Agronomy Research **14**(S2), 1328–1346, 2016

Gas chromatography–mass spectrometry study of lipids in northern berries

L. Klavins, J. Kviesis, I. Steinberga, L. Klavina and M. Klavins*

University of Latvia, Raina blvd. 19, Riga, LV-1586, Latvia
*Correspondence: maris.klavins@lu.lv

Abstract. Wild berries from forests and bogs of Northern Europe are an excellent source of natural antioxidants, vitamins and fatty acids, all of which are substances with high biological activity. This study investigates lipids extracted from fresh and powdered berries, using low-polarity solvents (chloroform, diethyl ether and others) and a mixture of chloroform and methanol. Berry lipids were analysed by gas chromatography–mass spectrometry. The following berries were analysed: blueberry (*Vaccinium myrtillus* L.), bilberry (*Vaccinium uliginosum* L.), two cultivars of highbush blueberry (*Vaccinium corymbosum* L.), lingonberry (*Vaccinium vitis-idaea* L.), cloudberry (*Rubus chamaemorus* L.), black crowberry (*Empetrum nigrum* L.), cranberry (*Vaccinium oxycoccos* L.) and rowanberry (*Sorbus aucuparia* L.). One hundred and elevencompounds were identified and quantified in the 9 species of analysed berries. The lipid fraction contained compound classes like fatty acids, sterols, triterpenoids, alkanes, phenolic and carboxylic acids and carotenoids. All fresh berries contained high amounts of C18 unsaturated fatty acids (for example, up to 102 μg g^{-1} of blueberries) and phytosterols (86 μg of β-sitosterol g^{-1} of blueberries), and high amounts of benzoic acid were found in lingonberries (164 μg g^{-1}). The analysed berry lipid profiles were compared using the principal component analysis and hierarchical cluster analysis. The two analyses showed that the lipid profiles of the studied berries reflect their taxonomy.

Key words: Northern berries, lipids, extraction, GC/MS, chemotaxonomy.

INTRODUCTION

Nowadays, there is a strong scientific evidence that diets based on plant foods are the healthiest (Kähkönen et al., 2001; Silva et al., 2013). Regular consumption of vegetables, fruits and berries is beneficial for the prevention and reduction of developing chronic diseases (Hooper & Cassidy, 2006).

Berries have high concentrations of polyphenols, antioxidants, vitamins and minerals (Nile & Park, 2014). A large number of studies have shown the health benefits of berries, especially their antioxidant activity (Kähkönen et al., 2001), role in cardiovascular health (Rodriguez-Mateos et al., 2013) their protection against cancer (Määttä-Riihinen et al., 2005), anti-inflammatory, cholesterol-lowering (Szakiel et al., 2012a; Joseph et al., 2014) and antimicrobial effects (Silva et al., 2013), and so on.

Despite the fact that the lipid content in berries is low, there are several studies indicating the presence of different low-polarity substance groups in berries (Zlatanov 1999; Yang et al., 2003; Hoed et al., 2009). Plants (and also berries) contain lipids that regulate protein synthesis, metabolic and many other processes in cells (Corte et al.,

2015). Lipids in berries have a significant function to protect the berry from outside factors (cuticular waxes), such as pathogens and environmental stresses (Corte et al., 2015). Lipids in berries can be found in the cytoplasm, or they can be bound to cellular membranes; moreover, significant amounts of lipids can be found in berry seeds (Järvinen et al., 2010). Many lipid groups in berries (unsaturated fatty acids, sterols, terpenoids and others) have high biological activity, and they differ from lipids in higher organisms (mammals); therefore, their consumption is important for human metabolism. For example, it has been shown that berry sterols (phytosterols) have the ability to reduce cholesterol levels in humans (Dulf et al., 2012).

Studies of lipids in berries began with analysis of cranberry composition and major lipid groups in these berries. Croteau and Fagerson (1969) investigated cranberry seed lipids and found neutral lipids, phospholipids, glycolipids and many free lipids, such as paraffins, aldehydes, alcohols, fatty acids, sterols and carboxylic acids (Croteau & Fagerson, 1971). More recent studies have concentrated on cutin composition and lipid content analysis in berry seed oils (Johansson et al., 1997; Zlatanov, 1999; Johansson et al., 2000; Oomah et al., 2000; Kallio et al., 2006; Hoed et al., 2009; Dulf et al., 2012). It has been found that seed oils contain high amounts of polyunsaturated fatty acids and phytosterols. However, the contents of other low-polarity substances is not so widely studied, and only a few studies have concentrated on the analysis of sterol composition in berries (Dulf et al., 2012; Szakiel et al., 2012a; Szakiel et al., 2012b) or tocopherols (Zadernowski et al., 2003; Matthaus & Ozcan, 2014). Non-traditional vegetable oils, such as berry oils, have become increasingly popular in health care because of their highly specific composition, high concentration of unsaturated fatty acids and antioxidants. Since polyunsaturated fatty acids cannot be synthesised in human body, they must be obtained from food (Hoed et al., 2009). Therefore, the concept of 'berry lipids' could have a high marketing potential. Another group of substances found in berry seed oils are triterpenoids and sterols (Yang et al., 2003). Studies have shown the anti-inflammatory, antiviral, wound-healing and anticarcinogenic properties of sterols and triterpenoids present in berry seed oils (Szakiel et al., 2012b).

Regular consumption of berries has an impact on inflammation markers and antioxidative capacity (Rodriguez-Mateos et al., 2013; Joseph et al., 2014). Berries contribute to healthy gut microbiome, may improve lipid profile of human plasma and reduce the risk of cardiovascular diseases (Yang & Kortesniemi, 2015).

The studies done on berry lipids until now have concentrated mostly on cranberries and blueberries, as these are the most common commercially cultivated berries worldwide. However, many wild berries common in Northern Europe and the Baltic countries are available for everyday consumption. Moreover, many of these berries have a significant potential for cultivation and thus also for isolation of biologically active extracts and substances. Considering the high biological activity of berry lipids, it is important to continue studies of their composition for the purposes of uncovering their potential uses, developing new processing approaches and supporting innovation in the applicability of berries and their extracts in food, as food additives, in cosmetics, etc.

The aim of this study was to analyse the lipid composition of the selected 9 species of wild and cultivated berries commonly growing in Latvia.

MATERIALS AND METHODS

Berry samples and their processing

Nine species of berries grown in Latvia were investigated: blueberry (*Vaccinium myrtillus* L.), bilberry (*Vaccinium uliginosum* L.), highbush blueberry (*Vaccinium corymbosum* L.), lingonberry (*Vaccinium vitis-idaea* L.), cloudberry (*Rubus chamaemorus* L.), black crowberry (*Empetrum nigrum* L.), cranberry (*Vaccinium oxycoccos* L.) and rowanberry (*Sorbus aucuparia* L.). Two cultivars of highbush blueberry (cv. Blue Ray and cv. Chippewa) were studied, while the rest are wild berries.

Blueberries, lingonberries and rowanberries were harvested in the summer/autumn period (July-October) of 2014 in Vidzeme (Latvia). The cultivated highbush blueberries (HB) were harvested in a local garden in the town of Saldus in mid-August 2014. Bilberries were harvested in Pieņu bog in July 2014. Cloudberries were harvested in the bogs belonging to Teiču Nature Reserve in September 2014. Black crowberries and cranberries were harvested in Kurzeme, the western part of Latvia, in forests surrounding the town of Liepāja in September 2014. After the berries have been harvested, they were washed with demineralised water to remove any possible contaminations (dirt, bugs, etc.), air-dried and transported to the storage where they were frozen to -20 °C. Berries were kept at -18°C and analysed within 5–7months.

Blueberries and lingonberries were dried by the company SilvExpo SIA, using Мусson-2 microwave lyophiliser drying equipment (by BÜCHI Labortechnik AG, Switzerland). Berries were dried in vacuum at a temperature not exceeding 50 °C until they contained a maximum of 8% total moisture. After the berries have reached a satisfactory moisture level, they were milled and sifted.

