PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY OF SWEET AND SOUR CHERRIES

DEJAN PRVULOVIĆ^{a*}, MILAN POPOVIĆ^a, DJORDJE MALENČIĆ^a, MIRJANA LJUBOJEVIĆ^a, GORAN BARAĆ^a, VLADISLAV OGNJANOV^a

ABSTRACT. Sweet and sour cherries are a valuable natural source of some bioactive compounds important in human health preservation. Total phenolics, tannins, flavonoids and anthocyanins, and antioxidant capacity in a fruits of a two selected sweet cherry genotypes (Szomolyai Gombolyii and Valerij Cskalov) and 2 sour cherry cultivars (Érdi bőtermő and Kántorjánosi) were investigated. Total phenolic content ranged from 76.05 up to 301.19 mg gallic acid equivalents/100 g fresh fruit weight and total tannins content ranged from 32.33 to 236.61 mg gallic acid equivalents/100 g fresh fruit weight. Total flavonoids were within the range 49.47-70.27 mg of rutin equivalents/100 g fresh fruit weight and total anthocyanins content were between 16.86 and 51.16 mg cyanidin 3-glucoside equivalents/100 g fresh fruit weight. Antioxidant activity of sweet and sour cherries is correlated with the total phenolics and total tannins content, and partially related with total anthocyanins, but not with the total flavonoids. Fruits of sour cherries contains more phenolics than fruits of sweet cherries and possess more potent antioxidant activity.

Keywords: antioxidant peroperties, fruit, phenolic compounds, Prunus avium L., Prunus cerasus L.

INTRODUCTION

Sweet (*Prunus avium* L.) and sour (*Prunus cerasus* L.) cherries are popular fruit crops across the temperate region of Europe. They are also economically and nutritionally important crops worldwide [1, 2, 3].

Apart from several essential dietary components, such as vitamins, minerals, protein and carbohydrate, cherries also contain other phytonutrients that may provide benefits beyond the prevention of dietary deficiencies such as compacting multiple disease states. The consumption of sweet or sour cherries reduce the risk of cancer, pain from arthritis and inflammation, symptoms of exercise-induced muscle diseases. The beneficial effects of cherries may be attributed to the presence of phenolics such as anthocyanins and melatonin that exert potent antioxidant capacity [4].

^a University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića, Nr. 8, 21000 Novi Sad, Serbia. * dprvulovic@yahoo.com

The genetic background is the first parameter with the potential to influence the antioxidant content in a commodity. Significant inter-cultivar variation in the phenolic content and antioxidant capacity has also been documented in cherries. The biosynthesis of polyphenolic compounds is triggered by exposure to stress conditions, as a natural defense system, generating a significant amount of the above variability [5].

Phenolics are diverse group of aromatic compounds with at least one hydroxyl group, which also include various derivates. Phenolics are important in determining the sensory quality of food such as colour, taste and flavor. The composition and concentration of phenolics are significantly influenced by the stage of maturity, cultivars, cultural practices, geographic origin, growing season, climate condition, postharvest storage condition, and food-processing procedures [6].

The objectives in this study were to comparatively analyze dietary functional phenolics of fresh sweet and sour cherries and to investigate the antioxidant capacity of these fruits.

RESULTS AND DISCUSSION

In cherries the ripening process is closely related to a change from the initial green colour into red, with degradation of chlorophyll and accumulation of different phenolic compounds and anthocyanins. Phenolic compounds are mostly concentrated in the skin and contribute to sensory and organoleptic qualities of fruits [6].

Table 1. Total phenolics, total tannins and total anthocyanins content of sweet and sour cherry cultivars

Cultivar	Phenolics ¹	Tannins ¹	Anthocyanins ²
Szomolyai Gombolyii	76.05 ± 4.85^{a}	32.33 ± 1.57 ^a	16.86 ± 1.92 ^a
Valerij Cskalov	110.96 ± 13.33 ^b	74.68 ± 11.04 ^b	33.25 ± 2.79 ^b
Érdi bőtermő	300.22 ± 32.44 ^c	236.61 ± 24.45°	38.18 ± 2.76 ^b
Kántorjánosi	301.19 ± 9.16 ^c	$227.00 \pm 5.03^{\circ}$	51.16 ± 4.66 ^c

The data are mean values ± standard error

² Expressed as mg of cyanidin-3-glucoside/100 g of fresh plant material.

