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Antioxidant content of dark colored berries

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Abstract. Blackberries (*Rubus caesius*), elderberries (*Sambucus nigra L)*, highbush blueberries (*Vaccinium corymbosum* L) and black currants (*Ribes nigrum*) were selected for research on their content of phenolic compounds, including anthocyanins and comparing their content in these berries. Samples were also assayed for ascorbic acid and soluble solid content. The unifying mark of these fruits is purple-black color, which indicates high content of anthocyanins. Analyses of these fruits showed that they contain high biological activity components that justify the uses of these fruits.

The research was done at the Department of Chemistry, Latvia University of Life Sciences and Technologies, year 2019. Berry samples were bought at the supermarket (highbush blueberries) or collected from garden in Jelgava, Latvia (blackberries, elderberries and black currants). All berries were at full maturity. The content of ascorbic acid, total phenols, anthocyanins, flavonoids as well as soluble solids was determined.

Descending order of ascorbic acid content in berries is: black currants > blackberries > elderberries > highbush blueberries. Elderberries had the highest content of anthocyanins, total phenols and flavonoids on average 161.5, 537.9 and 112.6 mg 100 g^{-1} FW, respectively. The content of soluble solids changes from 5.83 Brix (elderberries) to 13.67 Brix (black currants).

Key words: berries, bioactive compounds, phenolic compounds.

INTRODUCTION

Free radicals and other reactive species are capable to cause oxidation and biomolecular damages when oxidative processes exceed the antioxidative protection of the organism. Fruit, especially small berries, contain a wide variety of antioxidant compounds such as phenolics, mostly flavonoids and anthocyanins, that may help protect cellular systems from oxidative damage. Thanks to these compounds berries possess antioxidant, anticancer, antiinflammatory, and antineurodegenerative biological properties (Prior et al., 1998; Liu, 2003; Milivojevic et al., 2011).

Blackberries (*Rubus caesius*), elderberries (*Sambucus nigra L)*, highbush blueberries (*Vaccinium corymbosum* L) and black currants (*Ribes nigrum*) were selected for research on their content of phenolic compounds, including anthocyanins and comparing their content in these berries. The unifying mark of these fruits is purple-black color, which indicates high content of anthocyanins.

Anthocyanins classification is based on the number and position of hydroxyl and carboxyl groups in the flavinium nucleus. The most common anthocyanins found in plants, including berries, are pelargonidin, cyanidin, delphinidin, penonidin, petunidin and malvidin. Recent studies demonstrate that anthocyanin extracts display a wide range of biological activities, including antioxidant, antimicrobial, anti-inflammatory and anticarcinogenic activities; maintaining eye health and vision and neuroprotective effects (Ramos, 2008; Seeram, 2008).

Blackberries are popular not only in Europe, but also in the southern part of United States. Several reports have demonstrated that the most antioxidant capacity was due to these phenolics and ascorbic acid in blackberries (Pantelidis et al., 2007; Wang & Lin, 2000). Blackberry fruits are abundant in flavonoids, colorants, and organic acids. The blackberry residues exhibited a high amount of malic acid (5,706.37 mg 100 g^{-1} dry bases -db), phenols (4,016.43 mg GAE 100 g^{-1} db), and content of anthocyanins (364.53 mg 100 g^{-1} db) (Zafra-Rojas et al., 2018). These berries contain also phenolic compounds as anthocyanins, flavonols, chlorogenic acid, and procyanidins, which can have beneficial effects on human health (Moure et al., 2001, Siriwoham & Wrolstad, 2004).

Mertz and co-authors reported that ellagitannins and cyanidin-3-glucoside are major phenolic compounds in blackberries. The anthocyanins (cyanidin-3-rutinoside and cyanidin-3-malonyl glucoside), flavonols (quercetin and kaempferol glycosides) and flavan-3-ol (epicatechin) were also identified in blackberries. Hydroxycinnamic acids are minor compounds, and they are found as ferulic, caffeic and *p*-coumaric acid esters (Mertz et al., 2007).

