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Chemical Composition and Antioxidant Activity of Small Fruits

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1. Introduction

Small fruits contain significant levels of micronutrients and phytochemicals with important biological properties. Consumption of small fruits has been associated with diverse health benefits, such as prevention of heart disease, hypertension, certain forms of cancer and other degenerative or age-related diseases (Manach et al 2004, Santos-Buelga and Scalbert 2000, Hummer and Barney, 2002). These beneficial health effects of small berry fruits could mostly be due to their particularly high concentrations of natural antioxidants (Wang et al., 1996), including phenolic compounds, ascorbic acid and carotenoids. Because of the high contents and wide diversity of health-promoting substances in berries, these fruits are often referred to as natural functional products (Bravo, 1998; Joeph et al., 2000). The chemical composition of berry fruits has previously been shown to be affected by the environmental conditions under which these plants are grown. However, accumulating data suggest that the genotype has a profound impact on the concentration and qualitative composition of phytochemicals and other important constituents of berries. Studies have reported that standard cultivars of dark-fruited berries present a higher antioxidant content compared to vegetables or other foods (Wang et al., 1997). The influence of the genotype is of increasing interest, and several studies, particularly those addressing antioxidants, have been published on this topic (Connor et al., 2002, Lister et al., 2002).

Therefore, the aim of this study was to evaluate the quality parameters (the total amounts of phenolic compounds, anthocyanins and ascorbic acid as well as radical scavenging capacity) related to the fruits of *Sambucus, Aronia, Ribes, Hippophae rhamnoides* and *Rubus* cultivars.

2. Materials and methods

The amount of total phenolics in the fruit extracts was determined with the Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1977) using gallic acid as a standard. The reagent was prepared by diluting a stock solution (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) with distilled water (1:10, v/v). Samples (1 ml in duplicate) were aliquoted into test cuvettes, and 5 ml of Folin-Ciocalteu's phenol reagent and 4 ml of Na_2CO_3 (7.5%) were added. The absorbance of all samples was measured at 765 nm using a Genesys10 UV/VIS spectrophotometer (Thermo Spectronic, Rochester, USA) after incubation at 20°C for 1 h. The results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of fresh weight. The anthocyanins were evaluated spectrophotometrically (Giusti, Wrolstad, 2001).

The radical scavenging capacity against stable DPPH* was determined spectrophotometrically (Brand-Williams et al., 1995; Viskelis et al., 2010). Methanolic fruit extracts (5 g of homogenised berries were extracted with 50 ml of methanol) were tested for their antioxidant activity using the DPPH*. A stable DPPH* radical ($C_{18}H_{12}N_5O_6$, 2,2-diphenyl-1-picrylhydrazyl, M = 394.32 g/mol) was purchased from Sigma Aldrich Chemical, Germany and analytical reagent grade methanol was used (Penta, Czech Republic). When DPPH* (2 ml) reacts with an antioxidant compound (50 µl) that can donate hydrogen, the DPPH* is reduced, resulting in a colour change from from deepviolet to light-yellow. This change was measured every 1 min at 515 nm for 30 min. Radical scavenging capacity (RSC) was calculated by the following formula:

$$RSC=[(AB - AS) / AB] \times 100\%$$

where *A*B is the absorption of the blank sample (*t*=0 min), and *AS* is the absorption of the tested sample.

Total soluble solids (TSS) were determined by a digital refractometer (ATAGO PR-32, Atago company, Japan). The dry matter (DM) content was determined by the air oven method after drying at 105°C in a Universal Oven ULE 500 (Memmert GmbH+Co. KG, Schwabach, Germany) to a constant weight. The titratable acidity (TA) was measured by titrating 10 g of pulp that had been homogenised with 100 ml distilled water. The initial pH of the sample was recorded before titration with 0.1 N NaOH to a final pH 8.2. The acidity was expressed as the percentage of citric acid equivalent to the quantity of NaOH used for the titration. The carotenoid content, expressed as β-carotene, was estimated from the extinction value $E_{1\ cm}^{1\%}$ =2592 at 450 nm (Scot, 2001). The absorbance of the hexane solution was determined at 450 nm against a hexane blank in a Genesys10 UV/VIS spectrophotometer (Thermo Spectronic, Rochester, USA). The ascorbic acid content was measured by titration with 2,6-dichlorphenolindophenol sodium salt solution using chloroform for intensely coloured extracts (AOAC, 1990).

The pH of the fruits was measured using a pH meter. The free and total ellagic acid contents of raspberries and that of anthocyanins in black currants were quantified using a reversed-phase high-performance liquid chromatography technique (Koponen et al., 2007; Rubinskiene et al., 2005).

Color coordinates (L*, a*, b*) measurements were made with a portable spectrophotometer MiniScan XE Plus (Hunter Associates Laboratory, Inc., Reston, Virginia, USA). The spectrophotometer was set to measure total reflectance with illuminant C and a 10° observation angle. The parameters L*, a* and b* (lightness, red value and yellow value, respectively, on the CIEL*a*b* scale) were measured, converted into hue angle ($h° = \arctan(b^*/a^*)$) and chroma ($C = (a^{*2}+b^{*2})^{1/2}$). The spectrophotometer was calibrated on a standard white tile (X = 81.3, Y = 86.2, Z = 92.7) before each series of measurements.

Statistical analyses were performed using analysis of variance (ANOVA).

3. Black currant (*Ribes nigrum* L.) fruits

The use of black currants (*Ribes nigrum* L.) as a domesticated crop is comparatively recent and has occurred only within the last 400-500 years. The modern-day commercial cultivars of black currants are significantly advanced compared to their wild progenitors, and a range of desirable attributes have been selected within the available germplasm by breeders in Europe and elsewhere. The major objectives of further breeding projects for black currants are to obtain the following characteristics: combined resistance to fungal diseases, reversion and gall mites, suitability of bush habit for mechanical harvesting, high productivity, self-fertility, spring frost resistance or avoidance, winter hardiness, adaptation to minimal pruning and suitability for juice production and other products (Keep, 1975; Brennan et. al., 1993; Viskelis et al., 2008). These objectives are most readily achieved by crosses between regional forms of *R. nigrum* L. and species of different sections of the *Eucoreosma* subgenus (Brennan, 1990). Black currants are widely used to make juices, wines, soft drinks and various preserved products. They are associated with important high-value horticultural industries in many European countries, providing employment in agriculture as well as in food processing and confectionary production. The production and consumption of black currant products has recently been increasing in Poland, Germany, France, the Baltic States, Great Britain and Switzerland.

In terms of accumulated amounts of ascorbic acid, black currant berries are only surpassed by actinidia and rosehip fruits. In currant berries, the concentration of ascorbic acid varies from 100 to >250 mg/100 g of fruit (Mage, 1993; Brennan, 1996). In certain berries of wild species (*R. nigrum* spp. *Sibiricum*), the amount of ascorbic acid reaches up to 800 mg/100 g (Brennan, 1990). Some researchers state that the ascorbic acid in black currant berries stimulates the antioxidant activity of polyphenolic compounds (Lister et al., 2002). The resistance of different cultivars to abiotic factors and changes in bioactive substances in their berries during ripening-cropping were analysed under the conditions of the Lithuanian climate in different growing years from 2001-2008. Among the twenty-four cultivars analysed, the highest amount of ascorbic acid (vitamin C) was found in the berries of the long-vegetation cultivar 'Vakariai': 267 mg/100 g of berries (Fig. 1). A very high concentration of vitamin C was found to be characteristic of six other black currant cultivars: 'Tiben'<'Joniniai'<'Minaj Shmyriov'<'Ben Moore'<'Ceres'<'Ben Hope'. The amount of ascorbic acid in the above-listed cultivars ranged from 209 to 251 mg/100 g of fruit. The second group in terms of vitamin content included the following cultivars: 'Almiai'<'Ben Dorain'<'Bona'<'Tisel'<'Ben Alder'<'Ben Tirran'<'Ruben'. The ascorbic acid concentration in the berries of these cultivars ranged from 153 to 179 mg/100 g. The majority of the black currant cultivars analysed accumulated from 112 to 140 mg/100 g of vitamin C.

