

## Effect of climate and ripening on sour cherry Maraska and Oblačinska bioactive properties

### Abstract

The aim of this study was to examine the influence of climate conditions and ripening on total phenols (TP), total monomeric anthocyanins (TAC) and antioxidant capacity (AC) of Maraska and Oblačinska sour cherries. For that reason, TP using Folin-Ciocalteu reagent, TAC by pH-differential method and AC via DPPH method were determined in Maraska and Oblačinska sour cherry samples harvested in Zadar and Osijek area in 2014 at three maturity stages. Despite that statistical analysis showed significant differences in TP and AC between cultivars and growing locations, obtained results revealed considerable functional properties of both cultivars. Still, Maraska achieved higher TP, TAC and AC values compared to Oblačinska. Furthermore, Mediterranean climate conditions positively affected on bioactive compounds accumulation in both cultivars, where generally the highest contents of examined bioactive compounds were accomplished at the latest harvest date.

**Keywords:** sour cherry, cultivar, climate, ripening, bioactive compounds

### Introduction

Sour cherry is fruit characterized with many quality attributes, e.g., respectable dry matter content (Grafe and Schuster, 2014; Papp et al., 2010; Wojdyło et al., 2014), red color (Levaj et al., 2012; Pedisić et al., 2009; Viljevac et al., 2012) and exceptionally sour taste with intense overall aroma. It is also abundant with various phytochemicals that contributes to its sensory and nutritional properties (Ferretti et al., 2010), where phenolic compounds represent main group of bioactive compounds, especially anthocyanins, from which sour cherry red color originates (Ferretti et al., 2010). Many studies described polyphenols, including anthocyanins, as compounds that possess antioxidant properties with ability of scavenging and neutralizing radicals (Machlin and Bendich, 1987) and therefore have various health promoting effects (Prior, 2003; Szajdek and Borowska, 2008). The sour cherry richness with bioactive compounds positions this fruit with certainty as desirable for consumption, as well as processing.

Anthocyanins are often used as the main indicators of ripening, since initial green color changes to red due to degradation of chlorophyll and accumulation of anthocyanins during ripening process in red fruits (Ferretti et al., 2010). Results of Pedisić et al. (2010) study showed that in several investigated sour cherry ecotypes concentrations of anthocyanins varied during ripening, but they were determined in the highest amounts at the latest stage of maturity. Furthermore, content of polyphenols, as well as anthocyanins, greatly depends on genetic and environmental factors (Tomás-Barberán and Espín, 2001). Previous research confirmed differences in sour cherry bioactive properties influenced by cultivar (Pissard et al., 2016; Viljevac et

<sup>1</sup> Maja Repajić, Ph.D., Postdoctoral researcher, Branka Levaj, Ph.D., Full Professor, Faculty of Food Technology and Biotechnology University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia

<sup>2</sup> Rajko Vidrih, Ph.D., Full Professor, Janez Hribar, Ph.D., Full Professor, Biotechnical Faculty University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

<sup>3</sup> Boris Puškar, Ph.D., Krunoslav Dugalić, Ph.D., Assistant Professor, Croatian Centre for Agriculture, Food and Rural Affairs, Svetošimunska cesta 25, 10000 Zagreb, Croatia  
Zorica Jurković, Ph.D., Senior Scientific Researcher, Agricultural Institute Osijek, Južno predgrađe 17, 31000 Osijek, Croatia  
Corresponding author: mrepajic@pbf.hr

al., 2012; Viljevac Vuletić et al., 2017; Wojdyło et al., 2014), as well as climate conditions (Viljevac Vuletić et al., 2017). For example, in Viljevac Vuletić et al. (2017) different sour cherry cultivars grown in Mediterranean area had highest polyphenols content compared to the same cultivars grown in continental zone, while higher amounts of anthocyanins were determined in cherries from continental part combined with higher precipitation.



