

Antioxidant Properties of Sweet Cherries (*Prunus avium* L.) - Role of Phenolic Compounds

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Abstract—Sweet cherries (*Prunus avium* L.) contain various phenolic compounds which contribute to total antioxidant activity. Total polyphenols, tannins, flavonoids and anthocyanins, and antioxidant capacity in a fruits of a number of selected sweet cherry genotypes were investigated. Total polyphenols content ranged from 4.12 to 8.34 mg gallic acid equivalents/g dry fruit weight and total tannins content ranged from 0.19 to 1.95 mg gallic acid equivalent/g dry fruit weight. Total flavonoids were within the range 0.42-1.56 mg of rutin equivalents/g dry fruit weight and total anthocyanins content were between 0.35 and 0.69 mg cyanidin 3-glucoside equivalent/ g dry fruit weight. Although sweet cherry fruits are a significant source of different phenolic compounds, antioxidant activity of sweet cherries is not related only with the total polyphenolics, flavonoids or anthocyanins.

Keywords—antioxidant activity, polyphenols, *Prunus avium* L., sweet cherry

I. INTRODUCTION

SWEET cherries for fresh consumption are one of the most popular spring-summer fruits across the temperate regions of Europe. In numerous fruit production areas sweet cherries are also the first fresh fruits of the season [1, 2, 3, 4]. Fruit weight, skin colour and sweetness influence consumer acceptance of sweet cherry cultivars [5, 6, 7]. Increasing recent interest in nutraceuticals and functional foods has led plant breeders to initiate selection of crops with higher than normal phenolic antioxidant contents. Because of the health benefits attributed to various fruits, numerous studies have been conducted in recent years to evaluate their properties in terms of quality and bioactivity [8, 9]. Fruits are considered a natural source of antioxidants, including polyphenols and anthocyanins, compounds that can reduce the risk of degenerative diseases caused by oxidative stress, such as cancer, cardiovascular disease and stroke [10, 11, 12, 13]. Red fruits, including sweet cherries, are rich in these types of compounds. Sweet cherries have been reported to contain various phenolics and anthocyanins which contribute to total antioxidant activity [6, 9, 14]. Cherries are also thought to alleviate the pain associated with arthritis and gout [15].

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Antioxidant activity and phenolic composition were genotype dependent and influenced by climatic condition [16].

The objectives of our work were to quantify chemical attributes (the content of different phenolic compounds and antioxidant activity) of 17 sweet cherry cultivars of different ripening time, and to find correlations between them.

II. MATERIAL AND METHODS

A. Plant material

Fruits of sweet cherry cultivars were collected in 2010 from the productive orchard “Sloga” Kač in vicinity of Novi Sad, Serbia. Fruits of 17 red-coloured cultivars (Sándor, Katalin, Kavics, Rita, Margit, Peter, Linda, Aida, Alex, Carmen, Sunburst, Summit, New Star, Burlat (Bigarreau Burlat), Germerdorf 3, Hedelfinger and Majeve rana), were included in this study. Cherry fruits were picked at commercial maturity on the basis of fruit colour: Rita 8 days before Burlat; Sándor and Majeve rana 5 days before Burlat; Peter and Summit 10 days after Burlat; New Star and Margit 15 days after Burlat; Carmen 17 days after Burlat; Aida 18 days after Burlat; Sunburst 19 days after Burlat; Germerdorf 3, Hedelfinger and Kavics 20 days after Burlat; Linda and Katalin 27 days after Burlat and Alex 34 days after Burlat. Approximately 1 kg per cultivar of ripe sweet cherry fruits were harvested from trees. The fruits were selected according to uniformity of size, shape and colour and then transported to the laboratory for analysis. Fruits were air-dried at 45°C in an oven the constant weight. The edible parts of dried samples were ground to a fine powder with a mortar and pestle and used for biochemical analysis. All chemicals used were analytical degree.

B. Extraction and determination of total polyphenols, and tannins

One gram of plant material was extracted with 70% aqueous acetone solution (50 mL) by sonication for 20 minutes in an ultrasonic bath at ambient temperature. The extracts were rapidly vacuum-filtered through a sintered glass funnel and kept refrigerated before assay. All extractions were done in triplicate.

