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CONTENT OF SOME ANTIOXIDANTS IN THE FRUITS OF AUTOCHTHONOUS APPLE VARIETIES

Abstract

The content of vitamin C, total phenols, anthocyanins, flavan-3-ols and the antioxidant fruit activity of 7 apple varieties (Ubavo cvetka, Shareno blago, Prespanka, Tetovka, Karapasha, Kozharka, Bela Tetovka) were examined. Most of the autochthonous varieties had better properties and higher antioxidant activity compared to the standard variety Idared. The autochthonous variety Bela tetovka had the highest content of total phenols (7544.59 mg/kg FW), flavan-3-ols (1019.957 mg/kg FW) and antioxidant fruit activity (87.2% inhibition). A positive correlation was found between total phenols and flavan-3-ols with the antioxidant activity, as well as between flavan-3-ols and phenols. Moderate negative correlation was detected between the content of vitamin C and flavan-3-ols.

Keywords: *vitamin C, total phenols, anthocyanins, flavan-3-ols, 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 content of antioxidants depends on the genotype, environmental conditions, cultivation way, method of fruit storage and processing (Bassi et al., 2017).

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 (4-20 mg/100g pear, apple, plum, walnut, apricot, cherry, peach) or bigger quantities (50-1008.3 mg/100g citrus, strawberry, blueberry, black currant, pomegranate, hawthorn, rose hips and actinidia) (Latocha et al., 2010; Selamovska and Miskoska-Milevska, 2021; Rahman et al., 2008).

Polyphenols are very important compounds found in fruits, that have antioxidant, anti-inflammatory, anticancer and antimutagenic action, antiallergenic and antimicrobial action (Hertog et al., 1993; Goldner et al., 2015). They provide protection against infections, reduce the risk of chronic diseases, cardiovascular and neurodegenerative diseases (Vauzour et al., 2010). **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, including 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 and represent 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. They include flavan-3ols, mainly responsible for the astringency, bitterness, and nutrient structure (Ivanova and Dimovska, 2010). Flavon-3-ols include catechins and their derivatives (catechin, epicatechin, epigallocatechin, gallocatechin, epicatechin-3-O-gallate). 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. They belong to the group of phenols. 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. (Panche et al., 2016; Khoo et al., 2017).

Macedonia is plentiful in autochthonous fruit varieties which represent a rich starting material in the further selection process. The aim of this study is to detect and determine the content of specific biologically active substances (antioxidants) in the fruits of each autochthonous apple variety.

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*. The laboratory tests were performed in the oenological laboratory at the Institute of Agriculture in Skopje.

Seven autochthonous varieties of apple (Ubavo cvetka, Shareno blago, Prespanka, Tetovka, Karapasha, Kozharka, Bela Tetovka) were taken as material for analysis. Comparative studies were performed in relation to the standard variety Idared.

The content of vitamin C (mg%) was examined by the volumetric method, according to the method of Murray. The content of total phenols, anthocyanins and flavan-3-ols was determined by the spectrophotometric method, expressed in mg/kg FW.

Their content was determined with Agilent 8453 UV-VIS spectrophotometer. Determining of the antioxidant activity was performed as an anti-radical activity against the stable product DPPH (2,2-diphenyl-1-picrylhydrazil).

According to the 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 some autochthonous apple varieties were given.

Table 1. Results from the chemical analysis performed on fruits of autochthonous apple varieties

Variety	***Vitamin C (mg %)***	***Total phenols mg/kgFW***	***Antho-cyanins (mg/kgFW)***	***Flavan 3 ols mg/kgFW***	***Antioxidant activity (% inhibition)***
Ubavo cvetka	12.0	3668.59	19.63	291.420	53.1
Shareno blago	7.0	3475.31	8.22	366.399	52.6
Prespanka	11.0	5012.37	13.61	579.940	69.9
Tetovka	7.0	3518.51	2.90	450.609	50.9
Karapasha	7.0	4387.83	3.70	898.645	61.6
Kozharka	9.0	4975.00	6.15	393.035	63.2
Bela tetovka	9.0	7544.59	1.30	1019.957	87.2
Idared	13.0	2482.30	1.37	143.884	44.5
Average	**9.37**	**4383.06**	**7.11**	**517.980**	**60.37**

The content of vitamin C in the fruits of the apple varieties had average value of 9.37 mg%. The obtained results for the content of vitamin C were about 15.52% of the total antioxidant activity. Compared to the standard variety, all autochthonous varieties had smaller content of vitamin C, except Ubavo cvetka (12.0 mg%) and Prespanka (11.0 mg%). The standard variety Idared had the biggest content of vitamin C of the total antioxidant activity (29.2%), while Bela tetovka the smallest (10.3%). The data on the content of vitamin C in apple corresponded to the available literature data (Boyer and Liu, 2004) according to which, apple fruits contained an average of about 5.7 mg/100g of vitamin C, i.e. vitamin C participated with less than 0.4% of the total antioxidant activity. Vitamin C was a powerful antioxidant, but according to the authors, the antioxidant activity of apples was caused more by other antioxidant components.