**Figure 1** Sour cherry tree

**Slika 2.** Stablo višnje

Source/Izvor: private photography/vlastita fotografija

Sour cherry is popular and important fruit in Croatia. It is in the third place by production, after apples and mandarins, with a total production of 8770 tones in 2017, where 8716 tones were intensively produced (for market) and 54 tones were produced in extensive orchards, mainly for their own household consumption (Croatian Bureau of Statistics, 2018). Two main sour cherry cultivars grown for industrial processing are Maraska and Oblačinska (Jurković et al., 2008; Repajić et al., 2015). Maraska is mainly cultivated in Dalmatia region, being an autochthonous cultivar with long history of cultivation in that region (Levaj et al., 2010; Pedisić et al., 2010; Repajić, et al., 2015), while Oblačinska represents to continental cultivar (Repajić et al., 2015) which originates from south Serbia (Mihaljević et al., 2013). Both cultivars are characterized with favorable quality and processing suitability (small fruit, dark red color and high dry matter content) (Jurković et al., 2008; Repajić et al., 2015), as well as appreciable polyphenols content, with anthocyanins included (Levaj et al., 2010; Pedisić et al., 2010; Repajić et al., 2015; Viljevac et al., 2012; Viljevac Vuletić et al., 2017).

Hence, the aim of this study was to investigate the influence of growing location, climate conditions (Mediterranean, continental) and stage of ripening on total phenols, total monomeric anthocyanins and antioxidant capacity of sour cherries Maraska and Oblačinska.

## Materials and methods

### Fruit sample

Sour cherry (*Prunus cerasus* L.) cultivars Maraska (genotype Maraska) and Oblačinska (genotype 18) were harvested in two experimental orchards of Agricultural Institute Osijek in 2014 at three maturity stages. In orchards Vlačine (Zadar area, Croatia) and Tovljač (Osijek area, Croatia) (Figure 2) cherries were planted on *Prunus mahaleb* L. rootstock in randomized block design with three trees per block in four replicates. Orchard Vlačine is located at altitude 95 m, latitude 44° 8' N and longitude 15° 22' E, where trees were planted 5 x 4 m spacing on a deep red soil with partial stone debris without grass layer. In orchard Tovljač, lying on altitude 89 m, latitude 45° 32' N and longitude 18° 38' E, trees were grown on grass layered Eutric Cambisol soil with 4.8 x 3.5 m spacing. Both orchards were maintained with permanent cultivation without irrigation using usual agrotechnical measures.



Orchard Vlačine



Orchard Tovljač

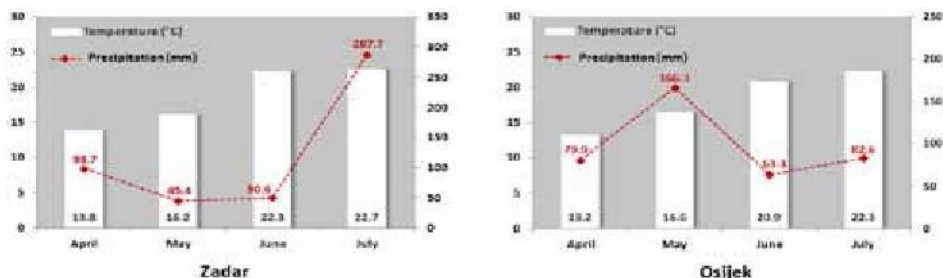
**Figure 2** Experimental orchards Vlačine and Tovljač

**Slika 2.** Eksperimentalni voćnjaci Vlačine i Tovljač

Source/Izvor: private photography, Agricultural Institute Osijek/vlastita fotografija, Poljoprivredni institut Osijek

### Climate conditions

Investigated sour cherry growing areas differed by the climate conditions, where cherries from Zadar area were grown in Mediterranean climate conditions, and Osijek area cherries were affected by the continental climate.



