

INFLUENCE OF THE GENOTYPE ON THE POLYPHENOLIC COMPOSITION AND ANTIOXIDANT ACTIVITY OF SOME BERRY FRUITS

Ana SELAMOVSKA ¹, Elizabeta MISKOSKA-MILEVSKA ², Milena TASESKA-
GJORGJIJEVSKI ^{*1}, Igor ILJOVSKI ²

¹Institute of Agriculture, “Ss. Cyril and Methodius” University, Skopje, R. North Macedonia,

²Faculty of Agricultural Sciences and Food – Skopje, “Ss. Cyril and Methodius” University,
Skopje, R. North Macedonia

Selamovska A., E. Miskoska-Milevska, M. Taseska-Gjorgjijevski, I. Iljovski (2024).
*Influence of the genotype on the polyphenolic composition and antioxidant activity of
some berry fruits*- Genetika, Vol 56, No.1, 143-155.

The objective of this study was to analyze the content of vitamin C, total phenols, total anthocyanins, flavan-3-ols and antioxidant activity in fruits of five berry fruit species: blackcurrant variety “Rosenthal”, white mulberry local ecotype, black chokeberry variety “Viking”, blackberry variety “Thornfree” and pomegranate autochthonous variety “Karamustafa”. Determination of vitamin C was performed by classical analytical method. Total phenols, total anthocyanins, flavan-3-ols and antioxidant activity were analysed by spectrophotometric methods. The obtained results from the analysis for berry fruits showed high antioxidant activity, over 70% and high content of polyphenols. According to the genotype, black chokeberry had the highest content of total phenols (23.9 mg g⁻¹ FW (fresh weight), anthocyanins (6.1 mg g⁻¹ FW) and flavan-3-ols (2.8 mg g⁻¹ FW). Blackcurrant had highest content of vitamin C (216.0 mg 100 g⁻¹ FW) and antioxidant activity (86.2% inhibition).

A positive correlation was found among all the studied fruit species. A strong positive correlation of vitamin C with antioxidant activity was determined, as well as between total phenols and total anthocyanins, i.e. flavan-3-ols. A weak negative correlation was only observed for catechin in relation to vitamin C, inhibitory antioxidant activity and vitamin C antioxidant activity.

Keywords: genotype, berry fruits, vitamin C, polyphenols, antioxidant activity

INTRODUCTION

Fruits are a rich source of antioxidants that have a preventive, curative and therapeutic effect on human health, destroying free radicals that cause cancer and degenerative diseases. The

Corresponding author: Milena Taseska-Gjorgjijevski, Institute of Agriculture, “Ss. Cyril and Methodius” University, Skopje, R. North Macedonia, e-mail: milenataseska2005@yahoo.com; tel: +389 75 462 403, ORCID: 0000-0003-0853-2442, A.Selamovska ORCID: 0000-0002-0981-9288, E. Miskoska-Milevska ORCID: 0000-0001-7368-6355, I. Iljovsk ORCID: 0009-0009-4787-4219

content of antioxidants depends on the genotype, environmental conditions, cultivation type, fruit storage method and processing (BASSI *et al.*, 2017). In order to meet the future needs of the population for food raw materials, food production must significantly increase (SIMIĆ *et al.*, 2023).

Vitamins are necessary for maintaining human life and health, ensuring growth and development of the body (NOŽINIĆ *et al.*, 2022; PETROVIĆ *et al.*, 2021; 2022; STEVANOVIĆ *et al.*, 2023). Vitamin C is one of the strongest antioxidants. It is mostly found in rose hips and kiwi fruits, hawthorn, blackcurrant, pomegranate, blueberry and wild strawberry and citrus fruits (RAHMAN *et al.*, 2008; AKIMOV *et al.*, 2020).

Polyphenols are very important compounds that have antioxidant, anti-inflammatory, anticancer effects, antimutagenic, antiallergenic, antimicrobial action, they provide protection against infections, reduce the risk of chronic diseases, cardiovascular and neurodegenerative diseases (VAUZOUR *et al.*, 2010). More than 40 phenolic compounds have been identified. The aronia berries are known as one of the richest natural sources of polyphenols such as flavanols, anthocyanins (300-2000 mg 100 g⁻¹ in fruit) and hidroxycinnamic acid (KULLING and RAWEL, 2008; ČUJIĆ *et al.*, 2013).

Flavonoids are a class of plant secondary metabolites, polyphenolic antioxidants, which belong to the group of soluble coloured pigments. They are classified into 12 subclasses in terms of chemical structure. They are most common in fruits (especially berry fruits) and grapes.

Anthocyanins are a group of over 500 different compounds. The main source of anthocyanins are fruits. In nature, cyanidin is a reddish-purple pigment and the major pigment in berry fruits species, little pelargonidin and peonidin. Petunidin has been detected in blackcurrant (SLIMESTAD and SOLHEIM, 2002). Anthocyanins are nutritious bioactive components, they protect the plant from UV radiation, help in their pollination, and on the other hand, they have a potential application in the prevention of the human body from cardiovascular diseases and cancer (DE PASCUAL-TERESA and SANCHEZ, 2008; RAUF *et al.*, 2019). Flavanols or catechins (flavan-3-ols) are subgroup of flavonoids that exist in a variety of chemical forms and derivatives. Flavan-3-ols, which are a large family of phenolic compounds, mainly responsible for the astringency, bitterness, and nutrient structure. They have antioxidant, antitumor, antibacterial, anti-inflammatory, antiallergic and vasodilatory cardioprotective, neuroprotective, antidiabetic actions. They are found in many fruits (RAUF *et al.*, 2019; DE PASCUAL-TERESA *et al.*, 2010; MURKOVIC, 2016).

The wealth of biologically active substances, the high content of phenolic components and the high antioxidant activity (PAZ and FREDES, 2015), put berry fruits among the top foods with a wide range of active substances useful for human health. The aim of this study was survey of phenolic status and antioxidant activity in five different berry fruit species and their comparison.

