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Original scientific paper

COMPARATIVE STUDIES OF THE CONTENT OF ANTIOXIDANTS IN FRUITS OF SOME AUTOCHTHONOUS CHERRY VARIETIES

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Abstract

The content of vitamin C, total phenols, anthocyanins, flavan-3-ols and the fruit antioxidant activity of 6 cherry varieties (Ohridska brza, Ohridska rana, Ohridska crna, Dolga shishka, Dalbazlija and Ohridska bela) was examined.

The fruits of autochthonous cherry varieties (*Prunus avium* L.) averagely contained 12.83 mg% vitamin C, 1386.25 mg/kg FW total phenols, 394.30 mg/kg FW anthocyanins, 69.150 mg/kg FW flavan-3-ols and had antioxidant activity 43.36% inhibition.

Some of the autochthonous cherry varieties compared to the proposed standard variety showed higher values in the examined parameters. The highest content of vitamin C and the fruit antioxidant activity were recorded in Dalbazlija and Dolga shishka varieties. The highest content of total phenols had the standard variety Burlat. High content of total phenols was also found in Ohridska rana, Dalbazlija, Ohridska brza and Ohridska crna varieties. Ohridska rana and Ohridska crna varieties had higher content of anthocyanins compared to the standard variety and other autochthonous varieties.

A moderate positive correlation was found between the content of anthocyanins and vitamin C. There was a moderate negative correlation between the content of flavan-3-ols and phenols. A very high positive correlation was found between the content of vitamin C and the antioxidant activity, while other chemical compounds had little or no effect on the antioxidant activity of cherry fruits.

Keywords: *Prunus avium* L.*, vitamin C, total phenols, anthocyanins, flavan-3-ols, antioxidant activity*

INTRODUCTION

Fruits are rich source of antioxidants that have a preventive and therapeutic effect on human health, destroying free radicals that cause cancer and degenerative diseases. The content of antioxidants according to Bassi et al. (2017) depends on the genotype, environmental conditions, cultivation type, fruit storage method and processing. Unlike fresh fruits, industrially processed agricultural products contain fewer natural antioxidants.

Vitamins are necessary for maintaining human life and health, ensuring growth and development of the body. Insufficient intake of certain vitamins in the body leads to various diseases called hypovitaminosis and avitaminosis. One of the strongest antioxidants is vitamin C. It is contained in smaller or bigger quantities in all fruits. It is mostly found in rose hips and actinidia (634.1-1008.3 mg/100g), hawthorn (500 mg/100g), black currant, pomegranate (300 mg/100g), wild strawberry and blueberry (80 mg/100g) and citrus fruits (50 mg/100g). In smaller quantities it is found in raspberry, chestnut (40 mg/100g), apricot, cherry, peach (20 mg/100g), plum, walnut (10 mg/100g) and pear (4 mg/100g) (Latocha et al., 2010; Selamovska and Miskoska-Milevska, 2021; Rahman et al., 2008).

Polyphenols are very important compounds in plants that have antioxidant,

anti-inflammatory, anti-cancer effects (Hertog et al., 1993; Goldner et al., 2015), anti-mutagenic, 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. **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, such as: flavonols, flavons, catechins, isoflavonoids etc. (Panche et al., 2016). They are most common in fruits (especially berry fruits) and grapes. Flavonoids are necessary for the production of vitamin C (Cook and Sammon, 1996). **Flavonols** belong to flavonoids, they are building blocks of proanthocyanidins. They are found in apples, strawberry fruits and grapes. Campferol, quercentin, myricetin, rutin and ficetin are especially present. **Flavanols** or catechins are the 3 hydroxy- derivatives of flavanones. These include flavan-3-ols, which are a large family of phenolic compounds, mainly responsible for the astringency, bitterness, and nutrient structure (Ivanova and Dimovska, 2010). They are mostly present in apple, blueberry, strawberry, and grape.