**Graph 1** Meteorological data (average day temperature and accumulated precipitation) for Zadar and Osijek area during sour cherry vegetation period

**Grafikon 1.** Meteorološki podaci (srednja dnevna temperatura i ukupne oborine) za područje Zadra i Osijeka tijekom vegetacijskog perioda višnje

In order to evaluate and compare different sour cherry growing circumstances influenced by the climate, meteorological data for Zadar and Osijek area during sour cherry vegetation period in year 2014 were collected and provided by the Meteorological and Hydrological institute of Croatia weather stations Zadar-Zemunik and Osijek-Klisa, and are presented in Graph 1.

#### *Sample preparation*

Approximately 100 g of each sour cherry cultivar was manually harvested at three maturity stages (from the end of June to the middle of July, at 7 days apart) from three different trees and then combined to get an average sample (300 g). After harvesting, cherries were packed in polyethylene bags and immediately transported at +4 °C to the laboratory and kept at -18 °C prior to analysis. For analysis purpose, cherries were thawed, manually depitted and homogenized using T-25 Ultra Turrax Homogenizer (IKA-Werke GmbH & Co. KG, Staufen im Breisgau, Germany). Homogenized cherries (2.5 g) were extracted with 20 mL of 1% formic acid (Sigma-Aldrich, Germany) in methanol (Sigma-Aldrich, Germany) (v/v) using ultrasound bath for 30 min at room temperature. Obtained extracts were centrifuged (Avanti JXN-26, Beckmans Coulter Inc., USA) at 8000 rpm for 10 min, filtered and filled up with the extraction solvent in a 25 mL volumetric flask. From each sour cherry sample three extracts (n=3) were prepared and used for the determination of total phenols, total monomeric anthocyanins and antioxidant capacity.

#### Determination of total phenols

Total phenols (TP) were determined according to the Folin-Ciocalteu method described by Singleton and Rossi (1965) and Kokalj et al. (2016), slightly modified. Briefly, 0.2 mL of sample (threefold diluted with extraction solvent), 2.54 mL of Folin Ciocalteu's reagent (Merck, Germany) diluted in deionized water (0.14 mL of Folin Ciocalteu's reagent in 2.4 mL of deionized water) and 0.42 mL of sodium carbonate solution (Merck, Germany) (20%, w/v) were mixed thoroughly and incubated for 60 minutes at room temperature. After addition of 0.91 mL of deionized water, the absorbance was measured against the deionized water set as blank at 765 nm. Calibration curve was obtained using different concentrations of gallic acid (Fluka, Switzerland) (3 - 150 mg/L;  $R^2 = 0.9997$ ) and TP were expressed as mg of gallic acid equivalents (GAE) per 100 g of sour cherry fresh weight.

#### Determination of total monomeric anthocyanins

Total monomeric anthocyanins (TAC) were determined using the pH differential method (Lee and al., 2005) as follows: 0.5 mL of sample was diluted with 2 mL of potassium chloride buffer (pH=1.0; 0.025M) and sodium acetate buffer (pH=4.5; 0.4M), respectively. The absorbance of the solutions was measured at 520 and 700 nm and content of TAC was calculated according to the following equation:

$$TAC = \frac{A \times MW \times DF \times 10^3}{\epsilon \times l} \quad [1]$$

where  $A = (A_{520nm} - A_{700nm})_{pH\ 1.0} - (A_{520nm} - A_{700nm})_{pH\ 4.5}$ ; MW = molecular weight of cyanidin-3-glucoside (449.2 g/mol); DF = dilution factor (5),  $10^3$  = factor for conversion from g to mg;  $\epsilon$  = molar extinction coefficient for cyanidin-3-glucoside (29 600 L/mol cm) and  $l$  = pathlength in cm. The TAC results were expressed as mg cyanidin-3-glucoside equivalents per 100 g of sour cherry fresh weight.

#### Determination of antioxidant capacity

The determination of antioxidant capacity (AC) was carried out using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method (Brand-Williams et al., 1995; Kokalj et al., 2016). For this analysis, the mixture of 0.04 mL of sample and 1.5 mL of freshly prepared 127 mM DPPH (Sigma-Aldrich, Germany) solution in methanol (w/v) was incubated for 15 minutes at room temperature before the absorbance was measured at 517 nm with methanol set as blank. Different concentrations of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Sigma-Aldrich, Germany) (22.43 – 256.34 mg/L;  $R^2 = 0.9985$ ) were used for calibration curve and AC was expressed as mmol of Trolox equivalents (TE) per 100 g of sour cherry fresh weight.