The ascorbic acid content in berries depends on their growing location. Higher levels of ascorbic acid were detected in the berries of cultivars introduced in Northern countries ('Minaj Shmyriov', 'Titania', 'Ojebyn', 'Ben Alder', 'Ben Lomond' and 'Ben Tirran') compared with cultivars from countries in Southern regions (Kampuse et al., 2002; Franceschi et al., 2002; Franke et al., 2004). Depending on their growing location, some black currant cultivars are characterised by higher fluctuations in ascorbic acid levels. For instance, the amount of ascorbic acid in 'Ojebyn' berries varies from 91.3 to 241.0 mg/100 g (Pecho et al., 1993; Rubinskienė, Viškelis, 2002). Some new cultivars (e.g. 'Bona', 'Tisel' and 'Tiben') have been bred from Scandinavian black currant varieties, and the content of ascorbic acid reaches up to 250 mg/100 g in the berries of these new cultivars (Żurawicz et al., 2000). Analysis of the variation of ascorbic acid during berry ripening showed that its content in the berries of individual cultivars depended on the stage of ripeness. The dynamics of ascorbic acid in the berries of cultivars of different earliness depended on the physiological properties of the cultivar and (for certain cultivars) on cultivation conditions. For all cultivars analysed, much higher amounts of ascorbic acid were found in underripe berries. A higher concentration of ascorbic acid was established on day 45 following bush blooming in black currant berries of early and medium-early cultivars, when the berry skin turned pink and the berry mass was increasing rapidly. The highest concentration of ascorbic acid in the berries of late black currant cultivars was established on days 50-55 following the initiation of blooming.

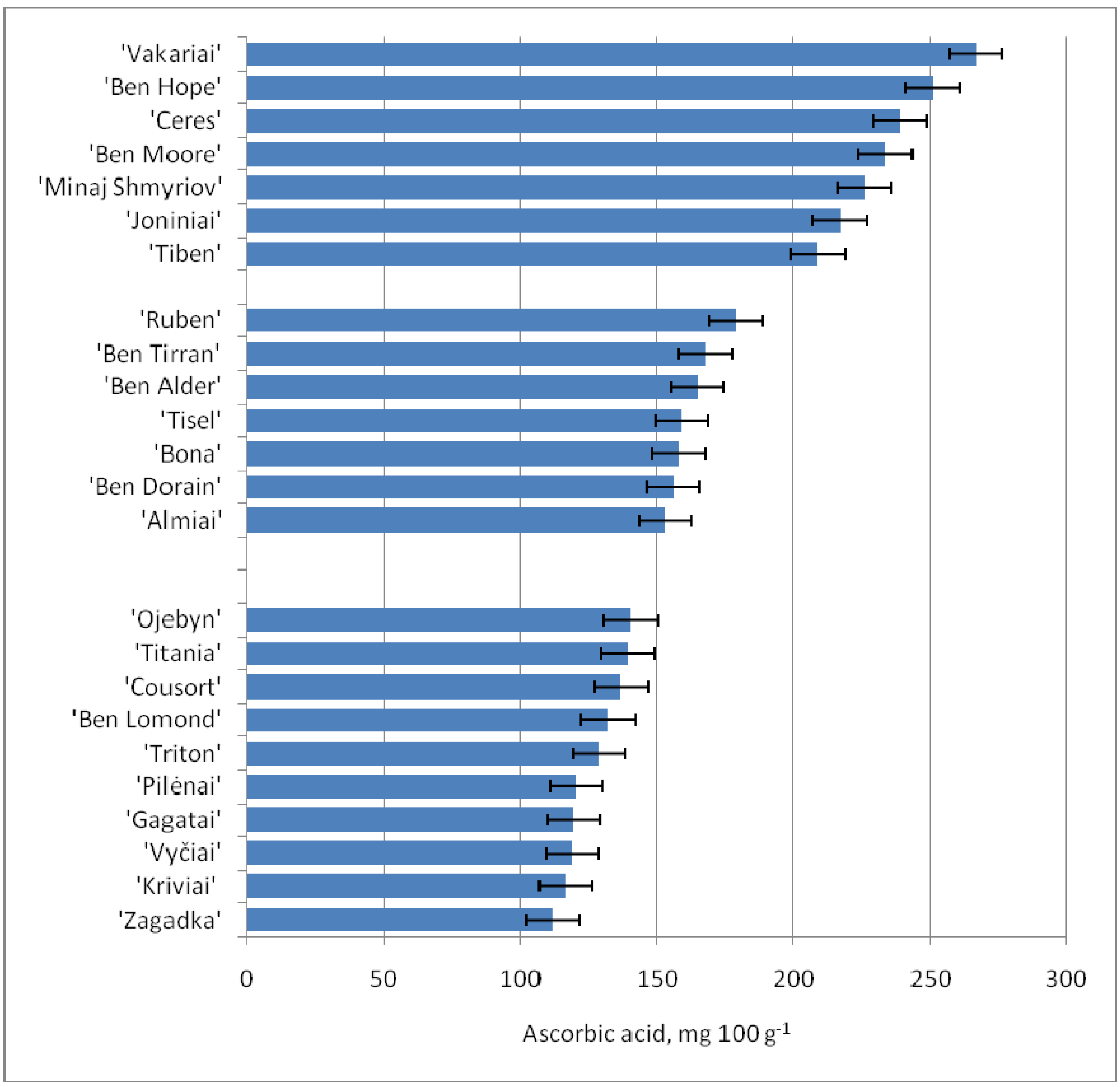

Fig. 1. Ascorbic acid content of black currant fruits

The synthesis of this vitamin is intensive in berries that are not fully ripened, whereas the level of ascorbic oxidase in fruits is low. A sudden decrease in ascorbic acid was recorded in black currants of early and medium-early cultivars that coincided with intense coloration of the berries (dark-brown and black berries). The decrease in the ascorbic acid concentration in berries suitable for consumption can reach 47-52%. The reduction of ascorbic acid during fruit ripening in late black currant cultivars is less pronounced; the ascorbic acid content in the ripe berries of cvs. 'Ben Moore', 'Vakariai', 'Ben Tirran' and 'Ben Alder' was found to be decreased by only 27.3-33.5%. Irrespective of the earliness of the cultivars, overripe berries showed the lowest ascorbic acid contents. The ascorbic acid content in overripe berries was from 14.7 to 29.5% lower. The average correlation (r=-0.52) was found between the ascorbic acid content and the concentration of anthocyanins in black currants. A similar relationship has been noted by other scientists (Brennan, 1990; Trajkovski et al., 2000).

Black currant berries grown under Lithuanian agro-climatic conditions accumulate from 400 to >900 mg of phenolic compounds in 100 g of berries (Fig. 2). The total content of phenolic compounds in most of the analysed cultivars ranged from 600 to 700 mg/100 g. Higher concentrations of phenolic compounds were accumulated in the Scandinavian black currant cultivars 'Ben More', 'Ben Tron', 'Ben Hope', 'Ben Tirran' and 'Tiben'. Additionally, higher amounts of phenolic compounds were found in underripe berries.

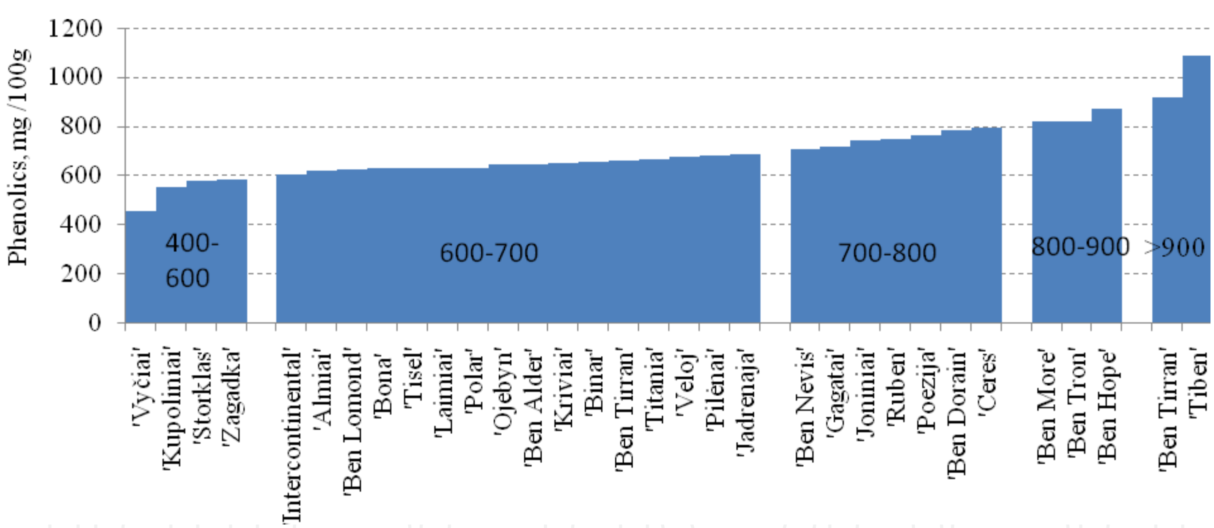

Fig. 2. The total content of phenolic compounds in the berries of different black currant cultivars grown in Lithuania (2005-2010)

The antioxidant activity of ripe berry extracts reaches 78% on average (Fig. 3). The free radical scavenging capacity of ripe and overripe berries was higher than that of underripe berries. A similar tendency was noted by Tabart and co-workers (2006).