Extraction of berry lipids

For the extraction of lipids from fresh berries, 50 g of the selected berries was crushed in mortar. The crushed berries were then mixed with 150 mL of Bligh-Dyer reagent (CH_3OH (Labscan)/$CHCl_3$ (Chempur), 2:1). After 2 minutes of stirring, 50 mL of $CHCl_3$ was added. The mixture was poured in a glass bottle with a cap and sonicated for 40 minutes; the water in the ultrasound bath (Cole – Parmer, USA) was changed every 20 minutes to avoid evaporation of the solvents and heating up. After the sonication, the samples were equilibrated to room temperature. 50 mL of H_2O was added, and the samples were filtered. The final ratio of the solvents in the mixture was CH_3OH:$CHCl_3$:H_2O, 2:2:1. The berry residues in the filter were extracted again with 100 mL $CHCl_3$ and sonicated for another 40 minutes as before. The process was repeated twice. All the extracts were pooled in a separation funnel to separate the CH_3OH:H_2O and $CHCl_3$ phases. The $CHCl_3$ phase was gathered and dried with Na_2SO_4 (Enola). The extract was filtered once again and rotary-evaporated at 40 °C max. until it formed thick syrup (Bligh & Dyer, 1959).

For the extraction of lipids from dried berries a similar approach to above was used, applying solvents with different polarities (hexane (polarity index 0.1), petroleum ether (polarity index 0.1), diethyl ether (polarity index 2.8), ethyl acetate (polarity index 4.4), and chloroform (polarity index 4.1)) (Labscan, Czech Republic). The weighed-out berry powder was mixed with 50 mL of the chosen solvent and sonicated for 20 minutes. The extract was left to shake for 24 hours, then sonicated for 20 minutes again and filtered. The filter paper together with the berry residues was extracted again with 50 mL solvent

by sonicating the mixture for 20 minutes, then filtering. The process was repeated twice. All extracts were pooled and evaporated until dry. The dry residue was then dissolved in 10 mL $CHCl_3$ and stored at -20 °C.

Sample preparation and gas chromatography–mass spectrometry analysis

The obtained berry extracts were evaporated to the amount of dry residue constituting ~20 mg in each sample. After the evaporation, the sample was kept in an exicator for 1 hour to make sure that it does not contain any residues of water. The dry sample was dissolved in 1 mL of acetonitrile (Sigma-Aldrich, USA), and 0.2 mL of N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) (Sigma-Aldrich, USA) was added. The sample was then heated at 60 °C for 1 hour. 0.5 mL internal standard was added to the sample. Palmitic acid methyl ester and dinonyl phthalate were used as an internal standard at the concentration of 150 mg L^{-1} (Sigma-Aldrich). The sample was mixed in a 1.5 mL chromatography vial.

The parameters and chromatography settings can be seen in Table 1. The identification of the compounds separated in the GC was done using Perkin Elmer TurboMass v 6.0.0.1811 software, which uses NIST MS Search 2.0 spectral library. Several spectra of substances that could not be identified using the built-in library were identified comparing with the previously published spectra by other authors: sterols were identified according to Brooks et al., 1968, and Yang et al., 2003, whereas fatty acids were identified according to Mjøs 2004.

Table 1. Equipment and parameters used in the gas chromatography–mass spectrometry analysis

Gas chromatograph–mass spectrometer Clarus 680/Clarus SQ8 (Perkin Elmer, USA)	
Column	Perkin Elmer Elite-5MS (5% diphenyl + 95% dimethyl polyoxane) 30 m x 0.25 mm x 0.25 μm. Working temperature range: +60 ° to +350 °C
Thermostat	Temperature programme: +75° (2 min) ramp to +130 °C with 20 °C min^{-1}, then to +300 °C with 4°C min^{-1} held for 15 min
Carrier	Helium; flow rate 2.0 mL min^{-1}; split ratio 1:4
Injector	Temperature +300 °C; injection done with auto-sampler; injected volume: 0.5 μL
Detector	Mass selective detector with quadrupole mass analyser; electron impact ionisation; energy: 70eV; ion source temperature: +300 °C; interface temperature: – +300 °C
Analysis time	62.25 min

Statistics

A triplicate determination for the Bligh-Dyer extraction was done to determine the standard deviation (SD) for the dry residue and selected compounds found in the fresh blueberries. Also, triplicate determination was done on the powdered blueberries and lingonberries. PCA analysis was done in order to group berry species based on compositional variability, and results visualized in component plot. PCA analysis was done to visualise the sample relations to each other. Hierarchical agglomerative cluster analysis was done to see how the chemical compounds link between the different analysed berries. Combining of clusters performed by Ward's method, were for each

cluster, the means for all variables and squared Euclidean distance are calculated. Accordingly to calculated summed distances clusters are merged where smallest increase of distances occurs. PCA and cluster analyses were done using Statistical Package for the Social Sciences (SPSS) software (IBM®, version 22).

RESULTS AND DISCUSSION

The objective of the study was analysis of freely available lipids in 9 species of northern berries belonging to the *Ericaceae*, *Empetraceae* and *Rosaceae* families. Containing high concentrations of biologically active substances, these berries are traditionally used in ethnomedicine in the Baltic countries, and they have a perspective for use as nutraceuticals and for isolation of biologically active extracts or individual substances (Yang et al., 2011).

Table 2. Summary of sample extractions and corresponding dry residues

Extraction type	Berries extracted, weight	Dry residue, mg g^{-1} berries
Bligh-Dyer (Bligh and Dyer, 1959) ($CH_3OH/CHCl_3$ 2:1)	Fresh blueberry 50 g	8.62
	Fresh bilberry 50 g	3.62
	Fresh lingonberry 50 g	5.68
	Fresh cloudberry 50 g	2.70
	Fresh black crowberry 50 g	4.64
	Fresh cranberry 50 g	3.72
	Fresh rowanberry 50 g	4.84
	Fresh highbush blueberry (HB)	2.36
	(cv. Blue Ray(LKM), cv. Chippewa(MKM)) 50 g each	3.06
Bligh-Dyer, 3 extractions	Fresh blueberry 50 g each extr.	8.43±0.32
Hexane	Powdered blueberry 5 g	46.22
Chloroform	Powdered blueberry 5 g	75.81
Petroleum ether	Powdered blueberry 5 g	40.15
Ethyl acetate	Powdered blueberry 5 g	52.53
Diethyl ether	Powdered blueberry 5 g	44.62
Diethyl ether, 3 extractions	Powdered blueberry 5 g each extr.	43.54±1.54
Diethyl ether, 3 extractions	Powdered lingonberry 5 g each extr.	32.03±0.52

To extract lipids from fresh berries, the main task was to dehydrate fresh, homogenised berries. To do this, an approach suggested by Bligh-Dyer (Bligh and Dyer, 1959) was used. A mixture of $CHCl_3$ and CH_3OH was used, ensuring simultaneous dehydration of the berry mass and transfer of lipids to the $CHCl_3$ phase. The yield of lipid fraction using Bligh-Dyer extraction was from 2.70 mg dry residue g^{-1} of cloudberries to 8.43 mg dry residue g^{-1} of blueberries. The relative standard deviation for Bligh-Dyer extraction was determined to be ± 3.8%.

Considering the possible biological activity of extracts and results of other studies, analysis of freely available lipids were done (Fig. 1, Tables 3 and 4).