The purple-black fruits of elderberries (*Sambucus* spp. L.) are one of the richest sources of anthocyanins and phenolic compounds among berries and have strong antioxidant capacity (Duymus et al., 2014). Elderberries are widely used in Europe for the productions of healthy food and beverages as well as medicinal products. These fruits have been used for generations in traditional herbal medicine as a remedy for colds, sinusitis, and herpes. Biological value of the fruit is in high level of vitamins, minerals, pectins, colour, cellulose, dietetic fibres, sugars, organic acids and low energetic value (Cejpek et al., 2009). Although berries are often recommended for fresh use, care should be taken when picking fresh elderberries. As fresh elderberries contain cyanogening glycosides, they are slightly toxic and can cause vomiting. The mild toxicity is destroyed by cooking (Vulic et al., 2008). Analysing infusions of elderberry fruits and flowers have shown, that the infusions prepared from elderflowers contained more abundant phenolic compounds than the elderberry infusions. The TPC (total phenolic) of these infusions ranged from 19.81 to 23.90 mg of gallic acid equivalents/g dry weight of sample (GAE/g DW) for elderberries and from 15.23 to 35.57 mg GAE/g DW for elderflowers, whereas the TFC (flavonoid) ranged from 2.60 to 4.49 mg of rutin equivalents/g dry weight of sample (RUTE/g DW) in elderberry infusions and from 5.27 to 13.19 mg RUTE/g DW in elderflower infusions (Viapiana & Wesolowski, 2017).

Blueberry fruits are rich in phenolic acids, flavonols and anthocyanins (Wang & Lin, 2000; Moyer et al., 2002). The content of phenolic compounds in berry fruits is affected by genetic differences among species and within the same species and maturity at harvest.

Ribera et al. (2010) reported that in mature blueberry fruits the content of antioxidant, anthocyanins and total phenolic increases. By contrast, Rodarte et al. (2008) found that phenolic compounds, flavonols and hydroxycinnamic acids concentration and antioxidant activity in highbush blueberry fruits decreased during ripening.

Many of the antioxidant characteristics associated with berries can be attributed to the anthocyanin content. Four anthocyanins: delphinidin-3-rutinoside, cyanidin-3-rutinoside, delphinidin-3-glucoside and cyanidin-3-glucoside make up 98% of blackcurrant anthocyanins, the remaining 2% comprise 11 other anthocyanins including petunidin and malvinidin glycosides. Other polyphenols in blackcurrant are quercetin, myricetin, kaempferol and isorhamnetin (Karjalainen et al., 2009).

Black currants compared to other berries, for example, strawberries and raspberries contain high levels of polyphenols (500–1,342 mg 100 g^{-1} of fresh weight) especially anthocyanins, phenolic acid derivatives (both hydroxybenzoic and hydroxycinnamic acids), flavonols (glycosides of myricetin, quercetin, kaempferol and isorhamnetin) as well as proanthocyanidins. These compounds have health promoting properties. Both myricetin and quercetin as described have neuroprotective activity. Furthermore, quercetin and isorhamnetin reduce blood pressure and improve blood flow evoking a potential protective function against development of vascular type of dementia (Vagiri et al., 2012).

Vitamin C is needed for the formation of blood vessels, bones and connective tissue, promoting absorption of iron and as an antioxidant (Hancock & Viola, 2005).

Black currant are considered to be a rich source of vitamin C. Vitamin C content in black currants in some examples ranging from 130–200 mg 100 mL^{-1} of juice to over 350–450 mg 100 mL^{-1} of juice (Mattila et al., 2011). Black currants are good sources of antioxidants like polyphenols, flavonols and vitamin C which make them a desirable addition to functional foods.

Several studies have been conducted in Latvia and Estonia analysing the chemical composition of berries (Albert et al., 2010; Soots et al., 2017; Karlsons et al., 2018; Klavins et al., 2019).

The aim of research was to analyse content of antioxidants in dark coloured berries – blackberries, elderberries, highbush blueberries and black currants.

MATERIALS AND METHODS

The research was done at the Department of Chemistry, Latvia University of Life Sciences and Technologies, year 2019. Berry samples were bought at the supermarket (highbush blueberries) or collected from garden in Jelgava, Latvia (blackberries, elderberries and black currants). 500 g of each fruit species were taken for analysis. All berries were at full maturity. Approximately 100 g of randomly selected fruits were purified and finely homogenized for 3 min. For determination of chemical parameters, three repetitions for each fruit species were performed.

Chemicals and spectral measurements

All the reagents used were with the analytical grade from Sigma Aldrich, Germany. UV spectrophotometer UV-1800 (Shimadzu Corporation, Japan) was used for the absorbance measurements.