Anthocyanins are considered to be powerful (efficient) antioxidants due to their specific structure and activity (Nakajima et al., 2004; Lohachoompol et al., 2004; Galvano, 2005; Fleschhut et al., 2006). Their biological activity has been demonstrated in many studies (Šarkinas et al., 2005; Cooke et al., 2005; Fleschhut et al., 2006).

The persistence of the biological activity of black currant anthocyanin extracts after the recovery procedure was evaluated according to their antibacterial effect (Liobikas et al., 2008). In our opinion, the high antioxidant activity persistence of anthocyanin extracts after recovery procedures indicates the potential of black currant berries to be a good natural source of anthocyanins. In addition, indicators of antioxidant activity and its persistence in recovered anthocyanin extracts support the assumption that these properties depend on the composition of anthocyanin complexes and their aglycones (anthocyanidins) accumulated in the plants of different cultivars (Matsumoto et al., 2002; Blando et al., 2004; Nakajima et al., 2004; Galvano, 2005; Fleschhut et al., 2006).

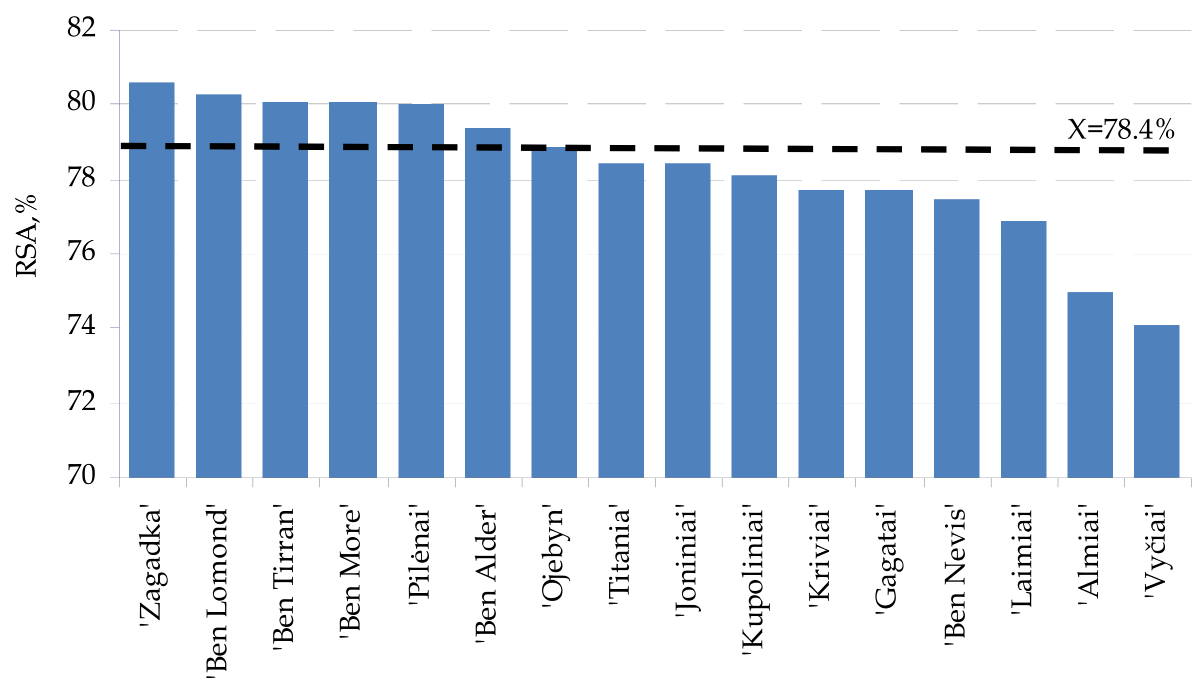

Fig. 3. Radical scavenging capacity of different black currant cultivars

A number of scientists (Kahkonen et al., 2003; Einbond et. al., 2004; Orak, 2006; Tian et al., 2006; Viskelis et al., 2007) are searching for ingredients that can enrich food products with natural antioxidants. Anthocyanins are highly valued as antioxidants and natural colourants (Betsui et al., 2004; Blando et al., 2004; Mazza, 2007). The reported total anthocyanin content of black currants ranges from 214 to 589 mg/100 g, which is 3 to 4 times higher compared to the anthocyanin content found in red raspberries and strawberries and 3 times higher compared with red currants (Mazza, 1993). Black currant berries cultivated under Lithuanian climatic conditions accumulate from 233.5 to 539.7 mg of anthocyanins in 100 g of fruit (Fig. 4). The berries of late and very late black currant cultivars are distinguished by their high amounts of anthocyanins (Rubinskiene et al., 2005). The highest concentrations of these compounds are accumulated in overripe berries. The amount of anthocyanins in overripe berries may be up to 34.5% higher than in ripe berries (Fig. 4). The anthocyanin content in berries depends on cultivar properties and ripening time (Shin et al., 2008; Wang et al., 2009).