The average content of total phenols in apple varieties was 4383.06 mg/kg FW. All of the autochthonous apple varieties had a higher content of total phenols compared to the standard variety Idared. The highest content of total phenols was found in Prespanka (5012.37 mg/kg FW) and Bela tetovka (7544.59 mg/kg FW). A positive correlation was found between the content of total phenols and the antioxidant activity of the fruits. Our results corresponded with Murillo et al. (2012), who also, found a positive correlation between polyphenol content and the antioxidant activity. Kaur et Kapoor (2005) found a positive correlation between the content of phenols and anthocyanins in some fruit species.

The average content of anthocyanins in the fruits of the autochthonous varieties was 7.11 mg/kg FW. Almost all of the apple autochthonous varieties, with the exception of Bela tetovka, had higher content of anthocyanins compared to the standard variety. The highest content of anthocyanins was detected in Ubavo cvetka (19.63 mg/kg FW) and Prespanka (13.61 mg/kg FW).

The accumulation of anthocyanins is primarily influenced by genetic and external factors (light, temperature, etc.). According to Honda et al. (2002), during the synthesis of anthocyanins in apple fruits, five genes were expressed, of which the level of expression correlated with the concentration of anthocyanins. Anthocyanins in apples were found in the cellular vacuoles of the epidermis and subepidermis (Dayton citation according to Tešović et al., 1999). The main anthocyanin component in the epidermis of apple fruits according to Sun and Francis (1967) was cyanidin-3-galactoside, followed by cyanidin-3-arabinoside and cyanidin-7-arabinoside. In the fruits of wild and cultivated apple varieties, Tešović et al. (2012) determined 3 anthocyanin components.

The average content of flavan-3-ols in the fruits of the autochthonous apple varieties was 517.98 mg/kg FW. All of the apple varieties had higher content of flavan-3-ols compared to the standard variety. The highest content of flavan-3-ols was observed in Bela tetovka (1019.957 mg/kg FW). There was a strong and statistically significant positive correlation between flavan-3-ols and phenols. Moderate negative correlation between vitamin C and flavan-3-ols was detected. The other compared parameters had no significant correlation. The variety Bela tetovka had low content of vitamin C, but high content of flavan-3-ols. These results were similar to the results of Boyer and Liu (2004). According to them, most of the content of antioxidants in apples was made of flavonoids.

The antioxidant activity of apple fruits had an average of 60.37% inhibition. All autochthonous apple varieties had higher antioxidative activity in fruits compared to the standard variety. Bela tetovka had the highest fruit antioxidant activity (87.2% inhibition). A strong and statistically significant correlation between the total phenols and flavan-3-ols and the antioxidant activity was determined. The variety Bela tetovka had the highest content of total phenols and flavan-3-ols and also had the biggest antioxidant activity in fruits.

The antioxidant fruit activity depended on the biotype, type and age of the plant material. The greatest antioxidant activity was found in strawberries, blackberries and red raspberries. 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 (Wang and Lin, 2000). In some apple and strawberry varieties the antioxidant activity was 12- 64 mM FRAP (Kaur and Kapoor, 2005).

In Tables 2, 3, 4 and 5, the statistical data and the correlation values of the examined parameters in the autochthonous varieties of apples were given.

Table 2. Maximum and minimum values, average value and standard deviation for each of the parameters

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. Deviat.
Vitamin C	8	0	8	7,0000	13,0000	9,3750	2,3867
Phenols	8	0	8	2482,3000	7544,5900	4383,0625	1532,3433
Anthocyans	8	0	8	1,3000	19,6300	7,1100	6,5160
Flavan-3-ols	8	0	8	143,8840	1019,9570	517,9861	301,2189
Antiox. activity	8	0	8	44,5000	87,2000	60,3750	13,4958

In the apple varieties, strong and statistically significant correlation of total phenols and flavan-3-ols and the antioxidant activity was found. This indicated presence of other chemical compounds that have inhibitory or intensifying effect on the tested compounds (phenols and flavan-3-ols). That meant, in case of increasing of the value of total phenols, as well as of flavan-3-ols, the antioxidant activity in the apple fruits would drastically increase. There was a strong and statistically significant correlation between the flavan-3-ols and phenols. This meant that the values of phenols, flavan-3-ols and the antioxidant activity in apple fruits were directly related. Apart from the moderate negative correlation between vitamin C and flavan-3-ols, the other compared parameters had no significant correlation (Table 3).

