaded when edible oil volatiles are analyzed, since a relatively high sample concentration is required. SFC combined with GC has been used successfully in the fractionation and characterization of aviation fuel [13] and gasolines [14].

In this study, an SFC–GC method was developed and applied for the analysis of the volatiles of cloudberry seed oil extracted with supercritical carbon dioxide. The parameters affecting the system performance are briefly discussed.

2. Experimental

2.1. Materials

Reference compounds of methyl caproate (caproic acid methyl ester) (Sigma, St. Louis, MO, USA), acetophenone (1-phenyl ethanone), *trans*-cinnamaldehyde (*trans*-3-phenyl-2-propen-1-al) and cinnamyl alcohol (3-phenyl-2-propen-1-ol) (Chem Service, West Chester, PA) were each dissolved in dichloromethane (Rathburn, Walkerburn, UK) to a concentration of approximately 1.2 mg ml^{-1}. Cloudberry (*Rubus chamaemorus*) seed oil was extracted with supercritical carbon dioxide (30 MPa, 40°C) using a pilot plant at Flavex (Rehlingen, Germany). Counter-current extraction of cloudberry seed oil was obtained on a 3 m×46 mm I.D. counter-current column (21 MPa, 40°C) at Flavex. A carbon dioxide mass flow-rate of 35 kg/kg of cloudberry oil resulted in an extract/raffinate ratio of 24/76. Approximately 950 mg of cloudberry seed oil and counter-current extract were diluted in 1 ml of dichloromethane corresponding to the total sample volume of 2 ml.

2.2. Methods

A supercritical fluid chromatograph (Lee Scientific Series 600, Dionex, Salt Lake City, UT, USA) was coupled with a gas chromatograph (HP 5890, Hewlett-Packard, Avondale, PA, USA) via a valve (Rheodyne 7030, Cotati, CA, USA) (Fig. 1). The valve was installed on the top of the SFC oven to heat the valve. The valve was used to introduce the SFC column flow either into the SFC frit restrictor (30 cm×50 μm I.D.×195 μm O.D., Dionex) connected to an FID or into a transfer line leading to the GC split/splitless injector. Deactivated fused-silica capillary tubing (50 cm×50 μm I.D.×195 μm O.D.) was used as the transfer line. The transfer line was covered with an aluminum foil such that it can be heated by SFC oven air. A frit restrictor (30 cm×50 μm I.D.×195 μm O.D., Dionex) was combined to the transfer line with a 1/16 in. zero dead volume butt connector (SGE, Austin, TX, USA) (1 in.=2.54 cm). The frit restrictor head was pushed through a GC injector septum 10 cm below the top and placed inside a GC trap column. A 2 m×0.32 mm I.D. SE-54 column (HNU Nordion, Helsinki, Finland) with a film thickness of 0.25 μm was used as the GC trap column. It was installed to the GC injector so that the frit restrictor head was approximately 5 cm inside the trap column. An analytical column (30 m×0.25 mm I.D. FFAP with a film thickness of 0.25 μm, Alltech Associate, Deerfield, IL, USA) was connected to the coiled trap column with a 1/16 in. zero dead volume butt connector (SGE). The trap column coil was kept in a water–methanol–ice bath (−2°C) during the collection of the volatile fraction from SFC. The GC injector temperature was held at 250°C. The split flow and purge flow-rates were 15 and 0.4 ml min^{-1}, respectively.

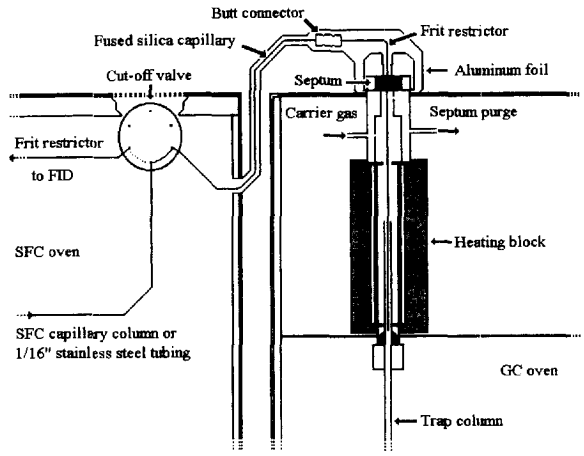

Fig. 1. Schematic drawing of the SFC–GC interfacing ("=inch; 1 inch=2.54 cm).

