# Polyphenol content and antioxidant activity of wild and cultivated blackberry (*Rubus Fruticosus L.*) juices

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ISSN 2712-1267 UDC: 615.279:[633.11-232:634.713 https://doi.org/10.7251/JCTE2001021K Original scientific paper

Paper received: 14 Oct 2020 Paper accepted: 07 Dec 2020 Paper available from 30 Dec 2020 at https://glasnik.tf.unibl.org/

Keywords:

cultivated blackberry, wild blackberry, juice, polyphenol content, antioxidant activity.

Blackberry fruits (Rubus fruticosus L.) have been valued as an excellent source of polyphenol content and antioxidant activity. In this study, blackberry juices of two cultivated blackberry cultivars and two wild blackberry cultivars were evaluated as potential sources of polyphenolic compounds and antioxidant activity. The content of polyphenols (total polyphenols, total flavonoids, flavonols, total and monomeric anthocyanins) and antioxidant activity (DPPH, ABTS, and OH radicals tests) were determined by the spectrophotometric method. The juices of wild blackberry varieties indicated the higher content of total polyphenols (2.16 - 2.25 mg GAE/g fw (fresh weight)), total anthocyanins (0.83 - 1.34 mg CyGE/g fw), and monomeric anthocyanins (0.70 - 1.14 mg CyGE/g fw), while the juice of cultivated blackberry variety had higher content of flavonoids (0.45 mg QcE/g fw) and flavonols (0.68 mg QcE/g fw). The results of the DPPH and the ABTS tests showed that the juices of wild blackberries possessed higher antioxidant activity compared to cultivated blackberries (p < p0.05). All blackberry juices showed high concentrations of total polyphenols, including flavonoids, and anthocyanins content. The antioxidant activities of wild variety samples were stronger than of cultivated varieties. Significant correlations were determined between the content of total polyphenols, total and monomeric anthocyanins and the capacities of inhibition DPPH and ABTS radicals.

## INTRODUCTION

Blackberry is an expensive fruit. It possesses extremely suitable chemical composition, as well as the possibility of growing without the use of pesticides. Wild blackberries grow up along streams and roads, in hedges, on the uncultivated and neglected field. Compared to cultivated varieties, wild blackberry fruits are smaller and with higher dry matter contents (Mladenović, 2014). Cultivated blackberries as a raw material showed excellent quality in production, while wild blackberries showed higher nutritional quality (Dujmović-Purgar et al., 2012; Takeda, 2017; Yilmaz et al., 2009; ). Blackberries are consumed fresh, processed into ice cream, jam, marmalade, and significant quantities are processed into juices (Koczka et al., 2018; Nikolić & Milivojević, 2010; Rao & Snyder, 2010). In north-west part of Bosnia and Herzegovina, wild blackberry species are present in almost all localities, while cultivated blackberry varieties, are intensively cultivated. Blackberries represent a very good source of polyphenolic compounds and are very important in human nutrition (Koca & Karadeniz, 2009; Zia-Ul-Haq et al., 2014). Blackberries contain polyphenols, flavonoids, lignins, condensed tannins, organic acids, anthocyanins, flavonols, chlorogenic acid, and procyanidins (Haminiuk et al., 2012; Veberic et al., 2014). A lot of these components obtained particular attention as potential protective factors against degenerative diseases (Dai et al., 2009; Farrukh et al., 2012). Flavonoids are polyphenolic compounds that make a large group of plant secondary metabolites (Cho et al., 2004; Panche et al., 2016). Anthocyanins are the most important group of water-soluble flavonoids, responsible for the red, blue and purple colours of flowers, vegetables, and fruits.

Based on the above, the beneficial effects of these compounds and the growing consumer request for functional foods that have positive effects on health, leads the food industry to express interest to develop bioactive berry-based juices (Guzmán-Gerónimo et al., 2020; Souza et al., 2015). Experts and nutritionists recommend the inclusion of fruits and fruit-based products, and especially juices in

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the everyday diet. Some studies also suggest the possibility of using blackberry juice as a preservative in the food industry, due to its antimicrobial action (Zia-UI-Haq et al., 2014).

The aim of this study was to determine the content of total polyphenolic components and the differences between juices of wild and cultivated blackberry varieties, and the differences in their antioxidant activities.

# MATERIALS AND METHODS

## **Chemicals and standards**

The chemicals and reagents used in this study were analytical and of higher purity, supplied from Lach-Ner s.r.o. (Neratovice, Czech Republic), Zorka, Šabac (Serbia) and J.T. Baker (Deventer, Netherlands). Other chemicals and reagents used in the experiments were analytical and of high purity. Spectrophotometric measuring was carried out with Lambda 25 UV/VIS spectrophotometer, producer: Perkin Elmer (USA).

