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PHENOLIC COMPOUNDS AND ANTIOXIDANT CAPACITY OF SWEET CHERRY FRUITS FROM VOJVODINA PROVINCE

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SUMMARY

Sweet cherries (Prunus avium L.) contain various phenolic compounds which contribute to total antioxidant activity. The present study was conducted to assess the antioxidant activity of 15 sweet cherry cultivars from Vojvodina province (north Serbia). The free radical scavenging properties of fruits were evaluated employing two different methodologies, including DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and ferric reducing antioxidant power (FRAP) assay. Strong correlations were found between total phenolics, tannins, flavonoids and anthocyanins and DPPH and FRAP assays. In particular, cultivar Peter showed the highest antioxidant capacity and possesses the highest amount of measured phenolic compounds. This investigation shows large variability among sweet cherry cultivars in measured chemical attributes.

Key words: *anthocyanins, antioxidant activity, total phenolics, Prunus avium* L., *sweet cherry.*

Abbreviations: DPPH - 2,2-diphenyl-1-picrylhydrazyl; FRAP - ferric reducing antioxidant power; FW – fresh weight: TA - total anthocyanins; TF – total flavonoids; TP – total phenolics; TT – total tannins;

INTRODUCTION

By consuming high amounts of fresh fruits, the risk of some chronic diseases can be reduced, which is attributed to the antioxidant compounds they contain. Sweet cherry is a valuable source of some bioactive compounds important for human health preservation (Tall et al., 2004; Yoo et al., 2010; Delgado et al., 2012). In numerous fruit production areas sweet cherries are also the first fresh fruits of the season. In recent years, the increasing interest in nutraceuticals and functional foods has led plant breeders to initiate selection of crops with higher than normal phenolic antioxidant contents (Southon, 2000; Dykes & Rooney, 2007; Cho et al., 2007; Serra et al., 2011). Red fruits, including sweet cherries, are rich in these types of compounds (Pantelidis et al., 2007). Phenolics are secondary plant metabolites characterized by having at least one aromatic ring with one or more hydroxyl groups attached. The distribution of phenolics differs depending on the plant species and the tissue, with many phenolics synthesized from carbohydrates via the shikimate and phenyl propanoid pathways (Lattanzio et al., 2006). In plants these molecules are generally involved in defense against aggression by pathogens, insects, herbivores, or ultraviolet radiation (Grassmann et al., 2002; Lattanzio et al., 2006; Báidez et al., 2006; Barbehenn and Constabel, 2011). Sweet cherries have been reported to contain various phenolics and anthocyanins which contribute to total antioxidant activity. Different cultivars of sweet cherries show a high variability of phenol compound levels and antioxidant capacity. Recent studies on natural products have indicated that the phenolic content and antioxidant capacity are related to the type and content of phenols (Usenik et al., 2008; Pérez-Sánchez et al., 2010, Prvulović et al., 2012).

There are two main antioxidant defense mechanisms in organisms: enzymatic defense and antioxidant defense involving different biomolecules including polyphenols. Phenolic reactions with reactive oxygen species (ROS) play an important role in preventing cancer (Kang et al., 2003; Moon et al., 2006). Some phenolic compounds in plant have been known to prevent DNA oxidative damage (Snijman et al., 2007), quench lipid peroxidation, and scavenge ROS (Caillet et al., 2011, Serra et al., 2011), such as hydrogen peroxide, hydroxyl and superoxide radicals.
The aim of this work was to determine the content of different phenolic compounds from fresh fruits of 15 different sweet cherry cultivars and to estimate their antioxidant activity.