Both a capillary column (10 m×50 μm I.D. SB-

Cyanopropyl-25 with a film thickness of 0.25 μm, Dionex) and a micropacked column (100×1 mm I.D., 5 μm, Deltabond Cyano, Keystone Scientific) were tested for the sample pre-separation by SFC. Both columns were connected directly to the SFC injector port. The sample introduction was performed using a pneumatic and electrically controlled valve (Valco Instruments, Houston, TX, USA) with an internal loop size of 1.0 μl. SFC-grade CO_2 (Scott Specialty Gases, Plumsteadville, PA, USA) was used as a carrier fluid in SFC. The SFC density program for sample pre-separation was from 0.18 to 0.63 g ml^{-1} with a rate of 0.01 g ml^{-1} min^{-1} at a constant temperature of 100°C. The GC temperature program for the separation of the volatile fraction was from 50 to 100°C with a rate of 5°C min^{-1}, and from 100 to 270°C with a rate of 3°C min^{-1}. The GC column head pressure was kept at 100 kPa during the GC run, and helium was used as a carrier gas. The SFC–GC transfer line was purified after every two analyses by CO_2 flow (36 MPa) and holding the GC oven at 250°C for 35 min.

Mass spectrometric (MS) determinations were conducted with a Finnigan MAT TSQ-700 triple quadrupole instrument (Finnigan MAT, San Jose, CA, USA) using chemical ionization (CI) and electron impact ionization (EI). Methane (5000 mTorr) was used as reactant gas at the ion source temperature of 120°C during CI operation (1 Torr=133.322 Pa). EI spectra were achieved using electron energy of 70 eV. Positively charged ions with m/z values of 50–400 were scanned with the first quadrupole using a scan time of 0.7 s. The GC–MS transfer line was kept at 230°C with both ionization modes.

3. Results and discussion

SFC was used for the separation of the volatile compounds of cloudberry oil from the low volatile compounds, such as triacylglycerols, which are difficult to elute from a GC column. The volatile compounds, which eluted at the beginning of the SFC programming, were introduced into GC, whereas the later eluting compounds were led to the FID of SFC (Fig. 1.) The volatiles were collected into the trap column and re-focused before being separated by GC. The GC injector was heated to compensate for the cooling of the restrictor end inside the trap column as a result of the rapid expansion of the SFC carrier fluid. The temperature of the trap column coil was kept at −2°C with a water–methanol–ice bath during the collection of the volatile fraction. Cooling baths providing lower temperatures were not used to avoid the condensation of CO_2 in the trap. Due to the relatively high trap temperature, a trap column with a stationary phase was used to ensure efficient collection of volatiles. The expansion of CO_2 from the restrictor outlet produced a large volume of gas, which resulted in a backflush of SFC effluent towards the trap column inlet due to the flow restriction of trap and GC analytical column. There-

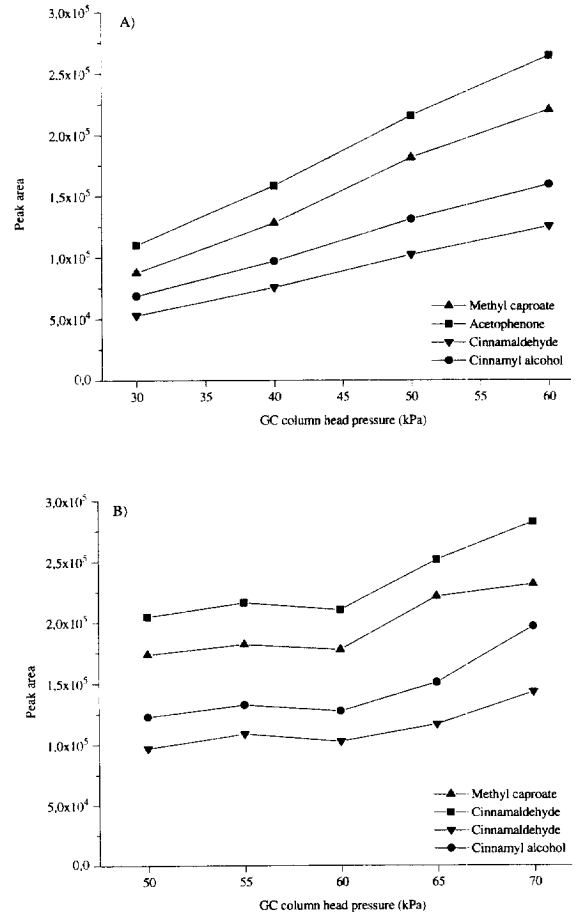

Fig. 2. Effect of GC column head pressure on the peak areas of the reference compounds with SFC–GC using (A) a capillary column, and (B) a micropacked column in SFC.