Total polyphenols in the acetone extracts were determined colorimetrically (Jenway 6505, UK) using Folin-Ciocalteu reagent [17]. Gallic acid (GAE) was used as a standard (covering the concentration range between 0.1 and 1.0 mg/mL) and results were expressed as milligrams of GAE per gram of dry plant material (DW).

Total tannins content was determined by the Folin-Ciocalteu procedure, after removal of tannins by their adsorption on insoluble matrix (polyvinylpyrrolidone) [18] (Hagermann 2000). Calculated values were subtracted from total polyphenol contents, and total tannin contents were expressed as milligrams of GAE per gram of DW.

C. Extraction and determination of flavonoids

Total flavonoids were determined after extraction of 1 g of dry plant material with 20 mL of extracting solvent methanol-water-acetic acid (140:50:10 by volume), for 60 minutes, according to the procedure of Marckam [19]. The amount of flavonoids was calculated as a rutin equivalent from the calibration curve of rutin standard solutions and expressed as milligrams of rutin per gram of DW.

D. Extraction and determination of anthocyanins

The quantification of total anthocyanins of dry cherries was evaluated by the pH differential method spectrophotometrically [20]. Anthocyanins were extracted after soaking in 20% (vol/vol) ethanol solution at 1:10 ratio (wt/wt) at 25°C for up to 10 days. Samples of extracts were filtrated through Whatman (Maidstone, UK) No. 1 filter paper. The filtered extracts in 5 mL aliquots were diluted either with 0.2 M KCl/0.2 M HCl (25:67 vol/vol) buffer to 100 mL and adjusted to pH 1.0 or with 1.0 M CH₃COONa/1.0 M HCl/water (10:6:9 by volume) to 50 mL and adjusted to pH 4.5. These diluted solutions were used for further spectrophotometrical analysis. The content of total anthocyanins was expressed as milligrams of cyanidin 3-glucoside (C3G) equivalents per gram of DW.

E. Measurement of antioxidant activity

The potential antioxidant activity of the test samples have been assessed based on scavenging activity of the 10% aqueous acetone sweet cherry extracts of the stable DPPH free radicals [21] (Abe 1998). DPPH-radical scavenging activity was expressed as % of neutralized free radicals, assuming that the sample with the higher percentage has higher scavenging capacity. All measurements were done in triplicate.

F. Statistical analysis

Results were expressed as mean of determinations of 3 independent samples made in triplicates. Statistical significance was tested by analysis of variance followed by comparison of means by Duncan's multiple range test ($P < 0.05$) calculated using STATISTICA for Windows version 9.0 (StatSoft, Tulsa, OK, USA). Stepwise multiple regression analyses were used to determine correlation among variables.

III. RESULTS AND DISCUSSION

The differences in total polyphenolic contents, total anthocyanins, antioxidant activity, and total flavonoid among sweet cherry genotypes were statistically significant (Table 1 and 2). The total polyphenolic contents of sweet cherry genotypes were in the range of 4.12-8.34 mg gallic acid per g

DW basis (Table 1). The highest total polyphenolic content was in the Majeve rana sweet cherry cultivar (8.34 mg/g), followed by the Hedelfinger (7.11 mg/g) and Aida (6.92 mg/g). The lowest content of total polyphenolic compounds was recorded in the Linda cultivar (4.07 mg/g). Serra *et al.* [13] detected from 4.40 to 13.09 mg GAE/g DW of sweet cherry fruits. Other authors [3, 4, 6, 9, 10, 22, 23, 24] found total polyphenol contents in a range from 0.41 up to 4.07 mg GAE/g fresh weight in sweet cherry fruits. Phenolic compounds serve in plant defense mechanisms, to counteract reactive oxygen species, in order to survive and prevent molecular damage, and damaging by microorganisms, insects and herbivores [25, 26, 27]. The difference in the sweet cherry genotypes in terms of total polyphenolics is due to genetic variations, as all genotypes were the same age and grown under the same ecological conditions. It is well-known that phenolic compounds contribute to fruit quality and nutritional value in terms of modifying colour, taste, aroma, and flavour, and also in providing beneficial-health effects [28]. Sweet cherries have relatively high polyphenol content, they are consumed fresh and therefore, can be considered as a relatively good source of dietary polyphenols.

