Food Chemistry 141 (2013) 3695–3702

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Antioxidant properties and polyphenolic compositions of fruits from different European cranberrybush (*Viburnum opulus* L.) genotypes

Vilma Kraujalytė [a], Petras Rimantas Venskutonis [a,*], Audrius Pukalskas [a], Laima Česonienė [b], Remigijus Daubaras [b]

[a] Department of Food Technology, Kaunas University of Technology, Radvilėnų pl. 19, Kaunas LT-50254, Lithuania
[b] Kaunas Botanical Garden of Vytautas Magnus University, Žilibero g. 6, Kaunas LT-46324, Lithuania

ARTICLE INFO

Article history:
Received 16 February 2013
Received in revised form 25 April 2013
Accepted 12 June 2013
Available online 20 June 2013

Keywords:
Viburnum opulus
Antioxidant activity
Polyphenolics
Chlorogenic acid
Catechins
Identification

ABSTRACT

Antioxidant properties of fruit juices of six *Viburnum opulus* genotypes were evaluated by DPPH·, ABTS·+ radical scavenging capacity (RSC), ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC) and Folin–Ciocalteu total phenolic content (TPC) assays. TPC varied in the range of 5.4–10.6 mg gallic acid equivalents/g, RSC (ABTS·+), FRAP and ORAC values were 31.9–109.8, 32.3–61.8 and 141.6–260.4 μmol trolox equivalents/g, respectively. *V. opulus* var. *sargentii* fruit juice was a remarkably stronger antioxidant than the other five *V. opulus* genotypes. The content of chlorogenic acid (the main phenolic compound in berry juices) depending on plant cultivar varied in the range of 0.54–6.93 mg/ml. The RSC of individual constituents was measured by the on-line HPLC–UV–DPPH· method: chlorogenic acid was the dominant radical scavenger in *V. opulus* P3 (74%), while epicatechin and catechin (the main antioxidants in *V. opulus* var. *sargentii)* contributed to 40% and 23% of the total RSC for the *sargentii* genotype. Nine constituents were identified in *V. opulus* juice by using ultra high performance liquid chromatography coupled to quadruple and time-of-flight mass spectrometers (UPLC–QTOF–MS). In general, the study demonstrated that *V. opulus* var. *sargentii* followed by *V. opulus* P3 and *V. opulus* var. *americanum* possessed the highest antioxidant capacity. The obtained results may assist in selecting the most valuable *V. opulus* genotypes for the production of fruits possessing strong antioxidant capacity and containing beneficial phenolic constituents.

1. Introduction

The relationship between the dietary intake of antioxidant-rich foods and reduced risk of various human diseases has been a widely discussed topic during the last few decades (Kris-Etherton et al., 2002). Although positive health effects of exogenous antioxidants have been questioned by many researchers, numerous scientific studies reported that the consumption of dietary polyphenolics is likely to improve redox status in humans. Therefore, various berries containing high amounts of bioactive phytochemicals, and particularly polyphenolic antioxidants, are the focus of many studies and are considered as valuable molecules which may reduce the risk of diseases, such as cardiovascular disorders, cancers and others (Kris-Etherton et al., 2002; Valko et al., 2007; Wang, Melnyk, Tsao, & Marcone, 2011).

The growing interest in natural food ingredients has been an important factor in expanding the studies of less known horticultural plants in recent years. Nevertheless, a large number of berry species remain under investigated, including the plants of the genus *Viburnum*, consisting of more than 230 species, which are grown for ornamental purposes and for their edible fruits. The majority of the *Viburnum* species are endemic; for instance, *Viburnum opulus* var. *opulus* is widespread in western, central, eastern and north eastern European regions and eastern Siberia; *V. opulus* var. *sargentii* is native to the Far East, e.g. Korea, Northern China and Japan (Česonienė, Daubaras, Venclovienė, & Viškelis, 2010). *V. opulus* var. *americanum* is native to North America, however botanically it was recognised as a variety quite similar to *V. opulus* (Česonienė, Daubaras, Viškelis, & Šarkinas, 2012).

Traditionally various anatomical parts of *V. opulus*, including bark, leaves, flowers and fruits have been used for food and medicinal purposes in Europe and Asia. The fruits have been used to treat heart, lung, kidney and stomach diseases and disorders (Velioglu, Ekici, & Poyrazoglu, 2006; Česonienė et al., 2010). Fruits of *V. opulus* have been also used in foods, mainly as an ingredient in sauces, jellies, marmalades and drinks (Rop et al., 2010; Velioglu et al., 2006).

It has been reported that the fruits of some *Viburnum* species contain high amount of polyphenolics (Rop et al., 2010; Venskutonis et al., 2012; Česonienė, Daubaras, & Viškelis, 2008; Česonienė et al., 2010, 2012), including phenolic acids (Velioglu et al., 2006) and anthocyanins (Jordheim, Giske, & Andersen, 2007; Velioglu

* Corresponding author. Tel.: +370 37 300188; fax: +370 37 456647.
E-mail address: rimas.venskutonis@ktu.lt (P.R. Venskutonis).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.06.054

et al., 2006), as well as organic acids, including ascorbic (Rop et al., 2010; Česonienė et al., 2008, 2010) and L-malic acids (Çam & Hişil, 2007). *In vitro* studies confirmed that berries of investigated *Viburnum* species or their extracts exhibit antioxidative effects (Sagdic, Aksoy, & Ozkan, 2006; Çam & Hişil, 2007; Česonienė et al., 2008). Bioactive substances of *V. opulus* fruits were also reported to possess antimicrobial effects against a wide range of human pathogenic bacteria; however, yeasts demonstrated low sensitivity or were resistant to the fruit juice (Česonienė et al., 2012). Moreover, fruit seeds of *V. opulus* were reported to be rich in linoleic and oleic fatty acids, constituting more than 90% of the total fatty acids (Yang, Ahotupa, Määttä, & Kallio, 2011).

V. opulus fruits exhibit some typical smell notes which are disliked by the consumers. Volatile and odour active compounds were characterised by SPME–GC–MS–olfactometry and it was reported that 3-methyl-butanoic and 2-methyl-butanoic acids followed by linalool and ethyl decanoate were the main odour active components (Kraujalytė, Leitner, & Venskutonis, 2012).

The formation of bioactive substances and their content in fruits and vegetables depends on various factors, such as genotypic differences, environmental conditions (climate, temperature, soil) and cultivation practices (Yang, Martinson, & Liu, 2009). Regardless of some previously published data on *V. opulus*, the scientific information on different plant cultivars is still scarce and rather fragmented. Therefore, more systematic studies are required in order to expand the possibilities for a wider application of *Viburnum* species and cultivars for foods, nutraceuticals and medicinal purposes. The aim of this study was to assess the antioxidant and phytochemical characteristics of several *Viburnum* genotypes cultivated in Lithuania by using a more systematic approach. To achieve this aim the main objectives were to evaluate oxygen radical absorbance and radical scavenging capacities, total content of polyphenolics, to identify and quantify the main antioxidant constituents in 6 locally grown *V. opulus* genotypes. From this point of view the results presented in this study are expected to expand the knowledge on possible variations in the composition of bioactive substances, determined by genetic variations within *V. opulus* species.