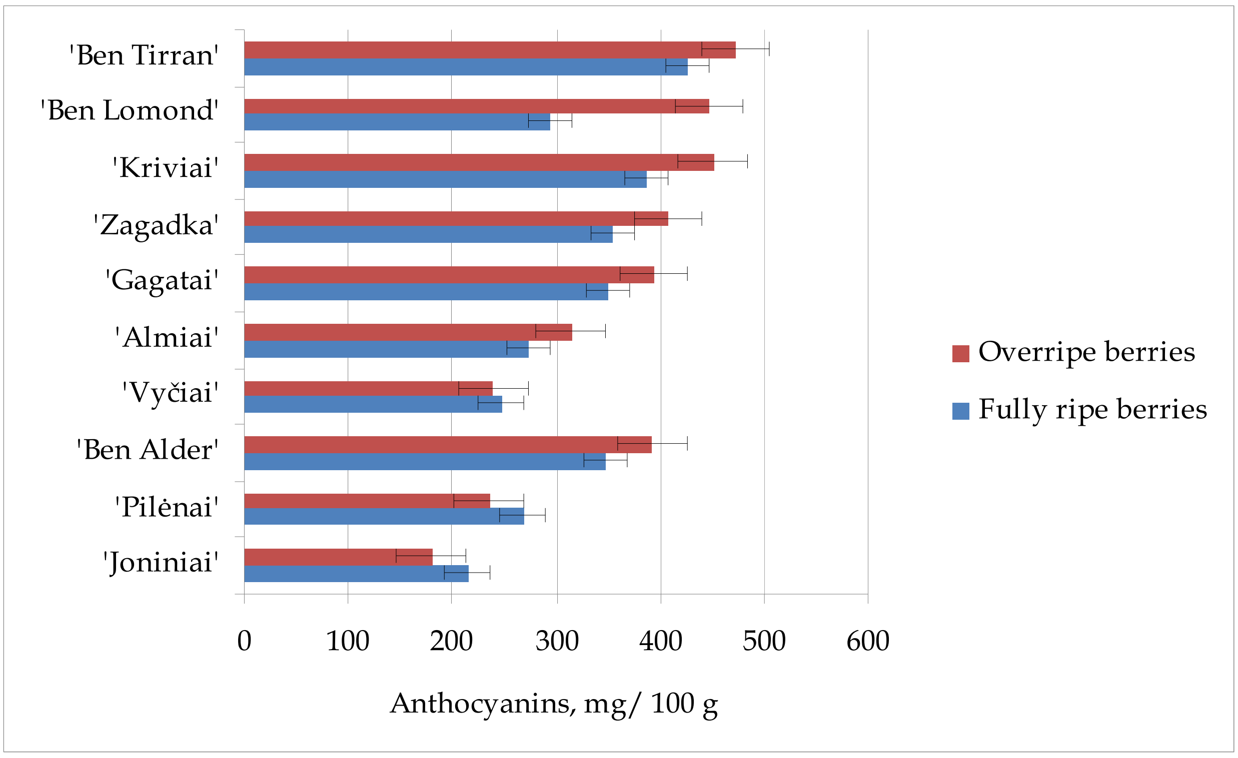

Fig. 4. Anthocyanin content of black currants in ripe and overripe berries

The four main black currant pigments were identified in berries of different maturity; these pigments are cyanidin-3-rutinoside (cy-3-rut), cyanidin-3-glucoside (cy-3glc), delphinidin-3-rutinoside (de-3-rut) and delphinidin-3-glucoside (de-3-glc) (Wrolstad, 2000; Slimestad, Solheim, 2002; Cacace, Mazza, 2003; Määttä-Riihinen et al., 2004). Both the quantitative and qualitative composition of anthocyanin pigments varies during black currant ripening (Rubinskienė et al., 2005; Bordonaba et al., 2010). Cyanidin and delphinidin rutinosides are dominant in ripe berries (Table 1). The amount of cy-3-rut increases considerably during berry ripening. A significantly higher amount of cy-3-rut accumulates in the berries of the 'Almiai' and 'Joniniai' cultivars: 53.08% and 48.33%, respectively. The amounts of anthocyanins in the berry extracts of Lithuanian black currant cultivars are distributed in following sequence: cy-3-rut, 44.14%; de-3-rut, 33.74%; de-3-glc, 11.24%; and cy-3-glc 8.40% (Tab.1). De-3-glc prevails among glucosides in the berries of Lithuanian cultivars. Higher amounts (by 9%) of the dominant pigment cyanidin-3-rutinoside were accumulated in Lithuanian black currant cultivars compared with other black currant berries investigated.

Howard and colleagues indicated that berry weight is closely correlated with the concentration of phenolic compounds in berries (Howard et al., 2003). During the ripening process, a strong negative correlation was detected between berry mass and the amount of anthocyanins accumulated in overripe black currant berries. The mass of overripe berries decreases, while their skin softens, and the concentration of anthocyanins significantly increases. Therefore, all of these quality parameters are correlated with each other (Table 2).

Cultivar	Berry maturity	Anthocyanins, %			
		Cyd-3-rut	Cyd-3-glu	Dpd-3-rut	Dpd-3-glu
'Joniniai'	I	39.06	3.86	48.31	8.77
	II	48.33	4.54	39.11	8.02
'Almiai'	I	36.40	6.06	44.31	13.23
	II	53.08	9.30	28.33	9.29
'Minaj Shmyriov'	I	30.36	4.02	46.49	19.13
	II	43.78	6.45	36.65	13.13
'Vakariai'	I	33.07	4.34	48.68	13.90
	II	38.76	11.30	33.46	16.42
'Ben Alder'	I	30.47	8.62	38.31	22.60
	II	36.63	11.9	31.14	20.33
Mean of cultivars	I	33.87	5.38	45.22	15.51
	II	44.12	8.71	33.74	13.44
$LSD_{05(men\ of\ cultivars)}$		3.002	1.924	1.969	1.641

I-reddish berries, II-black, mature berries

Table 1. Anthocyanin composition (%) in black currant berries of different cultivars;

Rate		Correlation coefficients			
X	Y	Beginning of the ripening	Half ripe	Fully ripe	Overripe
Berry weight, g	Anthocyanins content, mg/100 g	0.18	0.14*	0.46*	-0.63*
Skin strength, N /m2	Anthocyanins content, mg/100 g	-0.15*	0.26	0.54*	0.59*

* - coefficient significant at the probability level of 5%

Table 2. Correlation of the total amount of anthocyanins and physical parameters in black currant berries

4. Raspberry (*Rubus idaeus* L. and *Rubus occidentalis* L.) fruits

Fruits of eight red raspberry cultivars ('Meeker', 'Mirazh', 'Novokitaevskaja', 'Ottawa', 'Sputnica', 'Glen Moy', 'Norna' and 'Siveli'), two yellow raspberry cultivars ('Beglianka' and 'Poranna Rosa') and one black raspberry (*R. occidentalis* L.) cultivar ('Bristol') were investigated in this study.

The pH values of the investigated raspberry fruits varied from 2.96 to 3.35; the content of soluble solids ranged from 10.4 to 12.6 %, and the dry matter content ranged from 12.4 to 19.0 % (Table 3).

Raspberries are a good source of vitamin C (100 g of berries may provide up to 50% of the recommended daily allowance of vitamin C). In this study, the content of ascorbic acid in the investigated raspberry cultivars was found to range from 16.0 (cv. 'Bristol') to 32.1 mg/100 g f.w. (cv. 'Glen Moy') (Tab.3). These data are in agreement with data previously reported for raspberries by other researchers (Haffner et al., 2002; Pantelidis et al., 2007).

Phenolic compounds are the major group of phytochemicals found in berry fruits (Beattie et al., 2005). The total content of phenolics in the investigated raspberries ranged from 223.6 mg/100 g (cv. 'Poranna Rosa') to 690.5 mg/100 g (cv. 'Bristol') (Table 4). The black raspberry *R. occidentalis* exhibited a 1.7-fold higher content of total phenolics compared to the mean value of the investigated *R. idaeus* cultivars (408.8 mg/100 g).

It has previously been reported that the major phenolic compounds found in raspberry fruits are ellagitannins and anthocyanins, whereas hydroxycinnamic acids and flavonols (mainly quercetine glycosides) are the minor phenolic constituents of raspberries (Määttä-Riihinen et al., 2004b).

Cultivar	Dry matter %	Soluble solids %	Ascorbic acid mg/100 g f.w.	pH
Meeker	15.2±0.02e	11.3±0.14b	19.4±0.60bc	3.23±0.05g
Mirazh	15.2±0.11e	12.6±0.06h	19.6±1.90c	2.96±0.02a
Novokitaevskaja	13.2±0.32b	11.0±0.09f	21.4±1.60d	3.35±0.06i
Ottawa	15.7±0.60f	11.5±0.15c	27.2±2.00g	3.07±0.01bc
Sputnica	13.8±0.20c	12.0±0.01g	24.5±1.50ef	3.14±0.00f
Glen Moy	15.4±0.40ef	10.4±0.04a	32.1±0.50h	3.05±0.02b
Norna	12.9±0.04b	11.2±0.14b	25.1±2.00f	3.09±0.04cde
Siveli	14.2±0.22d	10.7±0.11d	18.3±1.80b	3.12±0.01ef
Poranna Rosa	12.4±0.30a	11.2±0.15b	22.0±2.20d	3.10±0.03def
Beglianka	14.1±0.14cd	10.9±0.03e	23.7±2.10e	3.06±0.05bc
Bristol	19.0±0.46g	11.6±0.05c	16.0±0.30a	3.30±0.06h

Values are the mean of three independent determinations ± standard deviation. Different letters in the same raw indicate significant differences (P≤0.05) between cultivars.

Table 3. Content of dry matter, soluble solids, ascorbic acid and pH of raspberry fruits

Cultivar	Free ellagic acid	Ellagitannins	Phenolics	Anthocyanins	RSC,
	mg/100g f.w.				%
Meeker	4.3±0.11c	236.1±12.35f	393.0±14.92d	50.3±2.40e	54.3±0.50c
Mirazh	3.5±0.10e	242.6±13.11c	410.0±15.10f	55.9±2.90b	54.0±2.85c
Novokitaevskaja	5.2±0.14i	219.3±9.96e	366.8±12.67c	56.9±2.00b	53.1±1.35bc
Ottawa	3.4±0.13b	248.9±14.00d	441.2±13.65b	43.6±2.50d	59.3±0.84de
Sputnica	4.7±0.12h	226.2±11.50b	444.8±14.79b	81.7±3.00c	58.6±1.80d
Glen Moy	3.1±0.07d	309.0±16.45g	525.8±19.00i	111.7±4.50f	63.3±2.54f
Norna	4.0±0.12f	225.3±10.27b	420.0±17.10g	82.2±2.20c	58.2±2.61d
Siveli	4.3±0.12c	242.8±14.14c	462.2±10.98h	56.4±3.00b	60.7±0.77e
Poranna Rosa	2.9±0.07a	147.8±10.24a	223.6±9.21a	3.5±0.02a	33.6±1.34a
Beglianka	3.4±0.05b	250.8±9.08d	400.7±16.50e	2.0±0.00a	52.3±0.63b
Bristol	4.1±0.13g	314.1±13.71h	690.5±18.40j	330.8±6.90g	81.5±0.70g

Values are the mean of three independent determinations ± standard deviation. Different letters in the same raw indicate significant differences (P≤0.05) between cultivars.

