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COMPARATIVE RESEARCH ON ANTIOXIDANT CONTENT IN THE FRUITS OF SELECT INDIGENOUS VARIETIES OF FRUITS, GRAPES AND VEGETABLES

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In this research study, we presented the results of the chemical analysis conducted on fruits of indigenous varieties and populations of several fruit species, namely cherries, pomegranates and apples, as well as grapes and vegetables such as peppers and tomatoes. Among the samples analyzed, the pepper populations exhibited the highest concentration of vitamin C, measured at 51.25 mg/100g. Conversely, the lowest levels of vitamin C were observed in apples and grapes, approximately 9 mg/100g. When considering the overall antioxidant activity, tomatoes and peppers displayed the largest proportion of vitamin C, accounting for over 75 mg/100g. Furthermore, the highest quantity of anthocyanins was found in cherries, specifically 394.30 mg/kg FW (FW = Fresh Weight). Pomegranate varieties exhibited the greatest content of total phenols, measuring 5359.43 mg/kg FW, along with the highest fruit antioxidant activity, showing 81.58 % inhibition. As for apple varieties, they contained the highest amount of flavan-3-ols, reaching 517.98 mg/kg FW.

Our analysis revealed a positive correlation between total phenols and antioxidant activity, as well as between total phenols and flavan-3-ols. Additionally, a moderate negative correlation was identified between vitamin C and flavan-3-ols.

Key words: autochthonous; fruit species; grapes; vegetables; antioxidants

INTRODUCTION

During a period when human health is increasingly endangered by pollution from both non-living and living factors, the consumption of biologically active compounds, known as antioxidants, plays a crucial role in maintaining well-being. Including foods that are abundant in antioxidants and other vital chemicals in one's diet can significantly decrease the risk of specific diseases. Agricultural products serve as a valuable source of antioxidants, which actively combat cancer-causing free radicals and degenerative ailments. The level of antioxidants present in these products varies based on factors such as genetic makeup, environmental conditions, cultivation techniques, storage methods, fruit ripeness, and fruit processing Bassi *et al.* [2], Lakra *et al.* [19].

In agricultural products, the antioxidant activity is attributable to a range of chemical compounds, including carotenoids, chlorophyll, phenols, lycopene, vitamin C, anthocyanin's, organic acids, flavan-3-ols and others. Silva-Beltran *et al.* [39], George *et al.* [11].

Vitamins play a vital role in sustaining human life and health, as well as promoting growth and development of the body. Inadequate intake of certain vitamins can result in various diseases known as hypovitaminosis and avitaminosis. Vitamin C, known for its potent antioxidant properties, holds significant importance in several physiological functions. It regulates iron and calcium levels, controls blood sugar, reduces high blood pressure and „bad" cholesterol levels, enhances the immune system, and participates in cellular metabolism through oxidoreduction processes. Fruits and vegetables serve as sources of vitamin C, albeit in varying quantities.

High levels of vitamin C have been found in rose hips and actinidia (634.1-1008.3 mg/100g), hawthorn (500 mg/100g), black currant (300 mg/100g), wild strawberry and blueberry (80 mg/100g), and citrus fruits (50 mg/100g) Latocha *et al.* [21], Selamovska and Miskoska-Milevska, [38]. In terms of tomatoes, cultivated varieties contain 20.78 mg/100g of vitamin C, while wild varieties contain 26.22 mg/100g Kurina *et al.* [18].

Polyphenols are highly significant chemical compounds, categorized into two main groups: flavonoids (including anthocyanin's, flavan-3-ols, monomers and polymers, flavones, and dihydroflavones) and non-flavonoids (comprising hydroxybenzoic acid, hydroxycinnamic acid, their derivatives, stilbene compounds, and phenolic acids) Ribéreau-Gayon *et al.* [36]. These compounds exhibit antioxidant, anti-inflammatory and anticancer effects Block *et al.* [4], Goldner *et al.* [12], along with antimutagenic properties Sochor *et al.* [40]. They also possess antiallergenic qualities, reduce the risk of chronic diseases, cardiovascular and neurodegenerative disorders Vauzour *et al.* [43], provide protection against infections and UV radiation, lower blood pressure, decrease the risk of heart attacks and strokes by 20 %, reduce the risk of diabetes, and improve bone function. Moreover, they are crucial for the biosynthesis of vitamin C. Certain polyphenols contribute to the quality, color, and taste of fruits, Von Baer *et al.* [44], while others serve as protective agents for plants against biotic factors such as predators and pests, as well as abiotic factors like frost and drought, B. Korunoska [3]. Additionally, some polyphenols play specific physiological roles in plant development, Macheix *et al.* [23] and so on.