Table 3. Peaks of the blueberry extract chromatogram (Fig. 1), concentration of the substances found and their typical mass fragmentation

Peak No.	Rt, min	Compound	C, μg g^{-1}	Typical mass fragments
1	4.70	Lactic acid	0.88	117(100), 73(90), 147(79), 191(16)
2	4.87	Hexanoic acid	0.53	75(100), 73(82), 173(58), 117(24)
3	5.83	Heptanoic acid	0.71	73(100), 75(92), 187(56), 117(48)
4	6.82	Benzoic acid	6.13	179(100), 105(69), 77(54), 135(44)
5	6.87	Octanoic acid	1.63	73(100), 75(67), 201(53), 117(51)
6	8.39	Nonanoic acid	0.61	73(100), 74(31), 117(86), 215(62)
7	10.15	Decanoic acid	1.12	73(100), 75(73), 117(62), 229(53)
8	10.73	Malic acid	4.61	73(100), 147(35), 233(16), 75(12)
9	12.16	Undecanoic acid	0.25	73(100), 75(70), 117(60), 243(50)
10	12.38	m-Hydroxybenzoic acid	0.30	267(100), 73(58), 193(43), 223(40)
11	14.36	Dodecanoic acid	0.64	117(100), 73(92), 257(73), 75(72)
12	18.18	D-fructose	18.24	73(99), 204(44), 217(30), 147(24)
13	18.95	Tetradecanoic acid	0.58	73(100), 117(68), 75(50), 285(43)
14	20.90	Phenyloctanoic acid	3.16	75(100), 91(99), 117(95), 73(93)
15	21.23	Pentadecanoic acid	0.44	73(100), 117(98), 75(68), 299(58)
16	23.52	Palmitic acid	38.46	117(100), 73(94), 75(67), 132(49)
17	25.66	Heptadecanoic acid	0.57	73(100), 117(68), 75(50), 327(43)
18	27.04	9,12-Octadecadienoic acid	26.44	73(100), 75(78), 67(61), 81(47)
19	27.20	9,12,15-Octadecatrienoic acid	102.10	73(100), 75(83), 79(75), 67(46)
21	27.34	Trans-11-octadecenoic acid	51.16	73(100), 75(87), 117(71), 129(56)
22	27.75	Octadecanoic acid	21.31	73(100), 117(83), 75(66), 129(43)
23	31.74	Eicosanoic acid	5.52	73(100), 117(93), 75(62), 369(57)
24	32.24	Butyl 9,12-octadecadienoate	7.96	73(100), 117(93), 75(62), 369(57)
25	32.35	Butyl 9,12,15-octadecatrienoic acid	19.34	73(100), 75(86), 117(78), 129(51)
26	34.52	α-Monopalmitin	0.33	28(100), 73(48), 371(46), 147(30)
27	35.43	Docosanoic acid	0.72	117(100), 73(97), 75(60), 132(52)
28	37.35	Tetracosan-1-ol	0.59	411(100), 75(97), 28(41), 43(41)
29	38.45	Squalene	0.82	57(100), 43(93), 82(73), 55(67)
30	38.89	Tetracosanoic acid	2.11	73(100), 117(92), 75(72), 43(50)
31	40.65	1-Hexacosanol	4.20	439(99), 440(36), 75(29), 57(16)
32	42.22	Octacosanal	2.76	57(100), 43(82), 82(68), 55(56)
33	43.54	α-Tocopherol (Vitamin E)	0.65	73(100), 502(94), 237(87), 236(65)
34	43.76	1-Octacosanol	0.40	468(100), 75(72), 57(61), 43(51)
35	45.16	Campesterol	12.74	73(100), 129(84), 43(43), 75(38)
36	45.33	1-Triacontanal	0.95	57(100), 43(86), 82(82), 55(64)
37	46.46	β-Sitosterol	84.64	129(100), 73(61), 43(49), 357(43)
38	46.70	β-Amyrin	11.44	218(100), 203(40), 73(34), 75(25)
39	47.31	α-Amyrin	5.30	218(100), 73(36), 189(31), 203(28)
40	49.81	Betulin	0.53	73(100), 203(67), 189(43), 75(41)
41	49.98	Oleanolic acid	14.85	203(100), 73(99), 202(78), 189(46)
42	51.10	Ursolic acid	20.96	203(40), 73(99), 202(56), 133(55)

Table 4. Lipid analysis of 9 chosen species of berries (ND – substance not detected; all values are expressed as μg of substance g^{-1} of berries

Rt, min	Compound	Cran-berry	Crow-berry	Cloud-berry	Lingon-berry	HB cv. Blue Ray	HB cv. Chippewa	Bil-berry	Blue-berry	Rowan-berry
4.70	Lactic acid	1.16	2.91	0.91	2.45	2.52	1.30	2.23	0.88	0.43
4.87	Hexanoic acid	0.95	0.44	1.10	1.51	1.25	0.54	0.83	0.53	0.40
5.58	m-Cresol	ND	ND	1.26	ND	ND	ND	ND	ND	ND
5.72	Benzyl alcohol	0.69	0.72	22.21	3.00	ND	ND	ND	ND	ND
5.83	Heptanoic acid	0.47	0.73	0.39	0.58	0.85	0.41	0.57	0.71	0.74
6.35	Pantoyl lactone	ND	ND	0.53	ND	ND	ND	ND	ND	ND
6.82	Benzoic acid	37.08	2.84	68.41	164.40	4.40	0.64	0.51	6.13	2.90
6.87	Octanoic acid	0.87	0.32	0.45	1.31	1.86	1.00	0.67	1.63	1.05
7.01	Phosphoric acid	3.09	ND	ND	3.31	ND	ND	0.65	13.67	2.13
7.04	Glycerol	ND	2.65	4.77	ND	0.18	0.14	0.08	0.28	ND
7.48	Phenylacetic acid	ND	0.49	ND	ND	0.13	0.25	0.27	0.23	ND
7.64	Succinic acid	1.91	0.82	4.56	0.54	0.11	0.17	0.22	0.32	2.89
7.73	Pyrocatechol	0.08	4.64	0.13	ND	ND	0.08	0.13	0.25	ND
7.81	Methylsuccinic acid	0.61	0.67	0.63	0.28	0.33	0.15	0.27	0.25	ND
8.03	Benzenepropanol	ND	ND	0.54	ND	ND	ND	ND	ND	ND
8.20	Fumaric acid	0.32	0.26	0.36	ND	ND	ND	0.12	0.08	ND
8.25	o-Toluic acid	ND	ND	ND	ND	0.59	0.13	ND	ND	0.25
8.39	Nonanoic acid	0.70	0.67	0.34	1.43	1.40	0.44	0.65	0.61	0.74
8.47	m-Toluic acid	ND	ND	ND	ND	0.40	0.08	ND	0.08	ND
9.13	Glutaric acid	0.39	0.21	0.48	ND	ND	0.09	0.22	0.22	ND
9.43	Hydrocinnamic acid	ND	0.18	0.21	0.24	0.13	0.08	0.13	0.19	0.21
9.60	2-Deoxytetronic acid	ND	0.42	ND	0.41	0.24	0.23	0.46	0.34	ND
9.65	Cinnamic acid	0.22	ND	8.54	1.42	ND	ND	ND	ND	ND
9.98	9-Decenoic acid	0.16	0.22	ND	0.37	0.18	0.10	0.17	0.20	ND
10.15	Decanoic acid	ND	0.46	0.51	0.79	2.22	1.51	0.48	1.12	1.00

Table 1 (continued)