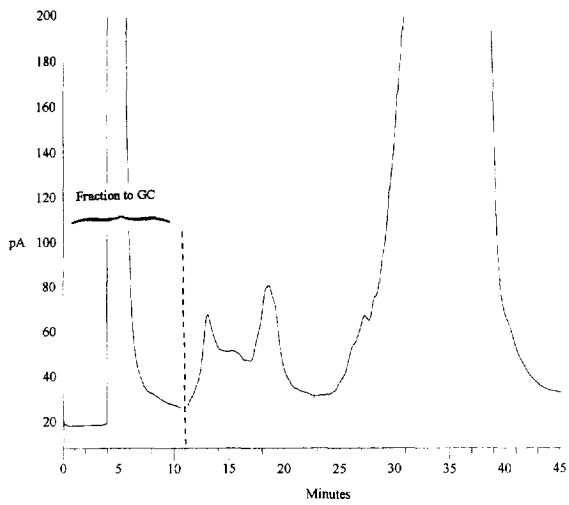

Fig. 3. Capillary SFC–FID chromatogram of cloudberry oil.

fore, a GC carrier gas flow, which was determined as a column head pressure, was required also during the collection of volatiles to ensure the introduction of the compounds into the trap.

The effect of GC column head pressure on the trapping efficiency was studied by using a reference mixture. The components of the reference mixture were selected on the basis of their presence in cloudberry aroma [15], and to represent the vapor pressure and polarity range of cloudberry volatiles. Since the volatiles corresponded to less than 4% of the total SFC–FID response of cloudberry oil components, both capillary and micropacked SFC columns were used for sample pre-separation. Capillary columns provide higher resolution and a lower column flow-rate than micropacked columns, whereas micropacked columns offer higher sample capacity. The effect of the GC column head pressure on the