# Preparation of blackberry juice

The samples were collected at the mature stage (period August–September) on two different localities in the north-west part of Bosnia and Herzegovina (Verići and Javorani). The samples of blackberry juices were obtained using a commercial juicer, type Coral PJ 500 (Fagor, Spain). The samples were marked: J-c1 – Chester Thornless (from Javorani), J-c2 – Čačnka bestrna (from Verići), J-w1 – wild blackberry (Javorani) and J-w2 – wild blackberry (Verići). After obtaining juices, the densities of all samples were determined using a pycnometer (Duran), reading at 20 °C. The values obtained for the relative density of blackberry juice were J-c1 (0.9808 g/ml), J-c2 (1,0003 g/ml), J-v1 (0.9781 g/ml) and J-v2 (0, 9801 g/ml).

# **Total polyphenolics content**

The total polyphenolics content in blackberry juices was identified by spectrophotometric method with *Folin-Ciocalteu* reagent described by Wolfe et al. (2003). The measurements were performed at the wavelength of 765 nm. The results were expressed as mg of gallic acid equivalent per gram of fresh juice (mg GAE/g fw). The total flavonoids contents were identified with method described by Woisky & Salatino (1998) at the wavelength of 420 nm. The results were expressed as mg of quercetin equivalent per gram of fresh juice (mg QcE/g fw). The total flavonols content was identified with the method described by Yermakov et al. (1987) at the wavelength of 510 nm. The results were expressed as mg of quercetin equivalent per gram of fresh juice (mg QcE/g fw). The total and monomeric anthocyanins

content was determined with the modified pHdifferential method described by Lee et al. (2005). The results were expressed as mg of cyanidin-3 glycoside equivalent per gram of fresh juices (mg CyGE/g fw). The degradation index (DI) was calculated as the ratio between total and monomeric anthocyanins.

## Determination of antioxidant capacity

# DPPH test

The antioxidant capacity of blackberry juices to inhibit 2,2-diphenyl-1-picrylhydrazyl (DPPH<sup>•</sup>) radical was determined by spectrophotometric method Liyana-Pathiranan & Shahidi (2005). The measurements were performed at the wavelength of 515 nm. The IC<sub>50</sub> is defined as the required amount of sample (fresh juice) to reduce 50% of the initial DPPH<sup>•</sup> concentration. The antioxidant capacity of fresh juices to inhibit DPPH<sup>•</sup> is presented as IC<sub>50</sub> values (mg/ml).

# **ABTS test**

Total antioxidant capacity to 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonik-acid) (ABTS<sup>++</sup>) was determined spectrophotometrically by Re et al. (1999). The measurements were performed at the wavelength of 734 nm. The antioxidant ability of fresh juice to inhibit ABTS<sup>++</sup> is presented as  $IC_{so}$  values (mg/ml).

## Neutralization of hydroxyl radical

Total antioxidant capacity to neutralize hydroxyl radicals (•OH) was determined by the modified spectrophotometric method (Gutteridge et al., 1987), described in the literature by Nađpal (2017). The method is based on measuring the degree of degradation of 2-deoxy-D-ribose under the effect of hydroxyl radicals generated in the Fenton reaction:

$$H_2O_2 + Fe_2 + \rightarrow Fe_3^{+} + \cdot OH + \cdot OH$$

The measurements were performed at the wavelength of 532 nm. The results of the neutralization ability of fresh juice to inhibit  $^{\circ}OH$  are presented as IC<sub>50</sub> values (mg/ml).

# Statistical analysis

All experiments were performed in three replicates (n = 3), and the results were expressed as mean  $\pm$  standard deviation (SD). The obtained results were performed using the software programs Excel, Microsoft Office 2010 and IBM SPSS Statistics 20. The significant difference between the arithmetic means (p ≤ 0.05) was calculated using multiple interval test (Duncan's test). Differences between variables were carried out using the analysis of variance (ANOVA) followed by Pearson's significant difference test (p ≤ 0.05).

#### **RESULTS AND DISCUSSION**

#### **Content of polyphenols**

In this research, wild blackberry juice showed a higher content of total polyphenols, total and monomeric anthocyanins, while the contents of total flavonoids and flavonols were higher in the samples of cultivated varieties (Table 1). The highest content of total polyphenols was determined in the sample J-w2 (2.25 mg GAE/g fw), with a statistically significant difference (p < 0.05). The highest contents of total flavonoids (0.45 mg QcE/g fw) and flavonols (0.68 mg QcE/g fw) were determined in the juices of cultivated blackberries from Javorani (J-c1), with a statistically significant difference (p < 0.05).