10.73	Butanedioic acid	41.68	ND	10.57	0.44	ND	ND	3.37	4.61	0.25
11.14	Salicylic acid	0.21	ND	0.17	1.61	ND	ND	0.14	0.16	ND
11.47	Terpinol	0.15	ND	ND	ND	ND	ND	ND	ND	ND
11.47	p-Anisic acid	ND	ND	0.18	0.33	ND	ND	ND	ND	ND
11.74	Vanillin	ND	0.26	0.11	0.08	ND	0.08	ND	0.16	ND
11.99	10-Undecenoic acid	ND	0.29	ND	ND	0.23	0.10	0.17	0.27	0.19
12.02	Trans-Cinnamic acid	0.48	ND	6.95	5.95	ND	ND	ND	ND	ND
12.16	Undecanoic acid	0.20	0.27	0.13	0.35	1.15	0.13	0.26	0.25	1.06
12.38	m-Hydroxybenzoic acid	0.26	0.29	0.18	0.52	0.28	0.16	0.29	0.30	0.28
12.73	β-Phenyllactic acid	0.14	0.21	0.23	ND	ND	ND	0.13	ND	ND
13.23	Pimelic acid	0.35	ND	0.17	0.25	ND	ND	ND	ND	ND
13.75	p-Salicylic acid	0.26	1.02	0.91	1.06	ND	0.15	0.14	0.17	0.36
13.97	Vanillic alcohol	ND	0.48	ND	ND	ND	ND	ND	ND	ND
14.00	4-Hydroxyphenylacetic acid	0.15	ND	0.21	ND	ND	ND	ND	ND	ND
14.36	Dodecanoic acid	0.87	0.83	1.47	1.92	1.72	0.36	1.06	0.64	5.19
15.40	Octanedioic acid	0.46	ND	0.53	0.37	ND	ND	ND	ND	ND
16.50	9-tridecenoic acid	0.36	ND	ND	0.63	ND	ND	0.41	0.21	ND
16.61	n-Tridecanoic acid	0.17	ND	ND	ND	ND	ND	ND	0.19	1.41
16.91	Vanillic acid	0.60	1.02	0.45	1.40	ND	ND	ND	ND	ND
17.71	Nonadioic acid	0.65	0.22	0.55	1.49	ND	ND	0.15	0.17	ND
18.18	D-fructose	ND	2.90	ND	ND	ND	ND	ND	ND	ND
18.21	Protocatechuic acid	ND	0.46	0.09	ND	ND	ND	0.08	ND	ND
18.23	Citric acid	1.01	ND	ND	0.25	ND	ND	ND	ND	ND
18.65	9-Tetradecenoic acid	ND	ND	0.51	ND	ND	ND	ND	ND	ND
18.95	Tetradecanoic acid	0.73	0.70	1.64	1.65	5.76	ND	0.41	0.58	9.72
20.05	Syringic acid	ND	1.58	ND	ND	0.12	0.09	ND	ND	ND
20.49	Ferulic acid	0.16	ND	0.68	ND	ND	ND	ND	ND	ND
20.90	Phenyloctanoic acid	0.39	0.38	0.38	0.96	0.48	0.20	0.35	3.16	ND

Table 1 (continued)

21.01	p-Coumaric acid	0.33	ND	5.32	1.10	ND	ND	ND	0.08	ND
21.23	Pentadecanoic acid	0.40	0.27	ND	0.62	2.31	0.26	0.27	0.44	1.55
22.69	9-Hexadecenoic acid	ND	ND	0.13	ND	ND	ND	ND	ND	ND
23.24	11-Hexadecenoic acid	ND	ND	4.44	ND	ND	ND	ND	ND	ND
23.52	Hexadecanoic acid	16.39	17.07	12.17	23.48	45.80	14.62	21.41	38.46	39.49
24.45	Isoferulic acid	0.08	ND	1.45	0.43	ND	0.23	ND	ND	ND
25.47	Caffeic acid	ND	ND	0.25	ND	ND	ND	ND	ND	ND
25.66	Heptadecanoic acid	0.46	0.37	0.30	0.64	1.41	0.43	0.44	0.57	1.53
27.04	9,12-Octadecadienoic acid	19.81	10.41	8.68	22.81	1.99	5.01	17.83	26.44	1.29
27.20	Trans-9-Octadecenoic acid	32.16	27.14	23.28	62.83	20.54	13.82	40.41	102.10	3.86
27.34	Trans-11-Octadecenoic acid	10.88	8.98	1.42	22.71	17.98	7.03	16.12	51.16	6.26
27.75	Octadecanoic acid	5.86	4.23	3.78	6.47	13.97	5.00	8.85	21.31	11.42
29.02	Linoleic acid	11.17	6.55	1.74	14.39	0.50	3.44	10.87	0.08	ND
29.79	Nonadecanoic acid	ND	0.19	ND	ND	0.60	0.35	0.24	0.18	1.11
31.19	11-Eicosenoic acid	0.53	0.35	0.43	0.40	0.65	0.20	0.19	ND	ND
31.74	Eicosanoic acid	2.63	3.33	1.05	13.31	2.16	4.81	4.21	5.52	1.57
32.24	Butyl 9,12-octadecadienoate	1.76	0.29	0.34	4.78	1.44	1.65	3.46	7.96	ND
32.35	Butyl 9,12,15-octadecatrienoic acid	3.44	0.30	0.58	7.98	2.73	2.71	7.25	19.34	ND
32.57	Butyl 11-octadecenoic acid	2.24	ND	0.13	0.76	0.82	0.64	1.26	5.28	ND
32.90	Pentacosane	ND	0.20	0.68	ND	ND	0.20	0.54	ND	ND
33.61	Heneicosanoic acid	0.38	0.22	0.08	0.24	0.34	0.28	0.29	ND	ND
33.83	1-Docosanol	0.14	2.71	0.87	1.13	0.18	0.15	ND	ND	0.77
34.52	α-Monopalmitin	0.37	0.56	0.39	0.72	0.27	0.24	0.30	0.33	0.46
35.34	Tetracosanal	ND	2.10	ND	0.46	ND	ND	0.48	ND	ND
35.43	Docosanoic acid	2.40	0.52	0.76	2.72	1.31	1.04	0.85	0.72	0.50
35.62	1-Tricosanol	ND	0.37	0.11	ND	ND	ND	ND	ND	0.20
36.55	Heptacosane	ND	0.45	3.65	0.31	ND	ND	1.42	0.42	0.48
37.19	Tricosanoic acid	0.43	0.22	0.15	0.62	0.26	0.28	0.47	ND	ND
37.35	Tetracosan-1-ol	0.33	14.50	2.43	1.63	0.36	0.26	0.33	0.59	1.91

Table 1 (continued)

37.93	α-Monostearin	0.32	0.48	0.25	0.70	0.13	0.10	0.32	0.35	0.12
38.45	Squalene	1.17	0.87	0.37	2.04	1.16	0.54	0.82	0.82	1.15
38.89	Tetracosanoic acid	3.35	1.74	0.75	2.50	0.24	0.72	5.91	2.11	ND
39.03	1-Pentacosanol	ND	0.54	0.22	ND	0.09	0.12	0.21	0.28	0.31
39.96	Nonacosane	ND	1.55	0.74	2.48	0.12	0.45	0.22	ND	4.48
40.52	Pentacosanoic acid	0.34	0.54	ND	0.24	ND	0.08	ND	ND	ND
40.65	1-Hexacosanol	ND	3.86	2.98	0.82	0.45	0.58	0.97	4.20	0.86
41.34	γ-Tocopherol	0.13	0.20	0.15	0.23	0.61	0.45	0.13	0.25	0.25
42.11	Hexacosanoic acid	2.00	0.49	0.40	2.92	ND	ND	0.68	ND	ND
42.22	Octacosanal	0.66	0.90	0.21	1.35	0.39	0.82	13.96	2.76	ND
43.32	Chlorogenic acid	ND	ND	ND	ND	0.24	1.37	ND	ND	ND
43.54	α-Tocopherol	1.13	0.77	3.51	2.22	1.44	1.19	0.78	0.65	3.34
43.76	1-Octacosanol	ND	1.17	1.10	0.21	0.56	0.72	1.72	0.40	0.86
45.16	Campesterol	2.98	1.05	0.52	3.91	0.51	0.36	0.47	12.74	ND
45.33	1-Triacontanal	1.30	0.31	ND	1.89	0.32	0.50	0.56	0.95	ND
45.88	α-Tocopherolhydroquinone	ND	ND	ND	ND	0.14	0.11	ND	ND	ND
46.46	β-Sitosterol	6.48	6.25	4.23	11.87	8.59	7.25	8.52	84.64	6.21
46.70	β-Amyrin	0.38	2.11	0.87	1.31	1.03	0.86	1.14	11.44	2.14
47.31	α-Amyrin	0.55	11.05	0.14	1.79	1.31	0.88	0.16	5.30	2.82
47.42	Cycloartenol	0.23	ND	1.57	2.39	ND	3.65	ND	ND	0.98
48.02	Lanosterol	ND	ND	ND	4.92	ND	ND	ND	ND	ND
48.02	Nonacosanoic acid	0.81	ND	0.40	ND	ND	ND	ND	ND	ND
48.05	Triacontanoic acid	0.81	ND	ND	ND	ND	ND	ND	ND	ND
48.63	Lupeol	0.20	1.37	ND	0.77	ND	ND	0.33	0.51	ND
48.82	Erythrodiol	ND	1.81	ND	0.80	ND	ND	ND	ND	ND
49.51	Uvaol	ND	3.61	ND	0.91	ND	ND	ND	ND	ND
49.81	Betulin	ND	ND	0.09	0.88	0.42	0.36	0.57	0.53	1.33
49.98	Oleanolic acid	9.98	11.32	1.09	6.67	0.52	1.42	0.11	14.85	7.02
51.10	Ursolic acid	53.18	44.15	6.24	30.54	1.31	3.94	26.76	20.96	4.94