The polyphenol content in fruits is influenced by various factors such as the genotype, fruit maturity, soil-climatic conditions, and cultivation methods, Rodríguez-Delgado *et al.* [37], López-Roca *et al.* [22], Fernandez-Mar *et al.* [10]. Phenolic compounds, particularly anthocyanin's, are synthesized in higher amounts under low air temperatures and insufficient soil moisture Ratiu *et al.* [34].

Flavans-3-ols, a group of phenolic compounds, play a significant role in the astringency, bitterness, and structural properties of food products Ivanova and Dimovska, [14]. Catechins and their derivatives, including catechins, epicatechins, epigallocatechins, gallocatechins, and epicatechins-3-O-gallate, are prominent flavans-3-ols found in apples, blueberries, strawberries, and grapes. Flavans- 3-ols have been associated with reducing high blood pressure, body mass, and the risk of type 2 diabetes, as well as providing protection against vascular diseases Raman *et al.* [33], Osakabe [28].

Anthocyanin's are water-soluble pigments responsible for the coloration of leaves, flowers, and fruit skins. They are most commonly found in berry fruit species (e.g., black currant, blueberry, strawberry, raspberry), grapes, and certain tropical fruits Paz and Fredes [30], Panche *et al.* [29]. Pelargonidine, cyanidin, peonidine, delphinidine, petunidine, and malvidin are the major anthocyanin's present in fruits and grapes. Anthocyanin's exhibit antioxidant, anti-inflammatory, antitumor, anticancer, antibacterial, antimicrobial properties, and have a hepatoprotective effect Pešić *et al.* [31], Rauf *et al.* [35], Mattioli *et al.* [26]. They are also involved in the management of chronic diseases, particularly cardiovascular diseases, diabetes, and Alzheimer's disease Ćujić *et al.* [8].

Previous studies by Cevallos-Casals *et al.* [7], Marić *et al.* [24], Jovančević and Božović [15] have identified several anthocyanin's in the fruits of various Prunus species, including cyanidin-3-glycoside, cyanidin-3-rutinoside, cyanidin-3-amnoglycoside, cyanidin-3-gentiobioside, peonidine-3-glycoside and peonidine-3-rutinoside.

Given the increasing interest in functional foods and the significance of local varieties, our research aimed to analyse and quantify specific bioactive substances (antioxidants) in the fruits of different species and varieties.

MATERIAL AND METHODS

The study was conducted as part of the scientific project titled **"Antioxidant activity of fruits from indigenous varieties and populations of fruits, vegetables, and grapes".** The laboratory tests were carried out at the oenological laboratory located at the Institute of Agriculture in Skopje. Fruit samples were collected for analysis from various indigenous fruit varieties, including 9 varieties of pomegranate (Zumnarija, Bejnarija, Valandovska kisela, Valandovska kiselo-slatka, Hidjas, Kisela, Lifanka, Ropkavec, Karamustafa), 7 varieties of apple (Ubavocvetka, Shareno blago, Prespanka, Tetovka, Karapasha, Kozharka, Bela tetovka), 6 varieties of cherry (Ohridska brza, Ohridska rana, Ohridska crna, Dolga shishka, Dalbazlija, Ohridska bela), 4 varieties of grapes (Belo zimsko, Stanushina, Crven valandovski drenok, Crn valandovski drenok), 2 varieties of tomato (Skopski jabuchar, Volovsko srce), and 3 varieties of pepper (Vezen blag piper, Vezen lut piper, Kavardjik).