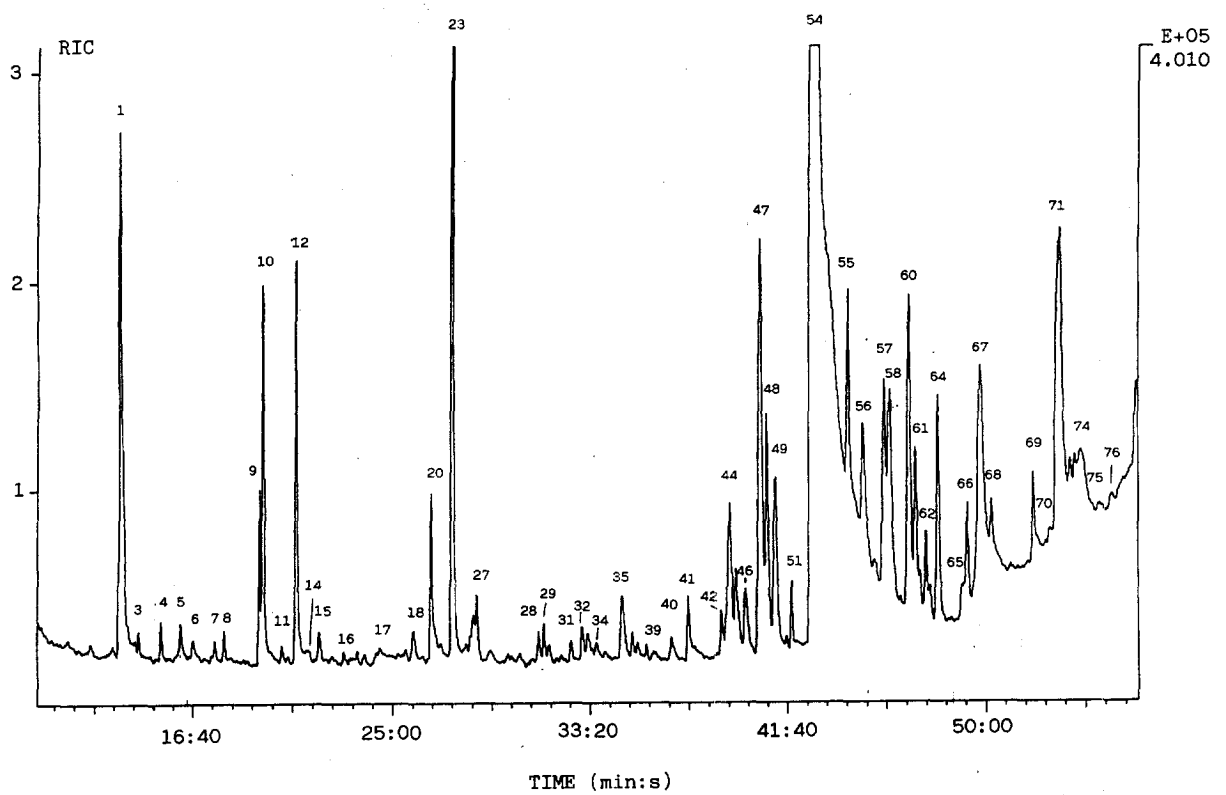

Fig. 4. Capillary SFC–GC–CI-MS reconstructed ion chromatogram of the volatile fraction of cloudberry oil. Due to lack of space, not all peak numbers given in Table 1 could be inserted in the figure; from the retention times given in Table 1, the positions of the peaks can be found.

peak areas of the reference compounds is shown in Fig. 2. The relative standard deviation (R.S.D.) of the peak areas was 3–5% with both columns. However, with the highest studied GC column head pressure the R.S.D. was twice as high. This was proposed to be a consequence of an incomplete retainment of the compounds in the trap as a result of too high combined flow of CO_2 and the GC carrier gas.

The proportions of the reference compounds were almost independent of the studied GC column head pressures. The use of a capillary column produced very similar relative amounts of the reference compounds (R.S.D.≈1–2%) to those obtained with GC using split injection. In addition, the repeatability of SFC–GC with the capillary column was better than with the micropacked column. A change in the GC injector split and purge flow-rates from the normally used values in GC split injection did not have an

Table 1
Identification of volatiles of cloudberry oil extracted with supercritical carbon dioxide based on the CI-MS and EI-MS spectra

