Environmental and Experimental Biology (2015) 13: 147–158 *Original Paper*

Polyphenols, carbohydrates and lipids in berries of *Vaccinium* species

Linards Klavins, Laura Klavina, Agnese Huna, Maris Klavins*

University of Latvia, Raina bulvaris 19, Riga LV-1586, Latvia

*Corresponding author, E-mail: maris.klavins@lu.lv

Abstract

Wild berries commonly found in the forests and bogs of Latvia are an excellent source of natural antioxidants, vitamins and fatty acids, all of which are substances with high biological activity. The aim of this study was to determine radical scavenging potential and total polyphenol, carbohydrate and flavonoid concentrations in polar solvents extracts, and also to conduct gas chromatographic-mass spectrometric analysis of lipid fraction composition in six types of berries of the Ericaceae family, genus *Vaccinium* (blueberry, bilberry, two cultivars of highbush blueberry, lingonberry and cranberry). Polarity of extrahents and procedure of extraction influenced the yield of biologically active substances in extracts. Polyphenol, flavonoid and carbohydrate extraction yields were optimised. In the berries analysed, 111 lipid compounds were identified and quantified. The lipid fraction contained compound classes like fatty acids, sterols, triterpenoids, alkanes, phenolic and carboxylic acids as well as carotenoids. All of the fresh berries studied contained high amounts of C18 unsaturated fatty acids (for example, up to 102 μg g^{-1} in blueberries) and phytosterols (86 μg g^{-1} of β-sitosterol in blueberries). High amounts of benzoic acid were found in lingonberry (164 μg g^{-1}).

Key words: berries, extraction, lipids, polyphenols, radical scavenging activity, *Vaccinium*.
Abbreviations: GC-MS, gas chromatography-mass spectrometry; GE, gallic acid equivalent.

Introduction

Currently there is a strong scientific evidence that regular consumption of vegetables, fruits and berries prevents and reduces the development of chronic diseases (Hooper, Cassidy 2006). 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) and protection against cancer (Arts, Hollman 2005), as well as anti-inflammatory, cholesterol-lowering (Patočka 2003; Joseph et al. 2014) and antimicrobial effects (Silva et al. 2013).

Berries are rich in secondary metabolites (Nile, Park 2014), phenolic acids, flavonoids (Häkkinen et al. 1999), tannins and lipids. Berries also have high concentrations of polyphenols, antioxidants, vitamins and minerals (Nile, Park 2014). Antimicrobial activity in berries is associated with high levels of phenolic acids and organic acids (Wu et al. 2008). Berries are known for their high content of different phenolic compounds with differing benzene ring substitution and conjugation with carbohydrates via glycosidic bonds. Polyphenols in berries have been widely studied (Riihinen 2005; Törrönen et al. 2012; Yang, Kortesniemi 2015). In recent studies, berry polyphenols have shown the abilities to control the activity of key enzymes in human metabolism, to regulate cellular receptors, signalling pathways and gene expression and even to repair oxidative damage of DNA (Kraft et al. 2008).

Lipids can be classified according to their physical properties, polarity of molecules, and functions in organism into neutral lipids (triglycerides), polar lipids (phospholipids), glycolipids, as well as fatty acids, fatty alcohols, waxes, sterols, terpenoids and other substances (Gunstone 1996). Despite the fact that the content of lipids in berries seems to be low, there are several studies indicating that different groups of low-polarity substance are present (Corte et al. 2015). Lipids in berries (cuticular waxes) have a significant function in protecting them from external impacts, such as pathogens and environmental extremes (Reina-Pinto, Yephremov 2009). Lipids in berries can be found not only in cytoplasm or bound to cellular membranes in pulp cells but also in seeds (Järvinen et al. 2010). Many of the lipid groups found in berries (unsaturated fatty acids, sterols, terpenoids and others) have high biological activity. Since they differ from lipids in mammals, their consumption has an important role in human metabolism. For example, it has been shown that plant (berry) sterols (phytosterols) reduce cholesterol levels in humans (Dulf et al. 2012).

The first studies of lipids in berries were on cranberries (Croteau, Fagerson 1969). 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 was observed that industrially produced seed oils contain high amounts of polyunsaturated fatty acids and phytosterols (Gomas et al. 2015). However, the contents of other low-polarity substances have not been so widely studied, and only a few studies have focused on sterol composition (Dulf et al. 2012; Szakiel et al. 2012) and tocopherols (Zadernowski et al. 2003; Matthaus, Ozcan 2014) in berries. Other groups of substances found in berry seed oils include triterpenoids and sterols (Yang et al. 2003). It has been shown that sterols and triterpenoids have significant biological activity. Studies have demonstrated the anti-inflammatory, antiviral, wound-healing and anticarcinogenic properties of sterols and triterpenoids in berry seed oils (Petronelli et al. 2009, which indicates a need for further study of berry lipids.

Considering the high biological activity of substances present in berries, it is important to determine their composition in order to discover new potential uses, to develop new processing approaches and to support innovation in the uses of berries and their extracts in food, as food additives, in cosmetics, etc. Already now, a wide variety of food supplements, vitamins, herbal teas and other berry processing products are commercially available, such as vitamins with blueberry extracts added for the improvement of vision, blueberry ω-3 fatty acids in capsules, cranberry extracts in capsules for urinary tract infections, hard candies with cranberry taste mixed with different herbs for soothing throat pain, vitamins with berry extracts for strengthening the immune system, and others (Canter, Edzard 2004; Tremblay et al. 2013). Scientists in the cosmetics industry have also appreciated the possible health benefits of berry extracts (Shahidi, Ambigaipalan 2015). Berry oils are widely used in moisturising creams, shampoos and conditioners. The beneficial health effects of berry extracts have been demonstrated, for example, the ability to reduce type 2 diabetes, relieve drye eye symptoms, and positive effects on the cardiovascular system by improving the plasma lipid profile and reducing blood pressure (Yang, Kortesniemi 2015).

The aim of this study was to determine the radical scavenging potential and total polyphenol, carbohydrate and flavonoid contents in berry extracts obtained with polar solvents, and to conduct gas chromatographic-mass spectrometric analysis of the lipid fraction composition in six types of berries of the Ericaceae family, genus *Vaccinium* (blueberry *Vaccinium myrtillus* L., bilberry *Vaccinium uliginosum* L., two cultivars of highbush blueberry *Vaccinium corymbosum* L., lingonberry *Vaccinium vitis-idaea* L., and cranberry *Vaccinium oxycoccos* L.).

Materials and methods

Berry samples and their processing

Six types of berries growing in Latvia were investigated: blueberry (*Vaccinium myrtillus* L.), bilberry (*Vaccinium uliginosum* L.), highbush blueberry (*Vaccinium corymbosum* L.), lingonberry (*Vaccinium vitis-idaea* L.), and cranberry (*Vaccinium oxycoccos* L.). Both cultivars of highbush blueberry (cv. 'Blue Ray' and cv. 'Chippewa') were cultivated, while the other were wild berries.