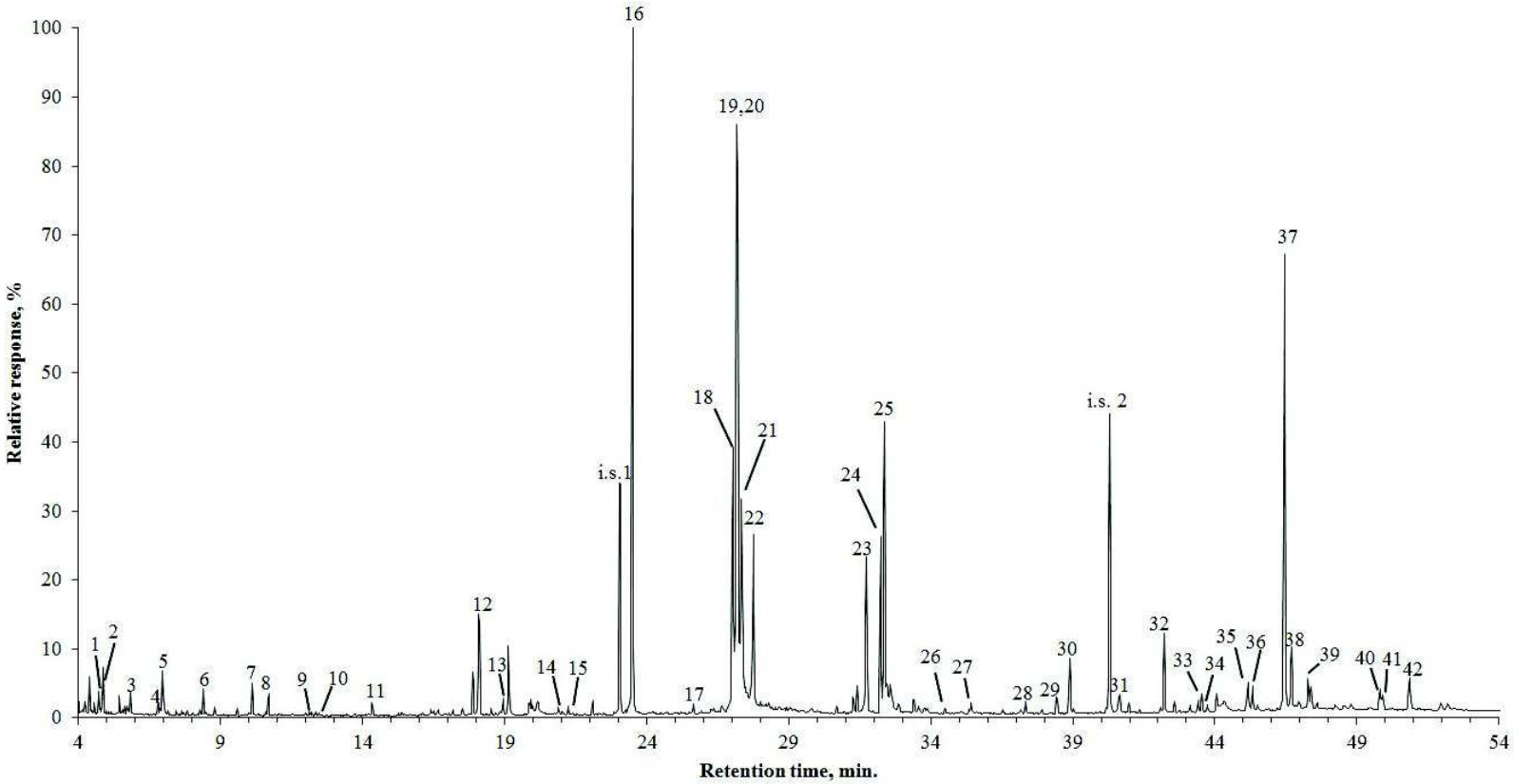

Figure 1. Chromatograms of blueberry Bligh-Dyer extract (peak numbers as indicated in Table 3).

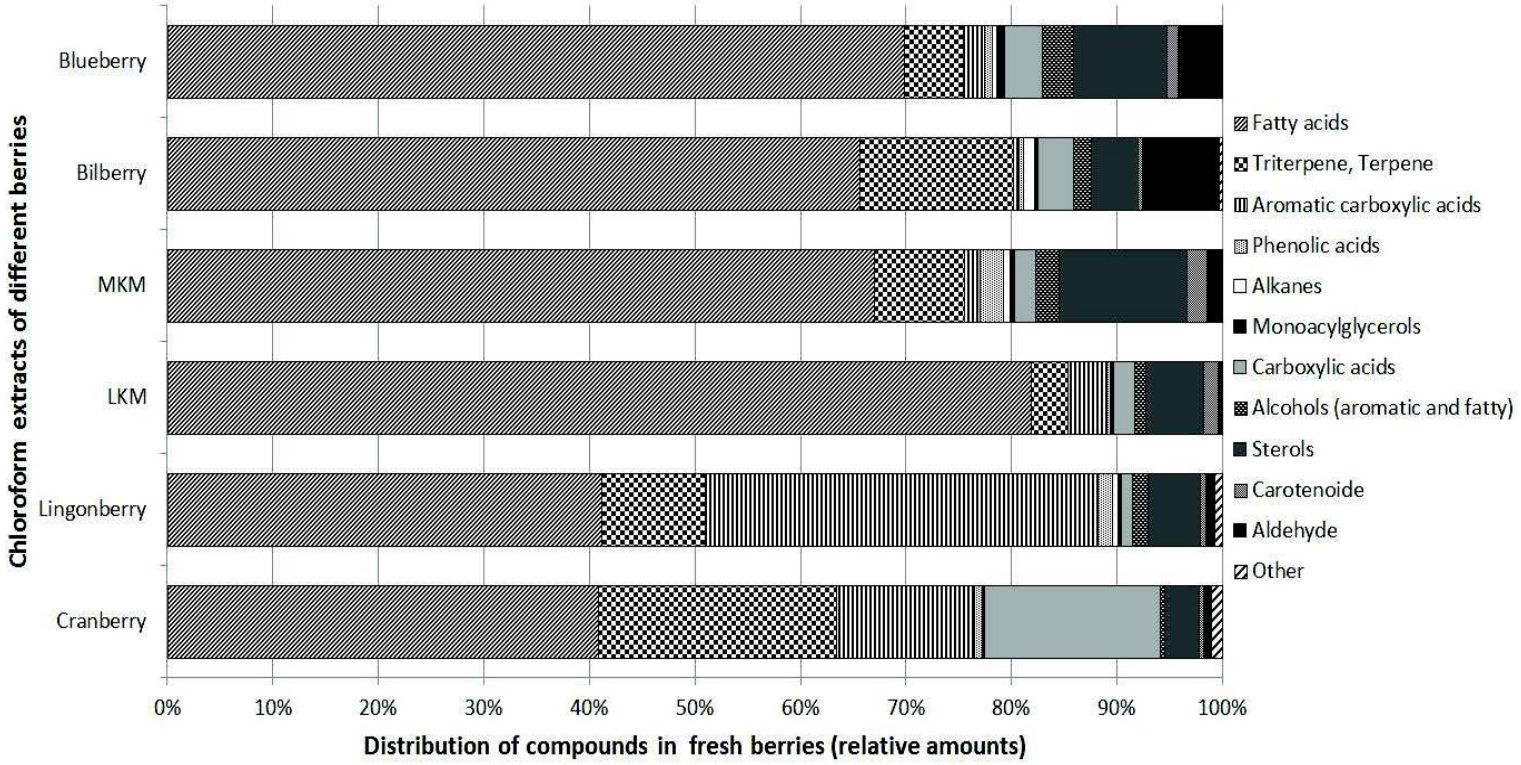

Figure 2. Distribution of compound classes in the studied berries.