The content of vitamin C (mg/100g), total phenols (mg/kg FW), anthocyanin's (mg/kg FW), flavan-3-ols (mg/kg FW), and antioxidant activity of the fruits (% inhibition) were measured. The vitamin C content (mg/100g) was determined using the volumetric method, which involved titration of the filtrate with 2, 6-dichlorophenol indophenol, following Murray's method. The end point of the titration was to achieve a faint pink colour. Samples with a higher degree of red staining were treated with 50 mg of activated carbon before titration, until complete decolourization was achieved.

The content of total phenols, anthocyanin's, and flavan-3-ols was determined using a spectrophotometric method and expressed in mg/kg FW. The determination was carried out using an Agilent 8453 UV-VIS spectrophotometer. Prior to analysis, samples were prepared by taking approximately 5 g of homogenized material and transferring it to a laboratory flask. Then, 20 ml of a preprepared extraction solution (methanol: water: hydrochloric acid in a ratio of 70:30:0.1) was added to the flask. The mixture was subjected to ultrasonic treatment for 15 minutes followed by 30 minutes of stirring on a magnetic stirrer. The resulting clarified solution was transferred to a 25 ml laboratory flask and topped up to the mark with the same extraction solution. Total phenols were determined using the Folin-Ciocalteu method. A blank sample was prepared using distilled water instead of the tested sample, while the other reagents remained the same.

The determination of total anthocyanin's content was conducted using the Acid ethanol method, with ethanol chloride solution used as a blank test. The measurement of anthocyanin's content was performed on a spectrophotometer at a wavelength of 550 nm. P-dimethylaminocinnamaldehyde (p-DMACA) was employed to quantify the total flavan-3-ols in the tested samples, with methanol used as a control. The absorbance was measured at a wavelength of 640 nm.

The method for determining antioxidant activity involved assessing antiradical activity against the stable product DPPH (2,2-diphenyl-1-picrylhydrazil). Ascorbic acid was used as a standard to prepare a series of standard solutions. Spectrophotometric analysis was performed at a wavelength of 517 nm. The absorbance of the samples was measured individually and from the obtained results, the antioxidant activities were calculated as percentages of inhibition.

Correlation analysis was conducted between the examined parameters using the XL-Stat test (2014), yielding a coefficient of determination (R^2) and various standard parameters such as maximum and minimum values, average value and standard deviation for each parameter. The results of the examined parameters were presented as average values for each species' varieties.

RESULTS AND DISCUSSION

Table 1 presents the results of the analysis of vitamin C, total phenols, anthocyanin's, flavan-3-ols and antioxidant activity in fruits of various fruit species (pomegranate, apple, cherry), grapes and vegetables (tomato and pepper).

On average, peppers exhibited the highest vitamin C content (51.25 mg/100g). Apples and grape varieties had the lowest vitamin C content (approximately 9 mg/100g). According to our findings, tomatoes and peppers contributed the largest proportion of vitamin C to the total antioxidant activity, accounting for over 75 %. These fruits were rich in vitamin C, highlighting the importance of including them frequently in the diet to obtain this vitamin. Apples had the lowest contribution of vitamin C to the total antioxidant activity (15.52 %). A moderate negative correlation was observed between the content of vitamin C and flavan-3-ols (Table 3). In this case, the species with the lowest vitamin C content (apples and grapes) exhibited the highest content of flavan-3-ols (517.98 mg/kg FW in apples and 130.23 mg/kg FW in grapes). Pomegranates also had a high flavan-3-ol content (122.51 mg/kg FW). The data on vitamin C content in apples align with existing literature Boyer and Liu, [6], which reports an average vitamin C content of approximately 5.7 mg/100g in apple fruits, contributing less than 0.4 % to the total antioxidant activity. Although vitamin C is a potent antioxidant, these authors suggest that the antioxidant activity of apples is primarily driven by other antioxidant components, such as total phenols and flavan-3-ols, given that apples have the highest content of these substances compared to other crops. Kurina *et al.* [18], found higher vitamin C content in wild tomatoes (26.22 mg/100g) compared to cultivated tomatoes (20.78 mg/100g). George *et al.* [11] reported an average vitamin C content in tomatoes ranging from 2.50 to 26.50 mg/100g.