2. Materials and methods

2.1. Fruits of Viburnum cultivars and production of berry juice

The bunches with mature fruits of *V. opulus* var. *sargentii*, *V. opulus* var. *americanum* and four *V. opulus* cultivars, namely 'Shukshinskaya', 'Krasnaya Grozd', 'Kiyevskaya Sadovaya' and 'P3' were harvested in October, 2011, from the collection of Kaunas Botanical Garden of Vytautas Magnus University (Lithuania). The bunches with fruits were picked randomly from different parts of the plant, combined and packed in plastic bags. The fruits were frozen and stored at −24 °C before further use. The samples of each genotype were defrosted at 24 °C during 2 h. The stalks were removed and the berries were crushed with a glass stick. The mash obtained was centrifuged at 10,000 rpm for 15 min. The supernatant of clear juice was poured into glass bottles, frozen at −24 °C and kept for a few days until further analysis.

2.2. Chemicals

2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH·, 95%), gallic acid, tetramethylchromane-2-carboxylic acid (Trolox 97%), anhydrous sodium carbonate, 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) were purchased from Sigma–Aldrich Chemie (Steinheim, Germany); 2,4,6-tripyridyl-s-triazine (TPTZ) and fluorescein (FL) was from Fluka Chemicals (Steinheim, Germany); aluminium trichloride hydrate and sodium acetate from Reachim (Riga, Latvia); 2.0 M Folin–Ciocalteu phenol reagent, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diamonium salt (ABTS), NaCl, KCl, Na_2HPO_4 and $K_2S_2O_8$ were from Merck (Darmstadt, Germany); KH_2PO_4 was from Jansen Chimica (Beerse, Belgium); methanol (99.8%) and acetic acid (98%) was from Lachema (Brno, Czech Republic), the reference substance (+)-catechin (98.3%) was purchased from Chromadex (California, USA) and chlorogenic acid (3-*O*-caffeoylquinic acid, 97%) from Roth (Karlsruhe, Germany).

2.3. Determination of soluble solids

The contents of soluble substances in fruit juices was measured by a PAL-3 refractometer (Ataga, Japan) at room temperature (18 °C) (AOAC Official Method). All analyses were carried out in triplicate.

2.4. DPPH· radical scavenging capacity

The free radical scavenging capacity (RSC) of fruit juices was measured using stable DPPH· radical as described previously (Brand-Williams, Cuvelier, & Berset, 1995), with some modifications. DPPH· is reduced to the corresponding hydrazine when it reacts with hydrogen donors, such as antioxidants. Fruit juice sample dilution and the ratio between the DPPH· was preliminary assessed by using 0.06 mM methanolic DPPH· solution and a series of juice diluted with distilled water (1:10, 1:20, 1:30, 1:40 and 1:50 w/v). The absorbance was measured every minute for monitoring radical quenching kinetics until the end point at 60 min. The dilution ratio of 1:20 (w/v) was chosen as the most convenient, due to a percentage of free radical not quenched at the steady state of reaction. Diluted juice sample (8 μl) and 320 μl DPPH· solutions were pippeted into a 96 well Fluostar Omega microplate reader (BMG Labtechnologies GmbH, Ortenberg, Germany) set at 515 nm and controlled by the MARS software. The percentage of inhibition was calculated according to the formula: $I = [(A_0 - A_i)/A_0] \times 100$, where I is DPPH· inhibition, %; A_0 is the absorbance of a blank sample (t = 0 min); A_i is the absorbance of berry juices sample (t = 60 min).

2.5. Determination of total phenolics content (TPC)

The TPC was measured with Folin–Ciocalteu reagent as originally described by Singleton, Orthofer, and Lamuela-Raventos (1999) with some modifications to reduce the consumption of reagents (Medina, 2011). Briefly, 10 μl of diluted juice sample (1:60 w/v) were mixed with 100 μl of a 10-fold diluted (v/v) Folin–Ciocalteu reagent, and after 4 min 100 μl of 7% Na_2CO_3 and 40 μl of distilled water were added. Soon after mixing all the reagents, the microplate was placed in the reader and shaken for 30 s. After incubation for 90 min, at room temperature, the absorbance of the mixtures was measured at 725 nm. All measurements were performed in triplicate. A series of gallic acid solutions in the concentration range of 0–250 mg/l were used for the calibration curve. The results were expressed in mg of gallic acid equivalents per g of berry juices (mg GAE/g).

2.6. $ABTS^{\cdot+}$ radical cation decolourisation assay

The RSC of fruit juices was also measured by $ABTS^{\cdot+}$ radical cation assay (Re et al., 1999). Firstly, phosphate buffered saline (PBS) solution was prepared by dissolving 8.18 g NaCl, 0.27 g KH_2PO_4, 1.42 g Na_2HPO_4 and 0.15 g KCl in 1 l of ultra pure water; if the pH was lower than 7.4 it was adjusted with NaOH. The $ABTS^{\cdot+}$ radical solution was prepared by mixing 50 ml of ABTS (2 mM) with 200 μl $K_2S_2O_8$ (70 mM) and allowing the mixture to stand in the dark at room temperature for 15–16 h before use. The working solution was prepared by diluting with PBS to obtain the absorbance of AU 0.700 ± 0.030 at 734 nm as measured on a Spectronic Genesys 8 spectrophotometer (Thermo Spectronic, Rochester, NY). Five μl of diluted fruit juice

sample (1:60 and 1:70 w/v depending on cultivar) were pippeted into the microplate wells and 300 μl $ABTS^{\cdot+}$ working solution added. The plate was placed in the Fluorstar Omega apparatus, shaken for 30 s and the absorbance was read after 30 min. All determinations were performed in quadruplicate. A series of Trolox solutions in the concentration range of 0–1450 μM were used for the calibration curve. The RSC was expressed as Trolox equivalent antioxidant capacity (TEAC, μmol Trolox/g juice).

2.7. Ferric reducing antioxidant power assay (FRAP)

This method was based on the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) under acidic (pH 3.6) conditions (for iron solubility) followed by a blue complex formation of Fe^{2+} and TPTZ (Benzie & Strain, 1996). Briefly, the working reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ at 10:1:1 (v/v/v). Ten μl of juice sample diluted at a ratio 1:10 (w/v) and heated to 37 °C in working reagent (300 μl) solutions were pippeted into the microplate wells, 30 μl H_2O added, the plate was placed in the Fluorstar Omega reader, shaken for a 30 s, and the absorbance was measured at 593 nm every min until 30 min. All determinations were performed in quadruplicate. A series of Trolox and Fe_2SO_4 solutions in the concentration ranges of 0–1500 μM and 0–1000 μM, respectively, were used for the calibration. The antioxidant power was expressed as Trolox equivalent antioxidant capacity (TEAC, μmol Trolox/g juice) and Fe^{2+} antioxidant capacity (Fe^{2+} μmol/g juice).