In recent years, berry powders (dried, lyophilised berries) are used more frequently in the food industry (Nile & Park 2014). To estimate the most abundant lipids in berry powders, single-solvent extraction was tested using 5 different solvents, considering their possible extraction efficiency, perspectives of their application at an industrial scale and environmental aspects of their application. Single-solvent extraction from berry powder gives much higher yields of dry residue than the use of $CHCl_3$ and CH_3OH mixture. The highest concentration of dry residue was found in the powdered blueberry extracts with diethyl ether, giving 75.81 mg dry residue g^{-1} of berries, while the highest yield of Bligh-Dyer dry residue was 8.62 mg g^{-1} of berries (Table 2). Repeatability for the single-solvent extraction was estimated by doing 3 separate extractions on 2 different berry powders: blueberry and lingonberry. The repeatability was ± 3.54% and ± 1.64% respectively, which is similar to the repeatability of Bligh-Dyer extraction.

The substances with highest concentrations in the blueberry extracts were C18 unsaturated fatty acids (26.44–102.10 mg 100 g^{-1} berries) (Table 4), which were also found in the previous studies (Johansson et al., 1997; Croteau & Fagerson, 1969; Dulf et al., 2012). Two of the peaks of unsaturated fatty acids overlapped (peaks 19 and 20, Fig. 1), and they were quantified as a single peak. The rest of the peaks had good separation.

Freely available lipids obtained using the Bligh-Dyer extraction method from the 9 chosen types of berries were determined. In total, 111 different substances were identified (Table 4) by comparing their mass spectra and retention index with the reference mass spectra and reference retention index (Mjøs, 2004). The highest numbers of substances were found in the cloudberry (86), lingonberry (79) and crowberry (78) extracts.

The lowest numbers of substances were found in the rowanberry (50), highbush blueberry cv. BlueRay (63) and blueberry (65) extracts. 70 substances were identified in the highbush blueberry cv. Chippewa extract and 73 and 75 substances – in the bilberry and cranberry extracts respectively. Substances like benzoic acid (0.64–164.40 μg g^{-1} berries), nonanoic acid (0.34–1.43 μg g^{-1}), m-hydroxybenzoic acid (0.16–0.52 μg g^{-1}), squalene (0.37–2.04 μg g^{-1}), α-tocopherol (0.65–3.51 μg g^{-1}) and β-sitosterol (4.23–84.64 μg g^{-1}) were found in all berries in various concentrations. Some of the substances were found in one berry type only – for example, lanosterol in lingonberries (4.92 μg g^{-1}), m-cresol in cloudberries (1.26 μg g^{-1}), uvaol in crowberries (3.61 μg g^{-1}) and chlorogenic acid in both cultivars of highbush blueberry (0.24–1.37 μg g^{-1}) (Table 4). The substance with the highest concentration was benzoic acid (164.40 μg g^{-1}) in lingonberries. Also, all of the C18 unsaturated fatty acids were in high concentrations (up to 102.10 μg g^{-1} of blueberries).

Single-solvent extractions were done on dry berry powders to find the best solvent for lipid extractions. Five solvents were used: hexane, petroleum ether, diethyl ether, ethyl acetate and chloroform. 22 peaks were identified and quantified (Table 5). The largest amount of substances was extracted using diethyl ether (2.9 mg g^{-1} berry powder) and hexane (1.5 mg g^{-1}), the least amounts were extracted with petroleum ether (0.36 mg g^{-1}). Hexane and diethyl ether extracts contained large amounts of β-sitosterol (341.47 and 334.31 mg g^{-1} berry powder). Diethyl ether extracts contained large amounts of C18 unsaturated fatty acids (101.98–818.52 μg g^{-1}) and malic acid (402.19 μg g^{-1}).

Table 5. Single-solvent extractions on powdered blueberry sample (all values expressed as μg of substance g^{-1} of berry powder

Substance	Hexane	Petroleum ether	Diethyl ether	Ethyl acetate	Chloroform
Benzoic acid	65.97	22.81	64.74	16.90	49.04
Nonanoic acid	2.75	1.92	2.77	2.65	2.65
Butanedioic acid	0.00	0.00	402.19	0.00	0.00
Dodecanoic acid	2.80	1.78	4.32	1.92	3.35
Citric acid	0.00	0.00	77.33	0.00	0.00
Glucofuranoside	13.85	2.10	10.51	3.18	9.12
Palmitic acid	82.23	16.13	121.34	1.83	118.75
9,12-Octadecadienoic acid	75.15	5.59	320.75	718.16	223.80
9, 12, 15-Octadecatrieonoic acid	298.11	10.94	818.52	105.18	37.24
trans-11-Octadecenoic acid	53.61	2.58	101.98	36.65	65.51
Octadecanoic acid	12.77	2.58	41.65	2.87	2.70
Butyl 9,12-octadecadienoate	87.43	27.08	97.48	309.95	90.98
Butyl 9,12,15-octadecatrienoate	261.20	65.93	296.93	38.41	323.27
Butyl octadecanoate	34.52	11.54	39.22	10.25	30.88
Heptacosane	13.70	8.76	8.31	1.73	6.83
Nonacosane	15.05	2.01	15.14	3.21	15.22
Octacosanal	62.53	18.23	77.55	2.42	77.70
Triacontanal	31.15	3.14	27.83	1.86	36.62
β-Sitosterol	341.31	132.43	334.47	52.08	321.37
β-Amyrin	59.78	14.51	57.93	13.54	57.90
α-Amyrin	16.36	7.13	15.04	26.91	18.22
Betulin	8.58	3.84	15.51	17.91	24.63
Total, μg g^{-1} of berries	1,538.85	361.02	2951.52	1367.6	1515.80

To evaluate whether the extractions give constant results, triplicate extraction of the berry powders using diethyl ether were done. Blueberry and lingonberry extracts were prepared, with triplicate determination for each. Lingonberry powder extract of diethyl ether contained high amounts of benzoic acid (289.39 ± 20.30 μg g^{-1}), C18:3 unsaturated fatty acid (417.76 ± 46.1 μg g^{-1}) and ursolic acid (447.21 ± 36.2 μg g^{-1}). Relative standard deviation (RSD) for the 19 identified and quantified compounds in lingonberry powder was estimated to be between 1.95% and 12.57%. Triplicate blueberry powder diethyl ether extracts contained high amounts of malic acid (105.10 ± 7.5 μg g^{-1}), C18:2 unsaturated fatty acid (565.40 ± 14.9 μg g^{-1}) and β-sitosterol (189.21 ± 21.7 μg g^{-1}). RSD for the 22 compounds with highest response found in blueberry diethyl ether extracts ranged from 1.3% to 12.2%.

Table 4 includes many substances that have not been described as part of berry lipids in any of the previous studies. For example, black crowberry lipids have not been described previously. Although several species of the chosen berries have been widely studied (especially the *Vaccinium* species), such a thorough description of free berry lipids has not been reported before. Substances like lactic acid, malic acid, nonanoic acid, decanoic acid and many others found in blueberry extracts have not been described previously. However, a few substances that could not be recognised were found in addition to the identified compounds. No spectra similar to those that have been found could be matched with any of the reference spectra.

