Bulgarian Journal of Agricultural Science, 29 (No 3) 2023, 446–451

Phenolic compounds and antioxidant activity of seven Bulgarian cultivars sweet cherry (*Prunus avium* L.)

Mariya Georgieva[1*], Simeon Krumov[2], Teodora Ivanova[3], Desislava Dimitrova[3] and Rositsa Titorenkova[1]

[1] *Bulgarian Academy of Sciences, Institute of Mineralogy and Crystallography "Acad. Ivan Kostov", 1113 Sofia, Bulgaria*
[2] *Agricultural Academy, Institute of Agriculture-Kyustendil, 2500 Kyustendil, Bulgaria*
[3] *Bulgarian Academy of Sciences, Institute of Biodiversity and Ecosystem Research, 1113 Sofia, Bulgaria*
[*] *Corresponding author:* mgeorgieva@imc.bas.bg

Abstract

Georgieva, M., Krumov, S., Ivanova, T., Dimitrova, D. & Titorenkova, R. (2023). Phenolic compounds and antioxidant activity of seven Bulgarian cultivars sweet cherry (*Prunus avium* L.). *Bulg. J. Agric. Sci., 29 (3)*, 446–451

The phenolic compounds and antioxidant activity of cherries from seven Bulgarian cultivars (Kyustendilska Chrustyalka, Cherna Konyavska, Bulgarian Bigarreau, Mizia, Stefania, Dima, and Vasinika), were evaluated in this paper. The presented results are part of studies, aimed at characterizing the qualities of Bulgarian sweet cherry varieties and comparing them with the world-spread industrial varieties. The total polyphenols in sweet cherry fruits examined in this study ranged from 1.08 to 4.11 mg GAE/g. Total flavonoids varied from 0.69 to 1.54 mg QE/g. The amounts of total monomeric anthocyanins were between 0.1 to 2.91 mg CR/g, being the highest for the cultivar Vasinika fruits. The Vasinika extract possessed the strongest antioxidant activity in both tests (FRAP and DPPH). Analysis of the antioxidant activity and polyphenol content of these Bulgarian cherry cultivars was carried out for the first time.

Keywords: anthocyanins; antioxidant activity; sweet cherry; total polyphenols
Abbreviations: ANOVA – analysis of variance; CR – cyanidin 3-rutinoside; DPPH – 2,2-diphenyl-1-picrylhydrazyl; FRAP – ferric ion reducing antioxidant power; GAE – gallic acid equivalents; QE – quercetin equivalents; TE – Trolox equivalents; TF – total flavonoids; TMA – total monomeric anthocyanins; TP – total polyphenols; UV-VIS – spectrophotometer, ultraviolet-visible spectrophotometer.

Introduction

Sweet cherry fruits (*Prunus avium* L.) contain a significant amount of dry matter, sugars, minerals, organic acids, dyes and tannins. The dominance of sugars determines the sweet taste, which combined with the aroma and low acidity, makes them especially desirable for fresh consumption (Georgiev et al., 2001). In recent years, there has been a growing interest in research, related to the content of natural biologically active substances with therapeutic and protective potential (Georgieva et al., 2017; Filaferro et al., 2022). Sweet cherry fruits possess a high quantity of polyphenols and anthocyanins, which determine their anti-cancer antioxidant and anti-inflammatory properties (Kaur & Kapoor, 2001; Seeram et al., 2002; Dragan et al., 2008; Pandey & Rizvi, 2009; Ferretti et al., 2010; Topalov et al., 2017, Uribe et al., 2023; Vega et al., 2023). Sweet cherry fruits comprise significant amounts of flavonoids, such as anthocyanins, flavan-3-ols and flavanols. The main anthocyanins include cyanidin3-O-rutinoside (red-purple) and cyanidin 3-O-glucoside (orange-red), followed by peonidin 3-O-rutinoside (orange-red) and peonidin 3-O-glucoside (orange-red color). Pelargonidin 3-O-rutinoside is in much lower quantities (Gonçalves et al., 2007). The total anthocyanin content was found to vary from 2 to 300 mg/100 g (Valero & Serrano, 2010). The total phenols content and antioxidant activity in fruits depend on genotype, phase of ripeness, cultivar technique, and the environmental conditions during the year of studies (Gonçalves et al., 2007; Faniadis et al., 2010; Magri et al., 2023; Ockun et al., 2022). Cultivars with lighter skin color are with a lower amount of anthocyanins from 2 to 47.1 mg 100/g, compared with the dark-colored ones, where the content varies from 82 to 297 mg (Gao & Mazza, 1995).