Analytical methods

The contents of soluble solids were determined by refractometer, Refractometer A. KRÜSS Optronic Digital Handheld Refractometer Dr301-95, calibrated at 20 °C with distilled water and expressed as ºBrix. Analysis were performed in six replicates and expressed as mean values ± standard deviation.

The content of ascorbic acid was analysed according to Moor et al. (2005), using iodometric titration based on an oxidation-reduction reaction between ascorbic acid and iodine. The endpoint was determined using 1% starch suspension till blue-black colour, which does not disappear during 30 seconds.

For extraction of total phenols, flavonoids and anthocyanins 1.0 ± 0.001 g of finely ground fruit samples was weighed into volumetric flasks, 10 mL of extractant (methanol, distilled water and hydrochloric acid 79:20:1 v/v/v or acidified methanol with 0.1% HCl) was added, shaked at 20 °C for 30 min in the dark and then centrifuged for 10 min at 5,000 rpm.

The content of total phenols was determined quantitatively using 2.5 mL of Folin Ciocalteu reagent, 0.5 mL of extract and 2 mL of 7.5% sodium carbonate (Singleton et al., 1999). The absorbance was measured at wave length 765 nm. The results were expressed as mg gallic acid equivalents (GAE) 100 g^{-1} FW of fruits.

For determination of anthocyanins the light absorption of acidified methanol extract was detected with a spectrophotometer at 530 nm.

Determination of flavonoids were performed using aluminum chloride colorimetric method with quercetin as standard (Kim et al., 2003). To 2 mL of H_2O was added 0.50 mL of extract and 0.15 mL of 5% $NaNO_2$. After 5 min 0.15 mL of 10% $AlCl_3$ solution and after 10 min 1 mL of 1M NaOH was added. The absorbance was measured after 15 min at 415 nm using 6705 UV/VIS YENWAY Spectrophotometer.

Statistical analysis

Analyses were conducted in three replicates and each one was measured for three repetitions. Data were expressed as mean of triplicates assay ± standard deviation; for mathematical data processing the value of $p < 0.05$ was regarded as statistically significant. One-way analysis of variance (ANOVA) was used to determine the significance of differences.

RESULTS AND DISCUSSION

Berries are rich in vitamins, organic acids, minerals and antioxidants, high in fibres, therefore berries are among the healthiest products. Daily use of these products improves health and very often, dark coloured berries are of particular interest due to the best dietary sources of different bioactive compounds, such as antioxidants. The bioactive compounds in berries contain mainly phenolic compounds (phenolic acids, flavonoids, such as anthocyanins and flavonols, and tannins) and ascorbic acid (Skrovankova et al., 2015). Statistics shows that only 37% of the Baltic population consumes fresh fruits and berries on a daily basis. Our study of content of bioactive compounds in dark colour berries shows that nutritionists should also pay attention to less commonly grown crops – elderberry and blackberry.

The obtained results (Fig. 1) showed that content of ascorbic acid in black currants ranged from 40.25 to 44.71 mg 100 g^{-1}, while it ranged from 10.14 to 14.95 mg 100 g^{-1} for elderberries and from 16.11 to 18.03 mg 100 g^{-1} or from 7.43 to 9.98 mg 100 g^{-1} for blackberries and highbush blueberries respectively. Our findings are similary to results reported in literature (Schulz & Chim, 2019).

Figure 1. The content of ascorbic acid mg 100 g^{-1} in dark coloured berries.

Therefore, black currants had the highest ascorbic acid content comparing with three other analysed samples. Significant differences ($p < 0.05$) were found in the ascorbic acid content comparing black currants and other analysed samples. We can conclude, that with an average content ranging from 8.71 to 12.54 mg 100 g^{-1} FW, the vitamin C level is not high enough to classify blueberries and elderberries as a rich source of vitamin C.

Content of bioactive compounds – anthocyanins, total phenols and flavonoids – in different berries is shown in Table 1.

Table 1. Content of bioactive compounds in berries

	Anthocyanins*	Total phenols**	Flavonoids***
Blackberries	76.83 ± 2.53	342.48 ± 5.58	58.77 ± 2.72
Highbush blueberries	28.35 ± 0.59	228.63 ± 5.59	44.16 ± 1.58
Elderberries	161.51 ± 0.07	537.96 ± 9.53	112.65 ± 0.44
Black currants	137.88 ± 2.08	513.54 ± 15.97	73.26 ± 2.37

*mg 100 g^{-1} FW; **mg GAE 100g^{-1} FW; ***mg QE 100g^{-1} FW.