Table 1. Content of vitamin C, total phenols, anthocyanin's, flavan-3-ols and the antioxidant activity of fruits of several fruit species (pomegranate, apple, cherry), grapes and vegetables (tomato and pepper)

Species	Vitamin C (mg/100g)	Total phenols (mg/kg FW)	Anthocyanin's (mg/kg FW)	Flavan-3-ols (mg/kg FW)	Antioxidant activity (% inhibition)	% vitamin C of total antiox. activity
Pomegranate	23.67	5359.43	323.78	122.51	81.58	29.01
Apple	9.37	4383.06	7.11	517.98	60.37	15.52
Cherry	12.83	1386.25	394.30	69.15	43.36	29.59
Grape	9.00	1037.19	206.96	130.23	40.86	22.03
Tomato	38.25	685.79	63.74	48.04	48.32	79.16
Pepper	51.25	1846.73	46.09	30.73	65.45	78.30
Average	**24.06**	**2449.74**	**173.66**	**153.11**	**56.66**	**42.46**

Table 2. General statistical values for the comparative values of the examined parameters (vitamin C, total phenols, anthocyanin's, flavan-3-ols, antioxidant activity) of fruits (pomegranate, apple, cherry), grapes and vegetables (tomato, pepper

Variables	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
Vitamin C	6	0	6	9.0000	51.2500	24.0617	17.3754
Total phenols	6	0	6	685.7900	5359.4300	2449.7417	1939.3541
Antocyans	6	0	6	7.1100	394.3000	173.6633	160.2637
Flavan-3-ols	6	0	6	30.7260	517.9800	153.1060	183.1157
Antiox. activity	6	0	6	40.8600	81.5800	56.6567	15.5443
% of vit. C from antiox. activity	6	0	6	15.5200	79.1600	42.2667	28.7098

Flavan-3-ols in grapes are primarily synthesized in the seeds (60 %) and stalks (20 %), with a smaller amount found in the fruit skin (approximately 15 %) Bourzeix *et al.* [5]. The typical flavan-3-ols present in grapes include (+) catechin, (-) epicatechin, (-) epicatechin-gallate and rarely (-) epicatechin-3-O-gallate Pineiro *et al.* [31]. The highest content of total phenols was observed in pomegranate (5359.43 mg/kg FW) and apple (4383.06 mg/kg FW), while tomato exhibited the lowest content (685.79 mg/kg FW). Pepper also showed a high content of phenols (1846.73 mg/kg FW). A moderate positive correlation was observed between total phenols and flavan-3-ols, while a very strong positive correlation was found between the content of total phenols and the antioxidant activity of the fruits (Table 4). Pomegranate, apple and pepper varieties displayed high levels of total phenols and exhibited the highest antioxidant activity (81.58 % inhibition in pomegranate, 65.45 % inhibition in pepper and 60.37 % inhibition in apple), Pešić *et al.* [32].

The high content of phenols contributes to sensory characteristics, enhances wine stability and exhibits antioxidant activity Landete [20], Alcalde *et al.* [1]. Krstić *et al.* [17] found a presence of 11.47 g/kg^{-1} total phenols in pepper and did not establish a statistically significant difference in the content of total phenols between hot and mild pepper populations. In our studies, cherries and pomegranate displayed the highest amount of anthocyanins (394.30 mg/kg FW and 323.78 mg/kg FW, respectively), while apples exhibited the lowest (7.11mg/kg FW). Grapes also showed a high content of anthocyanins (206.96 mg/kg FW).

The accumulation of anthocyanins is primarily influenced by genetic and external factors such as light and temperature. Previous studies have reported a high content of anthocyanins in pomegranate (15-270 mg/100g) by Ćujić *et al.* [8], Kaur and Kapoor [16] and Dumlu and Gùrkan [9] (2,100–4,400 mg/l). Apples were found to contain 0-60 mg/100g of anthocyanins, sour cherries 2–450 mg/100g Cevallos-Casals *et al.* [7] and black grapes 192 mg/100g [16]. According to Honda *et al.* [13], during the synthesis of anthocyanins in apple fruits, five genes were expressed and the level of expression correlated with the concentration of anthocyanin's.