MATERIAL AND METHODS

Plant material: Fruits of 15 red-colored sweet cherry cultivars were collected from the productive orchard "Sloga", Kać, in the vicinity of Novi Sad, north Serbia. Fruits of genotypes Aida, Alex, Burlat (Bigarreau Burlat), Carmen, Germerdorf 3, Hedelfinger, Katalin, Kavics, Margit, New Star, Peter, Rita, Sándor, Summit and Sunburst were included in this study. Sweet cherry fruits were picked at commercial maturity on the basis of fruit colour. 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 color and then transported to the laboratory for analysis. Ten grams of edible parts of fresh samples was extracted with 70% aqueous acetone solution (10 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.
Determination of total phenolics and tannins: Total phenolics (TP) of acetone extracts of sweet cherry fruits were determined by colorimetric method (Jenway 6505, UK) using Folin-Ciocalteu reagent (Saha et al., 2013). Gallic acid was used as a standard (covering the concentration range between 0.1 and 1.0 mg/mL) and results were expressed as mg of GAE per 100 grams of fresh plant material. The 30 μL of sample was combined with 0.5 mL H_2O, 0.1 mL of 3-fold diluted Folin-Ciocalteu reagent and, after 10 minutes, 2.8 mL of 10% Na_2CO_3. After 40 minutes in the dark absorbance was read at 725 nm. Total tannins (TT) content was determined by the Folin-Ciocalteu procedure, after removal of tannins by their adsorption on insoluble matrix PVPP (polyvinylpolypyrrolidone). Calculated values were subtracted from total phenolics content, and total tannin contents were expressed as milligrams of gallic acid equivalents (GAE) per 100 grams of fresh plant weight (FW).
Determination of total flavonoids: The total flavonoids (TF) content was determined spectrophotometrically. Briefly, 0.5 mL of plant extract was mixed with 3 mL of 2% $AlCl_3$ solution (Saha et al., 2013). Absorption readings at 415 nm were taken after 1 h. The amount of flavonoids was calculated as a rutin equivalent (RuE) from the calibration curve of rutin standard solutions and expressed as milligrams of rutin per 100 grams of FW.
Determination of total anthocyanins: Total anthocyanins (TA) content was measured with pH differential absorbance method (Kim et al., 2005). Briefly, absorbance of the extract was measured at 510 and 700 nm in buffers at pH 1.0 (hydrochloric acid-potassium chloride, 0.2 M) and 4.5 (acetate acid-sodium acetate, 1 M). Anthocyanins content was calculated using a molar extinction coefficient of 29,600 (cyanidin 3-glucoside) and absorbance of $A = [(A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}]$. Results were expressed as mg cyanidin 3-glucoside equivalents (C3G)/100 g FW.
DPPH radical scavenging activity: Scavenging of free radicals was tested in a DPPH (2,2-diphenyl-1-picrylhydrazyl) acetone solution (Lai & Lim, 2011). The degree of decoloration of solution indicates the scavenging efficiency of the substance added. DPPH is a stable free radical and accepts an electron or hydrogen to become a stable diamagnetic molecule. Test sample extract (35 μL) was added to 3 mL acetone solution of DPPH (0.2 nM). The mixture was vigorously shaken and kept in the dark for 30 min. The absorbance of the resulting solution was measured at 518 nm spectrophotometrically. DPPH-radical scavenging activity was expressed as % of neutralized free radicals, assuming that the sample with the higher percentage has higher scavenging capacity.
Ferric reducing antioxidant power (FRAP) assay: The FRAP assay was performed according to the method of Valentao et al. (2002). The fresh working solution was prepared by mixing 10 parts of 300 mM acetate buffer, pH 3.6 with 1 part of 10mM TPTZ (2,4,6-Tripyridyl-s-triazine) solution in 40mM HCl and 1 part of 20mM $FeCl_3.6H_2O$ solution and then warmed at 37°C before using. Trolox for calibration curve was dissolved in 70% acetone. Twenty μL of each sample solution and trolox were taken in separate glass test tubes and 2.9 mL of FRAP solution was added. The fruit samples were allowed to react with FRAP 30 minutes in the dark. Measurements of the colored products (ferrous tripyridyltriazine complex) were performed at 593 nm. The FRAP values were determined as mg of trolox equivalents (TE) per 100 g FW by computing with standard calibration curve constructed by different concentrations of trolox.

Statistical analysis. Results were expressed as mean of determinations of 3 independent samples made in triplicate. 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 12.0 (StatSoft, Tulsa, OK, USA). Stepwise multiple regression analyses were used to determine correlation among variables.

RESULTS AND DISCUSSION

The contents of TP and TT in 15 sweet cherry cultivars introduced in the north Serbia climate conditions are given in Table 1. TP content in the sweet cherries ranged from 67.45 mg of GAE per 100 g of fresh weight in cultivar Rita followed by Alex (71.35 mg/100g) and Sunburst (71.74 mg/100g). Peter had the highest TP content (147.31 mg/100g), followed by Katalin (137.55 mg/100g) and Carmen (126.90 mg/100g). The average content of TP of sweet cherry Peter was about two times higher than that of sweet cherry cultivar Rita. There is high variability in TT content among the sweet cherry cultivars used in this experiment, ranging from 29.02 mg GAE/100 g for cultivar Rita, and 39.73 mg/100 g for cultivar Sunburst, up to 89.91 mg/100 g for cultivar Katalin and 95.36 mg/100g for cultivar Peter (Table 1).

