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Season, location and cultivar influence on bioactive compounds of sour cherry fruits

Marija VILJEVAC VULETIĆ[1,*], Krunoslav DUGALIĆ[2], Ines MIHALJEVIĆ[1], Vesna TOMAŠ[1], Dominik VUKOVIĆ[1], Zvonimir ZDUNIĆ[1], Boris PUŠKAR[2], Zorica JURKOVIĆ[1]

[1]Agricultural Institute Osijek, Osijek, Croatia
[2]Croatian Centre for Agriculture, Food and Rural Affairs, Zagreb, Croatia
**Corresponding author: marija.viljevac@poljinos.hr*

ABSTRACT

Viljevac Vuletić M., Dugalić K., Mihaljević I., Tomaš V., Vuković D., Zdunić Z., Puškar B., Jurković Z. (2017): Season, location and cultivar influence on bioactive compounds of sour cherry fruits. Plant Soil Environ., 63: 389–395.

The aim of this study was to determine how different locations, years and cultivars influenced polyphenol and anthocyanin content in fruits of different sour cherry cultivars (*Prunus cerasus* L.). Fruits of five sour cherry cultivars were harvested in two locations (Osijek and Zadar) over three consecutive years (2010, 2011 and 2012). Factorial ANOVA showed that year and location significantly influenced the accumulation of polyphenols and anthocyanins in sour cherry fruits. 2010 was the best year with 9.89 mg/g of polyphenols and 5.08 mg/g of anthocyanins on average. Although year and location revealed a strong influence, cultivar is the principal source of variation, as it is proved by the polyphenol content in the range from 5.89 to 10.78 mg/g and the anthocyanin content in the range from 3.15 to 4.76 mg/g. Cv. Maraska appears to be the most significant source of bioactive compounds, while cvs. Heimanns Konservenweichel and Rexelle gave very similar but significantly lower contents of polyphenols and anthocyanins than cv. Maraska. Cv. Oblačinska had significantly the lowest contents of investigated bioactive compounds.

Keywords: antioxidants; climate conditions; environmental factor; growing season; orchard

Sour cherry (*Prunus cerasus* L.) is a popular fruit species in professional fruit growing as well as household or hobby horticulture. It is an important fruit in the Croatian fruit production and processing industry. The products, such as jams, juice concentrates, frozen sour cherries, alcoholic drinks and other, are rich in antioxidants such as polyphenols, especially anthocyanins (Levaj et al. 2010, Pedisić et al. 2010). There is a considerable interest in polyphenols because they display a broad spectrum of health-promoting benefits such as prevention of cardiovascular diseases, cancers, and osteoporosis as well as neurodegenerative diseases and diabetes mellitus (Scalbert et al. 2005). Polyphenolic molecules have hydrogen donor capability, which allows them to act as antioxidants. Moreover, they also have a reducing capability (Kállay et al. 2008). Polyphenols interact with cellular signalling pathways and related machinery that mediate cell function under both normal and pathological conditions (Vauzour et al. 2010).

In cherry fruits, polyphenols are meritorious for bitterness, astringency, colour, flavour, odour and oxidative stability (Pandey and Rizvi 2009). The nature and the distribution of phenolics differ by plant tissue, with many of the phenolics synthesized from carbohydrates via the shikimate and phenylpropanoid pathways (Ferretti et al. 2010). Fruit skin contains high amounts of polyphenolics

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and anthocyanins; substantial amounts are however present also in the flesh and pit (Chaovanalikit and Wrolstad 2004). The amounts and content of polyphenols in fruits vary tremendously. Variations are influenced by genotype, maturity, climatic conditions in season and location (Tomás-Barberán and Espín 2001, Šimunić et al. 2005, Pedisić et al. 2010). Data regarding to seasonal as well as geographical differences of sour cherry polyphenols and anthocyanins are, however, lacking. The aim of this study was to quantify total polyphenols and anthocyanins content and to determine how different cultivars, locations and years influence polyphenol and anthocyanin content in fruits of different sour cherry cultivars grown in Croatia.