All spectrophotometric assays were performed by Cecil Aurius Series CE 2021 UV/Vis spectrophotometer (Cecil Instruments Limited, UK) in three replicates ( $n=3$ ) and data are presented as the mean value  $\pm$  SD.

#### Statistical analysis

Factorial analysis of variance (ANOVA) was applied to investigate the influence of cultivar, location and maturity stage on sour cherry TP, TAC and AC. Furthermore, marginal means were compared by Tukey's HSD test and Pearson's correlation coefficient was calculated to examine the relationships between determined parameters. In addition, the Principal Component Analysis (PCA) was performed to evaluate possible cherries grouping by the applied sources of variation (cultivar, location, maturity stage). All tests were carried out using a statistical software Statistica ver. 8.0 (Statsoft Inc., Tulsa, USA) at p-level  $\alpha \leq 0.05$ .

## Results and discussion

Sour cherries Maraska and Oblačinska previously showed high abundance with polyphenols, including anthocyanins (Repajić et al., 2015; Viljevac et al., 2012; Viljevac Vuletić et al., 2017), which contents can be affected by the growing conditions, namely climate (Viljevac Vuletić et al., 2017), as well as ripening (Pedisić et al., 2010). Hence, the objective of this study was to examine the prospective TP, TAC and AC differences in Maraska and Oblačinska sour cherries grown in two climate areas (Mediterranean-Zadar area, continental-Osijek area) and harvested at three maturity stages.

Table 1 provides TP values obtained in investigated sour cherry samples. Determined TP levels ranged from 193.38 – 433.31 mg/100g fw and grand mean was 310.26 mg/100g fw (Table 2). Obtained results are generally in accordance with previous study (Repajić et al., 2015), where minor differences can be attributed to ecotype variations and different extraction procedure. In comparison with results obtained by Viljevac et al. (2012) and Viljevac Vuletić et al. (2017), our TP results are almost threefold lower probably due to the extraction procedure that was twofold shorter. As can be seen in Table 2, sour cherries significantly differed in TP values by the cultivar and location ( $p \leq 0.05$ ), where Maraska had almost double fold higher TP content (394.41 mg/100g fw) in regard to Oblačinska (226.11 mg/100g fw). Studies of Levaj et al. (2010), Repajić et al. (2015), Viljevac et al. (2012) and Viljevac Vuletić et al. (2017) also reported higher Maraska TP content in comparison with Oblačinska.

**Table 1** Total phenols (TP), total monomeric anthocyanins (TAC) and antioxidant capacity (AC) of Maraska and Oblačinska grown in two locations (Zadar, Osijek) and harvested at three maturity stages

**Tablica 1.** Ukupni fenoli (TP), ukupni monomerni antocijani (TAC) i antioksidacijski kapacitet (AC) Maraske i Oblačinske uzgojenih na dvije lokacije (Zadar, Osijek) i ubranih u tri stupnja zrelosti

Location	Cultivar	Maturity stage	TP (mg/100g fw)	TAC (mg/100g fw)	AC (mmol/100g fw)
Zadar	Maraska	1 <sup>st</sup>	394.17±40.00	69.25±14.13	0.950±0.031
		2 <sup>nd</sup>	403.96±88.92	77.12±9.45	1.013±0.122
		3 <sup>rd</sup>	433.31±46.18	81.31±28.22	1.077±0.104
	Oblačinska	1 <sup>st</sup>	221.93±22.18	75.61±4.24	0.651±0.059
		2 <sup>nd</sup>	246.13±16.59	81.35±18.49	0.747±0.104
		3 <sup>rd</sup>	251.17±10.37	67.71±11.21	0.799±0.044
Osijek	Maraska	1 <sup>st</sup>	415.74±17.60	89.42±18.89	0.996±0.051
		2 <sup>nd</sup>	384.22±8.59	85.58±2.53	1.012±0.058
		3 <sup>rd</sup>	335.04±62.05	83.14±18.96	0.889±0.044
	Oblačinska	1 <sup>st</sup>	222.27±14.93	74.35±7.82	0.651±0.060
		2 <sup>nd</sup>	221.78±7.74	68.65±7.94	0.649±0.013
		3 <sup>rd</sup>	193.38±21.50	65.84±3.21	0.563±0.035