Table 4. Content of free ellagic acid, ellagitannins, total phenolics, total anthocyanins, and radical scavenging capacity of raspberries

Two major ellagitannins (sanguiin H-6 and lambertianin C) and various ellagic acid derivatives (acylated and/or glycosylated ellagic acid moieties) have been identified in raspberries. However, the free ellagic acid levels are generally low in *Rubus* fruits, and most of the ellagic acid present is in the form of water-soluble ellagitannins within the vacuoles of plant cells (Mullen et al., 2003; Määttä-Riihinen et al., 2004b; Beekwilder et al., 2005). Ellagitanins can be hydrolysed with acids or bases to release hexahydroxydiphenoyl units, which spontaneously form ellagic acid; this reaction is commonly used for the detection and quantification of food ellagitannins (Häkkinen et al., 2000; Vrhovsek et al., 2008; Vekiari et al., 2008; Koponen et al., 2007).

Ellagic acid has received much attention because it has been shown to act as a potent chemopreventive agent (Stoner et al., 2008). Furthermore, ellagic acid/ellagitannins have been found to exhibit antiviral, antibacterial and vasorelaxation properties (Corthout et al., 1991; Goodwin et al., 2009; Nohynek et al., 2006; Mullen et al., 2002).

In this study, the concentration of free (non-tannin) ellagic acid in raspberry fruits ranged from 2.9 to 5.2 mg/100 g in cvs. 'Poranna Rosa' and 'Novokitaevskaja', respectively (Table 4).

The contents of ellagitannins reported in the literature for raspberries are variable, ranging from 20.7 to 329.6 mg/100 g f.w. (De Ancos et al., 2000; Häkkinen et al., 2000; Anttonen and Karjalainen, 2005; Koponen et al., 2007; Vrhovsek et al., 2008). This variation is partly due to the differences in the applied extraction conditions and hydrolysis procedures. However, the reported ellagitannin concentrations for raspberries are generally lower in earlier studies compared to more recent results. The reason for this is that in the earlier studies, ellagic acid was quantified as the only ellagitannin hydrolysis product. However, it was later observed that berry ellagitannins were degraded by acid hydrolysis into ellagic acid and one additional, less polar, derivative (Määttä-Riihinen et al., 2004b). More recently, Vrhovsek and co-workers (2006) identified two other ellagic acid derivatives in addition to ellagic acid after acid hydrolysis of raspberry ellagitannins. Therefore, in later studies, other conversion products of ellagitannin hydrolysis beside ellagic acid were also taken into account.

In the present study, ellagitannins were determined as ellagic acid equivalents after acid hydrolysis. The method previously described by Koponen and co-workers (2007) was applied for ellagitannin analysis. Three major ellagitannin hydrolysis products (ellagic acid, methyl sanguisorboate and methyl gallate) previously identified by Vrhovsek and co-workers (2006) were taken into account. The content of ellagitannins in the investigated raspberries varied from 147.8 to 314.1 mg/100 g f.w. ('Poranna Rosa' and 'Bristol', respectively) (Tab. 4.2). A strong positive correlation (r=0.92) was found between the total content of phenolics and ellagitannin content, showing that raspberry cultivars with a higher phenolic content have higher amounts of ellagitannins.

Red raspberries contain a wide spectrum of anthocyanins, and the major constituents are cyanidin-3-sophoroside, cyanidin-3-glucosylrutinoside and cyanidin-3-glucoside; smaller quantities of cyanidin-3-xylosylrutinoside, cyanidin-3,5-diglucoside, cyanidin-3-rutinoside, cyanidin-3-sambubioside, pelargonidin-3-sophoroside, pelargonidin-3-glucosylrutinoside, pelargonidin-3-glucoside and pelargonidin-3-rutinoside are also present (Mullen et al., 2002; Beekwilder et al., 2005; Kassim et al., 2009; Borges et al., 2010). Significant differences were reported within cultivars with respect to the relative amounts of individual anthocyanins (Beekwider et al., 2005). Moreover, significant year-to-year variations in the contents of anthocyanins in raspberries were noted by different authors (Koponen et al., 2007; Kassim et al., 2009).

Black raspberries accumulate considerably higher amounts of total phenolics, especially anthocyanins, than red raspberries (Wada and Ou, 2002; Weber et al. 2008; Cheplick et al.

2007) blackberries (Wang and Lin, 2000; Wada and Ou, 2002) and some black currant and blueberry genotypes (Moyer et al., 2002). The anthocyanins in black raspberry (*R. occidentalis* L.) consist mainly of cyanidin-3-rutinoside and cyanidin-3-xylosylrutinoside, and smaller amounts of cyaniding-3-glucoside, cyaniding-3-sambubioside and pelargonidin-3-rutinoside have been observed (Tian et al. 2006; Tulio et al. 2008).

In this study, the total anthocyanin content of the investigated red raspberries ranged from 43.6 to 111.7 mg/100 g f.w. (cvs. 'Ottawa' and 'Glen Moy', respectively) (Table 4). Our results are in the range previously reported for raspberries by other scientists (Wada and Ou, 2002; Weber et al. 2008).

Weber and co-workers (2008) analysed 64 raspberry genotypes and noted that some of the red raspberry genotypes presented quite low total anthocyanin contents (up to 40 mg/100 g) in comparison with others (up to 100 mg/100 g). A similar tendency was noted by Lugasi and co-workers (2011); they grouped red raspberry cultivars based on those exhibiting less than 45 mg/100 g of anthocyanins or greater than 55 mg/100 g of anthocyanins. Similarly, it was noted in our study that several of the investigated red raspberry cultivars ('Glen Moy', 'Norna' and 'Sputnica') presented higher total anthocyanin contents than the other red-fruited cultivars (Table 4).

The total anthocyanin content is one of the main criteria used to differentiate between the species *Rubus idaeus* and *Rubus occidentalis* (Suthanthangjai et al., 2005). In the current study, the concentration of anthocyanins in the back raspberry cv. 'Bristol' (330.8 mg/100 g) was almost five times higher than the mean value found in the investigated red-fruited cultivars (67.3 mg/100 g) (Table 4). The concentration of anthocyanins in black raspberries was similar to that reported by other researchers for black raspberries (Wang and Zheng, 2005; Weber et al. 2008).

Due to the presence of recessive genes that suppress the production of anthocyanin pigments, yellow raspberry cultivars contain very low amounts of pigments. In this study, the anthocyanin contents in the yellow raspberry cultivars were found to be 3.5 and 2.0 mg/100 g f.w. (cvs. 'Poranna Rosa' and 'Beglianka', respectively) (Table 4). Similar results for yellow raspberries were reported by other researchers (Anttonen and Karjalainen 2005; Pantelidis et al., 2006; Lugasi et al., 2011).

The amount of anthocyanins represented 47.9% of the amount of total phenolics in black raspberries, whereas in red raspberries, the anthocyanins constituted much lower percentages from 9.9 to 21.2%. This indicates that anthocyanins are not the major phenolic compounds found in red raspberries.

Phytochemicals and vitamins with antioxidant properties are considered to be largely responsible for the health–promoting properties of fruits and vegetables (Stoner et al., 2008). To a significant degree, the antioxidant effects of berry fruits are due to their high content of polyphenols (Szajdek and Borowska, 2008). The contribution of vitamin C to the antioxidant activity of raspberries seems to be relatively low; according to Beekwiler and co-workers (2005), the contribution of vitamin C to the total antioxidant activity of red raspberries was approximately 20%, and Borges and co-workers (2010) reported that the contribution of vitamin C to the detected antioxidant capacity of raspberries was 11%.

The radical scavenging capacity (RSC) of the investigated raspberries in the DPPH• reaction system ranged from 33.6 to 81.5% (cvs. 'Poranna Rosa' and 'Bristol', respectively) (Table 4). The RSC of black raspberry cv. 'Bristol' was 30% higher than the mean RSC value of investigated *R. idaeus* cultivars.