The environmental conditions of the cultivation of sweet cherries influence the quantity and type of polyphenols. Usenik et al. (2008) reported, that the total polyphenols content of cherry cultivars from Northern Europe varied from 44.3 (Lapins) to 87.9 mg/100 g (Ferprime). Ballistreri et al. (2013) and Tomás-Barberán et al. (2013) determined, that the concentration of total phenols ranged from 84,96 (Napoleona Grappolo) to 192 mg/100 g (Sonata) for cultivars grown in southern Europe. According to Serrano et al. (2005), the content of polyphenols is highest at the stage of consumable ripeness of fruits.

Topalov et al. (2017) demonstrated, that the quantitative content of total polyphenols and anthocyanins in sweet cherry fruits (Early Van, Van, Cartilage Bing, Bigato Burla, and wild cherry), depends on the altitude and the time of ripening of the fruits. The authors have been found late-maturing varieties contain fewer anthocyanins.

Krumov & Christov (2018) reported, that the biological properties of the cultivar play a substantial role in the transportability of sweet cherry fruits. Cultivars Mizia and Bing were characterized as "very good" and Vasinika had "good" transportable qualities. Picking the fruits from their stems was found to be relatively "easy" for the cultivar Vasinika, "easy to medium-hard" for Van, and "hard" for Mizia. Vasinika distinguishes itself by "easily" detaching fruits from stems with no juice leakage. These characteristics make it the best cultivar for mechanized harvesting.

Mizia, Stefania and Bulgarian bigarreau have very good resistance to cold and late spring frost and high fertility (Georgiev et al., 2001).

As far as we know, there are no studies on the content of biologically active compounds, such as polyphenols, flavonoids, anthocyanins and their antioxidant activity of sweet cherry fruits of Bulgarian cultivars.

The aim of the present study was to determine the content of total polyphenols, total flavonoids, total monomeric anthocyanins, and antioxidant activity in seven Bulgarian cultivars of sweet cherries, selected at the Institute of Agriculture – Kyustendil.

Materials and Methods

The studied sweet cherry cultivars were Kyustendilska chrustyalka (Romanka x mixed pollen from Early by Ville and Razdavichka belvitsa), Cherna Konyavska (Romanka x Razdavichka belvitsa), Bulgarian bigarreau (seedling № 34 x Kozerska), Mizia (Lambert x Kozerska), Stefania (Compact Lambert x Stella 35 B-11), Dima (Van x Stella), and Vasinika (Van x Stella). The cultivars have been created at the Institute of Agriculture – Kyustendil by Vasil Georgiev and Nikolay Christov in the period 1955-2017.

Ripe fruit samples were collected from the experimental orchard of the Institute of Agriculture – Kyustendil in 2020 year. The fruits were frozen immediately after harvesting and stored at –20ºC until analysis.

The soil in the experimental plantation is highly leached, slightly sandy-clay cinnamon forest soil with a neutral reaction. The average monthly air temperatures for the months of April to July in 2020, were below the region's multiannual

Table 1. Climate conditions in the Kyustendil region during the vegetative season. 2020

Month	Precipitation		Average monthly air temperature	Total temperature sum	Hydrothermal coefficient
	number	*mm*	*t°C*	∑*t*°C	*HTC*
Multiannual norm. 1904–2008					
April	–	*53.0*	11.2	–	–
May	–	65.0	15.4	–	–
June	–	64.0	19.1	–	–
July	–	60.0	21.8	–	–
2020					
April	7	63.5	8.9	242.3	2.62
May	6	25.8	9.6	376.3	0.68
June	11	41.8	17.2	458.2	0.91
July	5	62.9	19.4	562.9	1.11

norm, affecting the occurrence of specific phases of the annual plant cycle. Lower average temperatures, especially in the last 15 years, have been unusual in the context of constant global warming. There have been no reports of prolonged drought. For the Kyustendil region, the number and amount of precipitation during fruit ripening is about average. In June and July, the hydrometric coefficient is about 1, indicating that the time is relatively humid (Table 1).

Extraction

5 g of thawed fruits after removing the stones and stems were homogenized with 50 cm^3 0.1% (v/v) HCl /methanol solution and were left for 24 h at 4ºC.

Methods

The Folin-Ciocalteau method was used to measure total polyphenols (TP), which were represented as mg gallic acid equivalents (GAE) per gram of frozen fruits (Current protocols in Food Analytical Chemistry, 2011).