MATERIALS AND METHODS

Five berry fruit species: *Ribes nigrum* L. (blackcurrant variety "Rosenthal"), *Morus alba* L. (white mulberry local ecotype), *Aronia melanocarpa* (Michx) Elliot (black chokeberry variety "Viking"), *Rubus fruticosus* L. (blackberry variety "Thornfree") and *Punica granatum* L. (pomegranate autochthonous variety "Karamustafa"), were used as research material in this study.

In the conditions of the Skopje valley (41°58'12" N and 21°28'59" E, at 234 meters altitudes), the varieties ripened in the following order: blackcurrant (third decade of June), white mulberry (beginning of July), aronia (third decade of July), blackberry (second decade of August) and pomegranate (third decade of September).

Method for determination of vitamin C

The laboratory tests were performed in the oenological laboratory at the Institute of Agriculture in Skopje. The content of vitamin C (mg 100 g⁻¹ FW) was examined by volumetric method (SADASIVAM and BALASUBRAMANIAN, 1987)

Methods for determination of total phenols, anthocyanins and flavan-3-ols (catechins)

Sample preparation: Take approximately 5 g mass of the sample (the fruit) and let it macerate in a closed container with a solution of ethanol, water and hydrochloric acid in the ratio 70:30:0.1 for 24 h.

Determination of total phenols

Total phenols in the samples are determined according to the Folin-Ciocalteu method. 1 mL of the prepared sample is placed in a 10 mL flask. 5 mL of distilled water and 0.5 mL of Folin- Ciocalteu reagent are added (SLINKARD and SINGLETON, 1977). After 3 minutes, 1.5 mL of 20% solution of Na₂CO₃ is added and the flask is fill up to the mark with distilled water. Then the flask are placed in a water bath heated to 50 °C for 16 minutes. Then they are cooled and total phenols are measured on a spectrophotometer at 765 nm wavelength. In parallel with the samples, a blank sample is prepared, where distilled water is used instead of the tested sample and the rest of the reagents remain the same.

Determination of total anthocyanins

The content of total anthocyanins was performed according to the acid - ethanol method (SOMERS and EVANS, 1977) In a 10 mL flask, add 0.1 mL of the macerated sample and fill up to the mark with the solution of ethanol, water and hydrochloric acid. A solution of ethyl chloride is used as a blank sample. Determination of anthocyanins was carried out by spectrophotometer, at a wavelength of 550 nm.

Determination of flavan-3-ols

p-dimethylaminocinnamaldehyde (p-DMACA) is used for the quantification of flavan-3-ols in the samples (DI STEFANO *et al.*, 1989). In 10 mL flask, put 0.1 mL of sample, three drops of glycerol, 5 mL of p-DMACA and fill up to the mark with methanol. After 7 min, the absorbance at a wavelength of 640 nm was measured. Methanol is used as a control sample.

The content of total phenols, anthocyanins and flavan-3-ols was performed by Agilent 8453 UV-VIS spectrophotometer. These methods are significantly suitable for routine analyses. They are rapid and can be applied to monitor changes in phenols composition during fruit ripening.

Method for determination of antioxidant activity

Determining of the antioxidant activity (ORAC-Oxygen Radical Absorbance Capacity) was performed as an anti-radical activity against the stable product DPPH (2,2-diphenyl-1-picrylhydrazil). The determination was performed spectrophotometrically, at a wavelength of 517 nm. The antioxidant activity, especially for vitamin C, as well as the inhibitory antioxidant activity of all concentrations are expressed in percent (%).

Method for statistical analysis

To determine a significant difference from the mean values of the four concentrations, ANOVA and the Least Significant Difference (LSD) test at the $p < 0.05$ level were used for all fruit species samples. From the total concentration of each fruit species, the percentage of concentrations was determined and presented together with the percentage antioxidant activity of vitamin C and the inhibitory antioxidant activity of all concentrations. Also, the correlation between the examined fruit species, as well as for all examined concentrations, was determined through the Pearson correlation test and the scale for the height of the correlation coefficient (r) ($-1 = 1$ 0.85 - 1 for a strong correlation, 0.5 - 0.85 for moderate and 0.1 - 0.5 for weak correlation).

RESULTS AND DISCUSSION

The results of the chemical analysis of the fruits of some berries species (blackcurrant, white mulberry, aronia, blackberry, pomegranate) are presented in Table 1.

Vitamin C: The content of vitamin C showed statistically significance at a level ($p < 0.05$) in blackcurrant of 216.0 mg 100 g⁻¹ FW, which is also confirmed in other studies determined with production obtained in similar climatic conditions (228.0 mg 100 g⁻¹ FW) (PAUNOVIĆ *et al.*, 2017) Among the rest of the examined fruit species, the vitamin C content ranges from 19.2 mg 100 g⁻¹ FW in blackberry to 29.9 mg 100 g⁻¹ FW in white mulberry, which is a higher concentration than some available literature data (25.20 mg 100 g⁻¹ FW) (IQBAL *et al.*, 2010). According to the results, most of the antioxidant activity in blackcurrant and white mulberry is due to the vitamin C.