It should be noted that comparing the results to the contents of polyphenols in the whole fruit (Jazić et al., 2018), analyzed juice samples contained lower concentrations of polyphenolic compounds (30 – 50%), because during traditional extraction procedure most of the polyphenols remain in the pomace (Gawalek et al., 2017; Struck et al., 2016). One of the factors may be that flavonols usually accumulate in the surface tissues of the fruit, and remain in the pomace after juice extraction (Caridi et al., 2007). In comparison with the results shown in the work of Metzner Ungureanu et al. (2020), with blackberry juices from two different areas of Romania, lower values of total polyphenols were obtained. The content of total and monomeric

anthocyanins in the juices of cultivated blackberries was up to 50 % lower, compared to the content in the juice of wild blackberries. Cyanidin-3-glucoside is the major pigment in blackberry juices. Fan-Chiang & Wrolstad (2005) reported a range of cyanidin-3-glucoside content (5.12–8.65 mg/100 g fw) in 50 blackberry samples from diverse varieties, locations, or seasons. The anthocyanin degradation index (DI) is low in the analysed juices, which indicates that the anthocyanins in the juices were well-preserved. The colour of fruit and blackberry juice depends on the present natural pigments, which are mainly anthocyanins (Zia-UI-Haq et al., 2014).

#### Antioxidant activity

Better antioxidant activities against DPPH and ABTS radicals (lower  $IC_{50}$  values) were observed in the juices of wild blackberries with statistically significant difference (p < 0.05). Also, better antioxidant activities to inhibition hydroxyl radicals were found within the juices of wild blackberries, but without statistically significant difference (p > 0.05). The highest antioxidant activity against DPPH and ABTS radicals was present in sample from the locations Verići J-w2 (3.53 ± 0.09 mg/ml) and (0.50±0.02 mg/ml) respectively, against hydroxyl radicals was shown in the sample from Javorani J-w1 (12.91 ± 0.73) (Table 2). The higher activity of the wild blackberry juices was probably influenced by the high content of polyphenols. Similar results for antioxidant activity between samples

Table 1. The total contents of polyphenols, flavonoids, flavonols and total and monomeric anthocyanins of four blackberry juices.

Juice	Polyphenols (mg GAE/g)	Flavonoids (mg QcE/g)	Flavonols (mg QcE/g)	Total anthocyanins (mg CyGE/g)	Monomeric anthocyanins (mg CyGE/g fw)	DI (anthocyanin degradation index)
J-c1	1.98±0.07 <sup>ab</sup>	0.45±0.04ª	0.68±0.06ª	0.70±0.04ª	0.59±0.04ª	1.18±0.02ª
J-c2	1.88±0.14 <sup>b</sup>	0.22±0.01 <sup>b</sup>	0.26±0.02 <sup>b</sup>	0.72±0.04ª	0.60±0.04ª	1.20±0.03ª
J-w1	2.16±0.09 <sup>bc</sup>	0.37±0.03°	0.38±0.01°	0.83±0.04 <sup>b</sup>	0.70±0.06 <sup>b</sup>	1.18±0.04ª
J-w2	2.25±0.10°	0.39±0.02°	0.42±0.01°	1.34±0.06°	1.14±0.06°	1.18±0.01ª

The results were presented as mean  $\pm$  SD standard deviation (n=3).

<sup>ab.c</sup> The letters above each value indicate the significant differences of at least 5% (Duncan's test).

Table 2. Antioxidative characteristics of four blackberry ju	iices.
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		IC <sub>50</sub> (mg/ml)	
	DPPH	ABTS	ОН
J-c1	5.10±0.21ª	0.91±0.02ª	13.16±0.25ª
J-c2	4.34±0.16 <sup>b</sup>	0.80±0.03 <sup>b</sup>	13.53±0.19ª
J-w1	4.07±0.15 <sup>b</sup>	0.61±0.01°	12.91±0.73ª
J-w2	3.53±0.09°	0.50±0.02 <sup>d</sup>	13.19±0.14ª

The results were presented as mean  $\pm$  SD standard deviation SD (n=3).

<sup>a,b,c,d</sup> The letters above each value indicate significant differences of at least 5% (Duncan's test).

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	IC <sub>50</sub> DPPH	IC <sub>50</sub> ABTS	IC <sub>50</sub> <sup>OH</sup>
Total polyphenolics	-0.555	-0.693	-0.306
Flavonoids	0.275	0.012	-0.334
Flavonols	0.600	0.428	-0.259
Total anthocyanins	-0.847	-0.865	-0.040
Monomeric anthocyanins	-0.848	-0.857	-0,014
DI	-0.028	-0.221	-0.492

Table 3. Pearson correlation coefficient (the content of polyphenolic components and the antioxidative activity of four blackberry juices).