Table 1. Total phenolics and tannins content of different sweet cherry cultivars

Cultivar	Total phenolics[1]	Tannins[1]	Flavonoids[2]	Anthocyanins[3]
Aida	$117.23^{a,b} \pm 3.178$	$74.77^{b} \pm 0.700$	$31.58^{c} \pm 1.147$	$21.73^{c} \pm 1.534$
Alex	71.35 ± 3.489	$39.82^{f} \pm 3.771$	$26.76^{f} \pm 1.563$	$3.54^{g} \pm 3.169$
Burlat	$84.52^{c,d} \pm 6.787$	$50.24^{d,e} \pm 1.887$	$30.95^{c,d} \pm 1.774$	$16.30^{d,e} \pm 1.379$
Carmen	$126.90^{a} \pm 9.039$	$74.85^{b} \pm 1.249$	$35.07^{b,c} \pm 1.745$	$29.67^{b} \pm 5.476$
Germerdorf 3	$90.73^{b,c} \pm 9.852$	$56.70^{c} \pm 0.746$	$30.18^{c,d,e} \pm 1.487$	$24.56^{b,c,d} \pm 4.528$
Hedelfinger	$94.63^{b,c} \pm 7.896$	$50.35^{d,e} \pm 3.677$	$31.94^{c} \pm 1.280$	$11.50^{e,f} \pm 3.227$
Katalin	$137.55^{a} \pm 29.461$	$89.91^{a} \pm 8.722$	$28.50^{e.f} \pm 1.222$	$47.25^{a} \pm 3.585$
Kavics	$87.58^{c} \pm 2.556$	$50.50^{d} \pm 0.731$	$30.32^{c,d,e} \pm 1.359$	$24.336^{b,c,d} \pm 3.591$
Margit	$75.14^{d} \pm 2.869$	$41.037^{f} \pm 1.591$	$36.98^{b} \pm 1.392$	$21.28^{c,d} \pm 2.458$
New Star	$86.27^{c} \pm 2.705$	$48.14^{e} \pm 1.053$	$29.57^{d,e} \pm 1.379$	$26.68^{b,c,d} \pm 6.065$
Peter	$147.31^{a} \pm 23.437$	$95.36^{a} \pm 2.912$	$41.80^{a} \pm 2.601$	$29.02^{b,c} \pm 12.044$
Rita	$67.45^{e} \pm 1.753$	$29.02^{g} \pm 0.161$	$39.00^{a} \pm 0.455$	$16.16^{d,e,f} \pm 6.568$
Sándor	$85.25^{c} \pm 9.805$	$52.41^{c,d} \pm 1.976$	$29.30^{d,e,f} \pm 3.838$	$24.09^{b,c,d} \pm 5.006$
Summit	$78.14^{d} \pm 6.224$	$45.34^{e,f} \pm 3.503$	$25.48^{f} \pm 2.350$	$10.90^{e,f} \pm 1.835$
Sunburst	$71.47^{d,e} \pm 5.629$	$39.73^{f} \pm 1.199$	$28.07^{d,e,f} \pm 2.197$	$8.04^{f,g} \pm 2.212$

Data are mean ± SE values

[1] Expressed as mg of gallic acid equivalents (GAE)/100 g of fresh plant material.

[2] Expressed as mg of rutin (Ru)/100 g of fresh plant material.

[3] Expressed as mg of cyanidin-3-glucoside (C3G)/100 g of fresh plant material.

[a-g] Values without the same superscripts within each column differ significantly ($p < 0.05$)

In sweet cherry the ripening process is related to a change from the green into the red colour, with degradation of chlorophyll and accumulation of different phenolic compounds. Phenolic compounds are concentrated in the skin of sweet cherry fruits and contribute to sensory and organoleptic qualities (Ferretti et al., 2010). In plants, phenolic compounds serve as signal compounds, pigments, and defense mechanisms in order to survive and prevent molecular damage by ROS, and damaging by fungal pathogens and animals (Lattanzio et al., 2006, Boeckler et al., 2011). Results of our study are in agreement with works of other authors (Chaovanalikit & Wrosland, 2004, Kim et al., 2005, Usenik et al., 2010, Ferretti et al., 2010, Faniadis et al., 2010, Serradilla et al., 2011) who found TP contents in a range from 41.00 up to 407.00 mg GAE/100 g fresh weight in sweet cherry fruits. It is well known that genetic and agronomic or environmental factors play an important role in the phenolic composition and thus nutritional quality of fruits (Ferretti et al., 2010, Faniadis et al., 2010). The difference among the sweet cherry genotypes in terms of TP is due to genetic variations, as all genotypes were the same age and grown under the same ecological and agrotechnical conditions.