The content of vitamin C in fruits depends on several factors: genotype, fruit type, variety, climatic factors, way of keeping the fruits, etc. Autochthonous pomegranate varieties contain 23.67 mg 100 g⁻¹ FW vitamin C (SELAMOVSKA *et al.*, 2022a) Content of vitamin C in fresh fruit of blackcurrant is 137 mg 100 g⁻¹ FW while in strawberry is 60 mg 100 g⁻¹ FW. Frozen storage destroy about 28% of the vitamin C contents in black currant and 34% in strawberries (HÄGG *et al.*, 1995). Blackcurrant fruits grown in the south have higher contents of most ascorbic acid and soluble solids from those grown in Northern Sweden (VAGIRI, 2014). Purple mulberry have a lower content of ascorbic acid than some berry fruits (blackberry, raspberry, blueberry, strawberry). Fruits of purple mulberry (*Morus rubra* L.) have an average of 28.42 mg kg⁻¹ FW ascorbic acid (KOCA *et al.*, 2008). SELAMOVSKA *et al.* (2022b), found very high and statistically significant correlation between vitamin C and the antioxidant activity in some autochthonous cherry varieties. Authors found a moderate positive correlation between the content of anthocyanins and vitamin C in some fruit species and moderate negative correlation was found between content of vitamin C and flavan-3ols in some autochthonous apple varieties

(SELAMOVSKA *et al.*, 2022c). Vitamin C content varied between 115.85 mg 100g⁻¹ FW and 43.77 mg 100 g⁻¹ FW (OZRENK *et al.*, 2023).

Table 1. Results obtained by chemical analysis of fruits of five berry fruit species

	<i>Ribes nigrum</i>		<i>Morus Alba</i>		<i>Aronia melanocarpa</i>		<i>Rubus fruticosus</i>		<i>Punica granatum</i>	
	Black currant	%	White mulberry	%	Black chokeberry	%	Blackberry	%	Pomegranate	%
Vitamin C (mg 100g ⁻¹ FW)	216.0a	89.5	29.9b	99.5	24.2b	42.5	19.2b	65.3	25.0b	79.4
Total phenols (mg g ⁻¹ FW)	18.6ab	7.7	0.1d	0.3	23.9a	41.9	7.6c	26.0	5.9c	18.6
Total anthocyanins (mg g ⁻¹ FW)	5.8a	2.4	0.1c	0.2	6.1a	10.7	1.7b	5.7	0.5bc	1.6
Catechin (flavan-3-ols) (mg g ⁻¹ FW)	0.9b	0.4	0.01c	0.0	2.8a	4.9	0.9b	3.1	0.1c	0.4
Antioxidant activity Vitamin C (%)		250.6		41.7		31.7		24.4		28.9
Antioxidant activity inhibition (%)		86.2		71.6		76.4		78.6		86.3
Mean	60.3*		7.5		14.2		7.4		7.9	
Sum	241.3		30.1		57.0		29.4		31.5	

* Symbol indicates significant differences in mg/g measured data between berry fruits group $p < 0.05$, letters indicate significant differences in the between group ($p < 0.05$).

Phenols: The analysis of the total phenols showed that the highest content was obtained in *Aronia melanocarpa* (23.9 mg g⁻¹ FW) as well as in *Ribes nigrum* (blackcurrant) (18.6 mg g⁻¹ FW), in contrast to the other species where the phenols ranged in a concentration of 0.1 mg g⁻¹ FW in *Morus alba* (white mulberry), 5.9 mg g⁻¹ FW in *Punica granatum* (pomegranate) and 7.6 mg g⁻¹ FW in *Rubus fruticosus* (blackberry). The participation of total phenols in the antioxidant activity was from 0.14% in white mulberry to 31.26% in aronia. The fruits of *Aronia melanocarpa* are a rich source of phenols. Also, in other researches, the content of total phenols showed approximate results as the highest results obtained in our research, 21.9 mg g⁻¹ based on fresh weight (KALOUDI *et al.*, 2022).

According to VAGIRI (2014), the high content of phenolic components is influenced by several factors: genotype, ontogenetic development (dormant buds have the highest content of total phenols), position of the leaves (basal leaves have the highest content), temperature, cultivation conditions (location, technique of cultivation), time of harvest (fruits harvested later have more juice), way of keeping the fruits, etc. Autochthonous pomegranate varieties contain 5359.43 mg kg⁻¹ FW total phenols (SELAMOVSKA *et al.*, 2022a). Total phenolic contents of blackberries ranged from 2.61-10.56 mg g⁻¹ reported by several researches (MOYER *et al.*, 2002; SIRIWOHARN *et al.*, 2004). Wild blackberries fruits have higher phenolic content than cultivated

(KOCA and KARADENIZ, 2009). Blackcurrant have higher phenolic content, specially anthocyanins, phenols, favonols and proanthocyanidins compare to raspberry and strawberry (KARJALAINEN *et al.*, 2009). Purple mulberry have lower content of total phenolic than some small-berry fruits including raspberry, strawberry and blackcurrant. Fruits of purple mulberry content 1308 mg kg⁻¹ total phenols (KOCA and KARADENIZ, 2009) and 30.4-44.7 mg g⁻¹ DW (dry weight) in leaves of mulberry varieties (ZOU *et al.*, 2012; POLUMACKANYEZ *et al.*, 2021). Chokeberry have higher content of total phenols compare to blueberry, red currant, red raspberry, strawberry and blackberry (JURIKOVA *et al.*, 2017). The chokeberry leaves contained a large proportion of the total phenolic and flavonoid contents of the chokeberry fruits. The young leaves contained more phenols and flavonoids than the old leaves (DO THI and HWANG, 2014). Chokeberry fruits content 24.87 mg g⁻¹ total phenols (CIOCOIU *et al.*, 2013). KAUR and KAPOOR, 2005, found a positive correlation between the content of phenols and anthocyanins in some fruit species, while MURILLO *et al.*, 2012, found a positive correlation between polyphenol content and the antioxidant activity.

Anthocyanins: Due to the positive effect on human health, the interest in researching anthocyanins has been present for a long time. Aronia fruits show positive effects in people exposed to high radiation, as well as in people with cardiovascular diseases. In the research, anthocyanins were most abundant in chokeberry 6.1 mg 100 g⁻¹ FW and 5.8 mg 100 g⁻¹ FW in blackcurrant and/or deviation/variation by more than 10 times in relation to literature data. According to literature data, the total amount of anthocyanins in the test samples varies by more than 2.4 or 284-686 mg 100 g⁻¹ FW (DENEV *et al.*, 2018). The lowest content of anthocyanins was in white mulberry (0.05 mg g⁻¹ FW). The participation of total anthocyanins in the antioxidant activity was from 0.07% in white mulberry to 7.96% in chokeberry.