Anthocyanins are a group of over 500 different compounds that contribute to the red, purple, and blue colour of many plants, especially fruits and vegetables. Anthocyanin aglycones are known as anthocyanidins. The most important representatives of the group of anthocyanidins found in fruits and grapes are: pelargonidine, cyanidin, peonidine, delphinidine, petunidine and malvidin. They are most common in berry fruit species (black currant, blueberry, strawberry, raspberry), grapes and some tropical species, and to a lesser extent are found in medlar, mulberry, plum, apricot, pomegranate (Paz and Fredes, 2015; Panche et al., 2016; Khoo et al., 2017). Cevallos-Casals et al., (2002), Werner et al. (1989) in Prunus fruits established presence of several anthocyanins: cyanidin-3-glycoside, cyanidin-3-rutinoside, peonidin-3-glycoside and peonidin-3-rutinoside, while Tešović (1985, 1987) found the presence of cyanidin-3-ramnoglycoside, cyanidin-3-gentiobioside, cyanizide-3-monoglycoside and cyanidin-3-diglycoside.

Republic of N. Macedonia is plentiful in autochthonous varieties of fruit species which represent a rich starting material in further selection processes. The aim of this study is to detect and determine the content of specific groups of active substances (antioxidants) in the fruits of autochthonous cherry varieties.

MATERIAL AND METHODS

The study was part of the scientific project ***Antioxidant activity of fruits of autochthonous varieties and populations of fruits, vegetables and grapes,*** financed by the Ministry of Education and Science in 2021. The laboratory tests were performed in the oenological laboratory at the Institute of Agriculture in Skopje.

Six autochthonous varieties of cherry, *Prunus avium* L. (Ohridska brza, Ohridska rana, Ohridska crna, Dolga shishka, Dalbazlija and Ohridska bela) were taken as material for analysis from the region of Ohrid. Comparative studies were performed in relation to the standard variety Burlat.

The content **of vitamin C** (mg%) was examined by volumetric method, i.e. by titration of the filtrate with 2.6 dichlorophenol indophenol, (Shrestha et al., 2016) The end point of the titration was to obtain a faint pink colour. Samples with a higher degree of red staining in the final stage, before titration, were treated with 50 mg of activated carbon, until complete decolourization.

The content **of total phenols, anthocyanins and flavan-3-ols** was determined by spectrophotometric method, expressed in mg/kg FW. Their content was determined with Agilent 8453 UV-VIS spectrophotometer. Before proceeding to their determination, samples were prepared. Approximately 5 g of material was taken from the homogenized sample and transferred to laboratory flask, where 20 ml of pre-prepared solution (methanol: water: hydrochloric acid in a ratio of 70:30:0.1) was added. It was placed in an ultrasonic bath for 15 minutes and then for 30 minutes on a magnetic stirrer. The obtained

clarified solution was transferred to a 25 ml laboratory flask and filled up to the mark with the same extraction solution.

Determining of total phenols: Total phenols in the samples were determined by the Folin-Ciocalteu method (Slinkar and Singelton, 1977). A blank sample was prepared, where distilled water was used instead of the tested sample, while the other reagents remained the same.

Determining of total anthocyanins: Determining the content of total anthocyanins was performed according to the Acid ethanol method (Somers and Evans, 1977). Ethanol chloride solution was used as a blank test. The content of anthocyanins was measured on a spectrophotometer at a wavelength of 550 nm.

Determining of total flavan-3-ols: P-dimethylaminocinnamaldehyde (p-DMACA) was used to quantify total flavan-3-ols in the tested samples (Di Stefano et al., 1989). Methanol was used as a control sample. The absorbance was measured at a wavelength of 640 nm.

These methods are significantly suitable for routine analysis, they are fast and can be used to monitor changes in polyphenols during fruit ripening.

Determining of antioxidant activity: This method was performed as an anti-radical activity against stable product DPPH (2,2-diphenyl-1-picrylhydrazil). Ascorbic acid was used as standard for preparing a series of standard solutions. The determining was performed spectrophotometrically, at a wavelength of 517 nm. (Shetty et al., 1995) (Shori & Baba, 2014).