In the fruits of both wild and cultivated varieties, Tešović *et al.* [42] identified 17 anthocyanin's, including three in apples, four in plums, four in cherries, five in dogwoods, four in raspberries and five in blueberries. According to Mikulić - Petkovsek *et al.* [27], cyanidin-3-glycoside and cyanidin-3-rutinoside were the most common anthocyanin's found in Prunes species. In apple fruit skin, the main anthocyanin identified by Sun and Francis [41], was cyanidin-3-galactoside, followed by cyanidin-3-arabinoside and cyanidin-7-arabinoside. Various fruit species [42] contained similar anthocyanin's: apple and dogwood fruits contained cyanidin-3-arabinoside, plum and juniper fruits contained cyanidin-3-glycoside and peonidine-3-rutinoside. Kurina *et al.* [18] observed a higher anthocyanin's content in wild tomato varieties (125.30 mg/100g) compared to cultivated varieties (45.20 mg/100g). Markovski *et al.* [25] measured anthocyanin's content in pomegranate varieties ranging from 58.67 to 298.95 mg/l and phenol content from 1540.58 to 2614.59 mg/l.

Table 2 presents the general statistical values for the analyzed parameters (vitamin C, total phenols, anthocyanin's, flavan-3-ols, antioxidant activity) of fruits (pomegranate, apple, cherry), grapes and vegetables (tomato, pepper). Table 3 displays the correlation dependencies between the examined parameters. A moderate negative correlation was found between vitamin C and flavan-3-ols. A moderate positive correlation was observed between total phenols and flavan-3-ols. A very strong correlation was found between total phenols and antioxidant activity. In our case (Table 4), the statistical significance of the correlation between total phenols and antioxidant activity was determined (p= 0.0395). The highest coefficient of determination R^2 (Table 5) was found between the percentage of antioxidant activity attributed to vitamin C and the concentration of vitamin C, with approximately 89 % of the variation in the percentage of antioxidant activity explained by the concentration of vitamin C. Furthermore, 69 % of the variation in antioxidant activity was explained by the variations in the concentration of total phenols.

Table 3. Correlation dependencies between the examined parameters (vitamin C, total phenols, anthocyanin's, flavan-3-ols, antioxidant activity)

Variables	Vitamin C	Total phenol	Antocyans	Flavan 3 ols	Antiox. activity	% vitamin C of total antiox. activity
Vitamin C	**1**	-0.224	-0.4036	-0.558	0.3286	**0.9416**
Total phenols	-0.224	**1**	0.0669	0.561	**0.8330**	-0.4850
Antocyans	-0.403	0.066	**1**	-0.391	-0.0435	-0.4198
Flavan-3-ols	-0.558	0.561	-0.3918	**1**	0.1314	-0.6130
Antiox. activity	0.3286	**0.833**	-0.0435	0.131	**1**	0.0305
% of vit. C from antiox. activity	**0.9416**	-0.485	-0.4198	-0.613	0.0305	**1**

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

Table 4. The statistical significance of the above mentioned correlation between total phenols and the antioxidant activity

Variables	Vitamin C	Total phenol	Antocyans	Flavan 3 ols	Antiox. activity	% vitamin C of total antiox. activity
Vitamin C	**0**	0.6696	0.4275	0.2497	0.5249	**0.0050**
Total phenols	0.6696	**0**	0.8998	0.2462	**0.0395**	0.3296
Antocyans	0.4275	0.8998	**0**	0.4424	0.9348	0.4072
Flavan-3-ols	0.2497	0.2462	0.4424	**0**	0.8041	0.1957
Antiox. activity	0.5249	**0.0395**	0.9348	0.8041	**0**	0.9543
% of vit. C from antiox. activity	**0.0050**	0.3296	0.4072	0.1957	0.9543	**0**