The accumulation of anthocyanins is primarily influenced by genotype, fruit species and varieties, light, temperature, cultivated way, degree of heat treatment, way of keeping the fruits etc. Chokeberry (300-2000 mg 100 g⁻¹ FW), blueberry (300-698 mg 100 g⁻¹ FW), blackcurrant (130-476 mg 100 g⁻¹ FW), blackberry (82.5-325.9 mg 100 g⁻¹ FW), pomegranate (15-252 mg 100 g⁻¹ FW) were rich in anthocyanins (ĆUJIĆ *et al.*, 2013; DENEV *et al.*, 2018; CEVALLOS-CASALS *et al.*, 2022). Chokeberry (*Aronia melanocarpa*) have higher content of flavonoids including total anthocyanins and proanthocyanidins, and higher antioxidant activity compared to compared to blackcurrant (*Ribes nigrum*), red currant (*Ribes rubrum*), gooseberry (*Ribes grossularia*), strawberry (*Fragaria anannassa*), blackberry (*Rubus fruticosus*), blueberry (*Vaccinium* sp.) and red raspberry (*Rubus idaeus*) (JURIKOVA *et al.*, 2017; WU *et al.*, 2004). Autochthonous pomegranate varieties content average 323.78 mg kg⁻¹ FW anthocyanins (IQBAL *et al.*, 2010). The average content of anthocyanins in 20 varieties of pomegranate according to DUMLU and GÜRKAN, 2007 is 2,100-4,400 mg L⁻¹. Total content of anthocyanins in blackberries ranges from 0.12-3.26 mg g⁻¹ FW. Wild blackberries have higher anthocyanins content compared with cultivated blackberry varieties (MOYER *et al.*, 2002; SAPERS *et al.*, 1986) Purple mulberry have lower total anthocyanins than other berry fruits (blackberry, raspberry, blueberry and strawberry) (KOCA *et al.*, 2008).

RUGINA *et al.* (2012) found a positive correlation between total proanthocyanidin and anthocyanin with antioxidant activity in fruits of *Aronia melanocarpa*.

Flavan-3-ols: Catechin (flavan-3-ols) showed significant results in *Aronia melanocarpa* (2.8 mg g⁻¹ FW), in relation to other fruit species where the results ranged below 1 mg g⁻¹ FW. This is understandable due to the more bitter taste of the fruits compared to the other analyzed fruit species. The presence of flavan-3-ols was not observed in white mulberry. The participation of flavan-3-ols in the antioxidant activity was from 0% in white mulberry to 2.85% in aronia. Literature data shows catechin present from 0.47 to 0.86 mg g⁻¹ FW (ROP *et al.*, 2012) and content of epicatechin (flavan-3-ol) to the most significant level (124 mg 100 g⁻¹ FW), that is significantly higher than the available literature data (DENEV *et al.*, 2018). The content of catechins (flavan-3-ols) depends on several factors: genotype (species, variety), locality, temperature, etc. Among the berry fruits, raspberry (2-48 mg 100 g⁻¹ FW), strawberry (10-29 mg 100 g⁻¹ FW) and blackberry (3.3-23.8 mg 100 g⁻¹ FW) contain the most catechins (DE PASCUAL-TERESA and SANCHEZ, 2008). Autochthonous pomegranate varieties, content average 122.51 mg kg⁻¹ FW flavan-3-ols, more than autochthonous cherry varieties (69.15 mg kg⁻¹ FW), but less than autochthonous apple varieties (517.98 mg kg⁻¹ FW) (SELAMOVSKA *et al.*, 2022c). Blackcurrant cultivars grown in more northern and cooler regions have higher content of flavan-3-ols, phenols and flavonols compared to cultivars grown in southern and warmer regions (VAGIRI, 2014).

SELAMOVSKA *et al.* (2022a), found a moderate negative correlation between the flavan-3-ols and total phenols and a moderate positive, insignificant correlation between the content of flavan-3-ols and anthocyanins in some autochthonous pomegranate varieties.

Antioxidant activity: All investigated species showed high antioxidative activity, over 70%. Blackcurrant and pomegranate had the largest value, over 86%. According to ZHENG and WANG (2003), the chokeberry had significantly higher antioxidant activity related to the higher content of anthocyanins and phenolics than the other three berries. According to GAZDIK *et al.* (2008) the antioxidant activity of fruits of *A. melanocarpa* is mostly due to by the polyphenolic compounds. Antioxidant activity as a limitation or inhibition of nutrient oxidation by restraining oxidative chain reactions (GUCLU *et al.*, 2021) shows significant differences between *Ribes nigrum* (blackcurrant) and other examined fruit species.

The very high antioxidant activity of blackcurrants of 250.6%, in contrast to other examined fruit species (24.4-41.7%), is primarily due to the high content of vitamin C, which is also confirmed by literature data (MOYER *et al.*, 2002). In blackcurrant and white mulberry, the antioxidant activity is due to the high content of vitamin C, and a smaller part of the content of the polyphenolic composition (29.30% and 0.21%). In other berry species (blackberry and pomegranate) the antioxidant activity depends almost equally on the content of vitamin C (average 13.35%) and polyphenolic composition (average 10.25%). The antioxidant activity of the fruits depended on the genotype, type and age of the plant material. Autochthonous pomegranate varieties have high antioxidant activity (81.58%) (SELAMOVSKA *et al.*, 2022a). Chokeberry have higher antioxidant activity compare to blueberry (*Vaccinium corymbosum*), cranberry (*Vaccinium macrocarpon*) and lingonberry (*Vaccinium vitis-idaea*) (ZHENG and WANG, 2003). Fruits and leaves of fruit species had great antioxidant activity. Blackberries and strawberries had the highest ORAC values during the green stages, whereas red raspberries had the highest ORAC activity at the ripe stage. Also, compared with fruits, leaves were found to have higher ORAC values. As the leaves become older, the ORAC values and total phenolic

contents decreased (WANG and LIN, 2000). According to KOCA *et al.* (2008) purple mulberry has lower antioxidant activity than other berry fruits (*Rubus*, *Vaccinium* and *Ribes* species) (TOSUN and ARTIK, 1998; KAHKONEN, *et al.*, 2001)