The content of total phenolics (TP), total tannins (TT) and total anthocyanins (TA) in two sweet (Szomolyai Gombolyii and Valerij Cskalov) and two sour (Érdi bőtermő and Kántorjánosi) cultivars are given in Table 1. TP, TT and TA content varies among cultivars analyzed in the present study. Previous reported TP content were carried from 4.12 up to 229.00 in sweet

a,b,c the values without the same superscript within each column differ significantly (P < 0.05)

Expressed as mg of gallic acid equivalents /100 g of fresh plant material.

cherries [1, 3, 5, 6, 7, 8, 9] and from 70.70 to 754.00 mg gallic acid equivalents/100 g fresh weight in sour cherries [4, 10, 11, 12, 13]. Valerij Cskalov had the higher amount of TP content (110.96 mg GAE/100 g FW) than Szomolyai Gombolyii cultivar (76.05 mg GAE/100 g FW). Fruits of sour cherry genotypes had a threefold higher content of TP than sweet cherries. TP content in the fruit of Kántorjánosi sweet cherry cultivar was 301.19 mg GAE/100 g FW followed by the Érdi bőtermő cultivar with 300.22 mg GAE/100 g FW. 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 [14].

Great variability exist among the examined cherry cultivars, regarding their content in TT, ranging from 32.33 mg GAE/100 g FW (Szomolyai Gombolyii) and 74.68 mg GAE/100 g FW (Valerij Cskalov) for sweet cherry cultivars, up to 227.00 mg GAE/100 g FW (Kántorjánosi) and 236.61 mg GAE/100 g FW (Érdi bőtermő) for sour cherry cultivars (Table 1). Cherries from the cultivars that are abundant in TP contained also more TT. Tannins are widely distributed in the plant kingdom. The concentration of tannins varies with environmental conditions, plant genotype and tissue development stage. The biochemical activities of tannins range from beneficial antioxidants to damaging prooxidants and toxins. Tannins are feeding deterrents to many herbivores. Feeding deterrence is undoubtedly an important mechanism by which tannins protects from non-adapted animals. For adapted species, tannins can act as stimulants [15]. Tannins markedly affect the flavor and the astringency of fruit [1].

Anthocyanins are water-soluble pigments that contribute the blues, purples and reds in plant food [15]. In cherries, colour is mainly influenced by the concentration and distribution of different anthocyanins in the skin [2, 16]. The TA content of sour cherry genotypes were in range of 38.18 (Érdi bőtermő) and 51.16 (Kántorjánosi) mg cyanidin-3-glucoside equivalents (C3GE) /100 g FW basis (Table 1). The lower content of TA was recorded in sweet cherry genotypes: Szomolyai Gombolyii cultivar (16.86 mg C3GE/100 g FW) and Valerij Cskalov (33.25 mg C3GE/100 g FW). Results from this study are in agreement with results of other authors [9, 10, 11, 12, 18, 19, 20]. Anthocyanins are effective in scavenging reactive oxygen species, in inhibiting lipid peroxidation, in protecting against cardiovascular disease and express antitumor activity [11, 18, 20].

The genotype influences the extent of total flavonoid (TF) accumulation in the cherry fruits. The contents of flavonoids found in sweet and sour cherries are given in Table 2. The genotypes with high flavonoid contents are Valerij Cskalov, Kántorjánosi and Szomolyai Gombolyii with 70.27, 63.67 and 63.35 mg of rutin equivalents (RE)/100 g FW respectively. The cultivar with lowest TF content is Érdi bőtermő with 49.47 mg of RE/100 g FW. Flavonoids were found

to be important part of human diet and are considered as active principles in many medical plants [21]. Flavonoids have been known to reduce oxidative stress in biological systems due to their antioxidant capacities [4, 22]. 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 [23]. Flavonoids are reported to have antioxidant, anticancer, antiallergic, antiinflamatory and gastroprotective properties [16].

Table 2. Total flavonoids content and antioxidant activity of sweet and sour cherry cultivars

Cultivar	Flavonoids ¹	DPPH values ²
Szomolyai Gombolyii	63.35 ± 1.14 ^a	33.74 ± 1.69 ^a
Valerij Cskalov	70.27 ± 5.06 ^a	27.15 ± 1.26 ^a
Kántorjánosi	63.67 ± 17.69 ^a	9.30 ± 0.07 ^b
Érdi bőtermő	49.47 ± 6.91 ^b	9.10 ± 0.70 ^b

The data are mean values ± standard error

The antioxidant activity using DPPH method in sweet and sour cherry genotypes are shown in Table 2. All examined cherry extracts showed very high antioxidant activity. A statistical difference was found among genotypes. The IC $_{50}$ DPPH-values for investigated extracts varied in a wide range between 9.10 and 33.74 μ l extract. The highest antioxidant activity was observed in sour cherry genotype Érdi bőtermő, followed by Kántorjánosi genotype.