Blueberries and lingonberries were harvested in summer/autumn (July-October) of 2014 in Vidzeme (Latvia). Cultivated highbush blueberries were harvested in a local garden in the town of Saldus in mid-August 2014. Bilberries and cranberries were harvested in Pienu bog in July 2014. After harvesting, the berries were washed with demineralised water to remove any possible contaminations (dirt, bugs, etc.), dried to remove water and transported to the laboratory where they were frozen to –20 °C and stored at –18 °C. Analysis was conducted within 5 to 7 months.

The berries (blueberries and lingonberries) were dried in a Муссон-2 microwave lyophiliser (Büchi Labortechnik AG), in vacuum, at a temperature not exceeding 50 °C, until containing a maximum of 8% total moisture. After the berries had reached the satisfactory moisture level, they were milled and sieved through two sieves with 1 and 0.6 mm openings.

Extraction of berry lipids

For the extraction of lipids in fresh berries, 50 g of the selected berry were crushed in a mortar. The crushed berries were then mixed with 150 mL of Bligh-Dyer reagent (CH_3OH [Labscan] /$CHCl_3$ [Chempur], 2:1) (Bligh, Dyer 1959). After 2 min of stirring, 50 mL of $CHCl_3$ was added. The mixture was poured in a glass bottle with a cap and sonicated for 40 min. The water in the ultrasound bath (Cole-Parmer) was changed every 20 min to avoid evaporation of the solvents and overheating.

After sonication, the samples were equilibrated to room temperature. Then 50 mL of H_2O were added, and the sample was filtered. The final ratio of the solvents (CH_3OH/$CHCl_3$/H_2O) in the mixture was 2:2:1. The berry residues in the filter were extracted again with 100 mL $CHCl_3$ and, as before, sonicated for another 40 min. 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 residual water was removed with Na_2SO_4 (Enola) for 24 h. The extract was filtered once again and rotary evaporated at 40 °C max. until it had a thick syrup texture.

A simpler approach was used for the extraction of lipids from dried berries, 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).

Weighed out berry powder was mixed with 50 mL of the chosen solvent and sonicated for 20 min. The extract was left to shake for 24 h, then sonicated again for 20 min and filtered. The filter paper, together with the berry residues,

was extracted again with 50 mL solvent by sonicating the mixture for 20 min, then filtered. The process was repeated twice. All the extracts were pooled and evaporated until dry. The dry residue was then dissolved in 10 mL $CHCl_3$ and stored at –20 °C. The dry residue of the extracts was determined.

Determination of dry residue of lipid extracts

The samples were equilibrated to room temperature before withdrawing the sample for determination of the dry residue. An empty, dried glass vial was weighed three times on an analytical balance. Berry extract (1 mL) was withdrawn and put into the weighed glass vial using a volumetric pipette. The sample was left on a heating plate at 40 °C until the solvent had evaporated and then transferred to an exicator for at least 3 h. The glass vial was then weighed three times to determine the amount of dry residue of the extract.

Extraction of berry polyphenols and carbohydrates

Fresh frozen berries were crushed in a mortar and transferred to Petri plates (~ 150 g of fresh weight per sample). After cooling down to ~ –40 °C, the berry mass was put into a lyophyliser (Laboconco Free Zone) and dried for five days. The dry mass was stored in an exicator over P_2O_5.

A dried berry sample (0.1 g) was weighed in a 100 mL bottle, and 50 mL of solvent was added (0 to 96% ethanol, 20 to 100% methanol, 20 to 100% acetone, dioxane, 20 to 100% dimethylsulphoxide [DMSO]). The samples were treated with ultrasound in an ultrasound bath (Cole-Parmer) for 40 min and then for 20 min. The temperature in the ultrasound bath was kept constant at 40 °C by regularly adding cold water. After the treatment with ultrasound, the samples were shaken for 24 h at 140 rpm. After shaking, the samples were repeatedly treated in the ultrasound bath for 40 min and then for 20 min.

After the extraction, the samples were filtered and those with solvent concentrations of 0 and 20% were stored until analysis at –20 °C, and the rest of the samples at 4 °C.

Determination of total polyphenol concentration in berry extracts

Before analysis, berry extracts were kept at room temperature for ~1 h. An extract sample (1 mL) was withdrawn into a test tube, and 5 mL of 10% Folin-Ciocalteu reagent (Aldrich) was added. After 5 min, 4 mL 7.5% sodium carbonate (Aldrich) was added. The test tube was shaken thoroughly and kept in darkness at room temperature for 2 h. The absorption was then measured in a quartz cuvette (d = 1 cm) on a spectrophotometer (Hach-Lange DR 2800) at a 725 nm wavelength. The concentrations were calculated using a standard curve, which was expressed as μg g^{-1} gallic acid (gallic acid equivalent GE) per berry dry matter (Singleton et al. 1999). Three parallel measurements were carried out.

Determination of radical scavenging activity in berry extracts

A sample of extract (0.3 mL) of was added into a test tube and mixed with 3.6 mL of 4% 2,2-diphenyl-1-picrylhydrazyl (Aldrich) solution in 96% ethanol. The mixture was incubated for 20 min in darkness at room temperature. The absorption was measured in a quartz cuvette (d = 1 cm) with a spectrophotometer at a 517 nm wavelength. Three parallel measurements were carried out. The concentrations were calculated using a standard curve, which was expressed as μg g^{-1} gallic acid (gallic acid equivalent GE) per berry dry matter (GE g^{-1}).

Carbohydrate concentration analysis in berry extracts

Before analysis, the berry extracts were kept at room temperature for ~ 1 h. An extract sample (0.1 mL) was added into a test tube and diluted with distilled water to 1 mL. Afterwards, 1 mL of 5% phenol (Aldrich) solution was added, also immediately adding 5 mL of concentrated sulphuric acid (Aldrich). After 10 min, the test tubes with the samples were carefully shaken and left for 20 min at room temperature. The absorption was measured with a spectrophotometer (Hach-Lange DR 2800) at 490 nm. Three parallel measurements were carried out. The

Table 1. Equipment and parameters used in the gas chromatographic-mass spectrometric analysis with Clarus 680/ Clarus SQ8 (Perkin Elmer, USA)

Equipment	Conditions
Column	Perkin Elmer Elite-5MS (5% diphenyl + 95% dimethyl polyoxane) 30 m × 0.25 mm × 0.25 μm, working temperature range 60 to 350 °C
Thermostat	Temperature programme: 75 °C (2 min) ramp to 130 °C with 20 °C min^{-1}, then to 300 °C with 4 °C min^{-1} hold for 15 min
Carrier	Helium, flow rate 2.0 mL min^{-1}, split ratio 1:4
Injector	Temperature 300 °C, injection performed with an autosampler, 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

Table 2. Polyphenols, flavonoids, carbohydrates and radical scavenging activity in extracts of bilberry (*Vaccinium uliginosum* L.). Extraction was performed using treatment with ultrasound for 40 min