The RSC of the investigated raspberry cultivars was highly correlated with their total phenolics content (r= 0.99). This is in agreement with previously reported results showing that the antioxidant properties of berries correlate well with their phenolic content (Moyer et al., 2002; Anttonen and Karjalainen, 2005; Pantelidis et al., 2007; Poiana et al., 2010). Additionally, the RSC of raspberry fruits was highly correlated with their ellagitannin content (r=0.89), implying that the antioxidant activity of raspberries is largely due to the presence of ellagitannins. These findings are in agreement with previous reports (Mullen et al., 2002; Beekwider et al., 2005; Borges et al., 2010). In this study, a medium correlation (r=0.60) was found between the total anthocyanin content of the eight investigated red-fruited raspberries and their RSC. Beekwilder and co-workers (2005) reported that anthocyanins are responsible for approximately 25% of the antioxidant capacity of red raspberry fruits. Borges et al. (2010) found that red raspberry anthocyanins were responsible only for 16% of the total antioxidant capacity. This indicates that anthocyanins are not the dominant antioxidants in red raspberries. In contrast, for black raspberries, anthocyanins have been reported to be the major phenolic antioxidants (Tulio, 2008).

The fruit colour of raspberries is determined not only by the combination of the contents of anthocyanins present but also by the cellular environment in which the anthocyanins are suspended. Jennings and Carmichael (1980) have investigated the inheritance of anthocyanins in various *Rubus* species; these authors noted that when pelargonidin pigments were incorporated, some degree of orange colour was imparted to progenies. Melo et al. (2002) have shown that the colouration of raspberries is not based on co-pigmentation but is due mainly to effects of the pH in vacuoles. Because of these pH effects, berries with the same anthocyanin constitution may exhibit very different fruit colours.

Cultivar	L^*	a^*	b^*	C	h°
Meeker	37.29±0.17d	28.11±0.40d	9.57±0.23e	29.64±0.36d	18.80±0.55d
Mirazh	34.95±0.28b	25.95±0.13c	8.84±0.14d	27.41±0.10c	18.81±0.35d
Novokitaevskaja	34.81±0.09b	23.64±0.20b	7.48±0.12c	24.79±0.21b	17.55±0.22b
Sputnica	32.26±0.23c	20.26±0.38a	5.99±0.29a	21.13±0.42a	16.46±0.57a
Glen Moy	31.68±0.19a	20.40±0.47a	6.50±0.44b	21.41±0.58a	17.64±0.75b

Parameters: L* (lightness), a* (from (-) green to (+) red), b* (from (-) blue to (+) yellow), h°=arctan (b*/a*), C= [(a*)2+(b*)2] 0.5.
Values are the mean of three independent determinations ± standard deviation. Different letters in the same raw indicate significant differences (P≤0.05) between cultivars.

Table 5. Colour determination values (L^*, a^*, b^*, C, h°) of raspberry fruits

The colour of five raspberry cultivars was measured by the CIE$L^*a^*b^*$ method. The estimated CIE$L^*a^*b^*$ values are given in Table 5. A very strong negative correlation (r =-0.89) was found between the total anthocyanin content of the raspberries and the lightness coordinate L^*. Slightly lower negative correlations were found between the total anthocyanin content and the redness coordinate a* as well as between the total anthocyanins content and the chroma (colour saturation) parameter C (r=-0.84 and -0.83, respectively). The correlations between the total anthocyanin content and the yellowness coordinate b* and the hue angle h° of raspberries was -0.77 and -0.53, respectively. According to our results, the L* colour parameter was sufficiently accurate for screening the anthocyanin concentration of raspberries. It should be noted that the colour of homogenised berries was measured in this study. It has previously been shown that when the colour of the surface of the raspberry fruit was measured, the colour parameters were not good indicators of the anthocyanin levels of raspberries (Haffner et al., 2002).

5. Black chokeberry (*Aronia melanocarpa*) fruits

Black chokeberries accumulate extremely high amounts of anthocyanins and other polyphenolic substances (Valcheva-Kuzmanova et al. 2004; Oszmiański, Wojdylo 2005). *Aronia melanocarpa* represents one of the richest natural sources of anthocyanins, containing from 300 to 630 mg of anthocyanins in 100 g of berries. Black chokeberries were reported to accumulate higher amounts of anthocyanins as compared to black currants, blackberries and elderberries (Oszmianski, Sapis, 1988; Benvenuti et al., 2004). A strong correlation was found between fruit antioxidant activity and the total amount of polyphenolic substances (Bermúdez-Soto, Cevallos-Casalsem et al., 2006). The results of various studies support the positive impact of phenolic antioxidants on human health (Borowska et al., 2005). Currently, most European juice manufacturers include black chokeberry juice among their products, and the demand for black chokeberry concentrate is increasing. According to earlier investigations, the anthocyanins present in *Aronia melanocarpa* are a mixture of four different cyanidin glucosides: 3-galactoside, 3-glucoside, 3-arabinoside and 3-xyloside, of which cyanidin 3-galactoside is the major component (Oszmianski, Sapis, 1988; Wu et al., 2004; Jakobek et al., 2007). In our study, the fruits of black chokeberry cultivars accumulated from 634.6 ('Aron') to 868.9 mg/100 g ('Viking') of anthocyanins (Table 6). The amount of anthocyanins in overripe fruits of *Aronia melanocarpa* was lower by 5.3 to 38.5% (in cvs. 'Aron' and 'Viking', respectively). The highest amount of phenolic compounds, 3647.0 mg/100 g, was found to accumulate in fruits of cv. 'Viking'. As was observed by Rop and colleagues (2010) while investigating five cultivars of *Aronia melanocarpa* of different origins, 'Viking' berries are distinguished with respect to the abundance of the mentioned compounds. However, the amount of total phenolics in overripe berries of cv. 'Viking' was lower by 28% compared to ripe fruits (Table 6). This tendency was also observed by Parr and colleagues (2000), who analysed the variations of phenolic compounds in overripe garden plants. It is estimated that the total phenolic concentration in fruits decreases due to increased polyphenolic oxidase activity. Different authors reported detecting high amounts of total phenolics in black chokeberries, and some reported concentrations that were even higher than those found in our study (Benvenuti et al., 2004; Oszmianski and Wojdylo, 2005). Based on the examination of the phenolic contents and antioxidant activity of 92 plant extracts, Kahkonen and co-workers (1999) reported that among the edible plant materials investigated, remarkably high antioxidant activities and total phenolic contents were found in berry fruits, especially in black chokeberries (Walther, Schnell, 2009). The radical scavenging capacity of the analysed *Aronia melanocarpa* berries ranged from 83.6-86.7%, though the extracts of overripe berries scavenged only slightly greater amounts of free radicals (Table 6).

Cultivar	Berry maturity	Total anthocyanins content, mg/100 g	Total phenolic content, mg/100 g	RSC, %
'Viking'	I*	868.9	3647.0	84.1
	II**	534.4	2609.5	86.7
'Aron'	I	634.6	3123.0	84.1
	II	601.2	3028.7	84.8
Aronia var. cleata	I	701.4	3028.7	83.6
	II	617.9	3028.7	84.2
LSD_{05}		28.49	153.88	3.23

*fullyripe fruit
** overripe fruit

Table 6. Chemical composition and radical scavenging capacity of black chokeberries

The colour parameters of berries and homogenised berry purees are shown in Table 7. 'Viking' berries and their homogenised purees were distinguished based on their colour intensity. Negative values of the colour coordinate b* support the conclusion that greater amounts of anthocyanin pigments are accumulated in chokeberry skins. The pulp tissues present in berry puree changed a value of coordinate b* into positive side. Substantial differences between the colour coordinates a* and b* in berries and purees have a marked influence the hue angle (h°) values (Table 7). The variations of the hue values are the most prominent compared to those of the other analysed colour quality parameters. The h° values of homogenised berry purees did not show a significant difference.