Great variability exist among the examined sweet cherry cultivars, regarding their content in total tannins, ranging from 0.19 mg GAE/g DW for cultivar Gemerdorf 3, and 0.20 mg GAE/g DW for cultivar Alex, up to 1.90 mg GAE/g DW for cultivar Hedelfinger and 1.95 g GAE/g DW for cultivars Katalin and Sunburst (Table 1). Tannins are widely distributed

TABLE II
TOTAL FLAVONOIDS, AND ANTHOCYANINS CONTENT OF DIFFERENT SWEET CHERRY CULTIVARS

Cultivar	Flavonoids ^a	Anthocyanins ^b	DPPH values ^b
Hedelfinger	0.75 ± 0.08	0.64 ± 0.04	44.28 ± 1.47
Germerdorf 3	0.65 ± 0.123	0.49 ± 0.01	26.61 ± 0.59
Majeve rana	1.50 ± 0.16	0.45 ± 0.02	61.12 ± 0.64
Burlat	0.73 ± 0.04	0.69 ± 0.05	36.63 ± 0.31
New Star	0.63 ± 0.16	0.38 ± 0.04	28.14 ± 1.51
Sunburst	0.72 ± 0.15	0.52 ± 0.05	46.12 ± 1.48
Summit	1.06 ± 0.20	0.44 ± 0.04	9.32 ± 1.05
Margit	0.98 ± 0.17	0.58 ± 0.03	17.35 ± 0.62
Sándor	0.66 ± 0.08	0.47 ± 0.01	3.72 ± 0.36
Katalin	0.98 ± 0.16	0.63 ± 0.03	35.87 ± 0.62
Kavics	0.42 ± 0.13	0.35 ± 0.02	21.64 ± 0.33
Rita	0.51 ± 0.09	0.44 ± 0.02	25.97 ± 0.70
Peter	0.50 ± 0.12	0.46 ± 0.02	33.28 ± 1.89
Linda	0.78 ± 0.17	0.43 ± 0.022	21.00 ± 1.58
Aida	1.54 ± 0.17	0.40 ± 0.01	47.75 ± 1.07
Alex	4.79 ± 0.14	0.20 ± 0.07	39.43 ± 1.05
Carmen	5.63 ± 0.05	0.57 ± 0.15	37.38 ± 0.51

Data are mean ± SE values

^a Expressed as mg of rutin/g of dry plant material.

^b Expressed as mg of cyanidin-3-glucoside/g of dry plant material.

in the plant kingdom. The concentration of tannins varies with plant genotype, tissue developmental stage, and environmental conditions. The biochemical activities of tannins range from beneficial antioxidants to damaging prooxidants and toxins. Tannins are feeding deterrents to many invertebrate and vertebrate herbivores. Feeding deterrence is undoubtedly an important mechanism by which tannins protect plant from non-adapted animals. For adapted species, tannins can act as

stimulants [29]. Tannins markedly affect the flavor and the astringency of fruit [1].

The genotype influence the extent of total flavonoid accumulation in the sweet cherry fruits. The contents of flavonoids found in sweet cherries are given in Table 2. The genotypes with high flavonoid contents are Majeve rana, Aida, and Alex with 1.50, 1.54, and 1.56 mg of rutin equivalents/g DW respectively. The cultivar with lowest total flavonoid content is Kavics with 0.42 g of rutin equivalents/g DW. Flavonoids were found to be an important part of human diet and are considered as active principles in many medical plants [30]. Flavonoids have been known to reduce oxidative stress in biological systems due to their antioxidant capacities [9]. Most flavonoids are found in nature as *O*- or *C*-glycosides. The glycosylation is important to reduce the reactivity and to increase the water solubility of flavonoids, which in turn prevents their cytoplasmic damage and guarantees their storage in the cell vacuole [31]. The presence of glycosides attached to flavonoid aglycons, such as flavonol or anthocyanidin, decreases the antioxidant activity of flavonoid. The reason for this is the glycoside moiety, which interferes with the coplanarity of the flavonoid molecule, decreases the ability to delocalise electrons and by that decreases the antioxidant activity of flavonoid [32].