*Results are expressed as mean±SD.*

Considering growing location, cherries from Zadar area were described with significantly higher TP amounts (325.11 mg/100g fw) compared to TP content of Osijek area cherries (295.41 mg/100g fw). Both cultivars achieved higher TP amounts in Zadar area (Maraska = 410.48 mg/100g fw, Oblačinska = 239.75 mg/100g fw) than in Osijek (Maraska = 378.33 mg/100g fw, Oblačinska = 212.48 mg/100g fw). Obtained results are not unexpected, since Zadar area belongs to Mediterranean climate zone, which is mild and characterized with higher temperatures and more insolation hours, opposed to continental climate conditions, which are present in Osijek area. Observing the weather conditions of both locations during sour cherry vegetation period, it can be concluded that less precipitation in May and June, as well as higher June's temperatures in Zadar area positively affected on TP accumulation in cherries. Viljevac Vuletić et al. (2017) investigated bioactive compounds of several sour cherry cultivars also grown in areas of Zadar and Osijek during three consecutive years, among which Maraska and Oblačinska were tested. Results of their study showed the same TP trend between locations as observed in our investigation, likewise.

Furthermore, despite that TP levels did not significantly differed by the maturity stage, it can be seen that they were similar at first two harvesting periods (1<sup>st</sup> = 313.53 mg/100g fw, 2<sup>nd</sup> = 314.02 mg/100g fw) and they slightly decreased by the latest harvest (303.22 mg/100g fw). Maraska TP decreased for 5% by the latest maturity stage, while TP of Oblačinska remained constant. Study of Pedisić et al. (2007) also examined phenolic content during ripening of Maraska grown at two locations. Their study showed variations in TP between harvest dates, where cherries from Split showed the highest TP content at the 2<sup>nd</sup> harvest date, what is similar to our results, while the highest TP values in cherries from Zadar were recorded at the latest harvesting date.

**Table 2** Influence of location and maturity stage on Maraska and Oblačinska total phenols (TP), total monomeric anthocyanins (TAC) and antioxidant capacity (AC)**Tablica 2.** Utjecaj lokacije i stupnja zrelosti na ukupne fenole (TP), ukupne monomernu antocijane (TAC) i antioksidacijski kapacitet (AC) Maraske i Oblačinske