Cultivar	Preparation	L*, %	a*	b*	C	h°
'Viking'	fruit	24.86	0.31	-1.02	1.1	286.9
	puree	26.40	3.95	0.96	4.1	13.7
'Aron'	fruit	26.67	0.67	-0.24	0.7	340.3
	puree	26.53	4.38	1.03	4.5	13.2
Aronia var. cleata	fruit	21.59	0.53	-0.04	0.5	355.7
	puree	26.52	4.20	1.09	4.3	14.5
$LSD_{05(fruit)}$		1.22	0.03	0.02	0.04	16.38
$LSD_{05(puree)}$		1.32	0.21	0.05	0.22	0.69

Table 7. Colour determination values of black chokeberries and homogenised purees

6. Elderberry (*Sambucus nigra* L.) fruits

Elderberries are one of the richest sources of anthocyanins, and the content of anthocyanins in elderberries has been reported to be as high as 1000 mg/100 g (Bronnum-Hansen, Flink, 1986; Kaack, Austed, 1998). Direct evidence has only recently been provided showing that anthocyanins can be absorbed by humans. Cao and Prior (1999) showed that after oral administration of elderberry extract, cyanidins are absorbed in their glycosidic forms. Additional evidence is available concerning the absorption of anthocyanins, including cyanidin 3-glucoside, by mammals (Talavéra et al., 2004; Galvano et al., 2007). In this study, the investigated elderberry cultivars (overripe berries) were found to present high amounts of anthocyanins (709.7 mg/100 g, on average) (Table 8). However, elderberries accumulated 3% less anthocyanins on average compared to *Aronia melanocarpa* fruits (see Tables 8 and 6). Higher amounts of total anthocyanins and total phenolics were found in *Sambucus nigra* cv. 'Lacimiata' (Table 8).

The RSC of *Sambucus nigra* fruits was lower when compared with the RSC of black raspberry and *Aronia melanocarpa* cultivars (Viškelis et al., 2010). Kaack and Aused (1998) reported large differences (from 6 to 60 mg/100 g) in the ascorbic acid content in elderberries depending on the conditions in which the berries were grown. In our study, the average content of ascorbic acid in fruits of *Sambucus nigra* was 29.5 mg/100 g (Table 8).

Cultivar	Ascorbic acid, mg/100g	Anthocyanins, mg/100g	Phenolic compounds, mg/100g	RSC, %
Sambucus nigra 'Aurea'	30.8	505.0	800.2	66.6
Sambucus nigra 'Lacimiata'	28.2	914.3	1050.0	62.9

Table 8. Chemical composition and radical scavenging capacity of elderberry fruits

7. Sea buckthorn (*Hippophae rhamnoides* L.) fruits

Sea buckthorn is becoming increasingly popular due to its valuable fruits, which are suitable for nutritional and medical purposes (Li, Schroeder, 1996). Sea buckthorn fruits are an excellent source of bioactive phytochemicals, such as carotenoids, tocopherols, sterols, vitamin C, organic acids, and polyphenols (Arimboor et al., 2006; Ranjith et al., 2006). Food, medicine, veterinary and cosmetic industries commonly uses sea buckthorn in their products (Li, 2002; Li, Zeb, 2004). Sea buckthorn varies widely both between populations and between individuals within the same population (Yao, Tigerstedt, 1994; Tang, Tigersted, 2001). The content of valuable fruit constituents within populations also varies (Beveridge et al. 1999; Yang, Kallio et al. 2002). From a practical point of view, cultivated sea buckthorn varieties are more valuable because plants of selected genotypes are adapted to certain environmental conditions; their fruits contain higher amounts of biologically active substances. Mechanical harvesting remains a main problem in developing new cultivars (Trajkovski, Jeppson, 1999).

Valuable sea buckthorn cultivars were selected at the Altai Horticulture Institute (Kalinina, Panteleyeva, 1987). Some of these cultivars were tested at the Lithuanian Institute of Horticulture from 1984-1989. The selected cultivars yielded large fruits (average fruit weight was 0.83 g) that were relatively easily picked by hand. However, numerous plants of Siberian-bred sea buckthorn cultivars have died when grown in orchards. It appears that Lithuanian winters, with thaws and frosts, are detrimental to continental climate-adapted cultivars of sea buckthorn.

Under Lithuanian climate conditions better approved at the Moscow State University Botanical Garden bred sea buckthorn cultivars 'Avgustinka', 'Botanicheskaya', 'Podarok sadu' and 'Trofimovskaya'. The fruits of the tested sea buckthorn cultivars gained colour in the middle of August. The average fruit weight at that point was 0.57 g. Despite showing external signs of maturity, the fruit weight continued to increase and reached a maximum (0.81 g) in the middle of September. A similar tendency of increasing berry weight was noted by Raffo et al. (2004).

The firmness of sea buckthorn fruits decreased as they matured: on the 13th of August, the peel strength was 48.7 N/cm^2 on average, and on the 24th of September, it was almost two times lower (25.8 N/cm^2). Hand picking of sea buckthorn fruit gradually becomes more difficult as the required fruit detachment force usually remains the same, while the skin firmness decreases. Harvesting of sea buckthorn is influenced by weather conditions and should usually be completed in September.

In this study, the highest content of dry matter was found in fruits of cv. 'Trofimovskaya', which presented a 17.2% dry matter content on average (Table 9). The rest of the tested cultivars exhibited significantly lower amounts of dry matter. Changes in the dry matter content during fruit ripening were insignificant.

Cultivar	Dry matter	Soluble solids	Total sugars	Titratable acidity	Vitamin C	Carotenoids
	%				mg/100 g	
Avgustinka	15.7	9.2	3.5	1.9	66.3	15.6
Botanicheskaya	16.0	8.3	2.9	1.6	74.2	10.0
Podarok sadu	15.2	8.9	4.1	1.9	74.9	15.2
Trofimovskaya	17.2	9.7	4.2	1.7	109.3	15.2
LSD_{05}	0.91	0.51	0.80	0.17	13.09	3.84

Table 9. Biochemical composition of sea buckthorn fruits

The content of soluble solids varied from 2.85 to 22.74% depending on the origin and stage of maturity of the plants and the climate under which they were cultivated (Raffo et al., 2004; Antonelli et al., 2005; Dwivedi et al., 2005; Tiitinen et al., 2005). In this study, highest contents of soluble solids were found in fruits of cvs. 'Trofimovskaya' and 'Avgustinka' (9.7 and 9.2%, respectively) (Table 9). Lower precipitation and higher temperatures result in higher dry matter and soluble solid contents.

The highest content of total sugars was found in fruits of cvs. 'Podarok sadu' and 'Trofimovskaya' (4.1 and 4.2%, respectively) (Table 9). The total sugar content in ripening fruits increased initially and later remained stable or slightly decreased.

Sea buckthorn fruits are among the richest food sources of vitamin C (Beveridge et al. 2002). Under Lithuanian agroclimatic conditions, the greatest amount of vitamin C accumulated in fruits of cv. 'Trofimovskaya', with a content of 109.3 mg/100 g (Table 9). High vitamin C content (82 mg/100 g) in fruits of 'Trofimovskaya' was also reported by Univer et al. (2004). The vitamin C concentration ranges from 28 to 310 mg in 100 g of berries in the European subspecies *rhamnoides* (Rousi, Aulin, 1977; Jeppsson, Gao, 2000; Yao et al., 1992). Wild fruits of subsp. *Sinensis* (native to China) contain 5 - 10 times greater amounts of vitamin C than the fruits of the European subsp. *rhamnoides* (Kallio et al., 2002). In our study, the content of vitamin C decreased during fruit ripening. The same tendency was reported by Univer et al. (2004).

The highest titratable acidity was found for cvs. 'Avgustinka' and 'Podarok sadu' and was 1.9 % for both cultivars, whereas the lowest was observed for cv. 'Botanicheskaya' (1.6 %) (Table 9).

The lowest content of carotenoids (10.0 mg/100 g) was found in fruits of cv. 'Botanicheskaya' (Table 9). The other tested cultivars accumulated up to 15.6 mg/100 g of carotenoids. Andersson et al. (2009) established that the carotenoid content increased during sea buckthorn fruit ripening, exhibiting values of 1.5-18.5 mg/100 g of fresh weight depending on the cultivar, harvest time, and year.

The highest content of total phenolics of 237.9 mg/100 g was found in fruits of cv. 'Trofimovskaya', whereas fruits of cv. 'Avgustinka' exhibited the lowest content of total phenolics of 179.7 mg/100 g (Fig. 5). The radical scavenging capacity of cv. 'Trofimovskaya' was the highest among the investigated sea buckthorn cultivars (27.4%) (Fig. 6). Sea buckthorn possesses antioxidant properties due to its ascorbic acid, carotenoid and polyphenolic compound content (Ecclesten et al., 2002; Zadernowski et al. 2003; Cenkowski et al., 2006). Sea buckthorn generally shows lower antioxidant activity in DPPH$^{\bullet}$ reaction system compared with some other berries and small fruits (Li et al., 2009; Viskelis et al., 2010). It should be noted that the antioxidant activity of the lipophilic berry fraction was not evaluated in our study. Gao and co-workers (2000) reported that lipophilic sea buckthorn berry fractions were most effective if the comparison was based on the ratio between the antioxidant capacity and content of antioxidants. It has been reported that sea buckthorn berry juice has a higher lipophilic antioxidant capacity than tomato, carrot or orange juice (Müller et al., 2011).