According to XLStat test 2014 5.03, a correlation analysis was made between the examined parameters. The coefficient of determination R^2 and certain general standard parameters, such as maximum and minimum values, average value and standard deviation for each of the parameters were calculated.

RESULTS AND DISCUSSION

In Table 1, results from the chemical analysis performed on the fruits of the autochthonous cherry varieties were given.

Table 1. Chemical analysis on fruits of some cherry varieties

Variety	Vitamin C mg% FW	Total phenols mg/kg FW	Anthocyanins (mg/kg FW)	Flavan-3-ols mg/kg FW	Antioxidant activity (% inhibition)
Ohridska brza	11.0	1582.59	33.67	19.214	41.8
Ohridska rana	12.0	1604.91	1007.30	47.288	43.0
Ohridska crna	13.0	1488.41	742.36	100.564	40.2
Dolga shishka	19.0	1027.31	150.12	70.057	46.7
Dalbazlija	15.0	1589.00	61.34	37.717	50.7
Ohridska bela	7.0	785.32	67.18	104.966	36.9
Burlat	15.0	1626.24	698.42	104.282	44.2
Average	12.83	1386.25	394.30	69.150	43.36

The content of vitamin C in the fruits of the autochthonous cherry varieties had an average of 12.83 mg%. The varieties Dolga shishka (19.0 mg%) and Dalbazlija (15.0 mg%) had highest content of vitamin C, more than the standard variety and the other autochthonous varieties. The obtained results for the content of vitamin C were about 30% of the total antioxidant activity. In Dolga shishka variety, the content of vitamin C was about 40.7% of the total antioxidant activity, and in Dalbazlija variety was 29.6% of the total antioxidant activity. A moderate positive correlation was found between the content of vitamin C and anthocyanins. There was very high and statistically significant correlation between vitamin C and the antioxidant activity.

The content of total phenols in the fruits was 1386.25 mg/kg FW (fresh weight). The standard cherry variety Burlat had higher content of total phenols (1626.24 mg/kg FW), than all the autochthonous cherry varieties. High content of total phenols was also observed

in Ohridska rana (1604.91 mg/kg FW), Dalbazlija (1589.00 mg/kg FW), Ohridska brza (1582.59 mg/kg FW) and Ohridska crna (1488.41 mg/kg FW) varieties. A positive correlation was found between the content of total phenols and the fruit antioxidant activity.

According to Mikulic-Petkovsek et al. (2016), bird cherry had higher content of total phenols (11053 mg GAE/kg) than wild cherry (2373 mg GAE/kg). Kaur et 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.

The average content of anthocyanins in the fruits of the autochthonous cherry varieties was 394.3 mg/kg FW. The varieties Ohridska rana (1007.30 mg/kg FW) and Ohridska crna (742.36 mg/kg FW) had a higher content of anthocyanins compared to the standard variety and other autochthonous varieties. Due to the high content of anthocyanins in the varieties Ohridska crna and Ohridska rana, it was necessary to add a larger amount of activated carbon for decolourization.

The accumulation of anthocyanins is primarily influenced by genetic and external factors (light, temperature, etc.). Chokeberry (300-2000mg/100g), blueberry (300-698 mg/100g), blackberry (82.5-325.9 mg/100g), pomegranate (15-252 mg/100g) were rich in anthocyanins (Cevallos-Casals et al., 2002; Ćujić et al., 2013). According to Tešović et al. (2012), different fruit species contained the same anthocyanin components: apple and dogwood fruits contained cyanidin-3-arabinoside, plum and juniper fruits contained cyanidin-3-glycoside and peonidin-3-rutinoside. In the fruits of wild and cultivated cherry varieties, the authors determined 4 anthocyanin components. Mikulic-Petkovsek et al. (2016) found a very high content of total flavonols in *Prunus mahaleb* and *Prunus avium*, represented by 19 quercetin derivatives, 10 campherol derivatives and 2 isoramenethine derivatives. Among the anthocyanin components in the *Prunus* species, according to the authors, the most common were cyanidin-3-glycoside and cyanidin-3-rutinoside.