The anthocyanins contents of sweet cherry genotypes were in the range of 0.35-0.69 mg C3G equivalents per g DW basis (Table 2). The highest anthocyanins content was in the Burlat sweet cherry cultivar (0.69 mg C3G/g DW), followed by the Hedelfinger (0.64 mg C3G/g DW) and Katalin (0.63 mg C3G/g DW). The lowest content of total polyphenolic

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Cultivar	Flavonoids ^a	Anthocyanins ^b
Hedelfinger	0.75 ± 0.081	0.64 ± 0.040
Germerdorf 3	0.65 ± 0.123	0.49 ± 0.008
Majeve rana	1.50 ± 0.155	0.45 ± 0.021
Burlat	0.73 ± 0.044	0.69 ± 0.047
New Star	0.63 ± 0.163	0.38 ± 0.042
Sunburst	0.72 ± 0.148	0.52 ± 0.053
Summit	1.06 ± 0.197	0.44 ± 0.038
Margit	0.98 ± 0.168	0.58 ± 0.030
Sándor	0.66 ± 0.080	0.47 ± 0.009
Katalin	0.98 ± 0.161	0.63 ± 0.031
Kavics	0.42 ± 0.125	0.35 ± 0.019
Rita	0.51 ± 0.095	0.44 ± 0.016
Peter	0.50 ± 0.123	0.46 ± 0.019
Linda	0.78 ± 0.167	0.43 ± 0.024
Aida	1.54 ± 0.166	0.40 ± 0.013

Data are mean ± SE values

^a Expressed as mg of rutin/g of dry plant material.

^b Expressed as mg of cyanidin-3-glucoside/g of dry plant material.

compounds was recorded in the Kvcics cultivar (0.35 mg C3G/g DW). Anthocyanins, one of the mayor groups of flavonoids, are widespread natural phenolic compounds in plants. They are mainly distributed among flowers, fruits (particularly in berries), and vegetables and are responsible for their bright colours [33]. Accumulation of anthocyanins is closely connected with ripening stage of the fruits and is responsible for the red-purple colour of fruits [22]. Maturation

of red fruits is followed by the change of the initial green colour to red, violet or blackish colour, caused by accumulation of anthocyanins and by chlorophyll degradation [6]. In cherries, colour is mainly influenced by the concentration and distribution of different anthocyanins in the skin [2, 34]. Other authors also found that Burlat has a very high anthocyanin concentration and the high antioxidant activity [3].

The antioxidant activity using DPPH method in sweet cherry genotypes are shown in Table 1. A statistical significant difference was found among genotypes. The DPPH-values for investigated extracts varied in a wide range between 3.72% and 61.12% (Table 1). The highest antioxidant activity was observed in Majeve rana genotype at 61.12%, followed by Aida genotype (47.75%), Sunburst genotype (46.12%), and Hedelfinger genotype (44.28%).

The relationship between antioxidant capacity and different phenolic groups varied between cultivars (Table 3). There were very weak but statistically significant correlation between antioxidant capacity and total polyphenol and total flavonoid content ($r^2=0.39$ and $r^2=0.12$, respectively). In this study, no statistically significant correlation was observed between

TABLE III
CORRELATION BETWEEN DPPH-ASSAY AND INVESTIGATED PHEOLIC COMPOUNDS IN SWEET CHERRY FRUITS

DPPH	Correlation coefficient (<i>r</i>)	Coefficient of determination (r^2)
Total polyphenol content	0.62*	0.39*
Total anthocyanins content	0.10	0.01
Total flavonoids content	0.35*	0.12*

* Values marked with asterisk are statistically significant ($P>0.05$)

antioxidant activity and total anthocyanins content ($r^2=0.01$). Other authors also found that anthocyanins did not seem to be the only important polyphenols to influence the antioxidant activity of the fruits, when correlating with DPPH data [16, 35].

IV. CONCLUSION

As the conclusion, this investigation show large variability between sweet cherry cultivars in measured chemical attributes. Antioxidant activity of some cultivars depends on phenolics, in others on flavonoids, and also with some other compounds. Sweet cherry fruits are a significant source of different phenolic compounds, and could be considered a good source of natural antioxidants.

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