Extrahent	Extrahent concentration	Radical scavenging activity (GE g^{-1})	Total polyphenol concentration (GE g^{-1})	Carbohydrate concentration (glucose eq. µg g^{-1})	Flavonoids (µg quercetin g^{-1})
Ethanol	96%	252 ± 2	6.6 ± 0.2	35.5 ± 1.2	103 ± 1
	80%	243 ± 2	5.1 ± 0.2	15.5 ± 1.2	107 ± 1
	60%	234 ± 2	4.8 ± 0.2	27.5 ± 1.2	115 ± 1
	40%	233 ± 2	4.7 ± 0.2	16.5 ± 1.2	105 ± 1
	20%	213 ± 2	4.6 ± 0.2	22.4 ± 1.2	102 ± 1
	0%	202 ± 2	4.5 ± 0.2	21.2 ± 1.2	95 ± 1
Acetone	100%	261 ± 1	5.7 ± 0.2	67.4 ± 1.2	101 ± 1
	80%	243 ± 1	5.3 ± 0.2	118.5 ± 1.2	113 ± 1
	60%	231 ± 1	5.1 ± 0.2	130.5 ± 1.2	99 ± 1
	40%	232 ± 1	4.9 ± 0.2	61.5 ± 1.2	99 ± 1
	20%	191 ± 1	4.8 ± 0.2	38.5 ± 1.2	101 ± 1
Methanol	100%	236 ± 2	4.9 ± 0.2	19.5 ± 1.2	116 ± 2
	80%	245 ± 2	5.4 ± 0.2	31.8 ± 1.2	111 ± 2
	60%	235 ± 2	4.6 ± 0.2	17.5 ± 1.2	98 ± 2
	40%	236 ± 2	4.7 ± 0.2	38.3 ± 1.2	100 ± 2
	20%	235 ± 2	4.5 ± 0.2	21.5 ± 1.2	97 ± 2
DMSO	100%	284 ± 1	5.1 ± 0.2	16.5 ± 1.2	108 ± 1
	80%	264 ± 1	5.1 ± 0.2	26.6 ± 1.2	101 ± 1
	60%	224 ± 1	4.9 ± 0.2	35.5 ± 1.2	106 ± 1
	40%	214 ± 1	4.8 ± 0.2	38.3 ± 1.2	102 ± 1
	20%	174 ± 1	4.7 ± 0.2	25.9 ± 1.2	97 ± 1

carbohydrate amount was determined with a calibration curve using glucose solutions as a standard (Narwal et al. 2011).

Flavonoid concentration analysis in berry extracts

Before analysis, berry extracts were kept at room temperature for ~ 1 h. An aliquot (1.0 mL) of $AlCl_3$ (20 g L^{-1}) was added into a test tube with 1.0 mL of extract. After incubating for 1 h at room temperature, absorption was measured with a spectrophotometer (Hach-Lange DR 2800) at 415 nm.

Three parallel measurements were carried out. The concentrations were calculated using a quercetin dihydrate (Aldrich) standard curve, which was expressed as µg of quercetin per g (dry weight) of berry mass (Mitrovič et al. 2011).

Sample preparation and gas chromatography-mass spectrometry analysis

The obtained berry lipid extracts were evaporated, such that the amount of dry residue would correspond to ~20 mg in each sample. After evaporation, the sample was kept in an exicator for 1 hour to avoid any residual water. The dry sample was dissolved in 1 mL acetonitrile (Sigma-Aldrich), and 0.2 mL of N,O-bis (trimethylsilyl) trifluoroacetamide (Sigma-Aldrich) was added. The sample was heated at 60 °C for 1 h. Internal standard (0.5 mL) was added to each sample. Palmitic acid methyl ester and dinonyl phthalate were used as internal standard at a concentration of 150 mg L^{-1} (Sigma-Aldrich). Samples were mixed in a 1.5 mL chromatography vial.

The quantification was done using two standard curves: 11-point stigmasterol (Sigma-Aldrich) and 9-point stearic acid (Sigma-Aldrich). The parameters and chromatography settings used are presented in Table 1.

Identification of the compounds separated in the GC was performed using the Perkin Elmer TurboMass v 6.0.0.1811 software, using the NIST MS Search 2.0 spectral library.

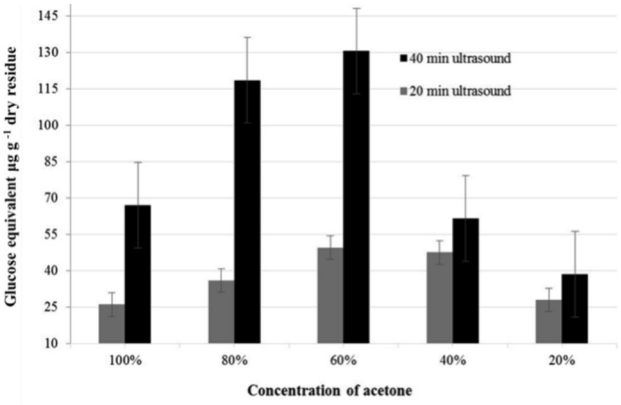

Fig. 1. Impact of ultrasound treatment (20 or 40 min) on the yield of carbohydrates.

Table 3. Flavonoids, carbohydrates and radical scavenging activity in extracts of berries belonging to the *Vaccinium* species. Extracts were obtained with ethanol, treatment with ultrasound was performed for 4 min

Type of berries	Radical scavenging activity (GE g^{-1})	Carbohydrate concentration (glucose eq. μg g^{-1})	Flavonoids (μg quercetin g^{-1})
Highbush blueberry 'Chippewa'	235 ± 1	47.1 ± 1.5	108 ± 1
Highbush blueberry 'Blue Ray'	233 ± 1	46.1 ± 1.5	98 ± 1
Blueberry	235 ± 1	45.3 ± 1.5	94 ± 1
Bilberry	248 ± 1	32.5 ± 1.5	88 ± 1
Lingonberry	321 ± 1	27.5 ± 1.5	54 ± 1
Cranberry	338 ± 1	68.3 ± 1.5	48 ± 1

The spectra of substances that could not be identified using the built-in library were compared with the previously published spectra from other authors. Sterols were identified using previous work from Brooks et al. (1968) and Yang et al. (2003).

Statistics

A triplicate determination for all extractions was performed to determine the standard deviation (SD) for the dry residue and selected compounds.

Results

Considering the high importance of polyphenols in the composition of berries due to their radical scavenging activity, the first objective of the study was to evaluate methods of polyphenol extraction. Also carbohydrate concentration in extracts was determined (Table 2).

The radical scavenging activity of bilberry extracts varied from 191 up to 284 GE g^{-1}, depending on the water/extrahent concentration, and varied in relation to the total polyphenol and flavonoid concentration (Fig. 1, Table 2). The variability of carbohydrate concentration in the obtained extracts was much higher (Table 2). The composition of the obtained extracts also greatly depended on berry species (Table 3). For example, the radical scavenging activities of cranberry and lingonberry extracts at optimal extraction conditions were significantly higher than those of other berries.