The average content of flavan-3-ols in the fruits was 69.15 mg/kg FW. The highest contents were measured in Ohridska bela (104.966 mg/kg FW), Burlat (104.282 mg/kg FW) and Ohridska crna (100.564 mg/kg FW). There was a moderate negative correlation between the flavan-3-ols and total phenols.

According to the results of the study, cherry fruits had 43.36% inhibition antioxidant activity. The highest antioxidant activity was recorded in Dalbazlija (50.7% inhibition) and Dolga shishka (46.7% inhibition) varieties. A positive correlation was found between the content of total phenols and the fruit antioxidant activity.

The antioxidant activity of the fruits depended on the biotype, type and age of the plant material. The greatest antioxidant activity was found in strawberries, blackberry and red raspberry. In some cherry varieties of the species *Elaeagnus umbellate*, the antioxidant activity was 4.32-9.49 mM TE/100g (Lachowicz et al., 2019). The antioxidant activity in some cherry varieties of the species *Elaeagnus umbellate* according to Mikulic-Petkovsek et al., (2016) was 7.26-31.54 mM/kg. According to Wang and Lin (2000), fruits and leaves of fruit species had great antioxidant activity. With aging, the content of total phenols in leaves reduced and their antioxidant ability decreased.

In Tables 2, 3, 4 and 5 statistical data (maximum and minimum values, average value, standard deviation) and the correlation values of the examined parameters in the autochthonous varieties of cherries were given.

Table 2. Maximum and minimum values, average value and standard deviation with the tested parameters

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
Vitamin C	7	0	7	7,0000	19,0000	13,1429	3,7607
Phenols	7	0	7	785,3200	1626,2400	1386,2543	337,9980
Anthocyans	7	0	7	33,6700	1007,3000	394,3414	407,6255
Flavan-3-ols	7	0	7	19214,0000	104966,0000	69155,4286	35271,0688
Antiox.activity	7	0	7	36,9000	50,7000	43,3571	4,4725

A moderate positive correlation was found between the content of anthocyanins and vitamin C. There was a moderate negative correlation between the flavan-3-ols and phenols, i.e. with increase of the value of flavan-3-ols, while the value of phenols decreased.

There was very high and statistically significant correlation between the content of vitamin C and the antioxidant activity, while other chemical compounds had little or no effect on the antioxidant activity in cherry fruits (Tab. 3).

Table 3. Correlations with tested parameters

Variables	Vitamin C	Phenols	Anthocyans	Flavan 3-ols	Antiox. Activity
Vitamin C	**1**	0,2263	0,0596	-0,1009	**0,7793**
Phenols	0,2263	**1**	0,4700	-0,4357	0,4041
Anthocyans	0,0596	0,4700	**1**	0,2870	-0,1507
Flavan-3-ols	-0,1009	-0,4357	0,2870	**1**	-0,4681
Antiox.activity	**0,7793**	0,4041	-0,1507	-0,4681	**1**

Values in bold are different from 0 with a significance level alpha=0.05

The values of p were lower than the given alpha = 0.05 when comparing the values of vitamin C and antioxidant activity (p = 0.0389), which indicated a statistically significant correlation (Tab. 4).

Table 4. p values with tested parameters

Variables	Vitamin C	Phenols	Anthocyans	Flavan 3-ols	Antiox. activity
Vitamin C	**0**	0,6255	0,8990	0,8295	**0,0389**
Phenols	0,6255	**0**	0,2873	0,3285	0,3686
Anthocyans	0,8990	0,2873	**0**	0,5327	0,7470
Flavan-3-ols	0,8295	0,3285	0,5327	**0**	0,2895
Antiox. Activity	**0,0389**	0,3686	0,7470	0,2895	**0**

Values in bold are different from 0 with a significance level alpha=0.05

The highest coefficient of determination was found between vitamin C and the antioxidant activity, i.e. about 60.7% of the variations in the value of the antioxidant activity were due to variations in the value of vitamin C (Tab. 5).