Some authors found positive correlation between the content of total phenols and the oxidant activity in fruits of some autochthonous pomegranate varieties (SELAMOVSKA *et al.*, 2022a), purple mulberry (KOCA *et al.*, 2008) and other berry fruits (KAHKONEN, *et al.*, 2001; WANG and LIN, 2000). Several studies (ČUJIĆ *et al.*, 2013; OVANDO *et al.*, 2009) indicate a positive correlation between the content of anthocyanins in fruits and high antioxidant activity.

Table 2. Relation between the examined species as determined by all the metrics of research

Blackcurrant	white mulberry	0.484098
	pomegranate	0.342295
White mulberry	pomegranate	0.938556
	blackberry	0.886053
Black chokeberry	blackberry	0.912287
	pomegranate	0.860983
Blackberry	pomegranate	0.991162
	aronia	0.912287
	white mulberry	0.886053
Pomegranate	blackberry	0.991162
	white mulberry	0.938556
	aronia	0.860983

$r = 0.85 - 1$ strong, $0.5 - 0.85$ moderate, $0.1 - 0.5$ weak correlation

A positive correlation was found among all the studied fruit species. The blackcurrant showed a weak correlation with the other fruit species: the highest coefficient was shown in relation to the white mulberry ($r=0.484098$). The white mulberry showed a strong correlation with respect to the pomegranate ($r=0.938556$). Aronia showed a strong correlation to blackberry ($r=0.912287$), while blackberry to pomegranate ($r=0.991162$) and black chokeberry ($r=0.912287$). Pomegranate showed the strongest correlation with blackberry ($r=0.991162$), white mulberry ($r=0.938556$), but also with chokeberry ($r=0.860983$). (Table 2). Vitamin C naturally showed a strong correlation ($r=0.99964$) with the antioxidant reaction, and a moderate one with total anthocyanins and IAA. A strong correlation was found between total phenolics and total anthocyanins and flavanols. The relationship between the antioxidant activity of vitamin C and antioxidant activity inhibitory (IAA) showed a moderate correlation. A weak negative correlation was only observed for catechin in relation to vitamin C, inhibitory antioxidant activity and vitamin C antioxidant activity (Table 3).

Table 3. Relation between the obtained values from the analysis of the five fruit species

	Vitamin C (mg 100g ⁻¹ FW)	Total phenols (mg g ⁻¹ FW)	Total anthocyanins (mg g ⁻¹ FW)	Catechin (flavan-3- ols (mg g ⁻¹ FW)	Antioxidant activity Vitamin C (%)	Antioxidant activity inhibition (%)
Vitamin C (mg 100g ⁻¹ FW)	1	0.40943	0.55994	-0.05501	0.99964	0.54225
Total phenols (mg g ⁻¹ FW)	0.40943	1	0.97499	0.86735	0.40466	0.26035
Total anthocyanins (mg g ⁻¹ FW)	0.55994	0.97499	1	0.79495	0.55886	0.22677
Catechin (flavan-3-ols (mg g ⁻¹ FW)	-0.05501	0.86735	0.79495	1	-0.05527	-0.15494
Antioxidant activity Vitamin C (%)	0.99964	0.40466	0.55886	-0.05527	1	0.52026
Antioxidant activity inhibition (%)	0.54225	0.26035	0.22677	-0.15494	0.52026	1

r = 0.85 - 1 strong, 0.5 - 0.85 moderate, 0.1 - 0.5 weak correlation

CONCLUSION

Fruits are a rich source of antioxidants that improve human health. The content of antioxidants in this study depends on the species and the genotype.

According to the genotype, black chokeberry had the highest content of total phenols, anthocyanins and flavan-3-ols. Blackcurrant had highest content of vitamin C and antioxidant activity. Berry fruits showed high antioxidant activity, over 70% and high content of polyphenols. White mulberry showed the lowest content of total phenols, anthocyanins, catechins (flavan-3-ols) and antioxidant activity.

A positive correlation was found among all the studied fruit species. A strong positive correlation of vitamin C with antioxidant activity was determined, as well as between total phenols and total anthocyanins, i.e. flavan-3-ols. A weak negative correlation was only observed for catechin in relation to vitamin C, inhibitory antioxidant activity and vitamin C antioxidant activity. Correlation values confirmed that phenolic compounds are the main micro constituents that contribute to the antioxidant activity of the fruits of these fruit species.

Received July 30, 2023

Accepted December 18, 2023

REFERENCES

- AKIMOV, M.Y., V.V., BESSONOV, V.M., KODENTSOVA, K.I., ELLER, O.A., VRZHESINSKAYA, N.A., BEKETOVA, O.V., KOSHELEVA, M.N., BOGACHUK, A.D., MALINKIN, M.A., MAKARENKO, L.V., SHEVYAKOVA, I.B., PEROVA, E.V., RYLINA, V.N., MAKAROV, T.V., ZHIDEHINA, V.A., KOLTSOV, A.N., YUSHKOV, A.A., NOVOTORTSEV, D.M., BRIKSIN, N.V., KHROMOV (2020): Biological value of fruits and berries of Russian production. *Vopr. Pitan.*, 89(4): 220-232.
- BASSI, M., G., LUBES, F., BIANCHI, S., AGNOLET, F., CIESA, K., BRUNNER, W., GUERRA, P., ROBATSCHER, M., OBERHUBER (2017): Ascorbic acid content in apple pulp, peel and monovarietal cloudy juices of 64 different cultivars. *Intern. Journal of Food properties*, 20 (3): 2626-2634.