Source of variation	TP (mg/100g fw)	TAC (mg/100g fw)	AC (mmol/100g fw)
Cultivar	p≤.01*	p=.06ns	p≤.01*
Maraska	394.41±9.00 <sup>b</sup>	83.08±3.35 <sup>a</sup>	0.989±0.016 <sup>b</sup>
Oblačinska	226.11±9.00 <sup>a</sup>	73.78±3.35 <sup>a</sup>	0.677±0.016 <sup>a</sup>
Location	p=.03*	p=.51ns	p≤.01*
Zadar	325.11±9.00 <sup>b</sup>	76.85±3.35 <sup>a</sup>	0.873±0.016 <sup>b</sup>
Osijek	295.41±9.00 <sup>a</sup>	80.01±3.35 <sup>a</sup>	0.793±0.016 <sup>a</sup>
Maturity stage	p=.74ns	p=.80ns	p=.31ns
1 <sup>st</sup>	313.53±11.02 <sup>a</sup>	78.82±4.11 <sup>a</sup>	0.812±0.020 <sup>a</sup>
2 <sup>nd</sup>	314.02±11.02 <sup>a</sup>	80.15±4.11 <sup>a</sup>	0.855±0.020 <sup>a</sup>
3 <sup>rd</sup>	303.22±11.02 <sup>a</sup>	76.33±4.11 <sup>a</sup>	0.832±0.020 <sup>a</sup>
Cultivar × location	p=.05*	p=.10ns	p≤.01*
Maraska × Zadar	410.48±12.73 <sup>b</sup>	77.41±4.74 <sup>a</sup>	1.013±0.023 <sup>c</sup>
Maraska × Osijek	378.33±12.73 <sup>b</sup>	88.75±4.74 <sup>a</sup>	0.966±0.023 <sup>c</sup>
Oblačinska × Zadar	239.75±12.73 <sup>a</sup>	76.29±4.74 <sup>a</sup>	0.732±0.023 <sup>b</sup>
Oblačinska × Osijek	212.48±12.73 <sup>a</sup>	71.27±4.74 <sup>a</sup>	0.621±0.023 <sup>a</sup>
Cultivar × maturity stage	p=.03*	p=.45ns	p=.04*
Maraska × 1 <sup>st</sup>	404.95±15.59 <sup>b</sup>	81.01±5.81 <sup>a</sup>	0.973±0.028 <sup>b</sup>
Maraska × 2 <sup>nd</sup>	394.09±15.59 <sup>b</sup>	82.95±5.81 <sup>a</sup>	1.012±0.028 <sup>b</sup>
Maraska × 3 <sup>rd</sup>	384.17±15.59 <sup>b</sup>	85.29±5.81 <sup>a</sup>	0.983±0.028 <sup>b</sup>
Oblačinska × 1 <sup>st</sup>	222.10±15.59 <sup>a</sup>	76.62±5.81 <sup>a</sup>	0.651±0.028 <sup>a</sup>
Oblačinska × 2 <sup>nd</sup>	233.96±15.59 <sup>a</sup>	77.34±5.81 <sup>a</sup>	0.698±0.028 <sup>a</sup>
Oblačinska × 3 <sup>rd</sup>	222.28±15.59 <sup>a</sup>	67.37±5.81 <sup>a</sup>	0.681±0.028 <sup>a</sup>
<b>Grand mean</b>	<b>310.26</b>	<b>78.43</b>	<b>0.833</b>

Results are expressed as mean±SE.

\*p≤0.05, ns = not significant (p>0.05)

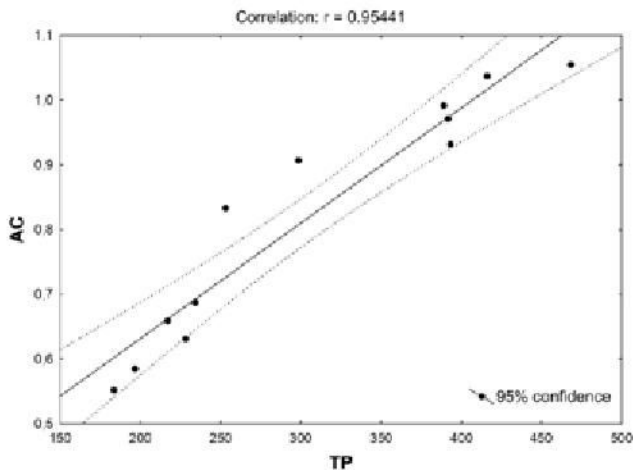
Values with different letters are statistically different at p≤0.05.

As anthocyanins are the most representative phenolic compounds in sour cherry (Wojdyło et al., 2014), TAC in sour cherry samples were also determined (Table 1), where their content ranged from 65.84 – 89.42 mg/100g fw (grand mean 78.43 mg/100g fw), what is similar to previously published results (Pedisić et al., 2007; Repajić et al., 2015). Compared to the results of Viljevac et al. (2012) and Viljevac Vuletić et al. (2017), TAC values in present study are 4-5-fold lower. As previously mentioned, these differences could be due to more exhaustive extraction used in earlier studies, as well as environmental conditions.