2.8. Oxygen radical absorbance capacity (ORAC)

ORAC method was performed as described by Prior et al. (2003) and Dávalos, Gómez-Cordovés, and Bartolomé (2004) by using fluorescein as a fluorescent probe. The reaction was carried out in 75 mM phosphate buffer (pH 7.4) and a stock solution of fluorescein was prepared according to Prior et al. (2003). Fruit juice samples were diluted from 1:600 to 1:800 (w/v) depending on the genotype. Juice sample (25 μl) and fluorescein (120 μl; 14 μM) solutions were placed in the 96 transparent flat-bottom microplate wells, the mixture was preincubated for 15 min at 37 °C followed by a rapid addition of AAPH solution as a peroxyl radical generator (26 μl; 240 mM) using a multichannel pipette. The microplate was immediately placed in the Fluorstar Omega reader, automatically shaken prior to each reading and the fluorescence was recorded every cycle (1 min × 1.1), total 80 cycles. The 485-P excitation and 520-P emission filters were used. At least three independent measurements were performed for each sample. Raw data were exported from the Mars software to Excel 2003 (Microsoft, Roselle, IL) for further calculations. Antioxidant curves (fluorescence versus time) were first normalised and from the normalised curves, the area under the fluorescence decay curve (AUC) was calculated as $AUC = 1 + \sum_{i=1}^{i=80} \frac{f_i}{f_0}$, where f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at time i. The final ORAC values were calculated by using a regression equation between the Trolox concentration and the net area under the curve (AUC). A series of Trolox solutions in the concentration range of 0–250 μM were used for the calibration. The antioxidant activity was expressed as Trolox equivalent antioxidant capacity (TEAC, μmol Trolox/g juice).

2.9. Determination of chlorogenic acid, catechin and epicatechin by LC/DAD/MS

The LC–MS system consisted of a Waters 1525 binary HPLC eluent pump (Waters Corporation, Milford, USA) and a manual Rheodyne 7125 injector (Rheodyne, Rohnert Park, USA). Separation of compounds was performed on a Supelco C18 column (5 μm, 250 × 4.6 mm i.d., Supelco Inc., Bellefonte, PA) using a linear gradient: from 10% to 100% of B (methanol), 0–50 min in A (10% of methanol, 0.2% of acetic acid); 50–55 min, 100% B; 55–58 min, from 100% to 10% of B in A; 58–60 min from 10% to 90% of B in A. The flow rate was 1.0 ml/min and 10 μl of the sample was injected. Chromatographic signals were recorded by a Waters 996 photodiode array detector (the scanning was performed in the range of 210–400 nm) and a Waters Micromass ZQ mass detector (Waters Corporation, Milford, USA). Electrospray ionisation in positive and negative modes were used to obtain mass spectra of the separated compounds. Chlorogenic acid, catechin and epicatechin were quantified in all analysed cultivars by using pure reference compound solutions in methanol in the concentration range of 10–1000 mg/l (R^2 = 0.996).

2.10. UPLC/ESI–QTOF–MS analysis

UPLC/ESI–QTOF–MS analysis was performed using an Acquity UPLC system (Waters, Milford, USA) combined with a Brüker maXis UHR-TOF mass spectrometer (Brüker Daltonics, Bremen, Germany). The Acquity UPLC was equipped with a binary solvent delivery system, an autosampler with a 10 μl sample loop, a photodiode array (PDA) detector and a column manager. An Acquity BEH C18 column (1.7 μm, 50 × 2.1 mm, i.d.) was used for separation of compounds and was maintained at 25 °C. The mobile phase was initially composed of 100% eluent A (1% v/v formic acid in ultra pure water) followed by an increase from 0% to 35% of eluent B (methanol) over 3 min. During the following 2 min, the eluent B was linearly increased from 35% to 100%, where it was maintained for 1 min. Finally, the initial conditions were re-introduced over 1 min, and the equilibration time was 1 min (the column was also equilibrated for 1 min before each run). The flow rate was 0.4 ml/min and the effluent was monitored at 254 nm. The effluent from the PDA detector was introduced directly into the UHR-TOF mass spectrometer equipped with an ESI source. Instrument control and data acquisition were achieved using the Compass 1.3 (HyStar 3.2 SR2) software. MS experiments were performed in negative ionisation mode. The capillary voltage was maintained at +4000 V with the end plate offset at −500 V. Nitrogen was used as the drying and nebulizing gas at a flow rate of 10.0 l/min and a pressure of 2.0 bar, respectively. Nitrogen was introduced into the collision cell as the collision gas. Peak identification was carried out by comparing the retention times with those of the corresponding peaks in chromatograms of standards or by the characteristic mass spectrometric fragmentation patterns and accurate masses.

2.11. On-line HPLC/UV/DPPH· assay

Diluted fruit juices (1:10 v/v) were analysed. The HPLC-separated analytes were transferred into the post-column reaction coil with circulating DPPH· solution. Thus, two chromatograms were recorded simultaneously; one of which showing UV absorbance of effluent at 280 nm before the reaction, while the second one recorded decrease in absorbance at 515 nm after reaction of effluent with the freshly prepared solution of DPPH· (6×10^{-5} M) in methanol, continuously supplied into a reaction coil (15 m, 0.25 mm ID) with a Agilent 1100 series pump (Agilent Technologies, USA) at 0.6 ml/min. The signals were acquired by a UV–VIS detector (SPD-20A, Shimadzu, Kyoto, Japan). HPLC conditions were as described in the LC/DAD/MS analysis.

2.12. Statistical analysis

Mean values and standard deviations of antioxidant activity from spectrometry measurements were calculated from at least

three replications, RSC, FRAP and TPC values were from 3 to 4 replicate measurements as indicated in each section using MS Excel 2003. Statistical analysis of the obtained results was performed by using one-way analysis of the variance (ANOVA), the differences between samples were evaluated by the Duncans' test that showed significant variation ($p < 0.05$). Analyses were performed using STATISTICA 8.0 software (2007).

3. Results and discussion

3.1. Antioxidant properties of fruit juices in electron/hydrogen transfer based assays

There are many assays for the assessment of antioxidant properties, the majority of them are based on electron transfer and hydrogen atom donation reactions. After comprehensive critical assessment of the most frequently used methods, Huang, Ou, and Prior (2005) concluded that ORAC, TPC measured with Folin–Ciocalteu reagent and one of the electron/hydrogen transfer assays should be recommended for representative evaluation of antioxidant properties. DPPH· assay is mainly attributed to the electron transfer assays, however the quenching of DPPH· radical to form DPPH-H is also possible. Other electron transfer based methods include the TPC assay using Folin–Ciocalteu reagent, $ABTS^{\cdot+}$ decolourisation assay and ferric ion reducing antioxidant power (FRAP). Following the above mentioned recommendation, all these methods were applied for the assessment of *V. opulus* antioxidant potential in our study.