A further objective of the study was analysis of freely available lipids by GC-MS in six types of berries of the Ericaceae family, genus Vaccinium. These berries contain high concentrations of biologically active substances, and they are traditionally used in ethnomedicine in the Baltic countries. Therefore, they are prospective for use as nutraceuticals and for isolation of biologically active extracts or individual substances. Fresh frozen and powdered (lyophilised) berries harvested in the summer/autumn season of 2014 in bogs and forests of Latvia were used for the extractions.

The method of lipid extraction from fresh berries required dehydrating of homogenized berries. To do this, an approach recommended by Bligh-Dyer was used (see Materials and methods). A mixture of $CHCl_3$ and CH_3OH was used, ensuring simultaneous dehydration of berry mass and transfer of lipids to the $CHCl_3$ phase. The yield of the lipid fraction using the Bligh-Dyer extraction was from 2.70 mg g^{-1} (cloudberries) to 8.43 mg g^{-1} (blueberries) (Table 4). The relative standard deviation for the Bligh-Dyer extraction was determined to be 3.8%.

To identify the most abundant lipids in berry powders, single-solvent extraction was used. Five solvents were tested for their possible extraction efficiency, perspectives of application at an industrial scale and the related environmental aspects. Single-solvent extraction from berry powder gave much higher yields of dry residue compared to using a mixture of $CHCl_3$ and CH_3OH. The highest concentration of dry residue was found in powdered blueberry extracts with diethyl ether, giving 75.81 mg dry residue per g of berries. The highest yield of Bligh-Dyer dry residue was 8.62 mg per g of berries (Table 4). Repeatability for the single-solvent extraction was estimated by doing three separate extractions of two different berry powders, blueberry and lingonberry. The repeatability was ± 3.54% and ± 1.64%, respectively, which is similar to the repeatability of the Bligh-Dyer extraction.

Considering the possible biological activity of extracts and the results of other studies, analysis of freely available lipids was performed. The chromatographic analysis of

Table 4. Summary of sample extractions (50 g of fresh berries) and corresponding dry residue

Extraction type	Berries	Dry residue (mg g^{-1})
Bligh-Dyer (Bligh, Dyer, 1959) ($CH_3OH/CHCl_3$ 2:1)	Blueberry	8.62 ± 0.32
	Bilberry	3.62 ± 0.15
	Lingonberry	5.68 ± 0.23
	Cranberry	3.72 ± 0.12
	Highbush blueberry cv. 'Blue Ray'	2.36 ± 0.10
	Highbush blueberry cv. 'Chippewa'	3.06 ± 0.13

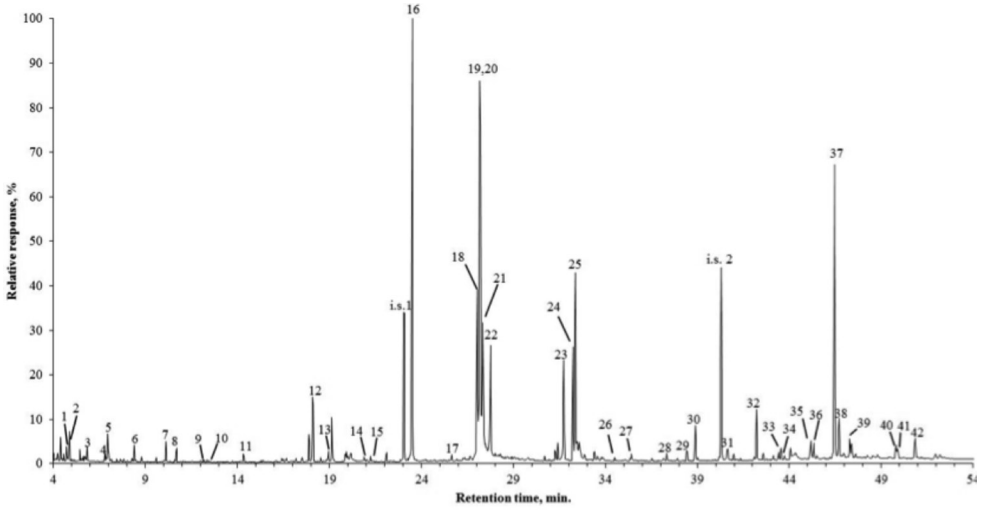

Fig. 2. Chromatogram of blueberry extract (peak numbers as indicated in Table 5).

extracts obtained using a Bligh-Dyer mixture demonstrated the presence of a large number of substances (Fig. 2). The substances were identified by the retention index and mass spectra, and compared with the NIST mass spectral library data. The mass spectra were matched with at least 85% confidence (Table 5). Spectra that were missing in the NIST library were identified using other references (see Materials and methods). Chromatographic runs had high repeatability, and the standard deviations between three parallel runs for the specific apparatus were less than 1%. Substances like carbohydrates were not identified, as this was not the main aim of the study.

Freely available lipids obtained from the six chosen types of berries using the Bligh-Dyer extraction methods were determined. In total, 111 different substances were identified (Table 6) by comparing their mass spectra and retention indices with the reference mass spectra and retention indices. The highest number of substances (79) was found in lingonberry extracts, and the lowest in highbush blueberry cv. 'BlueRay' (63) and blueberry (65). In total 70, 73 and 75 substances were identified in highbush blueberry cv. 'Chippewa', bilberry and cranberry respectively. Substances like benzoic acid (0.64 to 164.40 μg g^{-1}), nonanoic acid (0.34 to 1.43 μg g^{-1}), *m*-hydroxybenzoic acid (0.16 to 0.52 μg g^{-1}), squalene (0.37 to 2.04 μg g^{-1}), α-tocopherol (0.65 to 3.51 μg g^{-1}) and β-sitosterol (4.23 to 84.64 μg g^{-1}) were found in all of the berries in various concentrations. Some of the substances were found only in one type of berry, for example, lanosterol in lingonberry (4.92 μg g^{-1}) and chlorogenic acid in both cultivars of highbush blueberry (0.24 to 1.37 μg g^{-1}) (Table 6). The substance with the highest concentration was benzoic acid (164.40 μg g^{-1}) in lingonberry. All of the C_{18} unsaturated fatty acids were also found in high concentrations (up to 102.10 μg g^{-1} in blueberries).

The identified compounds of berry lipids can be divided into 11 classes of organic substances (Fig. 3). The largest class in each type of berries was fatty acids (up to 82% of the total lipids). Blueberry, bilberry and both cultivars of highbush blueberries (LKM and MKM) had very similar profiles of compound classes (Fig. 3). Lingonberry and cranberry had similar profiles: fatty acid (41 and 30%, respectively), triterpene (10 and 5%) and aromatic carboxylic acid (39 and 35%) classes. The high content of fatty acids in the berries is due to large quantity of seeds, in which energy is stored in the form of fatty acids. Blueberry, bilberry and both cultivars of highbush blueberries (cv. 'Blue Ray' and cv. 'Chippewa') are closely related, which can also be seen in their compound class profiles.