All the identified compounds of berry lipids can be divided into respective classes of organic substances, and a total of 11 classes of compounds were found (Fig. 2). The largest class in each type of berries except cloudberry was fatty acids (up to 82% of the total lipids). Blueberry, bilberry, both cultivars of highbush blueberries (LKM and MKM) and rowanberry have very similar profiles of compound classes (Fig. 2). Lingonberry and cloudberry have similar profiles: the fatty acid (41% and 30% respectively), triterpene (10% and 5%) and aromatic carboxylic acid (39% and 35%) classes give the same pattern for both types of berries. Black crowberry and cranberry also give similar patterns in composition profiles – both types of berries contain more triterpenes (35% and 25% respectively) than any other berries. Rowanberries are covered with waxy coating to protect from environmental stresses; they also have the highest amount of alkanes (5%), which are part of the berry cuticular waxes. Cloudberry and black crowberry extracts contained high amounts of alcohols and terpenes (Fig. 2).

The high content of fatty acids in the studied berries is due to the fact that berries have a lot of seeds, where the energy is stored in the form of fatty acids. Blueberry, bilberry and both cultivars of highbush blueberries (LKM and MKM) are closely related, which can also be seen in their compound class profiles.

Many of the berry lipids have high biological activity that might influence the application potential of lipid extracts.

The results of chromatographic analysis (Table 4) demonstrate significant differences amongst the studied berries. However, an open question is how the chemical composition reflects the biological taxonomy. Another open question is whether the growth environment can be considered as a major factor affecting the plant (berry) chemical composition.

Chemotaxonomy shows similarities in the chemical compositions of organisms in respect to their biological classification (Bisby et al., 1980). Chemotaxonomical analysis of the studied berries was done using the Principal Component Analysis (PCA) for the presence or absence of a chemical in the respective berry. Each compound was given a rank: 1 if the specific compound is present (component 2), 0 if absent (component 1). 2 components can explain the total variance of data by 61%. The PCA analysis shows two larger groups of berries, one consisting of cranberry, cloudberry and lingonberry, the second consisting of bilberry, blueberry, rowanberry, crowberry and both cultivars of highbush blueberries (Fig. 3). As expected, the two cultivars of highbush blueberry (LKM and MKM) are very similar to each other when their lipid composition is compared, as it is the same species of berries. Although distinguished as separate species, blueberry and bilberry are very closely related, and their lipid profiles are also similar. Cranberry and lingonberry come from the same genus, whereas cloudberry is from another family of plants. Cranberry, lingonberry and cloudberry have more similarities between each other than they have with, for example, any type of blueberries (positioned on the opposite sides of the axis).

Rowanberry and crowberry have similar lipid profiles with the blueberry species, even though this cannot be explained with the taxonomy of these berries, as rowanberry (*Rosaceae*) and crowberry (*Empetraceae*) belong to different families than blueberries (*Ericaceae*).

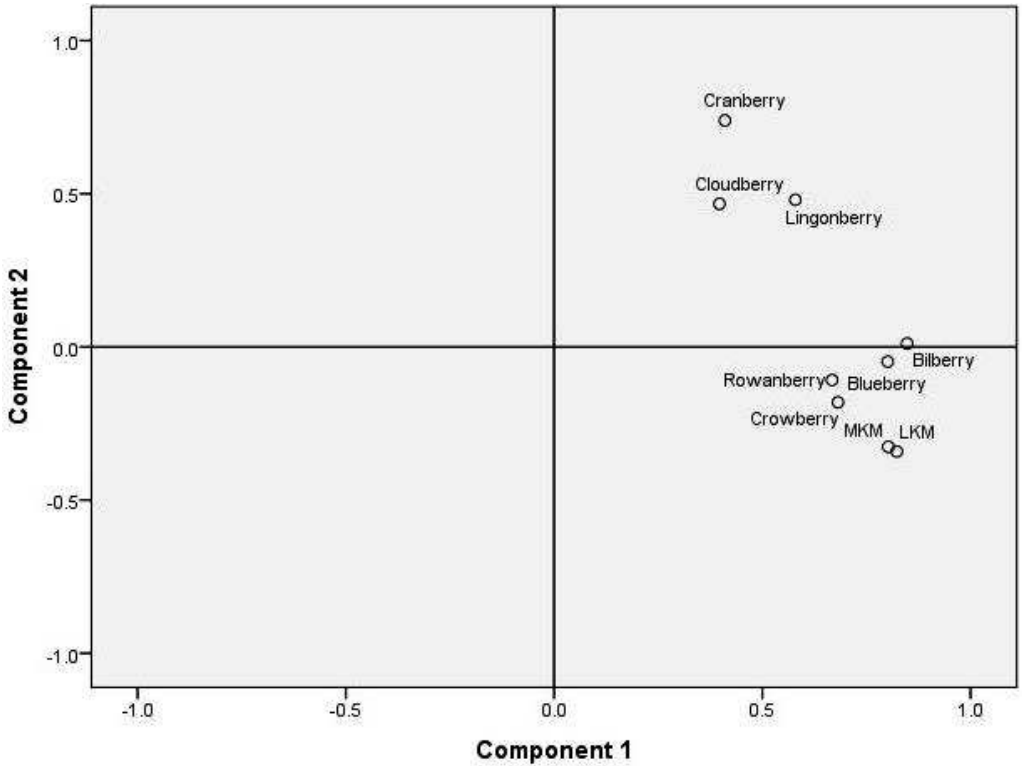

Figure 3. PCA analysis of the compounds present in the studied berries.

To support the results obtained by the PCA analysis even further, a hierarchical cluster analysis (HCA) was done to visualise the relations between different types of berries (Fig. 4). As confirmed by the PCA analysis, there are two distinct groups of berries, one consisting of cranberry, cloudberry and lingonberry, the second consisting of bilberry, blueberry, rowanberry, crowberry and both cultivars of highbush blueberries. In the dendrogram, it can be seen that rowanberries are remotely related to highbush blueberries, blueberries and bilberries, while not having many similarities with those berries. While PCA shows two base groups of berries, HCA demonstrates combination ways for each particular berry cluster, e.g. showing on this basis that in cranberry, lingonberry and cloudberry cluster cranberries are more similar to lingonberries within one cluster. Bilberries and blueberries, including both cultivars of highbush blueberry, show close relation between each other.

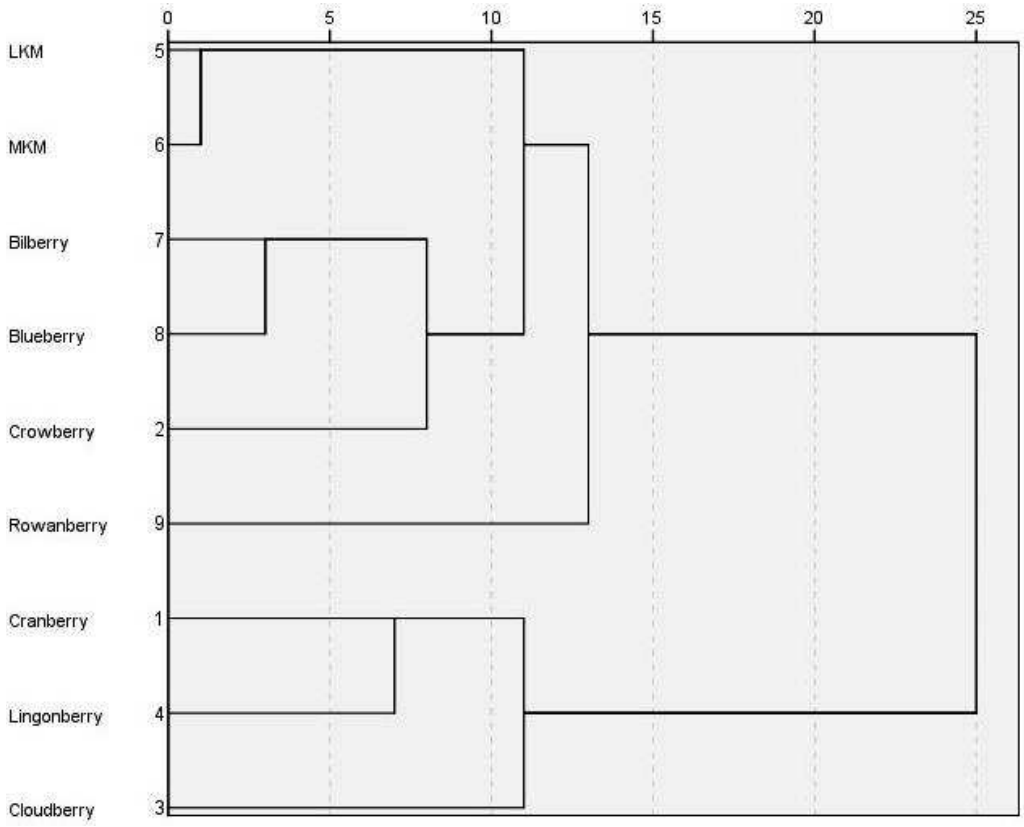

Figure 4. Hierarchical cluster analysis of the compounds present in the studied berries.