MATERIAL AND METHODS

Plant material and experimental design. The research was conducted with five sour cherry cultivars (Oblačinska, Maraska, Rexelle, Heimanns Konservenweichel and Kelleris 16) grown in experimental orchards of the Agricultural Institute Osijek in two locations of Croatia – Osijek (Slavonia region) and Zadar (Dalmatia region). Experimental orchard Tovljač in the Osijek location is situated at 89 m a.s.l. (45°32'N, 18°38'E) with Eutric Cambisol soil and continental climate conditions. Orchard Vlačine in the Zadar location lies at 95 m a.s.l. (44°8'N, 15°22'E) on deep red soil with partial stone debris in zone with Mediterranean climate. Both experimental orchards were planted in 2007. All of five sour cherry cultivars were grafted on *Prunus mahaleb* L. rootstock. Experiment was a completely random block design in four repetitions per cultivar with three sour cherry trees in block. Orchards were maintained with permanent cultivation without irrigation and grass layer. Trees were planted at 5 × 4 m spacing.

Climatic conditions over the three experimental years. In Osijek, over the three experimental seasons (from April to July), the year 2010 was characterized by the total rainfall of 457.4 mm, while years 2011 and 2012 in same period had less rainfalls, namely 225.3 mm and 256.5 mm, respectively (Figure 1a). In the contrary, location Zadar in the same periods had around two fold less rainfalls than location Osijek in years 2010 (207.3 mm) and 2011 (123.9 mm), while year 2012 was characterized by 192.6 mm of rainfall, similar as year 2010 (Figure 1b). Mean temperatures were relatively similar in both locations (Figure 1).

Extraction procedure. Sour cherry fruits were harvested in full maturity over three consecutive years (2010, 2011 and 2012) in two locations of Croatia. 500 g of sour cherry fruits were harvested from all three trees in one block and homogenated to get one pooled sample per block. Immediately after harvesting, fruits were frozen at –20°C. Defrosted sour cherries were de-pitted and homogenized in blender, weighed (~1 g), and total polyphenols and anthocyanins were extracted with 10 mL of acidified methanol (1% hydrochloric acid) for 60 min in an ultrasonic bath. After centrifugation at 4000 g for 15 min, supernatants were used for spectrophotometrical determination of total polyphenols and anthocyanins content.

Total polyphenol content. Total polyphenols were determined by the Folin-Ciocalteu method

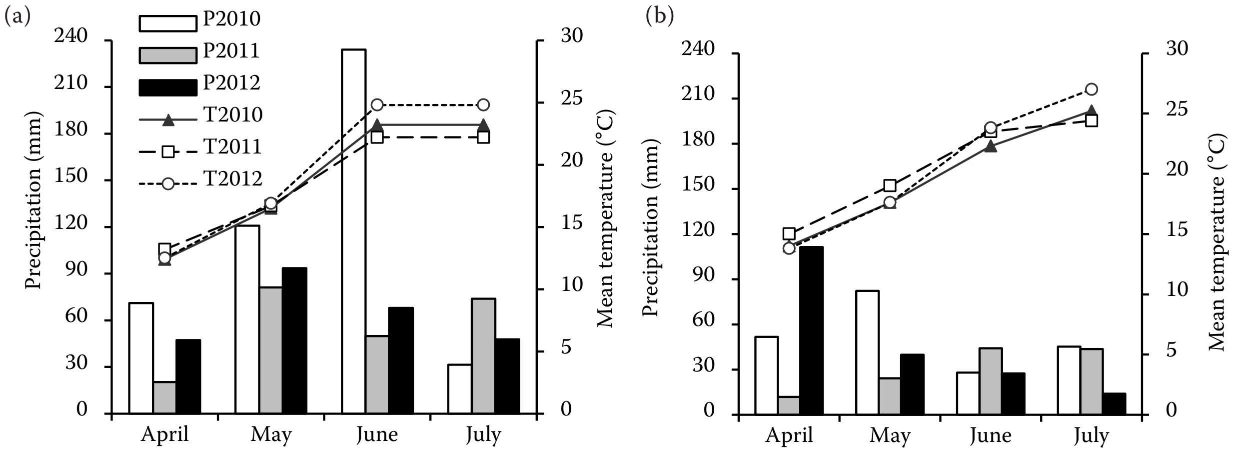

Figure 1. Temperatures (T) and precipitations (P) during the vegetation period from 2010 to 2012 at (a) Osijek and (b) Zadar

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(Slinkard and Singleton 1977) modified in the micro method as follows. An aliquot (100 μL) of diluted sour cherry extract (dilution factor 3) was mixed with 2 mL of sodium carbonate solution (2% w/v) and 100 μL of Folin-Ciocalteu reagent. After incubation at room temperature for 30 min in dark, the absorbance was read against the blank at 765 nm (Specord 200, Analytic Jena, Germany). Total polyphenols were expressed as mg of gallic acid equivalents per g of fruits fresh weight (FW). The measurement was carried out in four replicates and data are shown as a mean value.