Discussion

The composition of secondary metabolites, present in berries consist of substances with highly different polarities, ranging from polar substances like carbohydrates to sterols, fatty acids and even low polarity substances like waxes and alkanes (Yang et al. 2003; Kallio et al. 2006). To obtain maximal yields of biologically active substances, a sequence of different solvents should be used. For extraction of polar metabolites, solvents with high polarity (water, methanol, ethanol, acetone, dimethylsulphoxide and mixtures of solvents with water) were selected. From the perspective of application, solvents used in studies of polyphenols and similar substances in berries were chosen (Riihinen 2005; Törrönen et al. 2012).

An important parameter affecting the yield of extracts is the solvent/water ratio (from 0 to 100 %). This parameter does not have a major impact on the radical scavenging activity of extracts. With respect to the total polyphenol concentration, the highest yields can be obtained using pure solvents or solvents with minimal water admixture (Fig. 1, Table 2). High yields of carbohydrates or flavonoids in extracts for all studied solvents can be ensured by an optimal solvent/water ratio (Fig. 1).

Extraction yields are affected by extraction procedure and by the intensity of extraction process (treatment and intensity of ultrasound or microwave radiation) (Table 2). For example, increase of the period of ultrasound treatment from 20 to 40 min raised the yield of carbohydrates by more than twice (Fig. 1). Treatment with microwaves was even more efficient, increasing the yield of carbohydrates by 54%, polyphenols by 23 % and radical scavenging activity by 41%. All of the studied berries contained significant amounts of polyphenols and flavonoids and had high radical scavenging capacities (Table 3).

Fatty acids, fatty alcohols, waxes, sterols and terpenoids are considered to belong to lipids according to their physical properties, polarity and functions in organism

Table 5. Peaks of the blueberry extract chromatogram, concentration of the substances found and their typical mass fragmentation. i.s., internal standard

Peak No.	RT	Compound	C, μg g^{-1} berries	Typical mass fragments
1	4.701	Lactic acid	0.88	117(100), 73(90), 147(79), 191(16), 45(15)
2	4.866	Hexanoic acid	0.53	75(100), 73(82), 173(58), 117(24), 45(21)
3	5.829	Heptanoic acid	0.71	73(100), 75(92), 187(56), 117(48), 121(23)
4	6.819	Benzoic acid	6.13	179(100), 105(69), 77(54), 135(44), 180(12)
5	6.865	Octanoic acid	1.63	73(100), 75(67), 201(53), 117(51), 129(18)
6	8.387	Nonanoic acid	0.61	73(100), 74(31), 117(86), 215(62), 129(29)
7	10.147	Decanoic acid	1.12	73(100), 75(73), 117(62), 229(53), 129(31)
8	10.725	Malic acid	4.61	73(100), 147(35), 233(16), 75(12), 45(9)
9	12.155	Undecanoic acid	0.25	73(100), 75(70), 117(60), 243(50), 129(29)
10	12.375	*m*-Hydroxybenzoic acid	0.30	267(100), 73(58), 193(43), 223(40), 282(27)
11	14.356	Dodecanoic acid	0.64	117(100), 73(92), 257(73), 75(72), 129(38)
12	18.179	D-fructose	18.24	73(99), 204(44), 217(30), 147(24), 437(220)
13	18.949	Tetradecanoic acid	0.58	73(100), 117(68), 75(50), 285(43), 129(23)
14	20.902	Phenyloctanoic acid	3.16	75(100), 91(99), 117(95), 73(93), 129(80)
15	21.233	Pentadecanoic acid	0.44	73(100), 117(98), 75(68), 299(58), 129(41)
16	23.516	Palmitic acid	38.46	117(100), 73(94), 75(67), 132(49), 132(49)
17	25.661	Heptadecanoic acid	0.57	73(100), 117(68), 75(50), 327(43), 129(37)
18	27.036	9,12-Octadecadienoic acid	26.44	73(100), 75(78), 67(61), 81(47), 55(40)
19,20	27.201	9,12,15-Octadecatrieonate	102.10	73(100), 75(83), 79(75), 67(46), 41(39)
21	27.339	*trans*-11-Octadecenoic acid	51.16	73(100), 75(87), 117(71), 129(56), 55(54)
22	27.752	Octadecanoic acid	21.31	73(100), 117(83), 75(66), 129(43), 341(39)
23	31.74	Eicosanoic acid	5.52	73(100), 117(93), 75(62), 369(57), 132(43)
24	32.235	Butyl 9,12-octadecadienoate	7.96	73(100), 117(93), 75(62), 369(57), 132(43)
25	32.345	Butyl 9,12,15-octadecatrienoic acid	19.34	73(100), 75(86), 117(78), 129(51), 55(44)
26	34.518	α-Monopalmitin	0.33	28(100), 73(48), 371(46), 147(30), 43(22)
27	35.426	Docosanoic acid	0.72	117(100), 73(97), 75(60), 132(52), 43(51)
28	37.351	Tetracosan-1-ol	0.59	411(100), 75(97), 28(41), 43(41), 73(41)
29	38.451	Squalene	0.82	57(100), 43(93), 82(73), 55(67), 96(56)
30	38.891	Tetracosanoic acid	2.11	73(100), 117(92), 75(72), 43(50), 145(42)
31	40.652	1-Hexacosanol	4.20	439(99), 440(36), 75(29), 57(16), 103(16)
32	42.22	Octacosanal	2.76	57(100), 43(82), 82(68), 55(56), 96(54)
33	43.54	α-Tocopherol (vitamin E)	0.65	73(100), 502(94), 237(87), 236(65), 503(31)
34	43.76	1-Octacosanol	0.40	468(100), 75(72), 57(61), 43(51), 55(33)
35	45.163	Campesterol	12.74	73(100), 129(84), 43(43), 75(38), 55(37)
36	45.328	1-Triacontanal	0.95	57(100), 43(86), 82(82), 55(64), 67(57)
37	46.456	β-Sitosterol	84.64	129(100), 73(61), 43(49), 357(43), 75(38)
38	46.703	β-Amyrin	11.44	218(100), 203(40), 73(34), 75(25), 69(24)
39	47.309	α-Amyrin	5.30	218(100), 73(36), 189(31), 203(28), 190(24)
40	49.81	Betulin	0.53	73(100), 203(67), 189(43), 75(41), 129(35)
41	49.977	Oleanolic acid	14.85	203(100), 73(99), 202(78), 189(46), 75(27)
42	51.104	Ursolic acid	20.96	203(40), 73(99), 202(56), 133(55), 320(40)

Table 6. Lipid analysis of six types of studied berries. All values are expressed as μg of substance per g of berries. ND, substance not detected