3.1.1. Radical scavenging capacity (RSC) in DPPH· assay

Two indicators of antioxidant power may be obtained in the DPPH· assay, the total amount of radical scavenged during the whole reaction period and the speed of radical scavenging. The reaction between juice samples and DPPH· was monitored during 60 min (Fig. 1). It may be observed that all tested juices reacted with the radical rather slowly, and at the end of the reaction *V. opulus* var. *sargentii* demonstrated the highest RSC (77.5%), followed by the *V. opulus* var. *americanum* (73.2%) and *V. opulus* P3 (70.2%). The remaining three genotypes analysed displayed a similar RSC. It should be noted, that reaction kinetics are an important aspect in the evaluation of antioxidant activity, because the reaction mechanism between DPPH· and various compounds is different and depends on the molecular structure of the antioxidants. For example, ascorbic acid was shown to reduce DPPH· molecules very quickly, while many other tested antioxidants demonstrated slower reaction kinetics (Brand-Williams et al., 1995).

3.1.2. Total phenolics content (TPC)

The Folin–Ciocalteu method became very popular for the measurement of TPC, although the reaction involves all constituents possessing hydroxyl groups because the standard Folin–Ciocalteu reagent measures reducing capacity (Huang et al., 2005). Fruit juices of the studied *V. opulus* were found to be a good source of polyphenolics; however the TPC varied significantly between the studied genotypes (Table 1). The highest concentration of TPC in mg GAE/g juice was determined in *V. opulus* var. *sargentii* (10.6) followed by *V. opulus* var. *americanum* (8.6) and *V. opulus* P3 (7.7), while the lowest TPC was in *V. opulus* Kiyevskaya Sadovaja (5.4). TPC in *V. opulus* juices and fruits were measured previously (Çam & Hişil, 2007; Česonienė et al., 2008; Česonienė et al., 2010, 2012), however the results reported in these articles were quite different. Thus, Çam and Hişil (2007) reported that TPC in *V. opulus* was in the range of 3.3–3.5 mg/ml juice which is remarkably lower than in the juice studied in our work. Česonienė et al. (2008, 2010) determined that *V. opulus* var. *sargentii* and *V. opulus* var. *americanum* fruits accumulated more than 14 mg/g of TPC, which is higher compared to our results. In the most recent study it was reported that the TPC in *V. opulus* fruit juices varied from 8.04 to 11.7 mg/g (Česonienė et al., 2012). Most likely, the variations reported in TPC for the same *V. opulus* genotypes were dependent on climate conditions and other factors. In this study, a strong correlation was found between TPC and RSC in the DPPH· assay ($r = 0.93$), showing that cultivars with a higher phenolic content demonstrated stronger radical scavenging capacity. Such a correlation has been reported in numerous previously published articles and may be explained by the similarity in chemistry between the two assays: both belonging to the electron transfer based antioxidant activity assessment methods (Huang et al., 2005).

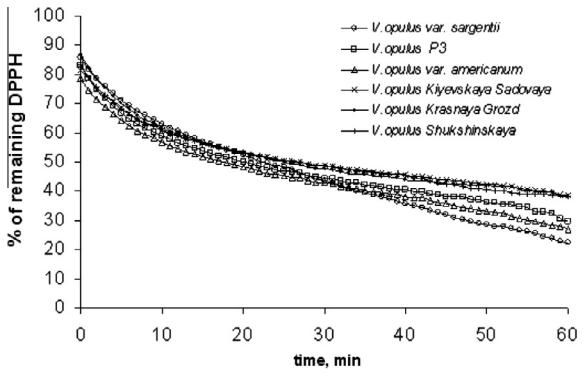

Fig. 1. Reaction kinetic of DPPH· radical scavenging by the juice of six *V. opulus* genotypes.

3.1.3. $ABTS^{\cdot+}$ radical cation decolourisation and ferric reducing antioxidant power (FRAP) assays

The basic principle of these two assays is quite similar; in the FRAP assay a ferric salt (Fe^{3+}) is used as an antioxidant and its redox potential (0.70 V) is comparable to that of $ABTS^{\cdot+}$ (0.68 V) (Huang et al., 2005), therefore the results of TEAC and FRAP are discussed together. The highest antioxidant activity in $ABTS^{\cdot+}$ was for *V. opulus* var. *sargentii* (109.8 μmol/g), while the weakest RSC was found for 'Kijevskaja Sadovaja' (31.9 μmol/g). A strong positive correlation ($r = 0.95$) was observed between TPC and $ABTS^{\cdot+}$ scavenging.

The reducing power in the FRAP assay may be expressed in Fe^{2+} equivalents and/or Trolox equivalents. Both values are informative, particularly for comparison with the results reported in other studies. Therefore the FRAP of fruit juice was calculated using both ways (Table 1). The highest FRAP values were obtained for *V. opulus* var. *sargentii* (109.8 μmol Fe^{2+}/g juice and 61.8 μmol TE/g juice) while the weakest reducing power was observed for the 'Shukshinskaya' cultivar (55.8 μmol Fe^{2+}/g juice and 32.3 μmol TE/g juice). A strong correlation ($r = 0.93$) was found between TPC and FRAP values. To the best of our knowledge, the antioxidant activity of *V. opulus* has not previously been evaluated by the FRAP assay, while $ABTS^{\cdot+}$ scavenging has been measured for the three year harvest *V. opulus* var. *edule* (Rop et al., 2010). In the mentioned study, the RSC of fruit extracts varied from 8.5 to 9.75 ascorbic acid equivalents/kg of fresh mass.

3.2. Oxygen radical absorbance capacity (ORAC)

The ORAC assay evaluates radical chain breaking antioxidant activity by H atom transfer and measures antioxidant inhibition induced by peroxyl radical oxidation. Basically, thermally generated peroxyl radical reacts with a fluorescent probe and forms a nonfluorescent probe. In the ORAC assay the highest TEAC values were also obtained for *V. opulus* var. *sargentii* juice (260.4 μmol/g) followed by the genotype P3 (190.0 μmol/g), while the lowest values were observed for the 'Shukshinskaya' cultivar

Table 1
The results of antioxidant activity *in vitro* ($ABTS^{\cdot+}$, FRAP, ORAC) and total phenolics (TPC) of juices of six *V. opulus* genotypes.