Total anthocyanin content. Total anthocyanins were estimated by a pH-differential method (Giusti and Wrolstad 2001). Two dilutions of extract (dilution factor 10) were prepared, one with potassium chloride buffer (0.025 mol/L; pH 1.0) and the other with sodium acetate buffer (0.4 mol/L; pH 4.5). Absorbance was measured simultaneously at 510 nm and 700 nm after l5 min of incubation at room temperature in the dark. The content of total anthocyanins was expressed in mg of cyanidin-3-O-glucoside equivalents per g of fruits fresh weight using a molar extinction coefficient (ε) of cyanidin-3-O-glucoside of 26 900 dm^3/mol cm and molar weight (MW) (449.2 g/mol). The measurement was carried out in four replicates and data are shown as a mean value.

Data analysis. Values of polyphenol and anthocyanin content per block were analysed by factorial analysis of variance (ANOVA) and the mean value for cultivar is the average of four blocks. All factors and their interactions were compared with the post-hoc least significant difference (*LSD*) test using pooled error. All statistical analyses were done with Statistica 7.1. software (StatSoft, Inc. 2005) and differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

A factorial analysis of variance across the two locations (Osijek and Zadar) and three consecutive but different years as to climate (2010, 2011 and 2012) on five investigated sour cherry cultivars showed highly significant differences in the total polyphenol and anthocyanin content between the years, locations, and cultivars as well as significant interactions of year with cultivar, year with location and year × cultivar × location (Table 1).

Total polyphenol content. The total polyphenol content of different sour cherry cultivars investigated during three years in two locations, expressed in mg/g, is shown in Table 2. Average values of the total polyphenol content over three investigated years in the Osijek location were between 4.87 and 12.76 mg/g (Table 2). The smallest polyphenol content was found for cv. Oblačinska but the highest for cv. Heimanns. The same cultivars in the Zadar location revealed slightly higher three-year average values of total polyphenol content in the range from 6.04 to 11.13 mg/g for cvs. Oblačinska and Maraska, respectively (Table 2). These values are higher than data reported by Khoo et al. (2011) in 34 sour cherry cultivars, in the range from 74 to 754 mg/100 g of total polyphenols. Other previous reports on the total polyphenol content of sour cherries varied from 78 to 500 mg/100 g (Kim et al. 2005, Bonerz et al. 2007, Dragović-Uzelac et al. 2009). Higher concentrations of polyphenols in our investigation as compared to other studies may be the result of differences in extraction and measuring protocols (Melicháčová et al. 2010) performed by a group of several authors in polyphenol determination but also it can be a consequence of cultivar impact, climatic conditions and agricultural measures in specific location were sampling is done.

Total anthocyanin content. Variability of total anthocyanin content among the investigated cultivars during three years in two locations is

Table 1. *F*-distribution and the probability levels in the factorial ANOVA testing for total polyphenol and anthocyanin content in five sour cherry cultivars during three experimental years (2010, 2011 and 2012) in two locations (Osijek and Zadar)

Effect	Total polyphenol content	Total anthocyanin content
Year	248.01***	596.40***
Location	10.63**	36.37***
Cultivar	411.89***	120.76***
Year × location	17.18***	21.35***
Year × cultivar	29.97***	35.68***
Location × cultivar	2.13ns	1.98ns
Year × location × cultivar	10.57***	17.20***

**$P \leq 0.05$; ***$P \leq 0.05$; ns – not significant

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Table 2. Total polyphenol content (mg/g) of five sour cherry cultivars over 3 years in two locations