Negative values of Pearson coefficient, and lower values  $IC_{50}$  mean higher antioxidative potential. Very good correlation (r < -0.8), good correlation (r < - 0.5), medium correlation (r < - 0.3) and weak correlation (r > - 0.3).

of wild and cultivated blackberry cultivars were obtained in studies by Sariburun et al. (2010) and Basu & Maier (2016), where blackberry fruit extracts were analized against ABTS radicals in four cultivated blackberry cultivars.

In the study by Vinčić (2017), lower potential against hydroxyl radicals in all tested juice samples of blackberry (Čačanska bestrna and Thornfree) was obtained, comparing to the results in Table 2. The differences between the antioxidant activities of blackberry juices can be due to different chemical composition, different varieties of blackberries, genetics, environmental, soil composition, climatic conditions and the intensity of fruit depigmentation (Castillo-Reyes et al., 2015; Lee et al., 2012).

## Correlations

The results showed in Table 3 indicated very good values of coefficient correlation between the content of total and monomeric anthocyanins, and DPPH and ABTS radicals. The good correlation coefficient was found between the content of total polyphenols and the antioxidant activity against DPPH and ABTS radicals.

Also, Milenković-Anđelković et al. (2015) and Basu & Maier (2016) found high correlation values between polyphenolic components and antioxidant activities in blackberry fruits. The correlations between total polyphenol content and inhibition of hydroxyl radicals showed low values weak correlation (r > -0.3).

# CONCLUSIONS

Based on the obtained results, it can be concluded that wild blackberry samples had a higher content of total polyphenols, total anthocyanins and monomeric anthocyanins compared to cultivated varieties. The cultivated varieties of blackberries had a higher content of total flavonoids and flavonols than the samples of juices of wild blackberries. Very good positive correlation was found between the contents of total and monomeric anthocyanins and antioxidant activities against DPPH and ABTS radicals. All tested juices showed significant contents of polyphenolic components, good antioxidant potential.

# **Conflict of interests**

The authors declare no conflict of interests

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# Sadržaj polifenola i antioksidativna aktivnost sokova divljih i kultiviranih sorti kupina (*Rubus Fruticosus L*.)

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Ključne reči: kultivisana kupina, divlja kupina, sok, sadržaj polifenola, antioksidativna aktivnost.

Plodovi kupine (Rubus fruticosus L.) su vrijednovani kao izvrstan izvor polifenolnih jedinjenia i antioksidativne aktivnosti. U ovoj studiji su ispitani sokovi dobijeni od dvije divlje i dvije kultivisane sorte kupina, kao potencijalni izvor polifenolnih jedinjenja i antioksidativne aktivnosti. Nakon izdvajanja sokova, kod svih uzoraka je određena gustina pomoću piknometra, a rezultati su prikazani kao mg/g svježe mase soka. Spektrofotometrijskom metodom je određen sadržaj polifenolnih jedinjenja (ukupni polifenoli, ukupni flavonoidi, ukupni flavonoli, ukupni i monomerni antocijani), kao i antioksidatvna aktivnost pomoću (DPPH, ABTS testova i sposobnosti neutralizacije OH radikala). Uzorci u radu su označeni kao J-c1 (kultivisana sorta sa područja Javorani), J-c2 (kultivisana sorta sa područja Verići), Jw1 (divlja sorta sa područja Javorani) i J-w2 (divlja sorta sa područja Verići). U radu je utvrđeno da uzorci soka divljih sorti kupine imaju veći sadržaj ukupnih polifenola (2,16 - 2,25 mg GAE/g s.m.), ukupnih antocijana (0,83 - 1,34 mg CyGE/g s.m.) i monomernih antocijana (0,70 - 1,14 mg CyGE/g s.m.), sa statistički značajnom razlikom, dok je kod uzorka soka kultivisane sorte kupine J-c1 utvrđen veći sadržaj flavonoida (0,45 mg QcE/g s.m.) i flavonola (0,68 mg QcE/g s.m.). Na osnovu dobijenih vrijednosti IC<sub>50</sub>, najsnažniju antioksidativnu aktivnost prema DPPH radikalima (IC<sub>50</sub>=3,53) i ABTS radikalima (IC $_{50}$  = 0,50) pokazao je uzorak J-w2, sa statistički značajnom razlikom (p < 0,05). Najsnažniju antioksidativnu aktivnost prema OH radikalima imao je uzorak J-w1 (IC<sub>50</sub> = 12,91), bez statistički značajne razlike (p  $\ge$  0,05). Svi ispitivani uzorci soka kupina su pokazali visoke koncentracije ukupnih polifenola, uključujući flavonoide, kao i sadržaj antocijana. Kod sokova divljih sorti kupina je utvrđena veća antioksidativna aktivnost u odnosu na sorte kultivisanih kupina. Kod svih ispitivanih uzoraka soka utvrđene su značajne korelacije između sadržaja ukupnih polifenola, ukupnih i monomernih antocijana i sposobnosti inhibicije DPPH i ABTS radikala.