A colorimetric method with aluminum trichloride was used to determine the total flavonoids (TF). The results were expressed as mg quercetin equivalents (QE), per g of frozen fruits (Gouveie & Castilho, 2011).

Total monomeric anthocyanins (TMA) were determined by a pH-differential method (Current protocols in Food Analytical Chemistry, 2011). The results were calculated, using a molar absorptivity of 7000 L/(mol.cm) and molecular weight of 326.2 g/mol, and expressed as equivalents of cyanidin 3–rutinoside (CR) in mg per g of the frozen fruits.

The antioxidant activity of fruit extracts was measured spectrophotometrically with UV-VIS spectrophotometer Cary 100 using DPPH (Brand-Williams et al., 1995) and FRAP assays (Benzie & Strain, 1996). Trolox as a water-soluble vitamin E analog was used as a reference in both methods and the antioxidant capacity values were expressed as μmol Trolox equivalents (TE) per g of the frozen fruits.

All analyses were carried out at least in triplicate.

Statistical analysis

Microsoft Excel was used to calculate the statistics. The analysis of variance was carried out using ANOVA techniques.

Results and Discussion

The obtained results are summarized and presented in Table 2. The presented values were obtained from three parallel measurements. It can be seen that the total polyphenols content are ranged from 1.08 to 4.11 mg GAE per g frozen fruits. The highest TP content was in the Vasinika (4.11 mg/g) cultivar, followed by the Kyustendilska chrustyalka (2.41 mg/g) and Mizia (2.10 mg/g). The Stefania cultivar was with the lowest TP (1.37 mg/g). Genetic, environmental, and agricultural factors affect the content of polyphenols and their antioxidant properties of fruits (Ferretti et al., 2010; Faniadis et al., 2010; Pissard et al., 2016).

Following the content of TMA in Table 2, the richest cultivar was Vasinika (2.91 mg/g), and the one with the smallest quantity of them was Stefania (0.1 mg/g*)*. The other cultivars varied in the range of 0.16 to 0.48 mg/g. Factors, such as season, climate, growth, fruit type, degree of ripeness, food preparation, storage, and processing, strongly affect the quantity and type of flavonoids (Marinova et al., 2005; Wang et al., 2008; Wang, 2006).

Table 2.Total polyphenol. Flavonoids anthocyanins and antioxidant capacity of different Bulgarian sweet cherry cultivars

Cultivar	Total polyphenols[a,e]	Total flavanoids[b,e]	Total monomeric anthocyanin[sc,e]	Antioxidant capacity[d,e]	
				FRAP	DPPH
Bulgarian bigarreau	1.76±0.03	0.69±0.02	0.35±0.09	3.62±0.03	26.45±0.13
ChernaKonyavska	1.86±0.03	0.98±0.03	0.35±0.03	7.77±0.02	26.05±0.12
Dima	1.73±0.03	0.92±0.02	0.16±0.04	6.01±0.08	25.69±0.06
Kyustendilskachrustyalka	2.41±0.03	1.08±0.02	0.48±0.12	7.25±0.08	25.38±0.11
Mizia	2.10±0.05	1.02±0.01	0.40±0.17	9.02±0.08	25.50±0.11
Stefania	1.37±0.04	0.83±0.01	0.10±0.02	3.92±0.14	26.68±0.18
Vasinika	4.11±0.05	1.54±0.22	2.90±0.13	11.41±0.22	31.30±0.09

[a] Results are expressed as mg gallic acid equivalents per g frozen fruits

[b] Results are expressed as mg quercetin equivalents per g frozen fruits

[c] Results are expressed as mg cyanidin 3-rutinoside per g frozen fruits

[d] Results are expressed as μmol Trolox equivalents per g frozen fruits

[e] Mean value ± SD ($n \geq 3$).

Table 3. Correlations between antioxidant capacity and individual phenolics' compound in studied cherry fruits.

	FRAP		DPPH	
	Correlation coefficient (r)	Coefficient of determination (r^2)	Correlation coefficient (r)	Coefficient of determination (r^2)
TMA	0.756	0.571	0.912	0.832
TF	0.935	0.874	0.667	0.445
TP	0.864	0.747	0.755	0.570

Antioxidant activity is the strongest in Vasinika for both tests. A significant difference in antioxidant activity of studied fruit extracts was found for different cherry cultivars with FRAP assay. The values of FRAP varied in the range of 2.9 to 11.4 µmol TE/g.