RT	RI calc.	Compound	MW (TMS)	Cranberry	Lingonberry	cv. Blue Ray	cv. Chippewa	Bilberry	Blueberry
4.70	1049	Lactic acid	234	1.16	2.45	2.52	1.30	2.23	0.88
4.87	1065	Hexanoic acid	188	0.95	1.51	1.25	0.54	0.83	0.53
5.58	1136	*m*-Cresol	180	ND	ND	ND	ND	ND	ND
5.72	1151	Benzyl alcohol	180	0.69	3.00	ND	ND	ND	ND
5.83	1162	Heptanoic acid	202	0.47	0.58	0.85	0.41	0.57	0.71
6.35	1204	Pantoyl lactone	202	ND	ND	ND	ND	ND	ND
6.82	1245	Benzoic acid	194	37.08	164.40	4.40	0.64	0.51	6.13
6.87	1249	Octanoic acid	216	0.87	1.31	1.86	1.00	0.67	1.63
7.01	1261	Phosphoric acid	314	3.09	3.31	ND	ND	0.65	13.67
7.04	1263	Glycerol	308	ND	ND	0.18	0.14	0.08	0.28
7.48	1296	Phenylacetic acid	208	ND	ND	0.13	0.25	0.27	0.23
7.64	1301	Succinic acid	262	1.91	0.54	0.11	0.17	0.22	0.32
7.73	1314	Pyrocatechol	254	0.08	ND	ND	0.08	0.13	0.25
7.81	1319	Methylsuccinic acid	276	0.61	0.28	0.33	0.15	0.27	0.25
8.03	1334	Benzenepropanol	208	ND	ND	ND	ND	ND	ND
8.20	1344	Fumaric acid	260	0.32	ND	ND	ND	0.12	0.08
8.25	1347	*o*-Toluic acid	208	ND	ND	0.59	0.13	ND	ND
8.39	1356	Nonanoic acid	230	0.70	1.43	1.40	0.44	0.65	0.61
8.47	1361	*m*-Toluic acid	208	ND	ND	0.40	0.08	ND	0.08
9.13	1400	Glutaric acid	218	0.39	ND	ND	0.09	0.22	0.22
9.43	1417	Hydrocinnamic acid	222	ND	0.24	0.13	0.08	0.13	0.19
9.60	1426	2-Deoxytetronic acid	336	ND	0.41	0.24	0.23	0.46	0.34
9.65	1429	Cinnamic acid	220	0.22	1.42	ND	ND	ND	ND
9.98	1446	9-Decenoic acid	242	0.16	0.37	0.18	0.10	0.17	0.20
10.15	1454	Decanoic acid	244	ND	0.79	2.22	1.51	0.48	1.12
10.73	1483	Butanedioic acid (malic acid)	350	41.68	0.44	ND	ND	3.37	4.61
11.14	1503	Salicylic acid	282	0.21	1.61	ND	ND	0.14	0.16
11.47	1519	Terpinol	316	0.15	ND	ND	ND	ND	ND
11.47	1519	*p*-Anisic acid	224	ND	0.33	ND	ND	ND	ND
11.74	1532	Vanillin	224	ND	0.08	ND	0.08	ND	0.16
11.99	1544	10-Undecenoic acid	256	ND	ND	0.23	0.10	0.17	0.27
12.02	1545	*trans*-Cinnamic acid	220	0.48	5.95	ND	ND	ND	ND
12.16	1552	Undecanoic acid	258	0.20	0.35	1.15	0.13	0.26	0.25
12.38	1562	*m*-Hydroxybenzoic acid	282	0.26	0.52	0.28	0.16	0.29	0.30
12.73	1579	β-Phenyllactic acid	310	0.14	ND	ND	ND	0.13	ND
13.23	1622	Pimelic acid	304	0.35	0.25	ND	ND	ND	ND
13.75	1636	*p*-Salicylic acid	282	0.26	1.06	ND	0.15	0.14	0.17
13.97	1644	Vanillic alcohol	298	ND	ND	ND	ND	ND	ND
14.00	1645	4-Hydroxyphenylacetic acid	296	0.15	ND	ND	ND	ND	ND
14.36	1658	Dodecanoic acid	272	0.87	1.92	1.72	0.36	1.06	0.64
15.40	1677	Octanedioic acid	318	0.46	0.37	ND	ND	ND	ND
16.50	1754	9-Tridecenoic acid	284	0.36	0.63	ND	ND	0.41	0.21
16.61	1761	*n*-Tridecanoic acid	286	0.17	ND	ND	ND	ND	0.19
16.91	1777	Vanillic acid (*m*-anisic acid)	312	0.60	1.40	ND	ND	ND	ND
17.71	1811	Nonadioic acid (azelaic acid)	332	0.65	1.49	ND	ND	0.15	0.17
18.18	1829	D-fructose	326	ND	ND	ND	ND	ND	ND
18.21	1834	Protocatechuic acid	370	ND	ND	ND	ND	0.08	ND
18.23	1835	Citric acid	480	1.01	0.25	ND	ND	ND	ND