Table 5. Coefficient of determination R^2 with tested parameters

Variables	Vitamin C	Phenols	Anthocyans	Flavan 3-ols	Antiox. activity
Vitamin C	**1**	0,0512	0,0036	0,0102	0,6073
Phenols	0,0512	**1**	0,2209	0,1898	0,1633
Anthocyans	0,0036	0,2209	**1**	0,0823	0,0227
Flavan-3-ols	0,0102	0,1898	0,0823	**1**	0,2191
Antiox. Activity	0,6073	0,1633	0,0227	0,2191	**1**

CONCLUDING REMARKS

Some of the autochthonous cherry varieties compared to the proposed standard variety showed higher values in the examined parameters. The varieties Dolga shishka and Dalbazlija had the highest content of vitamin C, more than the standard variety and the other autochthonous cherry varieties. The obtained results for the content of vitamin C were about 30% of the total antioxidant activity.

The highest content of total phenols had the standard variety Burlat. High content of total phenols was also found in Ohridska rana, Dalbazlija, Ohridska brza and Ohridska crna varieties.

The varieties Ohridska rana and Ohridska crna had higher content of anthocyanins compared to the standard variety and other autochthonous varieties.

The highest content of flavan-3-ols in the fruits were measured in Ohridska bela, Burlat and Ohridska crna varieties.

The varieties Dalbazlija and Dolga shishka had the highest antioxidant activity, more than the standard variety and the other autochthonous varieties.

A moderate positive correlation was found between the content of anthocyanins and vitamin C. There was a moderate negative correlation between the content of flavan-3-ols and total phenols. A very high positive correlation was found between vitamin C and the antioxidant activity, while other chemical compounds had little or no effect on the antioxidant activity of cherry fruits.

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СПОРЕДБЕНИ ПРОУЧУВАЊА НА СОДРЖИНАТА НА НЕКОИ АНТИОКСИДАНТИ ВО ПЛОДОВИТЕ ОД НЕКОИ АВТОХТОНИ СОРТИ ЦРЕШИ

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Резиме

Во трудот се испитувани содржината на витамин С, вкупни феноли, антоцијани, флаван-3-оли и антиоксидативната активност на плодовите од 6 сорти цреша (*охридска брза, охридска рана, охридска црна, долга шишка, далбазлија* и *охридска бела*).

Плодовите од автохтоните сорти цреша просечно содржеле 12.83 mg% витамин С, 1386.25 mg/kg FW вкупни феноли, 394.30 mg/kg FW антоцијани, 69.150 mg/kg FW флаван-3-оли и имале антиоксидативна активност на плодовите од 43.36% инхибиција.

Некои од автохтоните сорти цреша споредени со стандардната сорта покажале повисоки вредности во испитуваните параметри. Највисока содржина на витамин С и најголема антиоксидативна активност на плодовите биле регистрирани кај сортите *далбазлија* и *долга шишка*. Највисока содржина на вкупни феноли имала стандардната сорта *бурлат*. Висока содржина на вкупни феноли, исто така, била утврдена и кај *охридска рана, далбазлија, охридска брза* и *охридска црна*. *Охридска рана* и *охридска црна* имале повисока содржина на антоцијани, споредено со стандардната сорта и другите автохтони сорти.

Кај црешите е утврдена умерена позитивна корелација меѓу содржината на антоцијани и витаминот С. Утврдена е средна негативна корелација меѓу содржината на флаван-3-оли и вкупни феноли. Многу висока позитивна корелација е најдена меѓу содржината на витаминот С и антиоксидативната активност, додека другите хемиски компоненти имале мал или никаков ефект на антиоксидативната активност на плодовите цреши.

Клучни зборови: *цреша, автохтони сорти, витамин С, вкупни феноли, антоцијани, флаван-3-оли, антиоксидативна активност*