Cultivar	2010	2011	2012	Cultivar's average
Location Osijek				
Oblačinska	4.87 ± 0.74^{a}	7.39 ± 0.24^{e}	4.95 ± 0.38^{a}	5.73 ± 1.43^{A}
Maraska	12.54 ± 0.56^{kl}	10.20 ± 0.35^{h}	8.52 ± 0.57^{g}	10.42 ± 2.02^{D}
Rexelle	10.69 ± 0.21^{hi}	10.16 ± 0.36^{h}	8.54 ± 0.43^{g}	9.80 ± 1.12^{C}
Heimanns	12.76 ± 0.53^{l}	10.49 ± 0.04^{h}	8.33 ± 0.50^{fg}	10.52 ± 2.21^{D}
Kelleris 16	5.58 ± 0.96^{ab}	7.26 ± 2.18^{de}	5.54 ± 0.39^{ab}	6.13 ± 0.98^{AB}
Year's average	9.29 ± 3.80^{B}	9.10 ± 1.63^{B}	7.18 ± 1.78^{A}	
Location Zadar				
Oblačinska	6.85 ± 0.12^{cde}	5.61 ± 0.56^{ab}	5.67 ± 0.19^{ab}	6.04 ± 0.70^{AB}
Maraska	13.73 ± 0.48^{m}	11.43 ± 0.12^{ij}	8.24 ± 0.16^{fg}	11.13 ± 2.75^{E}
Rexelle	13.25 ± 0.38^{lm}	10.14 ± 0.17^{h}	7.55 ± 0.25^{ef}	10.31 ± 2.85^{D}
Heimanns	11.90 ± 0.59^{jk}	11.51 ± 0.31^{j}	7.55 ± 0.24^{f}	10.32 ± 2.40^{D}
Kelleris 16	6.78 ± 0.43^{cde}	6.57 ± 0 35^{cd}	6.18 ± 0.09^{bc}	6.51 ± 0.30^{B}
Year's average	10.50 ± 3.43^{C}	9.05 ± 2.78^{B}	7.04 ± 1.07^{A}	

Values are expressed as mean ± standard deviation of four blocks. Significant differences according to the *LSD* (least significant difference) test ($P \leq 0.05$) are designated by lower-case letters as well as entries with different upper-case letters within the same column and within the same row

shown in Table 3. Location Osijek is characterized by the anthocyanin content in a range of 3.20 to 4.93 mg/g with the smallest average three-year value for cv. Oblačinska and the highest for cv. Maraska, respectively. Very similar but slightly smaller values were found in samples from the Zadar location (Table 3). Particularly, in the range from 3.11 to 4.60 mg/g, cv. Oblačinska had the smallest and cv. Maraska the highest content of anthocyanins in a three-year average. The same as total polyphenol content, total anthocyanin content was slightly different in investigated genotypes than in results previously reported by other authors engaged in the same issues. It can be contributed

Table 3. Total anthocyanin content (mg/g) of five sour cherry cultivars over 3 years in two locations

Cultivar	2010	2011	2012	Cultivar's average
Location Osijek				
Oblačinska	3.90 ±0.51^{hi}	3.03 ± 0.12^{bcd}	2.68 ± 0.50^{b}	3.20 ± 0.63^{AB}
Maraska	5.96 ± 0.63^{mn}	4.96 ± 0.41^{jk}	3.86 ± 0.32^{ghi}	4.93 ± 1.05^{F}
Rexelle	6.42 ± 0.70^{o}	3.95 ± 0.47^{hi}	3.17 ± 0.29^{cde}	4.52 ± 1.70^{E}
Heimanns	6.26 ± 0.11^{no}	3.30 ± 0 04^{def}	2.93 ± 0.29^{bcd}	4.16 ± 1.82^{D}
Kelleris 16	4.77 ± 0.20^{j}	3.47 ± 0.60^{efg}	2.02 ± 0.19^{a}	3.42 ± 1.38^{B}
Year's average	5.46 ± 1.09^{E}	3.74 ± 0.76^{C}	2.93 ± 0.68^{B}	
Location Zadar				
Oblačinska	3.65 ± 0.03^{fgh}	2.85 ± 0.23^{bc}	2.82 ± 0.10^{bc}	3.11 ± 0.47^{A}
Maraska	5.34 ± 0.04^{kl}	5.62 ± 0.08^{lm}	2.84 ± 0.06^{bc}	4.60 ± 1.53^{E}
Rexelle	6.10 ± 0.14^{no}	3.60 ± 0.06^{fgh}	2.13 ± 0.04^{a}	3.94 ± 2.00^{CD}
Heimanns	5.04 ± 0.15^{jk}	4.22 ± 0.06^{i}	2.12 ± 0.05^{a}	3.79 ± 1.50^{C}
Kelleris 16	3.33 ± 0.12^{def}	2.92 ± 0.13^{bcd}	3.18 ± 0.10^{cde}	3.14 ± 0.21^{A}
Year's average	4.69 ± 1.17^{D}	3.84 ± 1.14^{C}	2.62 ± 0.47^{A}	