Genotype	TPC	$ABTS^{\cdot+}$	FRAP		ORAC	Soluble solids	Amount of chlorogenic acid
	mg GAE/g	TEAC, μmol Trolox/g	TEAC, μmol Trolox/g	Fe^{2+} μmol/g	TEAC, μmol Trolox/g	(%)	mg/ml juice
V. opulus var. *sargentii*	10.61 ± 0.42^{e}	109.81 ± 1.09^{d}	61.78 ± 0.75^{e}	109.76 ± 1.37^{e}	260.38 ± 7.38^{d}	8.1 ± 0.2^{a}	0.54 ± 0.12^{a}
V. opulus P3	7.78 ± 0.42^{c}	69.79 ± 8.15^{b}	53.23 ± 0.9^{d}	94.07 ± 1.64^{d}	189.98 ± 2.82^{c}	13.5 ± 0.2^{d}	6.93 ± 0.91^{c}
V. opulus var. *americanum*	8.67 ± 0.17^{d}	70.76 ± 2.21^{b}	51.48 ± 1.23^{c}	90.87 ± 2.25^{c}	198.27 ± 5.89^{c}	10.8 ± 0.1^{c}	3.68 ± 0.57^{b}
V. opulus 'Kiyevskaya Sadovaja'	5.47 ± 0.24^{a}	31.95 ± 0.94^{a}	32.33 ± 0.97^{a}	55.77 ± 1.77^{a}	141.63 ± 1.60^{b}	12.6 ± 0.1^{b}	4.52 ± 0.37^{b}
V. opulus 'Krasnaya Grozd'	5.72 ± 0.34^{a}	42.38 ± 4.82^{c}	35.35 ± 0.88^{b}	61.31 ± 1.62^{b}	143.25 ± 12.29^{b}	12.4 ± 0.1^{b}	5.94 ± 0.13^{c}
V. opulus 'Shukshinskaya'	6.30 ± 0.31^{b}	34.69 ± 3.21^{a}	35.94 ± 0.4^{b}	62.38 ± 1.17^{b}	127.37 ± 5.44^{a}	13.3 ± 0.2^{d}	1.31 ± 0.26^{a}

Results are expressed as a mean ± standard deviation (n = 3, 4). The values expressed with the different superscript letter within the same column differ significantly (one way ANOVA and Duncans' test, $P < 0.05$.

(127.4 μmol/g). The results obtained in our study are in the same range as previously reported for highbush cranberry (*Viburnum edule*), which possessed ORAC values from 145 to 222 μmol trolox/g fresh weight of wild berries collected in interior and south-central Alaska (Leiner, Holloway, & Neal, 2006). In our study, a strong correlation (r = 0.93) was found between TPC and ORAC.

V. opulus fruit juices possess higher antioxidant activities comparing to some other berries, such as blueberries, cranberries and chokeberries, with ORAC values of 76.9 (fw) 15.3 (juice) 160.2 (fw) μmol of TE/g, respectively (Zheng & Wang, 2003). To the best of our knowledge there were no results published about the antioxidant activity of *V. opulus* cultivars evaluated by the ORAC assay, i.e. the method which was recognised as one of the most important for the evaluation of antioxidant potential of foods (Huang et al., 2005).

3.3. Quantification of chlorogenic acid, catechin and epicatechin in V. opulus genotypes

Chlorogenic acid, as the main phenolic compound in fresh berries of *V. opulus,* has been reported previously (Altun & Yilmaz, 2007; Velioglu et al., 2006; Çam & Hişil, 2007). Chlorogenic acid is a water soluble secondary metabolite biosynthesised by the phenylpropanoid pathway from phenylalanine *via* cinnamic acid. It is the ester of caffeic and L-quinic acids (3-caffeoyl-quinic acid). Biological activities of chlorogenic acid are well documented and includes antioxidant properties (Sato et al., 2011), antimicrobial effects (Puupponen-Pimia et al., 2001) and antiviral activity (Wang et al., 2009). The beneficial roles of chlorogenic acid in human health has been demonstrated both *in vitro* and *in vivo*. It was shown to possess anti-obesity properties (Cho et al., 2010), antihypertensive effects (Zhao, Wang, Ballevre, Luo, & Zhang, 2011), antitumor effects and cytotoxicity in oral tumors (Jiang et al., 2000) and antimutagenic and anti-inflammatory effects (Jiang & Dusting, 2003). Chlorogenic acid occurs in some plant derived foods at relatively high levels, for example, 0.21–0.85 mg/ml in blueberry juices (Brambilla, Lo Scalzo, Bertolo, & Torreggiani, 2008), 0.04–0.43 mg/g in apple juice (Podsędek, Wilska-Jeszka, Anders, & Markowski, 2000), 0.19 mg/ml in prune juice (Donovan, Meyer, & Waterhouse, 1998) and 0.54 mg/ml in rowanberry juice (Gil-Izquierdo & Mellenthin, 2001).

In this study, LC–MS analysis of fruit juices from six *V. opulus* genotypes showed that chlorogenic acid was the main phenolic compound in 5 of them, although the content of chlorogenic acid varied significantly depending on the genotype (Table 1, Figs. 2 and 3). The highest concentration of chlorogenic acid was found in *V. opulus* P3 (6.9 mg/ml), while the lowest in *V. opulus* var. *sargentii* (0.54 mg/ml). Velioglu et al. (2006) reported that *V. opulus* juice contained 2.04 mg/g of chlorogenic acid, which constituted 54% of total phenolics. The results suggest that *V. opulus* fruits are potential sources of food grade chlorogenic acid.

Two important flavan-3-ol isomers, catechin and epicatechin were also quantified in berry juices by using catechin as a reference. The concentration of catechin varied from 0.02 mg/ml in juice of 'Shukshinskaya' genotype to 0.09 mg/ml in 'P3'. *V. opulus* var. *sargentii* juice contained 0.05 mg/ml of catechin. The concentration of epicatechin in juices of 5 *V. opulus* genotypes varied from undetectable levels ('Kiyevskaya Sadovaja') to 0.012 mg/ml ('Shukshinskaya') and 0.015 mg/ml ('P3' and 'Krasnaya Grozd'). The juice of *V. opulus* var. *sargentii* contained more than 10 times the amount of epicatechin (0.19 mg/ml) compared to other analysed genotypes.

3.4. Separation of individual radical scavenging compounds (on-line HPLV/UV/DPPH·) and analysis of composition (UPLC/ESI–QTOF–MS) of selected V. opulus genotypes

Fruits usually contain a complex mixture of various constituents; therefore, it is important to evaluate the contribution of individual phenolic constituents to the total antioxidant power. This task may be conveniently accomplished by the on-line methods, such as HPLC/UV/DPPH·-scavenging. For this purpose two genotypes were selected, *V. opulus* var. *sargentii* containing the smallest content of chlorogenic acid, but demonstrating the highest antioxidant activity and *V. opulus* P3, containing the highest amount of chlorogenic acid between analysed cultivars and also demonstrating high antioxidant activity. It is interesting to note that the percentage of soluble solids (Table 1) in *V. opulus* var. *sargentii* (8.1%) possessing the highest concentration of TPC and the strongest antioxidant potential was lower than in other genotypes (10.8–13.5%). This indicates that *V. opulus* var. *sargentii* may contain stronger individual antioxidants comparing to other cultivars.