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

	Correlation coefficient (r)	Coefficient of determination (r^2)		
Total polyphenol content	0.87*	0.75*		
Total tannins content	0.95*	0.89*		
Total anthocyanins content	0.87*	0.76*		
Total flavonoids content	0.28	0.08		
* Values marked with asterix are statistically significant (<i>P</i> >0.05)				

The relationship between antioxidant capacity and different phenolic groups varied between cultivars (Table 3). There were statistically significant correlation between antioxidant capacity and TP, TT and TA content (r^2 =0.75, r^2 =0.89 and r^2 =0.76 respectively). In this study, no statistically significant

 $^{^{}a,b,c}$ the values without the same superscript within each column differ significantly (P < 0.05)

¹ Expressed as mg of rutin/g of dry plant material.

² Expressed as IC₅₀ value (µl of sample).

correlation was observed between antioxidant activity and TF content (r^2 =0.08). Other authors also found that different phenolic groups influence the antioxidant activity of the fruits, when correlating with DPPH data [5, 8, 18, 20, 24].

CONCLUSIONS

As the conclusion, this investigation show large variability between sweet and sour cherry cultivars in measured chemical attributes. Antioxidant activity of both sweet and sour cherries depends on total phenolics, tannins and anthocyanins, but not on flavonoids. Cherries are a significant source of different phenolic compounds, and could be considered as a good source of natural antioxidants.

EXPERIMENTAL SECTION

Plant material

Fruits of sweet and sour cherry cultivars were collected in 2011 from the productive orchard "Sloga" Kać in vicinity of Novi Sad, Serbia. Fruits of 2 red-coloured sweet cherry cultivars (Valerij Cskalov and Szomolyai Gombolyii) and 2 sour cherry cultivars (Érdi bőtermő and Kántorjánosi) were included in this study. Cherry fruits were picked at commercial maturity on the basis of fruit colour. Approximately 1 kg per cultivar of ripe cherry fruits was harvested from trees. The fruits were selected according to uniformity of size, shape and colour and then transported to the laboratory for analysis.

Extraction and determination of phenolic compounds

Five grams of plant material was extracted with 70% acetone solution (5 ml) by sonication for 20 minutes in an ultrasonic bath at ambient temperature. The extracts were rapidly filtered and kept refrigerated before assay. All extractions were done in triplicate. Total phenolics (TP) in the acetone extracts were determined colorimetrically (Jenway 6505, UK) using Folin-Ciocalteu reagent [25]. Gallic acid (GA) was used as a standard (covering the concentration range between 0.1 and 1.0 mg/ml) and results were expressed as milligram of GAE/100 grams of fresh plant material (FW). Total tannins (TT) content was determined by the Folin-Ciocalteu procedure, after removal of tannins by their adsorption on insoluble matrix (polyvinylpolypyrolidone) [26]. Calculated values were subtracted from total polyphenol contents, and total tannins contents were expressed as mg GAE per 100 grams of FW. Total flavonoids (TF) were determined according to the procedure of Marckam [27]. The amount of flavonoids was calculated as a rutin equivalent (RE) from the calibration curve of rutin standard solution and expressed as mg of RE per

100 g of FW. The quantification of total anthocyanins (TA) of fresh sweet and sour cherries was evaluated by the pH differential method spectrophotometrically [28]. The content of TA was expressed ad mg of cyanidin 3-glucoside equivalents (C3GE)/100 g of FW.

Measurement of antioxidant activity

The potential antioxidant activity of the test samples have been assessed based on scavenging activity of the 70% aqueous acetone sweet and sour cherry extracts of the stable DPPH free radicals [29]. The antioxidant activity of the extracts was expressed as IC_{50} . The IC_{50} value was defined as the volume in μI of extract that reduce the presence of DPPH radicals in solution by 50% assuming that the sample with the smaller volume has higher scavenging capacity. All measurements were done in triplicate.

Statistical analysis

Results are expressed as mean of determinations of 3 independent samples made in triplicates. Statistical significance was tested by analysis of variance followed by comparison 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.

ACKNOWLEDGMENTS

This study was carried out within a project No. TR-31038 of Ministry of Education and Science of the Republic of Serbia.