Peak[a] no.	t_R (min)	Molecule	Compound name	Peak[a] no.	t_R (min)	Molecule	Compound name
1	13:45	$C_2H_4O_2$	Acetic acid	39	36:05	$C_9H_{18}O_2$	Nonanoic acid
2	14:12	$C_5H_8N_2$	1,3-Dimethyl-1(H)-pyrazole	40	36:44	$C_9H_{10}C_2$	2-Hydroxy-5-methylacetophenone
3	14:21	C_8H_{14}	3-Ethyl-1,4-hexadiene	41	37:26	$C_8H_7O_3$	Methyl 2-hydroxybenzoate
4	15:19	$C_7H_{10}O$	*trans,trans*-2,4-Heptadienal	42	38:52	$C_{10}H_{20}O_2$	Decanoic acid
5	16:08	C_7H_6O	Benzaldehyde	43	39:01	$C_9H_{10}O$	*trans*-Cinnamyl alcohol
6	16:42		Unknown (M_r=72)	44	39:13	$C_{17}H_{34}O_2$	Methyl 14-methylpentadecanoate
7	17:36	$C_4H_8O_2$	2-Methylpropanoic acid	45	39:27	$C_{10}H_{10}O_4$	Dimethylphthalate
8	17:59	C_4H_8O	3-Buten-2-ol	46	39:31	$C_{17}H_{32}O_2$	Methyl 9-hexadecenoate
9	19:26	$C_8H_8O_2$	Methyl benzoate	47	39:54	$C_{17}H_{32}O_2$	Methyl 11-hexadecenoate
10	19:34	$C_4H_8O_2$	Butanoic acid	48	40:16	$C_8H_{12}O_2$	Unknown
11	20:21	C_8H_8O	Acetophenone	49	40:32	$C_{18}H_{36}O_2$	Methyl 2-methylhexadecanoate
12	20:58	$C_9H_{10}O_2$	Ethyl benzoate	50	40:46		Unknown (M_r=310)
13	21:18		Unknown (M_r=168)	51	41:09		Unknown (M_r=310)
14	21:27	$C_{12}H_{24}O_2$	Ethyl decanoate	52	41:35	$C_8H_4N_2$	1,3-Benzene dicarbonitrile
15	21:55		Unknown (M_r=96)	53	41:48	C_8H_8O	2,3-Dihydrobenzofuran
16	22:57	$C_9H_{10}O_2$	Benzyl acetate	54	42:36	$C_7H_6O_2$	Benzoic acid
17	24:23	$C_{15}H_{24}$	α-Cedrene	55	44:12	$C_{12}H_{24}O_2$	Dodecanoic acid
18	25:32	$C_8H_{10}O$	α-Methyl benzenemethanol	56	44:52	$C_{19}H_{36}O_2$	Methyl 9-octadecenoate
19	25:48	$C_{15}H_{24}$	Cadinene	57	45:45	$C_{19}H_{34}O_2$	Methyl 9,12-octadienoate
20	26:35	$C_8H_{10}O$	Hexanoic acid	58	46:00	$C_{20}H_{38}O_2$	Ethyl 9-octadecenoate
21	26:47	$C_{13}H_{26}O_2$	Methyl dodecanoate	59	46:28	$C_{20}H_{38}O_2$	Ethyl 12-octadecenoate
22	27:02	$C_7H_8O_2$	4-Methoxyphenol	60	46:50	$C_{20}H_{36}O_2$	Ethyl 9,12-octadienoate
23	27:29	C_7H_8O	Benzyl alcohol	61	47:03	$C_{19}H_{32}O_2$	Methyl 9,12,15-octatrienoate
24	27:58	$C_{11}H_{14}O_2$	Ethyl benzenepropanoate	62	47:29	$C_{14}H_{12}O_2$	Benzyl benzoate
25	28:14	$C_6H_{10}O_2$	2-Hexenoic acid	63	48:02	$C_{14}H_{12}O_2$	Benzoin
26	28:26	$C_{14}H_{28}O_2$	Ethyl dodecanoate	64	47:39	$C_{20}H_{34}O_2$	Ethyl 9,12,15-octadecatrienoate
27	28:30	$C_8H_{10}O$	β-Phenylethyl alcohol	65	48:59	$C_{14}H_{28}O_2$	Tetradecanoic acid
28	30:50	$C_{11}H_{22}$	Undecene	66	49:12	$C_{16}H_{22}O_4$	Butyl 2-methylpropyl phthalate
29	31:08	$C_7H_{12}O$	[(2-Propenyloxy)methyl]-cyclopropane	67	49:53	$C_{18}H_{34}O_2$	Unknown (M_r=282)
30	31:22	C_6H_6O	Phenol	68	50:12	$C_9H_8O_2$	*cis*-Cinnamic acid
31	32:30	$C_9H_{12}O$	β-Phenylpropyl alcohol	69	51:57	$C_9H_8O_2$	*trans*-Cinnamic acid
32	32:50	$C_8H_{16}O_2$	Octanoic acid	70	52:41	$C_{12}H_{18}O_3$	Methyl 7-(2-furyl)heptanoate
33	33:15	$C_{15}H_{30}O_2$	Methyl tetradecanoate	71	52:58	$C_{16}H_{32}O_2$	Hexadecanoic acid
34	33:33	$C_{10}H_{10}O_2$	Methyl cinnamate	72	53:13		Unknown (M_r=378)
35	34:41	$C_{16}H_{32}O_2$	Ethyl tetradecanoate	73	53:32	$C_{16}H_{30}O_2$	Oxacycloheptadecan-2-one
36	35:06	$C_{11}H_{12}O_2$	Ethyl cinnamate	74	53:43	$C_{16}H_{30}O_2$	5-Dodecyldihydro-2(3H)-furanone
37	35:20		Unknown (M_r=254)	75	54:47	$C_{28}H_{58}$	Octacosane
38	35:41	$C_7H_{12}O_2$	1,5-Heptadiene-3,4-diol (?)	76	55:15	$C_{22}H_{46}O$	1-Docosanol

[a] The peak numbers refer to those in Fig. 4.

effect on the results. The overloading of the capillary column resulted in poor resolution in SFC (Fig. 3). However, high resolution was not required, since SFC was used only as a fraction introduction method for GC. The manual cut-off of the volatile fraction was more repeatable with the capillary column than with the micropacked column due to the more narrow sample zones. On the basis of system performance the capillary column was selected to be used in further studies. A GC column head pressure of 50 kPa produced intensive peaks of cloudberry oil volatiles with capillary SFC–GC for qualitative analysis of cloudberry oil volatiles, although a total recovery of the reference compounds was not achieved. The collection time of the cloudberry oil volatile fraction in the trap was determined on the basis of the SFC–GC–FID chromatogram: the aim was to minimize the amount of late eluting compounds, which would require a long isothermal period at the maximum temperature of the GC column to be eluted, without affecting the peak intensities of the volatiles. The optimum collection time from the beginning of the SFC programming was 11 min.