Values are expressed as mean ± standard deviation of four blocks. Significant differences according to the *LSD* (least significant difference) test ($P \leq 0.05$) are designated by lower-case letters as well as entries with different upper-case letters within the same column and within the same row

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to the different extraction and measuring protocols (Melicháčová et al. 2010) but also the strong impact of cultivar and environmental factors during the growing season cannot be discarded. Khoo et al. (2011) found variation in the anthocyanin content among 34 sour cherry cultivars in a range from 21 to 272 mg malvidin-3-glucoside equivalents/100 g of fresh fruit weight. Other authors reported the same or smaller anthocyanin content values compared to the above mentioned data (Pedisić et al. 2007, Siddiq et al. 2011, Mitić et al. 2012).

Influence of year on the polyphenol and anthocyanin content. The impact of year on the total polyphenol and anthocyanin content was significant according to the obtained results (Tables 1 and 4). Total polyphenols were the highest at 9.89 mg/g in 2010 harvest, by 8.19% higher than in 2011 and by 28.11% higher than in 2012. Among the investigated harvest years, total anthocyanin content was the highest in 2010, while harvests in 2011 and 2012 had 1.34 and 1.83-fold less anthocyanins than 2010. Both locations had higher amounts of rainfalls in 2010 than in other investigated years, so it is probable that more available water enabled increased polyphenol and anthocyanin synthesis. Alfaro et al. (2013) found a significant positive correlation between rainfalls and total polyphenols in murtilla fruits (*Ugni molinae* Turcz). Mitić et al. (2012) found an influence of season, in particular climatic conditions, on polyphenol and anthocyanin content in sour cherry cvs. Oblačinska, Cigančica and Marela grown in Serbia. Similar differences in polyphenol and anthocyanin content between seasons were also found in apricot (Leccese et al. 2012), almond (Bolling et al. 2010), hardy kiwifruit (Latocha et al. 2013), and other species.

Table 4. Mean values ± standard deviation of total polyphenol and anthocyanin content (mg/g) arranged by year, by location and by cultivar

		Total polyphenol content	Total anthocyanin content
Year	2010	9.89 ± 3.47^{c}	5.08 ± 1.14^{c}
	2011	9.08 ± 2.15^{b}	3.79 ± 0.91^{b}
	2012	7.11 ± 1.39^{a}	2.78 ± 0.57^{a}
Location	Osijek	8.52 ± 2.62^{a}	4.05 ± 1.36^{b}
	Zadar	8.86 ± 2.78^{b}	3.72 ± 1.24^{a}
Cultivar	Oblačinska	5.89 ± 1.02^{a}	3.15 ± 0.50^{a}
	Maraska	10.78 ± 2.20^{e}	4.76 ± 1.19^{d}
	Rexelle	10.06 ± 1.96^{c}	4.23 ± 1.69^{c}
	Heimanns	10.42 ± 2.07^{d}	3.98 ± 1.51^{b}
	Kelleris 16	6.32 ± 0.68^{b}	3.28 ± 0.89^{a}

Significant differences according to the *LSD* (least significant difference) test ($P \leq 0.05$) are designated by lower-case letters

Influence of geographical location on polyphenol and anthocyanin content. Geographical location, altogether with climatic conditions, had a strong impact on the polyphenol and anthocyanin content of different sour cherry cultivars (Tables 1 and 4). Also, the interaction of location with year showed significant differences in the total polyphenol and anthocyanin content, while the interaction of location with cultivar did not. However, the content of polyphenols was significantly higher in sour cherry fruits in the Zadar location. The total content of anthocyanins was found to be higher in sour cherry fruits from the Osijek location than the Zadar location. It may be assumed that fruits from the Zadar location should also have a higher anthocyanin content. Environmental influence on anthocyanins synthesis is very important. All flavonoids, especially anthocyanins are synthesized *via* the phenylpropanoid pathway with stimulation of light, which protects plants from harmful UV radiation acting as a protective filter (Solovchenko and Schmitz-Eiberger 2003). Jaakola et al. (2004) proved the activation of flavonoid biosynthesis by solar radiation in European blueberries (*Vaccinium myrtillus* L.).