RT	RI calc.	Compound	MW (TMS)	Cranberry	Lingonberry	cv. Blue Ray	cv. Chippewa	Bilberry	Blueberry
18.65	1845	9-Tetradecenoic acid	298	ND	ND	ND	ND	ND	ND
18.95	1853	Tetradecanoic acid	300	0.73	1.65	5.76	ND	0.41	0.58
20.05	1893	Syringic acid	342	ND	ND	0.12	0.09	ND	ND
20.49	1912	Ferulic acid	338	0.16	ND	ND	ND	ND	ND
20.90	1930	Phenyloctanoic acid	292	0.39	0.96	0.48	0.20	0.35	3.16
21.01	1935	*p*-Coumaric acid	308	0.33	1.10	ND	ND	ND	0.08
21.23	1944	Pentadecanoic acid	314	0.40	0.62	2.31	0.26	0.27	0.44
22.69	2010	9-Hexadecenoic acid	326	ND	ND	ND	ND	ND	ND
23.24	2034	11-Hexadecenoic acid	326	ND	ND	ND	ND	ND	ND
23.52	2045	Hexadecanoic acid	328	16.39	23.48	45.80	14.62	21.41	38.46
24.45	2087	Isoferulic acid	338	0.08	0.43	ND	0.23	ND	ND
25.47	2131	Caffeic acid	396	ND	ND	ND	ND	ND	ND
25.66	2140	Heptadecanoic acid	342	0.46	0.64	1.41	0.43	0.44	0.57
27.04	2204	9,12-Octadecadienoic acid	353	19.81	22.81	1.99	5.01	17.83	26.44
27.20	2212	*trans*-9-Octadecenoic acid	354	32.16	62.83	20.54	13.82	40.41	102.10
27.34	2218	*trans*-11-Octadecenoic acid	354	10.88	22.71	17.98	7.03	16.12	51.16
27.75	2238	Octadecanoic acid	356	5.86	6.47	13.97	5.00	8.85	21.31
29.02	2295	Linoleic acid	352	11.17	14.39	0.50	3.44	10.87	0.08
29.79	2334	Nonadecanoic acid	370	ND	ND	0.60	0.35	0.24	0.18
31.19	2414	11-Eicosenoic acid	382	0.53	0.40	0.65	0.20	0.19	ND
31.74	2443	Eicosanoic acid	384	2.63	13.31	2.16	4.81	4.21	5.52
32.24	2469	Butyl 9,12-Octadecadienoate	336	1.76	4.78	1.44	1.65	3.46	7.96
32.35	2474	Butyl 9,12,15-Octadecatrienoic acid	335	3.44	7.98	2.73	2.71	7.25	19.34
32.57	2485	Butyl 11-Octadecnoic acid	336	2.24	0.76	0.82	0.64	1.26	5.28
32.90	2504	Pentacosane	352	ND	ND	ND	0.20	0.54	ND
33.61	2535	Heneicosanoic acid	398	0.38	0.24	0.34	0.28	0.29	ND
33.83	2546	1-Docosanol	398	0.14	1.13	0.18	0.15	ND	ND
34.52	2585	α-Monopalmitin	474	0.37	0.72	0.27	0.24	0.30	0.33
35.34	2634	Tetracosanal	352	ND	0.46	ND	ND	0.48	ND
35.43	2639	Docosanoic acid	412	2.40	2.72	1.31	1.04	0.85	0.72
35.62	2650	1-Tricosanol	412	ND	ND	ND	ND	ND	ND
36.55	2704	Heptacosane	380	ND	0.31	ND	ND	1.42	0.42
37.19	2745	Tricosanoic acid	412	0.43	0.62	0.26	0.28	0.47	ND
37.35	2754	Tetracosan-1-ol	426	0.33	1.63	0.36	0.26	0.33	0.59
37.93	2785	α-Monostearin	502	0.32	0.70	0.13	0.10	0.32	0.35
38.45	2803	Squalene	410	1.17	2.04	1.16	0.54	0.82	0.82
38.89	2830	Tetracosanoic acid	440	3.35	2.50	0.24	0.72	5.91	2.11
39.03	2838	1-Pentacosanol	440	ND	ND	0.09	0.12	0.21	0.28
39.96	2898	Nonacosane	408	ND	2.48	0.12	0.45	0.22	ND
40.52	2945	Pentacosanoic acid	454	0.34	0.24	ND	0.08	ND	ND
40.65	2953	1-Hexacosanol	454	ND	0.82	0.45	0.58	0.97	4.20
41.34	2993	γ-Tocopherol	488	0.13	0.23	0.61	0.45	0.13	0.25
42.11	3034	Hexacosanoic acid	468	2.00	2.92	ND	ND	0.68	ND
42.22	3040	Octacosanal	408	0.66	1.35	0.39	0.82	13.96	2.76
43.32	3095	Chlorogenic acid	786	ND	ND	0.24	1.37	ND	ND
43.54	3111	α-Tocopherol (vitamin E)	502	1.13	2.22	1.44	1.19	0.78	0.65
43.76	3127	1-Octacosanol	482	ND	0.21	0.56	0.72	1.72	0.40
45.16	3249	Campesterol	472	2.98	3.91	0.51	0.36	0.47	12.74
45.33	3258	1-Triacontanal	436	1.30	1.89	0.32	0.50	0.56	0.95

RT	RI calc.	Compound	MW (TMS)	Cranberry	Lingonberry	cv. Blue Ray	cv. Chippewa	Bilberry	Blueberry
45.88	3291	α-Tocopherolhydroquinone	664	ND	ND	0.14	0.11	ND	ND
46.46	3327	β-Sitosterol	486	6.48	11.87	8.59	7.25	8.52	84.64
46.70	3345	β-Amyrin	498	0.38	1.31	1.03	0.86	1.14	11.44
47.31	3385	α-Amyrin	498	0.55	1.79	1.31	0.88	0.16	5.30
47.42	3393	Cycloartenol	498	0.23	2.39	ND	3.65	ND	ND
48.02	3425	Lanosterol	498	ND	4.92	ND	ND	ND	ND
48.02	3425	Nonacosanoic acid	510	0.81	ND	ND	ND	ND	ND
48.05	3427	Triacontanoic acid	524	0.81	ND	ND	ND	ND	ND
48.63	3466	Lupeol	498	0.20	0.77	ND	ND	0.33	0.51
48.82	3479	Erythrodiol	586	ND	0.80	ND	ND	ND	ND
49.51	3537	Uvaol	586	ND	0.91	ND	ND	ND	ND
49.81	3543	Betulin	586	ND	0.88	0.42	0.36	0.57	0.53
49.98	3562	Oleanolic acid	600	9.98	6.67	0.52	1.42	0.11	14.85
51.10	3590	Ursolic acid	600	53.18	30.54	1.31	3.94	26.76	20.96

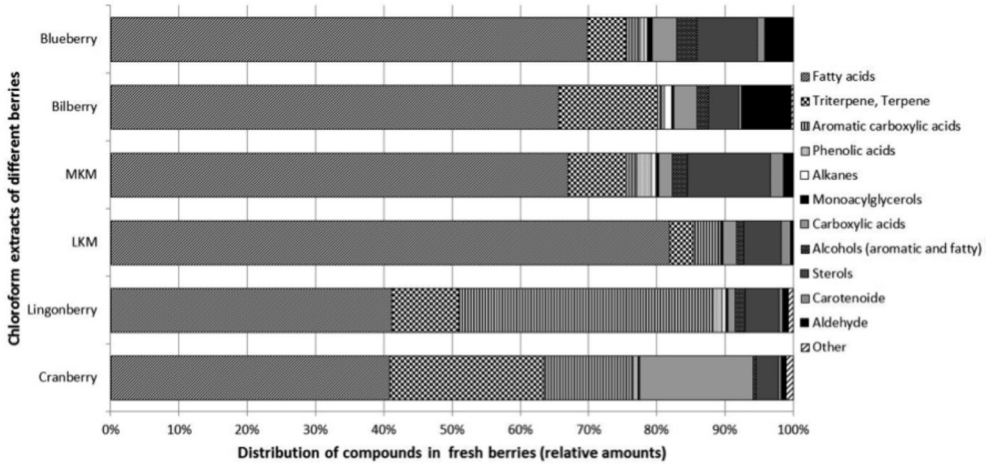

Fig. 3. Distribution of compound classes in the studied berries.

(Gunstone 1996). Such classification is widely used and is also supported by similar extraction results of these substances as obtained for low polarity solvents.

Extraction of studied berries with Blight-Dyer mixture ensured high yields of different low polarity substances will be obtained. Table 6 includes many substances that have not been described in berry lipids in previous studies. Although most of the berries have been widely studied, such a thorough description of free berry lipids has not been previously reported. Substances like lactic acid, malic acid, nonanoic acid, decanoic acid and many others found in blueberry extracts have not been described in previous studies (Häkkinen et al. 1999; Yang 2003). Highest concentrations in blueberry extracts were obtained for C_{18} unsaturated fatty acids (26.44 to102.10 μg g^{-1}) (Table 4), as also been found in previous studies (Croteau, Fagerson 1969; Johansson et al. 1997; Dulf et al. 2012).

Notably, besides the identified compounds, a few substances that could not be recognised were found. Some of the spectra that were found could not be matched with any of the available reference spectra, suggesting that further research is needed to identify the unknown substances.