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

Table 5. Percentage of determination R^2 between the examined parameters (vitamin C, total phenols, anthocyanin's, flavan-3-ols, antioxidant activity)

Variables	Vitamin C	Total phenol	Antocyans	Flavan-3-ols	Antiox. activity	% vitamin C of total antiox. activity
Vitamin C	**1**	0.0502	0.1629	0.3115	0.1080	0.8867
Total phenols	0.0502	**1**	0.0045	0.3153	0.6939	0.2352
Antocyans	0.1629	0.0045	**1**	0.1535	0.0019	0.1763
Flavan-3-ols	0.3115	0.3153	0.1535	**1**	0.0173	0.3757
Antiox. activity	0.1080	0.6939	0.0019	0.0173	**1**	0.0009
% of vit. C from antiox. activity	0.8867	0.2352	0.1763	0.3757	0.0009	**1**

CONCLUSIONS

Based on the analysis results of the chemical composition of fruits, including autochthonous varieties and populations of fruit species, grapes and vegetables, the following observations can be made: Autochthonous varieties of fruit species exhibited higher levels of total phenols, anthocyanin's, flavan-3-ols and displayed the highest antioxidant activity compared to autochthonous grape varieties, as well as tomato and pepper populations.

Among all fruit species, pomegranate showed the highest content of total phenols and the greatest fruit antioxidant activity. Apple varieties displayed the highest levels of flavan-3-ols. Cherries and pomegranates contained the highest amounts of anthocyanin's.

Grape varieties also demonstrated high levels of total phenols, anthocyanin's, and flavan-3-ols. In comparison to other fruit species and grapes, tomato and pepper fruits exhibited the highest vitamin C content.

Moreover, in tomato and pepper populations, vitamin C accounted for the largest proportion of the total fruit antioxidant activity (over 75 % inhibition).

Pepper populations contained higher amounts of vitamin C and total phenols and exhibited greater fruit antioxidant activity compared to tomato populations.

A very strong positive correlation was observed between the content of total phenols and the fruit antioxidant activity. Furthermore, a moderate positive correlation was found between total phenols and flavan-3-ols. Conversely, a moderate negative correlation existed between vitamin C and flavan-3-ols.

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КОМПАРАТИВНО ИСТРАЖУВАЊЕ НА СОДРЖИНАТА НА АНТИОКСИДАНТИТЕ ВО ПЛОДОВИТЕ ОД АВТОХТОНИ СОРТИ ОВОШЈЕ, ГРОЗЈЕ И ЗЕЛЕНЧУК

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Во овој научен труд ги презентиравме резултатите од хемиската анализа направена на плодови од автохтони сорти и популации од повеќе овошни видови, имено, цреши, калинки и јаболка, како и на грозје и зеленчук (некои сорти пиперки и домати). Меѓу анализираните примероци, популациите на пиперка покажаа највисока концентрација на витамин Ц, и тоа 51,25 mg/100 g. Спротивно на тоа, најниско ниво на витамин Ц е забележано кај јаболката и грозјето, околу 9 mg/100 g. Кога ќе се земе предвид целокупната антиоксидантна активност, доматите и пиперките имаат најголем удел на витамин Ц, со над 75 mg/100 g. Понатаму, најголемо количество антоцијани е пронајдено во црешите, поточно 394,30 mg/kg FW (FW = свеж примерок). Сортите калинка покажаа најголема содржина на вкупни феноли, околу 5359,43 mg/kg FW и со тоа покажаа највисока антиоксидантна активност (81,58 % инхибиција). Што се однесува до сортите јаболка, тие покажаа најголема содржина на флаван-3-оли, достигнувајќи 517,98 mg/kg FW. Нашата анализа откри позитивна корелација помеѓу вкупните феноли и антиоксидативната активност, како и помеѓу вкупните феноли и флаван-3-олите. Дополнително, беше идентификувана умерена негативна корелација помеѓу витаминот Ц и флаван-3-олите.

Клучни зборови: автохтони, овошни видови, грозје, зеленчук, антиоксиданти