The HPLC/UV/DPPH· chromatograms of *V. opulus* var. *sargentii* and *V. opulus* P3 fruit juices (Fig. 3) reveal the presence of several radical scavenging components at the detectable level; at least five major compounds in *V. opulus* var. *sargentii* juice and three (1 major and 2 minor) in *V. opulus* P3 (Table 2). As the reaction time in the on-line analysis is fixed and it may have an influence on the results, the previous study on the kinetic behaviour of reference phenols with DPPH· revealed, that epicatechin demonstrated more rapid reaction kinetics than chlorogenic acid and catechin, both of which showed the same kinetic behaviour (Bandonienė & Murkovic, 2002). In the same study by using the HPLC/UV/DPPH· method it was also shown that epicatechin demonstrated better RSC than chlorogenic acid, followed by catechin. The RSC of individual antioxidants depends on their chemical structure, mainly on the number and the location of hydroxyl groups in the molecule.

In UPLC–QTOF–MS analysis the compound **1** gave an m/z value of 191.0506 corresponding to the molecular ion formula $C_7H_{11}O_6$, therefore it was identified as quinic acid. The m/z of compound **2** (295.0669) correlates with the molecular ion formula $C_{10}H_{15}O_{10}$,

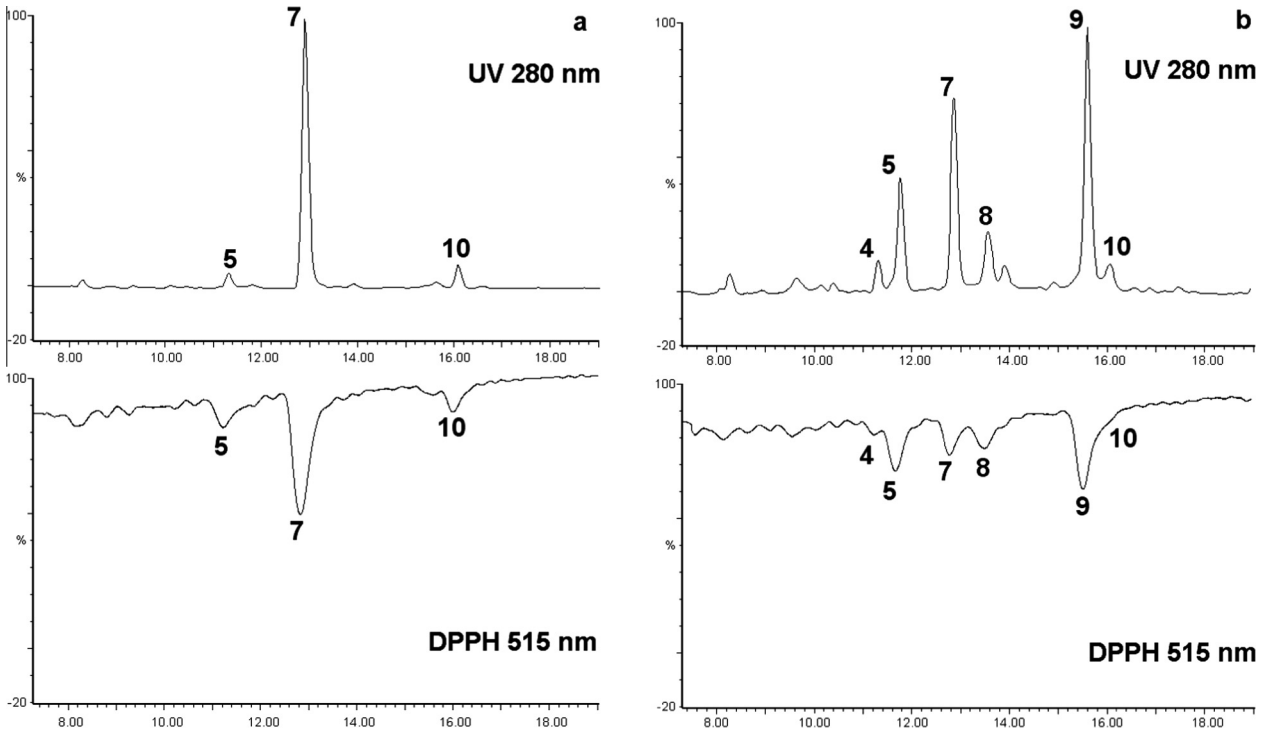

Fig. 2. UV and DPPH· radical quenching chromatograms of *V. opulus* P3 (a) and *V. opulus* var. *sargentii* (b) genotypes.

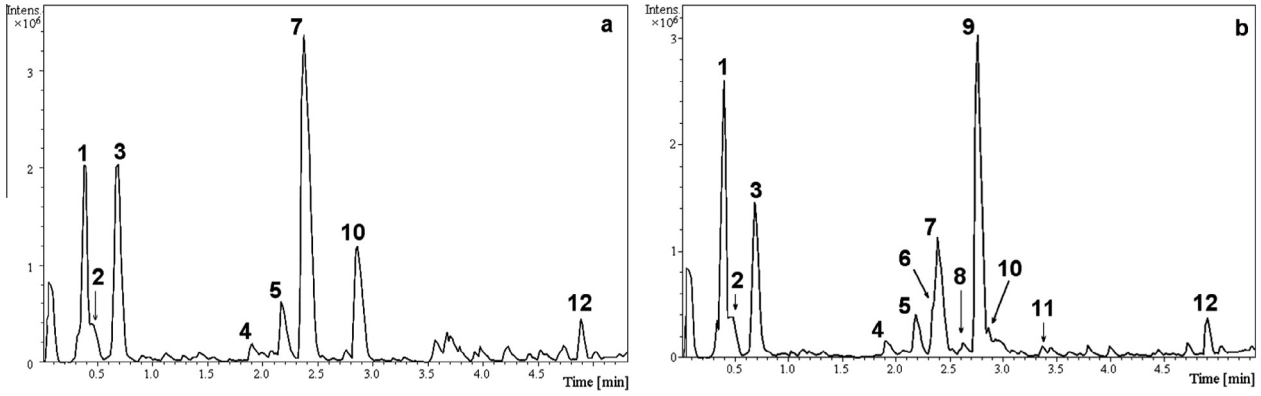

Fig. 3. UPLC/ESI-QTOFMS chromatograms of *V. opulus* P3 (a) and *V. opulus* var. *sargentii* (b) genotypes.

whereas the MS/MS fragmentation of this peak gave a fragment $m/z = 133.0143$ corresponding to a formula $C_4H_5O_5$. The peak **3** had an $m/z = 337.0776$ fitting the molecular formula of $C_{12}H_{17}O_{11}$. However, compounds **2** and **3** were not identified.

The analysis showed that the compounds **4**, **6** and **11** give similar m/z values, 577.1343, fitting the molecular ion formula $C_{30}H_{25}O_{12}$. MS/MS analysis of these peaks gave the same fragment at m/z 289.0713, fitting the formula $C_{15}H_{13}O_6$. This data suggests the presence of catechin dimers; however, the exact position of dimerisation could not be determined by MS. These compounds were also not demonstrating strong RSC.

The compound **5** with m/z of 289.0713, fitting molecular ion formula $C_{15}H_{13}O_6$ and perfectly matching retention time of the catechin standard, was identified as catechin (Fig. 3, Table 2). Although this compound in berry juices was detected in negligible quantities it demonstrated significant impact on antioxidant activity.