The identification of the sample compounds was accomplished with SFC–GC–MS using both CI and EI ionization modes. The vacuum pressure of the MS was slightly increased during the fraction collection as CO_2 was transferred through the GC column to the ion source of MS. However, the vacuum pressure reverted to the normal operating level before the MS data collection was initiated. The combined number of detected peaks with both ionization methods was 104. The molecular masses could be determined for 76 compounds with CI (Fig. 4). Only 69 compounds of these could be reliably identified according to their EI spectra (Table 1).

The two major groups of the identified components in the cloudberry seed oil carbon dioxide extract were aryl compounds and esters of fatty acids. The main component was benzoic acid representing approximately 58% of the total FID response. The other abundant aryl compounds were methyl benzoate (1%), ethyl benzoate (2%) and benzyl alcohol (6%). The results were comparable to those obtained with GC analysis of unheated press juice of cloudberry [15]. Esters of fatty acids having up to 18 acyl carbons were found in the volatile fraction of the cloudberry oil, whereas esters of fatty acids having more than six acyl carbons were not identified in the press juice [15]. A similar trend was also found in the case of free fatty acids. The most abundant aliphatic acids were acetic acid (2%) and butanoic acid (1%). The counter-current extracts of cloudberry oil have been reported to have a stronger and sweetened odor compared with the original oil [16]. In this study, the same compounds were identified in the cloudberry oil counter-current extract as in the SFE extract by SFC–GC. However, the volatiles were concentrated with counter-current extraction. In addition, the proportion of esters of fatty acids was higher, which was assumed to be responsible of the sweetened odor of the counter-current extract.

SFC–GC proved to be a useful method for the characterization of edible oil volatiles, which is complicated with other techniques. The SFC–GC method resulted in similar discrimination of the analytes as GC split injection. The quantitative analysis will require further studies.

References

[1] E. Selke, E.N. Frankel, J. Am. Oil Chem. Soc. 64 (1987) 749.
[2] J.M. Snyder, E.N. Frankel, E. Selke, K. Warner, J. Am. Oil Chem. Soc. 65 (1988) 1617.
[3] S.K. Raghavan, S.K. Reeder, A. Khayat, J. Am. Oil Chem. Soc. 66 (1989) 942.
[4] J.M. Snyder, T.L. Mounts, J. Am. Oil Chem. Soc. 67 (1990) 800.
[5] N.J. Engeseth, E.G. Perkins, B.P. Klein, J. Am. Oil Chem. Soc. 69 (1992) 641.
[6] S.B. Hawthorne, M.S. Krieger, D.J. Miller, Anal. Chem. 60 (1988) 472.
[7] C.R. Blatt, R. Ciola, J. High Resolut. Chromatogr. 14 (1991) 775.
[8] K. Hartonen, M. Jussila, P. Manninen, M.-L. Riekkola, J. Microcol. Sep. 4 (1992) 3.
[9] H. Kallio, K. Kerrola, P. Alhonmäki, J. Agric. Food Chem. 42 (1994) 2478.
[10] E. Stahl, W. Schilz, E. Schütz, E. Willing, in: G.M. Schneider, E. Stahl, G. Wilke (Eds.), Extraction with Supercritical Gases, Verlag Chemie, Weinheim, 1980, p. 94.
[11] M.-F. King, B.L. Hamilton, M.A. Matthews, D.C. Rule, R.A. Field, J. Agric. Food Chem. 41 (1993) 1974.
[12] P. Manninen, Y. Holm, R. Hiltunen, M.-L. Riekkola, J. High Resolut. Chromatogr. 13 (1990) 167.

[13] J.M. Levy, J.P. Guzowski, W.E. Huhak, J. High Resolut. Chromatogr. Chromatogr. Commun. 10 (1987) 337.
[14] J.M. Levy, J.P. Guzowski, Fresenius Z. Anal. Chem. 330 (1988) 207.
[15] T. Pyysalo, M. Suihko, E. Honkanen, Z. Lebensm. Unters. Forsch. 163 (1977) 25.
[16] P. Manninen, J. Pakarinen, H. Kallio, J. Agric. Food Chem. in press.