REFERENCES

- 1. M.J. Bernalte, E. Sabio, M.T. Hernández, C. Gervasini, *Postharvest Biology and Technology*, **2003**, *28*, 303.
- 2. B. Gonçalves, A.P. Silva, J. Moutinho-Pereira, E. Bacelar, E. Rosa, A.S. Meyer, *Food Chemistry*, **2007**, *103*, 976.
- 3. V. Usenik, J. Fabčič, F. Štampar, Food Cehmistry, 2008, 107, 185.
- 4. D. -O. Kim, H.J. Heo, Y.J. Kim, H.S. Yang, C.Y. Lee, *Journal of Agricultural and Food Chemistry*, **2005**, *53*, 9921.
- 5. D. Faniadis, P.D. Drogoudi, M. Vasilakakis, Scientia Horticulturae, 2010, 125, 301.
- 6. G. Ferretti, T. Bacchetti, T. Belleggia, D. Neri, Molecules, 2010, 15, 6993.

- 7. A. Chaovanalikit, A. Wrolstad, Food Chemistry and Toxicology, 2004, 69, 73.
- 8. M.J. Serradilla, A. Martín, A. Hernandez, A. López-Corrales, M. Lozano, M. de Guía Córdoba, *Journal of Agricultural and Food Chemistry*, **2010**, *58*, 9157.
- 9. D. Prvulović, Dj. Malenčić, M. Popović, M. Ljubojević, V. Ognjanov, *World Academy of Science, Engineering and Technology*, **2011**, 59, 1149.
- 10. V. Dragović-Uzelac, B. Levaj, D. Bursać, S. Pedisić, I. Radojčić, A. Bišo, *Agriculturae Conspectus Scientificus*, **2007**, *72*, 279.
- 11. S. Melicháčová, M. Timoracká, J. Bystrická, A. Vollmannová, J. Čéry, *Acta Agriculturae Slovenica*, **2010**, 95, 21.
- 12. G.M. Khoo, M.R. Clausen, B.H. Pedersen, E. Larsen, *Journal of Food Composition and Analysis*, **2011**, *24*, 772.
- 13. M.N. Mitić, M.V. Obradović, D.A. Kostić, R.J. Micić, E.T. Pecev, *Chemical Industry & Chemical Engineering Quartely*, **2012**, *18*, 53.
- 14. G.A. Boeckler, J. Gershenzon, S.B. Unsicker, *Phytochemistry*, **2011**, 72, 1497.
- 15. R.V. Barbehenn, C.P. Constabel, Phytochemistry, 2011, 72, 1551.
- 16. L. Dykes, L.W. Rooney, M, Cereal Foods World, 2007, 52, 105.
- 17. L. Esti, F. Cinquanta, E. Sinesio, E. Moneta, M. Di Matteo, *Food Chemistry*, **2002**, *76*, 399.
- 18. Zs. Veres, I. Holb, J. Nyéki, Z. Szabó, J. Remenyik, M.G. Fári, *International Journal of Horticultural Science*, **2006**, *12*, 45.
- 19. E. Kállay, M. Stéger-Máté, M. Mester-Ficzek, G. Sándor, G. Bujdosó, M. Tóth, *Acta Biologica Szegediensis*, **2008**, *52*, 217.
- 20. N. Papp, B. Szilvássy, Z. Szabó, J. Nyéki, É. Stefanovits-Bányai, A. Hegedűs, *International Journal of Horticultural Science*, **2008**, *14*, 59.
- 21. G.A. Cooper-Driver, *Phytochemistry*, **2001**, *56*, 229.
- 22. D. Prvulović, M. Popović, Dj. Malenčić, M. Ljubojević, V. Ognjanov, *Research Journal of Agricultural Science*, **2011**, 43, 198.
- 23. F. Cuyckens, M. Claeys, Journal of Mass Spectrometry, 2005, 40, 364.
- 24. A.T. Serra, R.O. Duarte, M.R. Bronze, C.M.M. Duarte, *Food Chemistry*, **2011**, *21258*, 318.
- 25. G.T. Kroyer, *Inovative Food Science and Emerging Technologies*, **2004**, 5, 101.
- 26. H.P.S. Makkar, "Quantification of Tannins in tree Foliage-a Laboratory Manual", FAO/IAEA Working Document, Vienna, **2000**.
- 27. K.R. Marckam, "Methods in Plant Biochemistry", Academic Press, London, **1989**, 16.
- 28. S.C. Shen, K.C. Tseng, F.T. Chao, S.B. Wu, Food Quality, 2007, 30, 202.
- 29. N. Abe, T. Murata, A. Hirota, *Bioscience, Biotechnology, Biochemistry*, **1998**, 62, 661.