The m/z of compound **7** (353.0874) was fitting molecular ion formula $C_{16}H_{17}O_9$, whereas the MS/MS fragmentation of this peak gave a fragment $m/z = 191.0559$ corresponding to a formula $C_7H_{11}O_6$. The obtained data were in accordance to the MS spectral characteristics of 3-*O*-caffeoylquinic acid (chlorogenic acid); the structure of this compound was also confirmed by a good match of its retention time with chlorogenic acid standard. Chlorogenic acid was one of the main compounds present in both samples, however, judging from the negative peak area in the chromatogram it was remarkably more important in *V. opulus* P3 (74% of the total radical scavenging peak area) than in *V. opulus* var. *sargentii*, where its peak constituted 15% of the total radical scavenging peak's area.

Peak **8**, demonstrating strong DPPH· RSC, was found only in *V. opulus* var. *sargentii*. This peak had an $m/z = 865.1977$, fitting molecular ion formula $C_{45}H_{37}O_{18}$, whereas MS/MS fragmentation produced the ions of $m/z = 577.1339$ ($C_{30}H_{25}O_{12}$) and 289.0713 ($C_{15}H_{13}O_6$). The UV maxima of this peak were similar to catechin, 239 and 279 nm. All this data suggests that this compound might be a catechin trimer and therefore it was tentatively identified as procyanidin C1. The percentage of negative peak area in the HPLC/UV/DPPH· chromatogram for this compound was 11% indicating that procyanidin C1 might be an important contributor to

Table 2
Characterisation of individual phenolic compounds in juices of selected *V. opulus* genotypes by using UPLC/ESI-QTOF-MS and the antioxidant activity of individual phenolic constituents in *V. opulus* juices by using on-line HPLC/UV/DPPH· scavenging tests.

Peak no.	Compound	UPLC/ESI-Q-TOF			HPLC/UV/DPPH·				
		RT (min)	MS $[M-H]^-$ m/z	Formula [M−H]	RT (min)	*V. opulus* P3		*V. opulus* var. *sargentii*	
						Peak, % UV	Peak, % DPPH·	Peak, % UV	Peak, % DPPH·
1.	Quinic acid	0.37	191.0506	$C_7H_{11}O_6$	–	–	–	–	–
2.	Not identified	0.47	295.0669	$C_{10}H_{15}O_{10}$	–	–	–	–	–
3.	Not identified	0.69	337.0776	$C_{12}H_{17}O_{11}$	–	–	–	–	–
4.	Catechin dimer	1.90	577.1343	$C_{30}H_{25}O_{12}$	11.31	–	–	6	3
5.	Catechin	2.17	289.0713	$C_{15}H_{13}O_6$	11.76	8	14	19	23
6.	Catechin dimer	2.34	577.1343	$C_{30}H_{25}O_{12}$		–	–	–	–
7.	Chlorogenic acid (3-*O*-Caffeoylquinic acid)	2.38	353.0874	$C_{16}H_{17}O_9$	12.86	83	74	23	15
8.	Procyanidin C1	2.62	865.1977	$C_{45}H_{37}O_{18}$	13.56	–	–	12	11
9.	Epicatechin	2.76	289.0714	$C_{15}H_{13}O_6$	15.59	–	–	32	40
10.	Neochlorogenic acid (5-*O*-caffeoylquinic acid)	2.86	353.0876	$C_{16}H_{17}O_9$	16.06	9	12	8	8
11.	Catechin dimer	3.36	577.1343	$C_{30}H_{25}O_{12}$	–	–	–	–	–
12.	Not identified	4.89	397.2566	$C_{22}H_{36}O_6$	–	–	–	–	–

the total RSC of *V. opulus* var. *sargentii*. Peak **6** was present in a substantial amount only in *V. opulus* var. *sargentii*, whereas in P3 sample it was detected in traces. From the HPLC/UV/DPPH· chromatogram it is obvious, that this compound, constituting 42% of the total radical scavenging negative peak area, has a major influence on the RSC of *V. opulus* var. *sargentii* juice.

Peak **9** had an $m/z = 289.0714$ fitting molecular formula of $C_{15}H_{13}O_6$ and therefore indicating that this compound should have a similar structure to catechin. As it had the same UV absorption maxima as catechin, it was tentatively identified as epicatechin; however for unambiguous structure identification NMR data would be needed.

Compound **10** was detected in both fruit juice samples; however in *V. opulus* var. *sargentii* it was present in a very small amount. This compound had little RSC. The peak gave $m/z = 353.0876$ fitting molecular ion formula $C_{16}H_{17}O_9$, and fragment ion $m/z = 191.0560$, corresponding to $C_7H_{11}O_6$. This data suggests the structure of caffeoyl quinic acid. Since chlorogenic acid has already been identified in the *V. opulus* juice, the remaining possibilities are 4- or 5-*O*-caffeoylquinic acid. As 4-*O*-caffeoylquinic acid gives MS^2 fragment at $m/z = 173$, but not at $m/z = 191$, the compound is most likely 5-*O*-caffeoylquinic acid.

The unidentified peak **12** gave $m/z = 397.2566$ fitting molecular ion formula $C_{22}H_{36}O_6$.

Velioglu et al. (2006) based on HPLC/UV data of *V. opulus* juices besides chlorogenic acid, as the main phenolic compound, reported several other constituents, namely (+)-catechin, (−)-epicatechin, cyanidin-3-glucoside, cyanidin-3-rutinoside and six different glucosides of quercetin. However, in the mentioned study mass spectrometry was not applied for the identification of phenolic compounds and therefore identification may be considered only as a preliminary. The identification of *V. opulus* constituents in our study is based on more comprehensive data.

4. Conclusion

V. opulus genotypes analysed are good sources of valuable biologically active substances possessing antioxidant and other beneficial health properties. However, the phytochemical composition of berry fruits is quite complex and depends on the plant genotype and other factors. Assessment of antioxidant potential by the *in vitro* methods demonstrated that *V. opulus* var. *sargentii* possesses the highest antioxidant potential between all investigated genotypes. The LC–MS and on-line HPLC/UV/DPPH· analyses revealed that the phytochemical profile of *V. opulus* var. *sargentii* is remarkably different compared to the other *V. opulus* genotypes analysed. This study expands our knowledge about the content of valuable phytochemicals in different *V. opulus* genotypes, and may assist in the selection of the most promising cultivars for further cultivation and application of fruits in the production of potential food ingredients, nutraceuticals, etc.

Acknowledgement

This study was funded by the Research Council of Lithuania, Grant No. SVE-04/2011.

References

Altun, M. L., & Yilmaz, B. S. (2007). HPLC method for the analysis of salicin and chlorogenic acid from *Viburnum opulus* and *V. lantana*. *Chemistry of Natural Compounds, 43*, 205–206.

Bandonienė, D., & Murkovic, M. (2002). On-line HPLC–DPPH screening method of evaluation of radical scavenging phenols extracted from apples (*Malus domestica* L.). *Journal of Agricultural and Food Chemistry, 50*, 2482–2487.