Total phenolic content is known to influence antioxidant and other health-related bioactivities (Milivojevic et al., 2011). In this study the elderberries had the highest amount of total phenols ranged from 528.43 to 547.49 mg GAE 100 g^{-1} FW, confirming that elderberries are an important source of health-related compounds. Significantly lower amounts of total phenols were observed in highbush blueberries and blackberries (228.63 and 342.48 mg GAE 100 g^{-1} FW, respectively). Significant differences were not found in the total phenolic when comparing between elderberries and black currants fruits.

Higher phenols content confirm reports of Mikulic-Petkovsekaet al. (2016) and Lee & Finn (2007), but are lower then results mentioned by Pedro Silva et al. (2017), Coklar & Akbulut (2017) and Ferreira et al. (2020).

Data obtained by Acosta-Montoya et al. (2010) corroborate low phenols content in highbush blueberries and blackberries.

Quantitatively elderberries were richest in anthocyanins and flavonoids (161.51 mg 100 g^{-1} and 112.65 mg QE 100g^{-1} FW respectively). The very high anthocyanins content of elderberries, 2-fold greater than blackberries and 6-fold greater than blueberries, showed it's potential as an important source of bioactive compounds and possible use for humans health. These results are significantly lower than that mentioned in the literature (Lee & Finn, 2007; Silva et al., 2017; Ferreira et al., 2020). Differences between the present results and those reported in the literature may be associated with different environmental and growing conditions, as well as the use of different solvents used for extraction.

From the results showed in Table 1 it can be seen that the highbush blueberries exhibited the lowest concentrations of anthocyanins (average 28.35 mg 100 g^{-1} FW) compared to blackberries, black currants and elderberries.

Berries are unique food due to source of vitamins, enzymes, minerals, carbohydrates, pectins and other valuable substances. Flavonoids are biologically active substances, which have not historically been classified as nutrients, however, have recently been increasingly mentioned in terms of healthy nutrition to maintain health. These compounds could be found in almost all fruits and vegetables, moreover dark coloured berries are a good source of flavonoids in the diet.

Descending order of flavonoids content in analysed berries is: elderberries > blackberries > black currants > highbush blueberries. Overall, we can conclude that elderberry is rich in all known antioxidants and recommended for people's health.

The total soluble solids (TSS) content, measured as °Brix, represents one of the main quality parameters of berries and it also allows monitoring the stage of fruit maturation (Salvador et al., 2015). The TSS of berries was evaluated using a digital hand held digital refractometer and the obtained results are shown in Fig. 2. By studying, significant differences ($p < 0.05$) were found between the samples regarding the content of total soluble solids. The black currants had the highest mean Brix value (average 13.67), whereas blackberries the lowest (4.93).

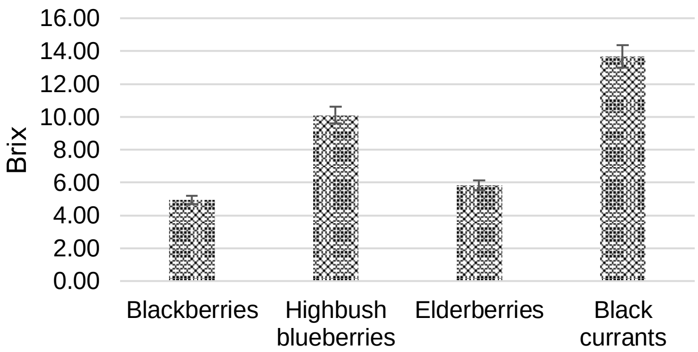

Figure 2. The content of soluble solids in dark coloured berries.

Studies showed that the soluble solids content of the elderberries was lower than those of reported by Salvador et al., 2015 or Ferreira et al., 2020. These differences could be due to different stage of maturity and different climatic conditions were the plants were grown. There are reports (Moggia et al., 2016; Schulza et al., 2019) indicating higher content of TSS of blacberries – about 8 Brix, but our results are similar to results found in Acosta-Montay et al. (2010) research.

CONCLUSIONS

Berries are a good source of vitamins, minerals and antioxidants in our diet. The results of the study confirmed that the dark colour berries are rich in antioxidants because of the high content of phenols, flavonoids and anthocyanins. The analysis of obtained results showed that the elderberries are the most valuable in this respect, since they contained the most of these biologically active compounds in significant amount. If you need to increase the amount of ascorbic acid in your diet, black currants are recommended.

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