CONCLUSIONS

Northern berries contain significant amounts of lipids, depending on the berry species, extraction conditions and solvents used in the extraction process. A lipid profile study was done for 9 species of berries common in Northern Europe and Latvia. 111 compounds were identified, many of which have not been described before as a part of berry lipids. The black crowberry lipid profile has not been studied before. The berry lipids found can be divided into 11 classes of organic compounds, for example, fatty acids, sterols, triterpenoids, carboxylic and phenolic acids and alkanes. The major group of lipids found in the studied berries was fatty acids. However, in respect to the functional significance and potential for application, sterols, triterpenoids and phenolic acids are of special interest. Lipids of some berries contain substances specific for the relevant species, for example, a high concentration of benzoic acid characterises the lipid pool of lingonberries.

The chemotaxonomic analysis of berry lipid profiles reflect their taxonomy using the principal component analysis (PCA) and hierarchical cluster analysis. as it demonstrated relations between the species (different cultivars) and genus. At the same time, no strict relation was seen between the families of berries, probably due to environmental factors (climatic conditions, growth substrate, harvest time, etc.). Berry

lipids are mainly extracted from berry seeds, and unsaturated fatty acids are used in many commercial products. This study has demonstrated the presence of a wide array of biologically active compounds (phytosterols and triterpenoids and others) that can support new fields of application of berry lipids, for example, as functional food, biopharmacy.

ACKNOWLEDGEMENTS. This study has been carried out with the support of the Latvia National research program 'ResProd'.

REFERENCES

Bisby, A., Vaughan, J.G. & Wright, C.A. 1980. *Chemosystematics: principles and practice.* London: Academic Press, 257 pp.

Bligh, E.G. & Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* **37**, 911–917.

Brooks, C.J.W., Horning, E.C. & Young, J.S. 1968. Characterization of sterols by gas chromatography- mass spectrometry of the trimethylsilyl ethers. *Lipids* **3**, 391–401.

Corte, A.D., Chitarrini, G., Iole, M.D.G., Masuero, D., Soini, E., Mattivi, F. & Vrhovsek, U. 2015. A rapid LC-MS/MS method for quantitative profiling of fatty acids, sterols, glycerolipids, glycerophospholipids and sphingolipids in grapes. *Talanta* **140**, 52–61.

Croteau, R. & Fagerson, I.S. 1969. Seed lipids of the American cranberry (*Vaccinium macrocarpon*). *Phytochemistry* **8**, 2219–2222.

Croteau, R. & Fagerson, I.S. 1971. The chemical composition of the cuticular wax of cranberry. *Phytochemistry* **10**, 3239–3245.

Dulf, F.V., Andrei, S., Bunea, A. & Socaciu, C. 2012. Fatty acids and phytosterol contents of some Romanian wild and cultivated berry pomaces. *Chemical Papers* **66**, 925–934.

Hoed, V.V., Clercq, N.D., Echim, C., Andjelkovic, M., Leber, E., Dewettinck, K. & Verhe, R. 2009. Berry seeds: a source of speciality oils with high content of bioactives and nutritional value. *Journal of Food Lipids* **16**, 33–49.

Järvinen, R., Kaimainen, M. & Kallio, H. 2010. Cutin composition of selected northern berries and seeds. *Food Chemistry* **122**, 137–144.

Johansson, A., Laakso, P. & Kallio, H. 1997. Characterization of seed oils of wild, edible Finnish berries. *Zeitschrift für Lebensmittel Untersuchung und Forschung,* A., **204**, 300–307.

Johansson, A., Laine, T., Linna, M.M. & Kallio, H. 2000. Variability in oil content and fatty acid composition in wild northern currants. *European Food Research and Techno*logy **211**, 277–283.

Joseph, S., Edirisinghe, I. & Burton-Freeman, B. 2014. Berries: anti-inflammatory effects in humans. *Journal of Agricultural and Food Chemistry* **62**, 3886–3903.

Kähkönen, M.P, Hopia, A. & Heinonen, M. 2001. Berry phenolics and their antioxidant activity. *Journal of Agricultural and Food Chemistry* **49**, 4076–4082.

Kallio, H., Nieminen, R., Tuomasjukka, S. & Hakala, M. 2006. Cutin composition of five Finnish berries. *Journal of Agricultural and Food Chemistry* **54**, 457–462.

Määttä-Riihinen, K., Kähkönen, M., Törrönen, R. & Heinonen, M. 2005. Catechins and procyanidins in berries of *Vaccinium* species and their antioxidant activity. *Journal of Agricultural and Food Chemistry* **53**, 8485–8491.

Matthaus, B. & Ozcan, M.M. 2014. Fatty acid, tocopherol and squalene contents of *Rosaceae* seed oils. *Botanical Studies* **55**, 1–6.

Mjøs, S.A. 2004. Two-dimensional fatty acid retention indices. *Journal of Chromatography* **1061**, 201–209.

Nile, S.H. & Park, S.W. 2014. Edible berries: bioactive components and their effect on human health. *Nutrition* **30**, 134–144.

Oomah, B.D., Ladet, S., Godfrey, D.V., Liang, J. & Girard, B. 2000. Characteristics of raspberry (*Rubus idaeus* L.) seed oil. *Food Chemistry* **69**, 187–193.

Rodriguez-Mateos, A., Heiss, C., Borges, G. & Crozier, A. 2013. Berry (poly)phenols and cardiovascular health. *Journal of Agricultural and Food Chemistry* **62**, 3842–3851.

Silva, S., Costa, E.M., Pereira, M.F., Costa, M.R. & Pintado, M.E. 2013. Evaluation of the antimicrobial activity of aqueous extracts from dry *Vaccinium corymbosum* extracts upon food microorganism. *Food Control* **34**, 645–650.

Szakiel, A., Paczkowski, C., Koivuniemi, H. & Huttunen, S. 2012a. Comparison of the triterpenoid content of berries and leaves of lingonberry *Vaccinium vitis-idaea* from Finland and Poland. *Journal of Agricultural and Food Chemistry* **60**, 4994–5002.

Szakiel, A., Paczkowski, C., Pensec, F. & Bertsch, C. 2012b. Fruit cuticular waxes as a source of biologically active triterpenoids. *Phytochemical Reviews* **11**, 263–284.

Yang, B., Ahotupa, M., Määttä, P. & Kallio, H. 2011. Composition and antioxidative activities of supercritical CO_2- extracted oils from seeds and soft parts of northern berries. *Food Research International* **44**, 2009–2017.

Yang, B., Koponen, J., Tahvonen, R. & Kallio, H. 2003. Plant sterols in seeds of two species of *Vaccinium* (*V. myrtillus* and *V. vitis-idaea*) naturally distributed in Finland. *European Food Research Technology* **216**, 34–38.

Yang, B. & Kortesniemi, M. 2015. Clinical evidence on potential benefits of berries. *Current Opinions Food Science* **2**, 36–42.

Zadernowski, R., Naczk, M. & Amarowicz, R. 2003. Tocopherols in sea buckthorn (*Hippophae rhamnoides* L.) berry oil. *Journal of American Oil Chemists Society* **80**, 1, 55–58.

Zlatanov, M.D. 1999. Lipid composition of Bulgarian chokeberry, black currant and rose hip seed oils. *Journal of the Science of Food and Agriculture* **79**, 1620–1624.