Table 3. Correlations between tested parameters

Variables	Vitamin C	Phenols	Anthocyans	Flavan 3 ols	Antiox. activity
Vitamin C	**1**	–0,1935	0,3731	–0,4721	–0,1518
Phenols	–0,1935	**1**	–0,1469	**0,8117**	**0,9885**
Anthocyans	0,3731	–0,1469	**1**	–0,3105	–0,1130
Flavan-3-ols	–0,4721	**0,8117**	–0,3105	**1**	**0,8284**
Antiox. Activity	–0,1518	**0,9885**	–0,1130	**0,8284**	**1**

Values in bold are different from 0 with a significance level alpha=0,05

The value of p (0.0001 – 0.0111) was significantly below the given alpha = 0.05, which showed high statistically significant correlation of the values of total phenols and flavan-3-ols on the antioxidant activity, as well as high correlation between phenols and flavan-3-ols. (0.0144). There was a value overlap of the mentioned parameters which was almost absolute (Table 4).

Table 4. p values between tested parameters

Variables	Vitamin C	Phenols	Anthocyans	Flavan three ols	Antiox. activity
Vitamin C	**0**	0,6462	0,3626	0,2376	0,7197
Phenols	0,6462	**0**	0,7286	**0,0144**	**< 0,0001**
Anthocyans	0,3626	0,7286	**0**	0,4542	0,7900
Flavan-3-ols	0,2376	**0,0144**	0,4542	**0**	**0,0111**
Antiox. Activity	0,7197	**<0,0001**	0,7900	**0,0111**	**0**

Values in bold are different from 0 with a significance level alpha=0,05

The highest coefficient of determination was between the phenols and the antioxidant activity, i.e. about 97.7% of the variations in the value of the antioxidant activity were explained by the variations in the value of phenols. Also, the high coefficient of determination between the flavan-3-ols and the antioxidant activity of 68.6%, as well as the coefficient of determination of 65.9% between the flavan-3-ols and phenols should be mentioned (Table 5).

Table 5. Coefficient of determination R^2

Variables	Vitamin C	Phenols	Anthocyans	Flavan 3 ols	Antiox. activity
Vitamin C	**1**	0,0374	0,1392	0,2228	0,0230
Phenols	0,0374	**1**	0,0216	0,6589	0,9772
Anthocyans	0,1392	0,0216	**1**	0,0964	0,0128
Flavan-3-ols	0,2228	0,6589	0,0964	**1**	0,6862
Antiox. activity	0,0230	0,9772	0,0128	0,6862	**1**

CONCLUSION

According to the values obtained for the examined parameters, the autochthonous apple varieties compared to the proposed standard variety Idared showed higher values. This indicated that these varieties had high quality properties, were characterized by

high antioxidant activity for the species and had a strong positive effect on human health. The autochthonous variety Bela tetovka had highest content of total phenol, flavan-3-ols and antioxidant fruit activity. The standard variety Idared had the lowest content of total phenols, anthocyanins, flavan-3-ols and antioxidant fruit activity, but the highest content of vitamin C.

A strong positive correlation of total phenols and flavan-3-ols and the antioxidant activity was determined. There was a strong positive correlation between flavan-3-ols and phenols. Moderate negative correlation between vitamin C and flavan-3-ols was observed. The other compared parameters had no significant correlation.

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САДРЖАЈ НЕКИХ АНТИОКСИДАНАТА У ПЛОДОВИМА АУТОХТОНИХ СОРТИ ЈАБУКА

Сажетак

Испитивани су садржај витамина С, укупни феноли, антоцијани, флаван-3-оли и антиоксидативна активност плодова у седам аутохтоних сорти јабука (Лепоцветка, Шарено благо, Преспанка, Тетовка, Карапаша, Кожара, Бела Тетовка). Већи део аутохтоних сорти имају боље карактеристике и више антиоксидативних активност у односу на стандардну сорту Ајдаред. Аутохтона сорта Бела Тетовка има највиши садржај укупних фенола (7544,59 mg/kg FW), флаван-3-ола (1019,957 mg/kg FW) и антиоксидативну активност плодова (87,2% инхибиције). Утврђена је већа позитивна корелација између укупних фенола и флаван-3-ола са антиоксидативном активношћу, као и између флаван-3-ола и фенола. Детерминисана је умерена негативна корелација између садржаја витамина С и флаван-3-ола.

Кључне речи: *витамин С, укупни феноли, антоцијани, флаван-3-оли, антиоксидативна активност*