- CEVALLOS-CASALS, B.A., D.H., BYRNE, L., CISNEROS-ZEVALLOS, W.R., OKIE (2002): Total phenolic and anthocyanin content in red-fleshed peaches and plums. *Acta Horticulturae*, 592: 589-592.
- CIOCIU, M., L., BADESCU, A., MIRON, M., BADESCU (2013): The involvement of a polyphenol-rich extract of black chokeberry in oxidative stress on experimental arterial hypertension. Hindawi Publishing Corporation. ID 912769.
- ĆUJIĆ, N., T., KUNDAKOVIĆ, K., ŠAVIKIN (2013): Anthocyanins-Chemistry and Biological activity. *Lek. Sirov.*, 33: 19-37.
- DENEV, P., M., KRATCHANOVA, I., PETROVA, D., KLISUROVA, Y., GEORGIEV, M., OGNYANOV, I., YANAKIEVA (2018): Black Chokeberry (*Aronia melanocarpa* (Michx Eliot) Fruits and Functional Drinks Differ Significantly in Their Chemical Composition and Antioxidant Activity. *Journal of Chemistry*, ID 9574587.
- DE PASCUAL-TERESA, S., B.S., SANCHEZ (2008): Anthocyanins, from plant to health. *Phytochem. Rev.*, 7: 281-299.
- DE PASCUAL-TERESA, S., D.A., MORENO, C., GARCIA-VIGUERA (2010): Flavanols and anthocyanins in cardiovascular health: A Review of current evidence. *Intern. Journal of Molecular Sciences*, 11: 1679-1703.
- DI STEFANO, R., M.C., CRAVERO, N., GENTILINI (1989): Methods for the study of wine polyphenols. *The winemaker*, 5: 83-90.
- DO THI, N., E.S., HWANG (2014): Bioactive compound contents and antioxidant activity in aronia (*Aronia melanocarpa*) leaves collected at different growth stages. *Prev. Nutr. Food Sci.*, 19(3): 204-212.
- DUMLU, M.U., E., GÜRKAN (2007): Elemental and nutritional analysis of *Punica granatum* from Turkey. *J. Med.Food.*, 10 (2): 392-395.
- GAZDIK, Z., V., REZNICEK, V., ADAM, O., ZITKA, T., JURIKOVA, B., KRSKA, J., MATUSKOVIC, J., PLSEKL, J., SALOUN, A., HORNA (2008): Use of liquid chromatography with electrochemical detection for the determination of antioxidants in less common fruits. *Molecules*, 13: 2823-2836.
- GUCLU, G., H., KELEBEK, S., SELLI (2021): Antioxidant activity in olive oils. *Olives and Olive Oil in Health and Disease Prevention (Second Edition)*; Academic Press: 313-325.
- HÄGG, M., S., YLIKOSKI, J., KUMPULAINEN (1995): Vitamin C content in fruits and berries consumed in Finland. *Journal of Food Composition and Analysis*, 8 (1): 12-20.
- IQBAL, M., K., MIR KHAN, M.S., JILANI, M., MUNIR KHAN (2010): Physico-Chemical Characteristics of Different Mulberry Cultivars Grown Under Agro-Climatic Conditions of Miran Shah, North Waziristan (Khyber Pakhtunkhwa), Pakistan. *J. Agric.Res.*, 48(2).
- JURIKOVA, T., J., MLCEK, S., SKROVANKOVA, D., SUMCZYNSKI, J., SOCHOR, I., HLAVACOVA, L., SNOPEK, J., ORSAVOVA (2017): Fruits of Black Chokeberry *Aronia melanocarpa* in the Prevention of Chronic Diseases. *Molecules*, 22: 944.
- KAHKONEN, M.P., A.I., HOPIA, M., HEINONEN (2001): Berry phenolics and their antioxidant activity. *J. Agric. Food Chem.*, 49: 4076-4082.
- KALOUDI, T., D., TSIMOGIANNIS, B., OREOPOULOU (2022): *Aronia Melanocarpa*: Identification and Exploitation of Its Phenolic Components. *Molecules*, 27(14): 4375.
- KARJALAINEN, K., M., ANTTONEN, N., SAVIRANTA, H., HILZ, D., STEWART, G.J., MCDUGALL, P., MATTILA, R., TORRONEN (2009): A review on bioactive compounds in blackcurrant (*Ribes nigrum* L.) and their potential health-promoting properties. *Proceedings of the first International Symposium on Biotechnology of fruit species*, Dresden, Germany. *ISHS Acta Hort.*, 839: 301-307.
- KAUR, C., H.C., KAPOOR (2005): Antioxidant activity of some fruits in Indian diet. *ISHS Acta Horticulturae*, 696: 99.
- KOCA, I., N.S., USTUN, A.F., KOCA, B., KARADENIZ (2008): Chemical composition, antioxidant activity and anthocyanin profiles of purple mulberry (*Morus rubra*) fruits. *Journal of Food, Agriculture & Environment*, 6 (2): 39-42.