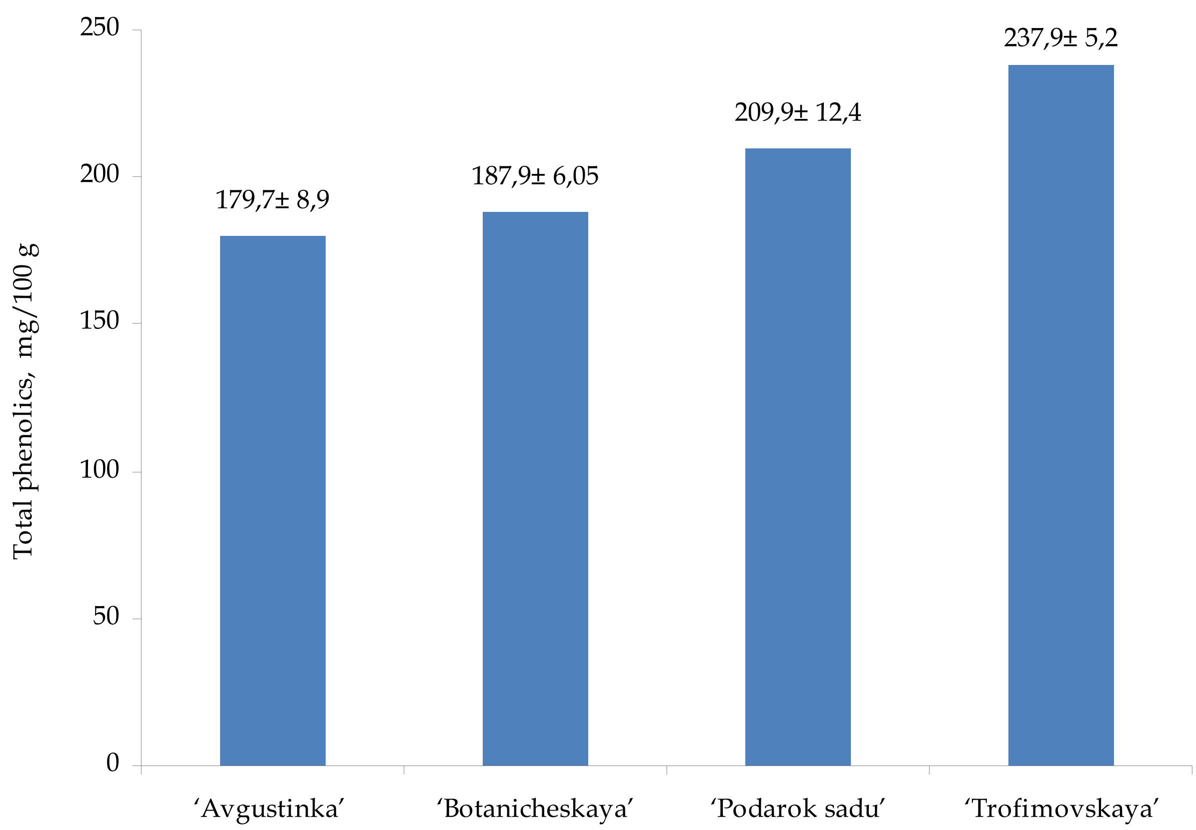

Fig. 5. Total phenolics content of sea buckthorn fruits

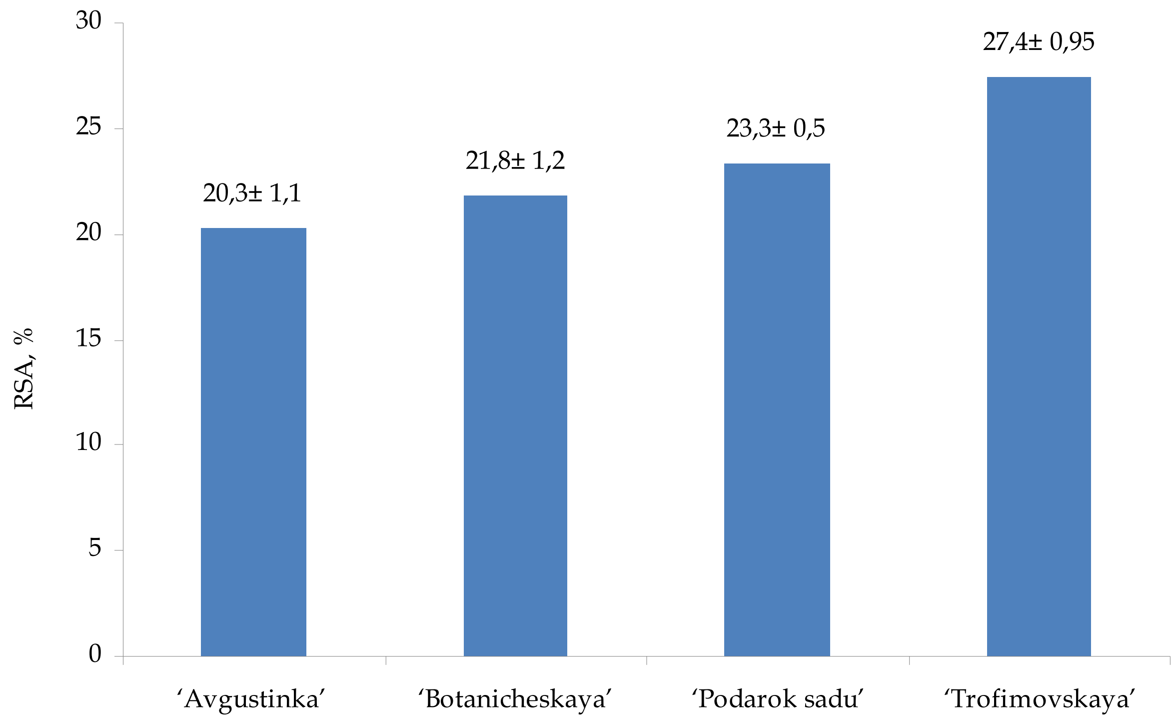

Fig. 6. Radical scavenging capacity of sea buckthorn fruit extract.

8. Conclusions

The results obtained in this study show that the chemical composition and antioxidant capacity of investigated berry fruits varies significantly due to genetic factors. Based on phenolic compounds concentration the investigated berry species may be ranked as follows: *Aronia melanocarpa*>*Sambucus nigra* L.>*Ribes nigrum* L.>*Rubus occidentalis* L.>*Rubus idaeus* L.> *Hippophae rhamnoides* L. Antioxidant capacities varied significantly among berries and cultivars investigated in this study and were highly correlated with phenolic compounds content. Among all berry species tested, fruits of *Aronia melanocarpa* were found to have the highest amounts of anthocyanins, followed by *Sambucus nigra* L., *Ribes nigrum* L., and black raspberry *Rubus occidentalis* L. The highest ascorbic acid content among investigated berry species was found in black currant fruits, followed by the sea buckthorn fruits.

The results indicate that analyzed berry species are rich sources of biologically active substances and posses potent antioxidant activities. The study expands the knowledge about variation in the content of biologically active compounds in different berry species.

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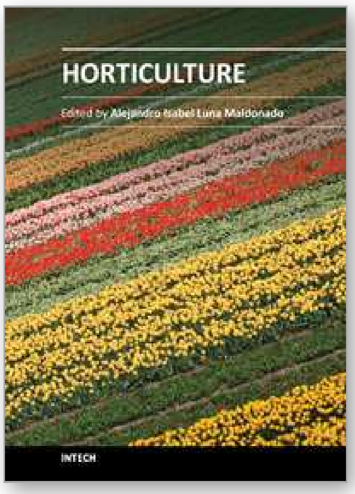

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This book is about the novel aspects and future trends of the horticulture. The topics covered by this book are the effect of the climate and soil characteristics on the nitrogen balance, influence of fertilizers with prolongation effect, diversity in grapevine gene pools, growth and nutrient uptake for tomato plants, post-harvest quality, chemical composition and antioxidant activity, local botanical knowledge and agrobiodiversity, urban horticulture, use of the humectant agents in protected horticulture as well as post-harvest technologies of fresh horticulture produce. This book is a general reference work for students, professional horticulturalists and readers with interest in the subject.

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