Tannins are widely distributed in the plant kingdom. Woody plants tend to synthetize more tannins than herbaceous plants. The concentration of tannins varies with plant genotype, tissue developmental stage, and environmental conditions. Tannins can defend plants against herbivores by deterrence or toxicity. The biochemical activities of tannins range from beneficial antioxidants to damaging prooxidants and toxins (Pérez-Sánchez et al., 2010).

Table 1 also presents the analytical data for the TF and TA content of the studied sweet cherry cultivars. The genotypes with high flavonoid contents are Peter, Rita, and Margit with 41.80, 39.00, and 36.98 mg RuE/100g FW, respectively. The cultivars with the lowest total flavonoid content are Summit with 25.48 and Alex with 26.76 mg

RuE/100g FW. Sweet cherries from the cultivars that are abundant in TP, TT and TF also contained more TA. The TA contents of sweet cherry genotypes were in the range of 3.54-47.25 mg C3G per 100 g FW basis (Table 2). The highest TA content was in Katalin sweet cherry cultivar (47.25 mg C3G/100g FW), followed by Carmen (29.07 mg C3G/100g FW) and Peter (29.02 mg C3G/100g FW). The lowest content of TA compounds was recorded in Alex cultivar (3.54 mg C3G/100g FW) and Sunburst cultivar (8.04 mg C3G/100 g FW).

Flavonoids play a variety of significant roles in plants. Flavonoids act as signal molecules, detoxifying agents, stimulants for germination of spores, phytoalexins, pollinator attractants and have many other functions (Lattanzio et al., 2006). Flavonoids have been known to reduce oxidative stress in biological systems due to their antioxidant capacities (Wang et al., 2008). Most flavonoids are found in nature as *O*- or *C*-glycosides. The glycosylation is important for reducing the reactivity and increasing the water solubility of flavonoids, which in turn prevent their cytoplasmic damage and guarantee their storage in the cell vacuole (Cuyckens & Claeys, 2005). 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 delocalize electrons and thereby decreases the antioxidant activity of flavonoid (Heim et al., 2002). Variation in the content of flavonol and other flavonoids in fruits is strongly influenced by extrinsic factors such as fruit type and growth, season, climate, degree of ripeness, food preparation, storage and processing (Marinova et al., 2005; Wang et al., 2008). In human nutrition flavonoids have powerful antioxidant and anti-inflammatory properties and flavonoid consumption could be a potential strategy for reducing cognitive decline in older adults (Hooper et al., 2008; Devore et al, 2012).

Anthocyanins are phenolic compounds found throughout the plant kingdom, being responsible for the blue to red colors found in leaves, flowers and fruits (Serradilla et al., 2011). It has been shown that anthocyanins are strong antioxidants with free radical scavenging properties attributed to the phenolic hydroxyl groups found attached to ring structures. Different hydroxylation and glucosylation may modulate their antioxidative properties (Kim et al., 2005). Results obtained in this experiment are in line with findings of other authors who reported that content of TA in different sweet cherry cultivars could vary in a range from 1.6 up to 510 mg C3G/100 g FW (Kim et al., 2005; Ferretti et al., 2010; Usenik et al., 2010; Serradilla et al., 2011; Serra et al., 2011).

Antioxidant capacity is an important fruit quality parameter. Total antioxidant capacity of sweet cherries was measured by DPPH and FRAPS assays (Table 2). In the present study, all extracts were found to be effective scavengers against DPPH radical. A statistically significant difference was found among genotypes. The DPPH-values for investigated extracts varied in a wide range between 39.76 % and 78.71% of neutralized free radicals. The highest antioxidant activity was observed in Peter genotype at 78.71%, followed by Katalin genotype (78.65%), and Carmen genotype (75.05%), while Alex (39.76%), Summit (44.33%) and Sunburst (44.42%) genotypes expressed the lowest capacity to scavenge DPPH radicals. Antioxidant activity of 15 sweet cherry cultivars was in a range from 16.30 (Summit) and 26.70 (Sunburst) up to 84.17 (Aida) and 87.87 mg TE/100 g FW (Peter).