- KOCA, I., B., KARADENIZ (2009): Antioxidant properties of blackberry and blueberry fruits grown in the Black Sea region of Turkey. *Scientia Horticulturae*, 121: 447-450.
- KULLING, S.E., H., RAWEL (2008): Chokeberry (*Aronia melanocarpa*). A review on the characteristic components and potential health effects. *Planta Med.*, 74: 1625-1634.
- MOYER, R.A., K.E., HUMMER, C.E., FINN, B., FREI, R.E., WROLSTAD (2002): Anthocyanins, phenolics and antioxidant capacity in diverse small fruits: *Vaccinium*, *Rubus* and *Ribes*. *J. Agric. Food Chem.*, 50: 519-525.
- MURILLO, E., G., BRITTON, A., DURANT (2012): Antioxidant activity and polyphenol content in cultivated and wild fruits grown in Panama. *J. Pharm Bioallied Sci.*, 4(4): 313-317.
- MURKOVIC, M. (2016): Phenolic compounds: Occurrence, Classes and Analysis. *Encyclopedia of Food and Health*: 346-351.
- NOŽINIĆ, M., Ž., LAKIĆ, V., POPOVIĆ (2022): Medicinal properties and main indicators of seed and oil quality of flaxseed - *Linum usitatissimum* L. *Agriculture and Forestry*, 68 (3): 57-69.
- OVANDO, A.C., L.P., HERNANDEZ, E.P., HERNANDEZ, J.A., RODRIGUEZ, C.A., GALAN-VIDAL (2009): Chemical studies of anthocyanins: A review. *Food Chemistry*, 113: 859-871.
- OZRENK K., A., M., TAS, N., GUNDOGDU, S., KESKIN, ERCISLI (2023). Physicochemical Substances and bioactive components of wild cornelian cherry (*Cornus mas* L.) fruits in Erzincan province of Eastern Turkey. *Genetika*, 55 (1): 95-110.
- PAZ, R., C., FREDES (2015): The Encapsulation of Anthocyanins from Berry-Type Fruits. *Trends in Foods. Molecules*, 20: 5875-5888.
- PAUNOVIĆ, S.M., M., NIKOLIĆ, R., MILETIĆ, P., MAŠKOVIĆ (2017): Vitamin and mineral content in blackcurrant (*Ribes nigrum* L.) fruits as affected by soil management system. *Acta Sci. Pol. Hortorum Cultus*, 16(5): 135-144.
- PETROVIĆ, B., P., VUKOMANOVIĆ, M.V., POPOVIĆ, Z., JOVOVIĆ, M., NIKOLIĆ, LJ., ŠARČEVIĆ-TODOSIJEVIĆ, S., JOVIĆ (2021): Herbal Remedies in the Treatment of Anxiety Disorders. Chapter 7. Ed. Emerald M. Book Title: *An Introduction to Medicinal Herbs*. NOVA Science publishers, USA: 205-236.
- PETROVIĆ, B., P., VUKOMANOVIĆ, V., POPOVIĆ, LJ., ŠARČEVIĆ-TODOSIJEVIĆ, M., BURIĆ, M., NIKOLIĆ, S., ĐORĐEVIĆ (2022): Significance and efficacy of triterpene saponin herbal drugs with expectorant action in cough therapy. *Agriculture & Forestry*, 68 (3): 221-239.
- POLUMACKANYEZ, M., M., WESOŁOWSKI, A., VIAPIANA (2021): *Morus alba* L. and *Morus nigra* L. leaves as a promising food source of phenolic compounds with antioxidant activity. *Plant Food's for Human Nutrition*, 76: 458-465.
- RAHMAN, M.M., M.R., KHAN, M.M., HOSAIN (2008): Analysis of vitamin C (ascorbic acid) contents in various fruits and vegetables by UV-spectrophotometry. *Bangladesh Journal of Scientific and Industrial Research*, 42(4): 417-424.
- RAUF, A., M., IMRAN, T., ABU-IZNEID, J., UL-HAQ, S., PATEL, X., PAN, S., NAZ, S.A., SILVA, F., SAEED, S.H., RASUL (2019): Proanthocyanidins: A comprehensive review. *Biomedicine & Pharmacotherapy*, 116: 108999.
- ROP, O., J., MLCEK, T., JURIKOVA, M., VALSIKOVA, J., SOCHOR, B., REZNICEK, D., KRAMAROVA (2012): Phenolic content, antioxidant capacity, radical oxygen species scavenging and lipid peroxidation inhibiting activities of extracts of five black chokeberries (*Aronia melanocarpa* (Michx.) Elliot) cultivars. *Journal of Medicinal Plants Research*, 4 (22): 2431-2437.
- RUGINA, D., Z., SCONTA, L., LEOPOLD, A., PINTEA, A., BUNEA, C., SOCACIU (2012): Antioxidant activities of chokeberry extracts and the cytotoxic action of their anthocyanin fraction on hela human cervical tumor cells. *J. Med. Food.*, 15: 700-706.
- SADASIVAM, S., T., BALASUBRAMANIAN (1987): Practical Manual in Biochemistry. Tamil Nadu Agricultural University, Coimbatore, India, 14.