A correlation between antioxidant activity and individual phenolic' compounds in studied cherry fruits was done statistically. The results are shown in Table 3. There was a clear correlation between anthocyanins and DPPH assay, respectively, total flavonoids and FRAP assay. The obtained coefficients of determination corroborate this correlation between anthocyanins and DPPH assay and the flavonoids and FRAP assay. Other coefficients of determination (between total flavonoids and DPPH assay; total anthocyanins and FRAP assay), were not so high. The presence of other compounds with an antioxidant activity, but not polyphenols is the possible reason for such. This correlates well with earlier results (Faniadis et al., 2010; Prvulović et al., 2019).

Vasinika was found to be the richest of total anthocyanins and with a 39% quantity of flavonoids as is shown in Figure 1. Stefania and Dima had approximately 50-60% total flavonoids, but the anthocyanins were with low concentration. Kyustendilska chrustyalka, Mizia, Cherna Konyavska, and Bulgarian bigarreau had respectively, around 20% anthocyanins and 40 to 53% of flavonoids. The composition and concentration of metabolites (including secondary metabolites such as polyphenols) are affected by genetics, environmental factors, and cultivation techniques, and therefore, vary widely from year to year (Faniadis et al., 2010; Goncalves et al., 2004).

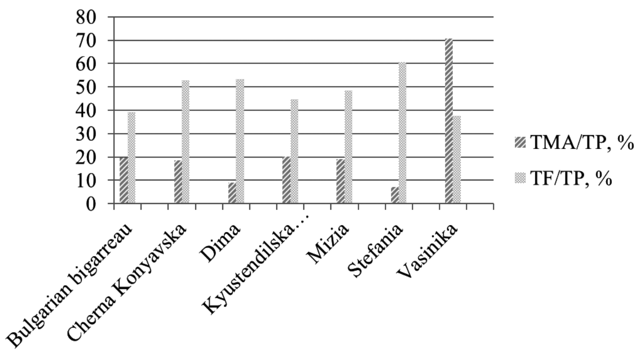

Fig. 1. Relative content of total anthocyanins and flavonoids in sweet cherry cultivars

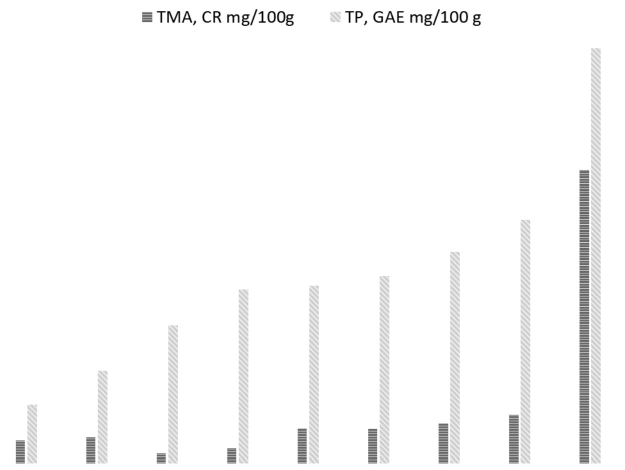

Fig. 2. Amount of TMA and TF in Bulgarian cultivars sweet cherry. Bing and Van (Hayaloglu & Demir. 2015)

The studied Bulgarian cultivars were compared with certain cultivars (Hayaloglu & Demir, 2015), presented in Figure 2. The values of TP and TMA were recalculated for 100 g frozen fruits for the purposes of comparison.

Conclusions

Both polyphenols content and antioxidant activity of seven Bulgarian sweet cherry cultivars were precisely determined. The acquired results regarding the polyphenol and anthocyanin contents were further, compared with the literature data for the world-spread *Bing* and *Van*. The amount of polyphenols in studied sweet cherry fruits is 1.5 to 3 times more than the variety *Bing* and 2.3 to 7 times more than the variety *Van*.

The total monomeric anthocyanins vary in the Bulgarian sweet cherries, as the cultivars, Stefania and Dima contain smaller quantities, compared to *Bing* and *Van*. The Bulgarian Bigareau has comparable anthocyanin contents with the *Bing* and *Van*, and the cultivar Vasinika and Kyustendilska chrustyalka have significantly higher values of the measured total anthocyanins.

Our results reveal the cultivar Mizia can be one of the preferred, because of its relatively high amounts of TP, TF, and TMA and possessing its resistance to cold and high fertility.