Conclusions

Berries of the *Vaccinium* species contain significant amounts of lipids, depending on the berry species, extraction conditions, and solvents used in the extraction process. A lipid profile study has been made for six types of berries common in Northern Europe and Latvia. In total, 111 compounds were identified, many of which had not been reported as a part of berry lipids.

The berry lipids found can be divided into 11 classes of organic compounds, including fatty acids, sterols, triterpenoids, carboxylic and phenolic acids and alkanes. The major group of lipids found in the studied berries was fatty acids. However, with respect to the functional significance and application potential, sterols, triterpenoids

and phenolic acids have special interest. Lipids of some berries contain substances specific for each species, for example, a high concentration of benzoic acid is found in lipid pool of lingonberries.

Berry lipids are mainly extracted from berry seeds, and their unsaturated fatty acids are used in many commercial products. Importantly, this study demonstrated the presence of a wide array of biologically active compounds (phytosterols and triterpenoids), which could support new fields of application for berry lipids. The possible application fields include cosmetics, pharmacy and the food industry.

Acknowledgements

This study was carried out with the support of the ResProd National Research Programme.

References

Arts I.C., Hollman P. 2005. Polyphenols and disease risk in epidemiologic studies. *Am. J. Clin. Nutr.* 81: 317–325.

Bligh E.G., Dyer W.J. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37: 8.

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.

Canter P.H., Edzard E. 2004. Anthocyanosides of *Vaccinium myrtillus* (Bilberry) for night vision – a systematic review of placebo-controlled trials. *Surv. Ophthalmol.* 49: 38–50.

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.

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

Gornas P., Rudzinska M., Seglina D. 2014. Lipophilic composition of eleven apple seed oils: A promising sourceof unconventional oil from industry by-products. *Ind. Crops Prod.* 60: 86–91

Gunstone F.D. 1996. *Fatty Acid and Lipid Chemistry*. Springer, New York. 252 p.

Häkkinen S., Heinonen M., Käarenlampi S., Mykkänen H., Ruuskanen J., Törrönen R. 1999. Screening of selected flavonoids and phenolic acids in 19 berries. *Food Res. Int.* 32: 345–353.

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. *J. Food Lipids* 16: 33–49.

Hooper L., Cassidy A. 2006. A review of the health care potential of bioactive compounds. *J. Sci. Food Agric.* 86: 1805–1813.

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

Johansson A., Laakso P., Kallio H. 1997. Characterization of seed oils of wild, edible Finnish berries. *Z. Lebensm. Unters. Forsch.* 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. *Eur. Food Res. Technol.* 211: 277–283.

Joseph S., Edirisinghe I., Burton-Freeman B. 2014. Berries: anti-inflammatory effects in humans. *J. Agric. Food Chem.* 62: 3886–3903.

Kähkönen M.P, Hopia A., Heinonen M. 2001. Berry phenolics and their antioxidant activity. *J. Agric. Food Chem.* 49: 4076–4082.

Kallio H., Nieminen R., Tuomasjukka S., Hakala M. 2006. Cutin composition of five Finnish berries. *J. Agric. Food Chem.* 54: 457–462.

Kraft B.T.F., Dey M., Rogers R.B., Ribnicky D.M., Gipp D.M., Cefalu W.T., Raskin I., Lila M.A. 2008. Phytochemical composition and metabolic performance enhancing activity of dietary berries traditionally used by native North Americans. *J. Agric. Food Chem. 5*6: 654–660.

Matthaus B., Ozcan M. M. 2014. Fatty acid, tocopherol and squalene contents of Rosaceae seed oils. *Bot. Stud.* 55: 1–6.

Mitrovič T., Stamenkovič S., Cvetkovič V., Tošič S., Stankovič M., Radojevič I., Stefanovič O., Čomič L., Dačič D., Čurčič M., Markovič S. 2011. Antioxidant, antimicrobial and antiproliferative activities of five lichen species. *Int. J. Mol. Sci.* 12: 5428–5448.

Narwal S.S., Szajdak L., Sampietro D.A. 2011. *Research Methods in Plant Sciences*. Vol. 1. *Soil Allelochemicals*. Studium Press, Houston.

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 Chem*. 69: 187–193.

Patočka J. 2003. Biologically active pentacyclic triterpenes and their current medicine signification. J. *Appl. Biomed.* 1: 7–12.

Petronelli A., Pannitteri G., Testa U. 2009. Triterpenoids as new promising anticancer drugs. *Anti-Cancer Drugs* 20: 880–892

Reina-Pinto J.J., Yephremov A. 2009. Surface lipids and plant defenses. *Plant Physiol. Biochem.* 47: 540–549.

Riihinen K. 2005. *Phenolic Compounds in Berries*. Kuopio University Publications: C. Natural and Environmental Sciences 187.

Rodriguez-Mateos A., Heiss C., Borges G., Crozier A. 2013. Berry (poly)phenols and cardiovascular health. *J. Agric. Food Chem.* 62: 3842–3851.

Shahidi F., Ambigaipalan P. 2015. Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects – a review. *J. Funct. Foods* 18: 820–897

Singleton V.L., Orthorfer R., Lamuel-Raventos R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods of Enzymology* 299: 152-178.

Szakiel A., Paczkowski C., Koivuniemi H., Huttunen S. 2012. Comparison of the triterpenoid content of berries and leaves of lingonberry *Vaccinium vitis-idaea* from Finland and Poland. *J. Agric. Food Chem.* 60: 4994–5002.

Törrönen R., McDougall G.J., Dobson G., Stewart D., Hellstro J., Mattila P., Pihlava J.M, Koskela A., Karjalainen R. 2012. Fortification of blackcurrant juice with crowberry: Impact on polyphenol composition, urinary phenolic metabolites, and postprandial glycemic response in healthy subjects. *J. Funct. Foods* 4: 746–756.

Tremblay F., Waterhouse J., Nason J., Kalt W. 2013. Prophylactic

neuroprotection by blueberry-enriched diet in a rat model of light-induced retinopathy. *J. Nutr. Biochem*. 24: 647–655.

Wu C. H. V., Qiu X., Bushway A., Harper L. 2008. Antibacterial effects of American cranberry (*Vaccinium macrocarpon*) concentrate on foodborne pathogens. *Food Sci. Techn.* 41: 1834–1841.

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 Res. Int.* 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. *Eur. Food Res. Technol.* 216: 34–38.

Yang B., Kortesniemi M. 2015. Clinical evidence on potential benefits of berries. *Curr. Opin. Food Sci.* 2: 36–42.

Zadernowski R., Naczk M., Amarowicz R. 2003. Tocopherols in sea buckthorn (*Hippophae rhamnoides* L.) berry oil. *JAOCS* 80: 55–58.

Zlatanov M.D. 1999. Lipid composition of Bulgarian chokeberry, black currant and rose hip seed oils. *J. Sci. Food Agric.* 79: 1620–1624.

Received 27 June 2015; received in revised form 5 December 2015; accepted 15 December 2015