Also, it is found in strawberries that higher temperatures increase the content of flavonols and anthocyanins (Josuttis et al. 2012). Although both locations, Osijek and Zadar, have lot of sunny days during the year, combination of high temperatures and higher precipitations during the season contributed to higher anthocyanin content in Osijek than in the Zadar region. Similarly, differences between day and night temperatures can affect the anthocyanin accumulation in some fruits (Tomás-Barberán and Espín 2001).

Influence of cultivar on the polyphenol and anthocyanin content. Although the influence of seasonal climatic conditions and geographic region/location on the total polyphenol and anthocyanin content of sour cherry fruits was proved, according to some studies the impact of cultivar is probably more significant than the above-mentioned

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variables (Bolling et al. 2010, Vagiri et al. 2013). Five investigated sour cherry cultivars significantly differed among themselves in total polyphenol and anthocyanin content according to three-year data in two locations by the *LSD* test (Table 4). The smallest polyphenol content was found for cv. Oblačinska but the highest for cv. Maraska. Cv. Kelleris 16 with 6.32 mg/g of total polyphenols was similar to Oblačinska, while cvs. Rexelle and Heimanns had approximate polyphenol content as cv. Maraska. The average phenolic content of three years in two locations and five investigated sour cherry cultivars varied 1.8-fold. Regarding to anthocyanins content, it is also found to be the smallest in cv. Oblačinska, but the highest in cv. Maraska. Other cultivars showed results similar as for polyphenol content but the average anthocyanin content of three-year study in two locations and with five investigated sour cherry cultivars showed less variation (1.5-fold). This cultivar-depended variation obtained in our investigation is smaller than those obtained for plum, peach and nectarine cultivars (2.0, 2.7 and 4.8-fold, respectively) found by Gil et al. (2002), for 67 cultivars of apple (5.2-fold) by Wojdylo et al. (2008) and for almond (2.7-fold) by Bolling et al. (2010).

Interaction year × location × cultivar. Year-to-year variation in certain bioactive compounds is well investigated through a lot of species (Bolling et al. 2010, Latocha et al. 2013, Vagiri et al. 2013). In our investigation, a significant interaction between year, cultivar and location was found (Table 1). The smallest amount of polyphenols was found for cv. Oblačinska in 2010 in the Osijek location and the highest for cv. Maraska in 2010 in the Zadar location (Table 2). Coefficient of variation ranged from 4.67% to 27.67% for cultivars during the three-year period in both locations. Much bigger variation coefficient was found for the anthocyanin content in the range from 6.56% to 50.82%, with the minimal anthocyanin content in cv. Kelleris 16 in 2012 at the Osijek location and maximal in Rexelle in 2010 also in Osijek (Table 3). It was already shown in our study that environmental growing conditions influence the level of synthetized phenolics, but genetic background of cultivar is important in response of cultivar to biotic (disease) and abiotic (moisture, temperature, radiation) stresses in certain environmental conditions (Howard et al. 2003).

In conclusion, sour cherry fruits are characterized by considerable variability in polyphenol and anthocyanin contents caused by year, i.e. climatic condition of a specific season as well as geographical location and cultivar. A strong influence of year on the content of polyphenols and anthocyanins is immanent and was caused by higher rainfalls during the 2010 season. Geographic location in combination with higher precipitations influenced the fortified biosynthesis of anthocyanins in Osijek more than in Zadar location. All presented results clearly indicate that the most important source of variability is cultivar. However, despite the variation caused by season or location, the same cultivar, precisely cv. Maraska, revealed the highest polyphenol and anthocyanin content; similarly cv. Oblačinska obtained the smallest contents of polyphenols and anthocyanins. Our data on cultivar-specific polyphenol and anthocyanin profiles may also recommend a choice of cultivar for orchard establishment in order to have good quality of sour cherry fruits rich with bioactive compounds.

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