Table 2. Antioxidant capacity of different sweet cherry cultivars

Cultivar	DPPH values[1]	FRAP values[2]
Aida	$69.79^{b} \pm 3.646$	$84.17^{a} \pm 3.524$
Alex	$39.76^{g} \pm 3.351$	$30.50^{g} \pm 1.883$
Burlat	$51.94^{d,e} \pm 2.151$	$38.10^{e} \pm 1.558$
Carmen	$75.05^{a} \pm 1.988$	$33.37^{f} \pm 1.223$
Germerdorf 3	$51.52^{d,e,f} \pm 3.837$	$31.13^{g} \pm 1.650$
Hedelfinger	$50.41^{d,e,f} \pm 3.768$	$57.83^{c} \pm 2.213$
Katalin	$78.65^{a,b} \pm 8.869$	$30.10^{g,h} \pm 3.963$
Kavics	$51.85^{d,e} \pm 3.345$	$67.20^{b} \pm 2.223$
Margit	$49.70^{e} \pm 1.093$	$47.30^{d} \pm 0.245$
New Star	$48.11^{e,f} \pm 2.361$	$34.17^{e,f,g} \pm 3.417$
Peter	$78.71^{a} \pm 5.795$	$87.87^{a} \pm 7.450$
Rita	$51.45^{d,e} \pm 3.174$	$27.97^{h} \pm 0.772$
Sándor	$55.65^{c,d} \pm 2.527$	$35.80^{e} \pm 1.349$
Summit	$44.33^{f,g} \pm 3.303$	$16.30^{i} \pm 0.572$
Sunburst	$44.42^{f} \pm 0.924$	$26.70^{h} \pm 1.374$

Data are mean ± SE values

[1] Expressed as % of neutralized DPPH free radicals.

[2] Expressed ad mg of Trolox equivalents/100 g of fresh plant material

[a-i] Values without the same superscripts within each column differ significantly ($p < 0.05$)

A correlation between DPPH and FRAP activity and individual compounds of phenolics was statistically analyzed. Overall DPPH activity (Table 3) was found to have a strong correlation ($p < 0.001$) with total phenolics (0.771), total tannins (0.747) and total anthocyanins (0.548), and a statistically significant correlation ($p<0.016$) with total flavonoids (0.357). The results are consistent with the previous report that phenolic compounds confer a high antioxidant activity of sweet cherries.

Table 3. Correlation between DPPH-assay and investigated phenolic compounds in sweet cherry fruits

DPPH	Correlation coefficient (r)	Coefficient of determination (r^2)	p
Total polyphenol content	0.771*	0.594*	0.000
Total tannins content	0.747*	0.558*	0.000
Total anthocyanins content	0.548*	0.300*	0.000
Total flavonoids content	0.357*	0.127*	0.016

However, a correlation of FRAP values with phenolics (Table 4) were relatively lower than those found for DPPH although statistically very significant ($p<0.001$ for TP (0.472), TT (0.510) and TF (0.486), and $p<0.019$ for TA (0.349)).

Table 4. Correlation between FRAP-assay and investigated phenolic compounds in sweet cherry fruits

FRAP	Correlation coefficient (r)	Coefficient of determination (r^2)	p
Total polyphenol content	0.472*	0.223*	0.001
Total tannins content	0.510*	0.260*	0.000
Total anthocyanins content	0.349*	0.122*	0.019
Total flavonoids content	0.486*	0.236*	0.001

The obtained correlation coefficients are not high, therefore it can be concluded that phenolic components are not the only compounds responsible for antioxidant activity in sweet cherry determined by FRAP assay. A positive correlation between the amount of phenolic compounds in samples and antioxidant capacity is supported by findings of other researchers (Faniadis et al., 2010; Usenik et al., 2010, Prvulović et al., 2012).

CONCLUSION

The results of our research show large variability among the cultivars in their chemical attributes. Our results suggest that the antioxidant capacity of sweet cherries is related to phenolics, flavonoids or anthocyanins. Antioxidant activity is cultivar-specific and depends on different chemical compounds. The results indicated that Peter cultivar is the richest source of bioactive ingredients with the highest antioxidant capacity.

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