The high percentage of anthocyanins (78%) in the Vasinika variety determines its high antioxidant activity. It has the best assessment for polyphenol compounds as flavonoids and anthocyanins and antioxidant activity. This study provides a basis for more detailed research content of bioactive compounds of these cultivars.

Acknowledgements

The study is funded by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life", approved by DCM # 577/17.08.2018.

The authors acknowledge the technical support from the project PERIMED BG05M2OP001-1.002-0005 /29.03.2018 (2018–2023).

References

Ballistreri, G., Continella, A., Gentile, A., Amenta, M., Fabroni, S. & Rapisarda, P. (2013). Fruit quality and bioactive compounds relevant to human health of sweet cherry (*Prunus avium* L.*)* cultivars grown in Italy. *Food Chem., 140*(4), 630-638.

Benzie, I. & Strain, J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem.,239*(1)*,* 70-76.

Brand-Williams, W., Cuvelier, M. & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft und Technologie, 28*(1), 25-30.

Current Protocols in Food Analytical Chemistry (2011). *John Wiley & Sons, Inc*., F1.2.1-F1.2.13.

Dragan, S., Iosif, G. & Socaciu, C. (2008). Functional nutrition with natural bioactive components in the methabolic syndrome, *Ed. Timişoara Eurostampa*.(Ro).

Faniadis, D., Drogoudi, P. D. & Vasilakakis, M. (2010). Effects of cultivar, orchard elevation, and storage on fruit quality characters of sweet cherry (*Prunus avium* L.*). Scientia Horticulturae, 125*(3)*,* 301–304.

Ferretti, G., Bacchetti, T., Belleggia, A. & Neri, D. (2010). Cherry Antioxidants: From Farm to Table. *Molecules, 15*(10),6993-7005.

Filaferro, M., Codeluppi, A., Brighenti, V., Cimurri, F., González-Paramás, A., Santos-Buelga, C., Bertelli, D., Pelatti, F. & Vitale, G. (2022). Disclosing the Antioxidant and Neuroprotective Activity of an Anthocyanin-Rich Extract from Sweet Cherry (*Prunus avium* L.) Using In Vitro and In Vivo Models. *Antioxidants, 11*(2), 211.

Gao, L. & Mazza, G. (1995). Characterization, quantitation, and distribution of anthocyanins and colorless phenolics in sweet cherries. *J. Agric. Food Chem., 43*(2), 343–346.

Georgiev, V., Borovinova, M. & Koleva, A. (2001). Sweet cherry. *Zemizdat,* Sofia, 352, (Bg).

Georgieva, G., Dobreva, D., Panayotova, V. & Merdzhanova, A. (2017). Phenolic compounds in edible plants. Proceedings from the Sixth Scientific Session of the Medical College of Varna, *6*(2), 509-517, (Bg).

Goncalves, B., Landbo, A. K., Knudsen, D., Silva, A. P., Moutinho-Pereira, J., Rosa, E. & Meyer, A. S. (2004). Effect of ripeness and postharvest storage on the phenolic profiles of cherries (*Prunus avium* L.). *J. Agr. Food Chem., 52*(3), 523-530.

Gonçalves, B., Silva, A. P., Moutinho-Pereira, J., Bacelar, E., Rosa, E. & Meyer, A. S. (2007). Effect of ripeness and postharvest storage on the evolution of colour and anthocyanins in cherries (*Prunus avium* L.). *Food Chem., 103*(3), 976–984.

Gouveie, S. & Castilho, P. (2011). Antioxidant potential of Artemisia argenteaL'Hér alcoholic extract and its relation with the phenolic composition. *Food Research Int., 44*(6), 1620-1631.

Hayaloglu, A. A. & Demir, N. (2015). Physicochemical characteristics, Antioxidant Activity, Organic Acids and Sugar Contents of 12 Sweet Cherry (*Primus Alvium* L.*)* Cultivars Grown in Turkey. *J. Food Sci., 80*(3), 564-570.

Kaur, C. & Kapoor, H. (2001). Antioxidant in fruits and vegetables the milleium's health*. Int. J. Food Sci. Technol., 36*(7), 703-725.

Krumov, S. & Christov, N. (2018). Mechanical properties of the fruits of some perspective sweet cherry cultivars. University of Agronomic Sciences and Veterinary Medicine of Bucharest. Faculty of Horticulture, *Bucharest.Scientific Papers. Series B, Horticulture. l. LXII,* Bucharest, 21-24.