Benzie, F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP assay. *Analytical Biochemistry, 239*, 70–76.

Brambilla, A., Lo Scalzo, R., Bertolo, G., & Torreggiani, D. (2008). Steam-bleached highbush blueberry (*Vaccinium corymbosum* L.) juice: Phenolic profile and antioxidant capacity in relation to cultivar selection. *Journal of Agricultural and Food Chemistry, 56*, 2643–2648.

Brand-Williams, W., Cuvelier, M. N., & Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und-Technologie, 28*, 25–30.

Çam, M., & Hişil, Y. (2007). Comparison of chemical characteristics of fresh and pasteurised juice of gilaburu (*Viburnum opulus* L.). *Acta Alimentaria, 36*, 381–385.

Česonienė, L., Daubaras, R., Venclovienė, J., & Viškelis, P. (2010). Biochemical and agro-biological diversity of *Viburnum opulus* genotypes. *Central European Journal of Biology, 6*, 864–871.

Česonienė, L., Daubaras, R., & Viškelis, P. (2008). Evaluation of productivity and biochemical components in fruit of different *Viburnum* accessions. *Biologija, 54*, 93–96.

Česonienė, L., Daubaras, R., Viškelis, P., & Šarkinas, A. (2012). Determination of the total phenolic and anthocyanin contents and antimicrobial activity of *Viburnum opulus* fruit juice. *Plant Foods for Human Nutrition, 67*, 256–261.

Cho, A. S., Jeon, S. M., Kim, M. J., Yeo, J., Seo, K. I., Choi, M. S., et al. (2010). Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in high-fat diet-induced-obese mice. *Food and Chemical Toxicology, 48*, 937–943.

Dávalos, A. D., Gómez-Cordovés, C., & Bartolomé, B. (2004). Extending applicability of the oxygen radical absorbance capacity (Orac-fluorescein) assay. *Journal of Agricultural and Food Chemistry, 52*, 48–54.

Donovan, J. L., Meyer, A. S., & Waterhouse, A. L. (1998). Phenolic composition and antioxidant activity of prunes and prune juice (*Prunus domestica*). *Journal of Agricultural and Food Chemistry, 46*, 1247–1252.

Gil-Izquierdo, A., & Mellenthin, A. (2001). Identification and quantitation of flavonols in rowanberry (*Sorbus aucuparia* L.) juice. *European Food Research and Technology, 213*, 12–17.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*, 1841–1856.

Jiang, F., & Dusting, G. J. (2003). Natural phenolic compounds as cardiovascular therapeutics: Potential role of their anti-inflammatory effects. *Current Vascular Pharmacology, 1*, 135–156.

Jiang, Y., Kusama, K., Satoh, K., Takayama, F., Watanabe, S., & Sakagami, H. (2000). Induction of cytotoxicity by chlorogenic acid in human oral tumor cell lines. *Phytomedicine, 7*, 483–491.

Jordheim, M., Giske, N. H., & Andersen, Ø. M. (2007). Anthocyanins in Caprifoliaceae. *Biochemical Systematics and Ecology, 35*, 153–159.

Kraujalytė, V., Leitner, E., & Venskutonis, P. R. (2012). Chemical and sensory characterisation of aroma of *Viburnum opulus* fruits by solid phase microextraction–gas chromatography–olfactometry. *Food Chemistry, 132*, 717–723.

Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., et al. (2002). Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *American Journal of Medicine, 113*(9B), 71–88.

Leiner, R. H., Holloway, P. S., & Neal, D. B. (2006). Antioxidant capacity and quercetin levels in Alaska wild berries. *International Journal of Fruit Science, 6*, 83–91.

Medina, M. B. (2011). Simple and rapid method for the analysis of phenolic compounds in beverages and grains. *Journal of Agricultural and Food Chemistry, 59*, 1565–1571.

Podsędek, A., Wilska-Jeszka, J., Anders, B., & Markowski, J. (2000). Compositional characterisation of some apple varieties. *European Food Research and Technology, 210*, 268–272.

Prior, R. L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., et al. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC(FL)) of plasma and other biological and food samples. *Journal of Agricultural and Food Chemistry, 51*, 3273–3279.

Puupponen-Pimia, R., Nohynek, L., Meier, C., Kahkonen, M., Heinonen, M., Hopia, A., et al. (2001). Antimicrobial properties of phenolic compounds from berries. *Journal of Applied Microbiology, 90*, 494–507.

Re, R., Pellegrini, R. N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.

Rop, O., Reznicek, V., Valsikova, M., Jurikova, T., Mlcek, J., & Kramarova, D. (2010). Antioxidant properties of European cranberrybush fruit (*Viburnum opulus* var. *edule*). *Molecules, 15*, 4467–4477.

Sagdic, O., Aksoy, A., & Ozkan, G. (2006). Evaluation of the antibacterial and antioxidant potentials of cranberry (gilaburu, *Viburnum opulus L.*) fruit extract. *Acta Alimentaria, 35*, 487–492.

Sato, Y., Itagaki, S., Kurokawa, T., Ogura, J., Kobayashi, M., Hirano, T., et al. (2011). *In vitro* and *in vivo* antioxidant properties of chlorogenic acid and caffeic acid. *International Journal of Pharmaceutics, 403*, 136–138.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology, 299*, 152–178.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology, 39*, 44–84.

Velioglu, Y. S., Ekici, L., & Poyrazoglu, E. S. (2006). Phenolic composition of European cranberrybush (*Viburnum opulus* L.) berries and astringency removal of its commercial juice. *International Journal of Food Science and Technology, 41*, 1011–1015.

Venskutonis, P. R., Kraujalytė, V., Stackevičiene, E., Labokas, J., Česoniene, L., & Daubaras, R. (2012). Phytochemical characterisation of highbush blueberry (*Vaccinium covilleanum*) and European cranberry bush (*Viburnum opulus*) accessions grown in Lithuania. *Pharmaceutical Biology, 50*, 551.

Wang, S., Melnyk, J. P., Tsao, R., & Marcone, M. F. (2011). How natural dietary antioxidants in fruits, vegetables and legumes promote vascular health. *Food Research International, 44*, 14–22.

Wang, G. F., Shi, L. P., Ren, Y. D., Liu, Q. F., Liu, H. F., Zhang, R. J., et al. (2009). Anti-hepatitis B virus activity of chlorogenic acid, quinic acid and caffeic acid in vivo and in vitro. *Antiviral Research, 83*, 186–190.

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

Yang, J., Martinson, T. E., & Liu, R. H. (2009). Phytochemical profiles and antioxidant activities of wine grapes. *Food Chemistry, 116*, 332–339.

Zhao, Y., Wang, J., Ballevre, O., Luo, H., & Zhang, W. (2011). Antihypertensive effects and mechanisms of chlorogenic acids. *Hypertension Research, 35*, 370–374.

Zheng, W., & Wang, S. Y. (2003). Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. *Journal of Agricultural and Food Chemistry, 51*, 502–509.