- SAPERS, G.M., K.B., HICKS, A.M., BURGHER, D.L., HARGRAVE, S.M., SONDEY, A., BILYK (1986): Anthocyanin patterns in ripening thornless blackberries. *J. Am. Soc. Hort. Sci.*, *111*: 945-950.
- SELAMOVSKA, A., V., GJAMOVSKI, M., TASESKA-GJORGJIEVSKI, D., NEDELKOVSKI, K., BELESKI, K., BANDJO-ORESHKOVIKJ, G., MILANOV, B., KORUNOSKA (2022a): Comparative studies of the content of some antioxidants in the fruits of autochthonous pomegranate varieties. *Agro-knowledge Journal*, *23* (4): 221-228.
- SELAMOVSKA, A., V., GJAMOVSKI, M., TASESKA-GJORGJIEVSKI, D., NEDELKOVSKI, K., BANDJO-ORESHKOVIKJ, B., KORUNOSKA, R., DJOLJEVSKA-MILENKOVSKA (2022b): A comparative studies of the content of antioxidants in fruits of some autochthonous cherry varieties. *Journal of Agriculture and Plant Sciences*, *20* (1): 33-40.
- SELAMOVSKA, A., V., GJAMOVSKI, M., TASESKA-GJORGJIEVSKI, D., NEDELKOVSKI, K., BANDJO-ORESHKOVIKJ (2022c): Content of some antioxidants in the fruits of autochthonous apple varieties. XXVI International ECO conference, Ecological movement, Novi Sad, Serbia. *Safe Food*, 172-179.
- SIMIĆ, D., S., JANKOVIĆ, V., POPOVIĆ, J., IKANOVIĆ, S., STANKOVIĆ, S., RAKIĆ, P., STEVANOVIĆ (2023): Investigation of the nitrogen and phosphorus content in arable agricultural land in Serbia. *Journal of Agricultural Sciences, Belgrade*, *68*(4):449-459.
- SIRIWOHARN, T., R.E., WROLSTAD, C.E., FINN, C.B., PEREIRA (2004): Influence of cultivar, maturity and sampling on blackberry (*Rubus* L. hybrids) anthocyanins, polyphenolics and antioxidant properties. *J. Agric. Food Chem.*, *52* (26): 8021-8030.
- SLIMESTAD, R., H., SOLHEIM (2002): Anthocyanins from blackcurrants (*Ribes nigrum* L.). *J. Agric Food Chem.*, *50* (11): 3228-3231.
- SLINKARD, K., V.L., SINGLETON (1977): Total Phenol Analysis: Automation and Comparison with Manual Methods. *Am J Enol Vitic*, *28*: 49-55.
- SOMERS, C., M.E., EVANS (1977): Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO₂, "chemical age". *Journal of the Science of Food and Agriculture*, *28*(3): 279-287.
- STEVANOVIĆ, A., J., BOŠKOVIĆ, V., ZEČEVIĆ, V., PEŠIĆ, M., ČOSIĆ, L.J., ŠARČEVIĆ TODOSIJEVIĆ, M., BURIĆ, V., POPOVIĆ (2023): Variability and heritability of technological characteristics of *Amaranthus* leaves and seeds" to *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *51*(2): 13128.
- TOSUN, I., N., ARTIK (1998): A study on the chemical composition of blackberry (*Rubus* L.). *Gida*, *23* (403): 41324.
- VAGIRI, M.P. (2018): Phenolic Compounds and Ascorbic Acid in Blackcurrant (*Ribes nigrum* L.). Variation due to Genotype, Ontogenetic stage, Harvest date and Location. Doctoral Thesis. Swedish University of Agricultural Sciences, Alnarp. <https://cdn.lifehacker.ru/wp-content/uploads/2018>.
- VAUZOUR, D., G., RODRIGUEZ-MATEOS, G., CORONA, M., ORUNA-CONCHA, J.P.E., SPENCER (2010): Polyphenols and Human Health: Prevention of Disease and Mechanisms of Action. *Nutrients*, *2*: 1106-1131.
- WANG, S.Y., H.S., LIN (2000): Antioxidant activity in fruits and leaves of blackberry, raspberry and strawberry varies with cultivar and developmental stage. *J. Agric. Food Chem.*, *48* (2): 140-6.
- WU, X., L., GU, R.L., PRIOR, S., MCKAY (2004): Characterization of anthocyanins and proanthocyanidins in some varieties of *Ribes*, *Aronia* and *Sambucus* and their antioxidant capacity. *J. Agricultural Food Chem.*, *52* (26): 7846-56.
- ZHENG, W., S.Y., WANG (2003): Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries and lingonberries. *J. Agric Food Chem.*, *51* (2): 502-509.
- ZOU, Y., S., LIAO, W., SHEN, F., LIU, C., TANG, Y.O., CHEN, Y., SUN (2012): Phenolics and Antioxidant activity of Mulberry leaves depend on cultivar and harvest month in Southern China. *Inter. Journal of Molecular Sciences*, *13*(12): 16544-16553.

**UTICAJ GENOTIPA NA POLIFENOLNI SASTAV I ANTIOKSIDATIVNU
AKTIVNOST NEKIH VRSTA JAGODASTOG VOĆA**

Ana SELAMOVSKA ¹, Elizabeta MISKOSKA-MILEVSKA ², Milena TASESKA-
GJORGJIJEVSKI ^{*1}, Igor ILJOVSKI²

¹Poljoprivredni institut, "Univerzitet Sv. Kiril i Metodij", Skoplje, Severna Makedonija

²Fakultet za poljoprivredne nauke i hrana, "Univerzitet Sv. Kiril i Metodij", Skoplje,
Severna Makedonija

Izvod

Cilj ovog rada je da se ispita sadržaj vitamina C, ukupnih fenola, antocijana, flavan-3-ola, kao i antioksidativna aktivnost plodova pet vrsta jagodičastog voća: sorte ribizle „Rosenthal“, lokalnog ekotipa bele dudinje, sorta aronije „Viking“, sorta kupine „Thornfree“ i autohtona sorta nara „Karamustafa“. Vitamin C je određen klasičnim analitičkim metodama. Ukupni fenoli, ukupni antocijani, flavan-3-oli i antioksidativna aktivnost analizirani su spektrofotometrijskim metodama. Prema dobijenim rezultatima, jagodičasto voće pokazuje da ima visoku antioksidativnu aktivnost, preko 70% i visoki sadržaj ukupnih polifenola. Prema genotipu, aronija ima najveći sadržaj ukupnih fenola (23,9 mg g⁻¹ FW (masa svežeg uzorka), antocijana (6,1 mg g⁻¹ FW) i flavan-3-ola (2,8 mg g⁻¹ FW). Crna ribizla ima najveći sadržaj C vitamina (216,0 mg 100 g⁻¹ FW) i najvišu antioksidativnu aktivnost (86,2% inhibicije). Utvrđena je pozitivna korelacija među svim proučavanim vrstama. Utvrđena je jaka pozitivna korelacija C vitamina sa antioksidativnom aktivnošću, kao i između ukupnih fenola i ukupnih antocijana, odnosno flavan-3-ola. Slaba negativna korelacija je primećena samo za katehin u odnosu na vitamin C, inhibitornu antioksidativnu aktivnost i antioksidativnu aktivnost vitamina C.

Primljeno 30.VII.2023.

Odobreno 18 XII.2023.