Magri, A., Malorni, L., Cozzolino, R., Adiletta, G., Siano, F., Picariello, G., Cice, D., Capriolo, G., Nunziata, A., Matteo, M. & Petriccione, M. (2023). Agronomic, Physicochemical, Aromatic and Sensory Characterization of Four Sweet Cherry Accessions of the Campania Region. *Plants*, *12*(3), 610.

Marinova, D., Ribarova, F. & Atanassova, M. (2005). Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *J. Chem. Technol. Metall., 40*(3), 255-260.

Ockun, M., Gercek, Y., Demirsoy, H., Demirsoy, L., Macit, I. & Oz, G. (2022). Comparative evaluation of phenolic profile and antioxidant activity of new sweet cherry (*Prunus avium* L.) genotypes in Turkey. *Phytochem Anal, 33*(4), 564-576.

Pandey, K. & Rizvi, S. (2009). Plant polyphenols as dietary antioxidants in human health and disease.*Oxidative Medicine and Cellular Longevity, 2*(5), 270-278.

Pissard, A., Lateura, M., Baetenb, V., Mageinc, H., Duponta, P., Tabartd, J., Pincemaile, J. & Kevers, C. (2016). Determination of total phenolic compound content and antioxidant activity in cherry species and cultivars. *J. of Berry Research, 6*(1), 81–91.

Prvulović, D., Malenčić, Đ., Ljubojević, M., Barać, G. & Ognjanov, V. (2019). Phenolic Compounds and Antioxidant Capacity of Sweet Cherry Fruits from Vojvodina Province. *Contemporary Agriculture, 68*(1-2), 1-6.

Seeram, N. P., Momin, R. A., Nair, M. G. & Bourquin, L. D. (2002). Cyclooxygenase inhibitory and antioxidant cyaniding glycosides in cherries and berries. *Phytomedicine, 8*(5), 362-369.

Serrano, M., Guillen, F., Martinez-Romero, D., Castillo, S. & Valero, D. (2005). Chemical Constituents and Antioxidant Activity of Sweet Cherry at Different Ripening Stages*. J. Agric. Food Chem., 53*(7), 2741-2745.

Tomás-Barberán, F. A., Ruiz, D., Valero, D., Rivera, D., Obón, C., Sánchez-Roca, C. & Gil, M. (2013). Health benefits from pomegranates and stone fruit, including plums, peaches, apricots and cherries. In: Skinner, Hunter (Eds.), Bioactives in Fruit: Health Benefits and Functional Foods, first ed*. John Wiley & Sons, Ltd,* 125–167.

Topalov, M., Dermenjiev, T. & Phortunov, S. (2017). Wild and Cultivated Cherries – Antioxidant Capacity Analysis. *Eur. Sci. J., 13*(33), 44-52.

Uribe, E., Pasten, A., Mejias, N., Vega-Galvez, A. (2023). Valorization of Sweet Cherries (*Prunus avium*) Using Different Drying Methods for Obtaining a Healthy Dried Product. *ACS Food Sci. Technol. 3*(2), 301–309.

Usenik, V., Fabĉiĉ, J. & Ŝtampar, F. (2008). Sugars, organic acids, phenolic composition, and antioxidant activity of sweet cherry (*Prunus avium* L.). *Food Chem., 107*(1), 185–192.

Valero, D. & Serrano, M. (2010). Postharvest Biology and Technology for Preserving Fruit Quality. *CRC Press,* Boca Raton, Florida.

Vega, E., García-Herrera, M., Cuidad-Mulero, M., Dias, M., Matallana-González, M., Cámara, M., Tardío, J., Molina, M., Pinela, J., Pires, T., Barros, L., Fernández-Ruiz, V. & Morales, P. (2023). Wild sweet cherry, strawberry and bilberry as underestimated sources of natural colorants and bioactive compounds with functional properties. *Food Chem*, *414,* 135669, 1-12.

Wang, S. Y., Chen, C. T., Sciarappa, W. & Camp, M. J. (2008). Fruit quality, antioxidant capacity, and flavonoid content of organically and conventionally grown blueberries. *J. Agric. Food Chem., 56*(14), 5788-5794.

Wang, S. Y. (2006). Effect of Pre-harvest Conditions on Antioxidant Capacity in Fruits. *Acta Hort., 712,* 299-306.

Received: **November, 18, 2021**; *Approved*: **January, 07, 2022;** *Published:* **June, 2023**