Although investigated sources of variation did not show significant influence on the TAC of cherries, tested cultivars differed in TAC amounts, where Maraska showed slightly higher TAC

content (83.08 mg/100g fw) than Oblačinska (73.78 mg/100g fw). Additionally, TAC generally showed dissimilar trend to TP regarding location, where higher TAC were achieved in Osijek area. Viljevac Vuletić et al. (2017) also reported higher TAC in sour cherries from Osijek area. They explained this occurrence by the fact that high temperatures in combination with higher precipitation during the season contributed to anthocyanins accumulation in Osijek, although both locations have lot of sunny days during the year.

However, observing the cultivars individually, Oblačinska gained higher TAC in Zadar area (Zadar = 76.29 mg/100g fw, Osijek = 71.27 mg/100g fw), unlike Maraska, which TAC content was higher in samples cultivated in Osijek area (Zadar = 77.41 mg/100g fw, Osijek = 88.75 mg/100g fw). TAC levels slightly varied through the ripening (Table 2), where marginally decrease was noticed at the latest maturity stage. Maraska showed constant increase of TAC during the ripening (from 81.01 – 85.29 mg/100g fw), but TAC of Oblačinska decreased by the latest maturity stage (from 76.62 – 67.37 mg/100g fw). Pedisić et al. (2007, 2010) also documented variations in anthocyanins content during sour cherry ripening. These authors mentioned that, as anthocyanins are subjected to constant biosynthesis during ripening, their quantity is constantly changing and can be affected with various effects, e.g., the position of the fruit on the tree, climate conditions in period between harvesting.



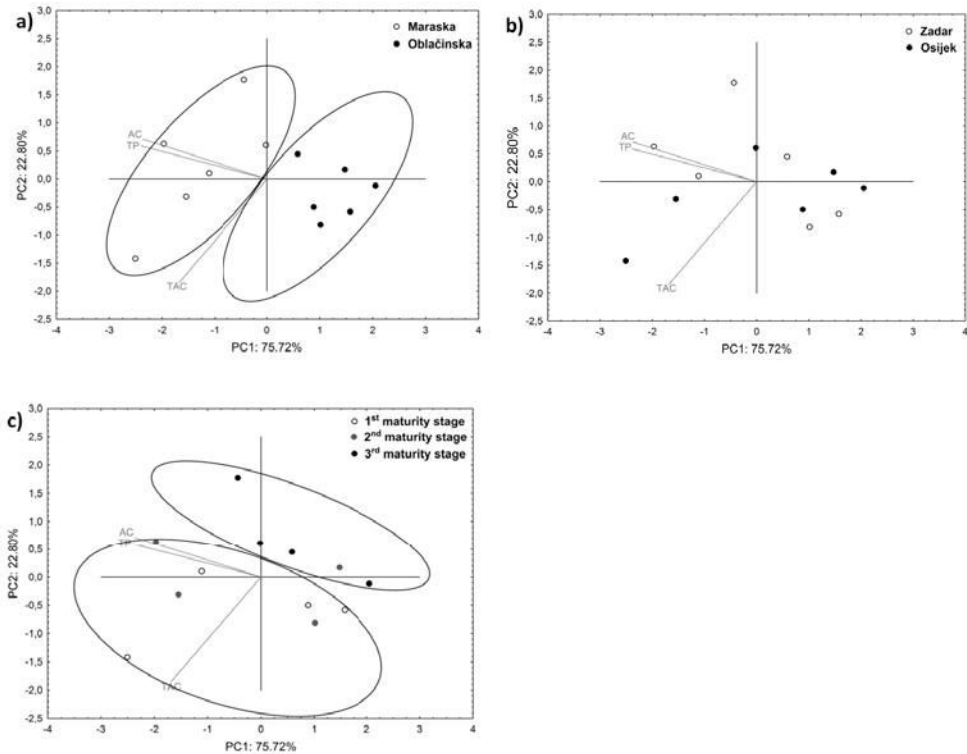
**Figure 3** Correlation between Maraska and Oblačinska total phenols (TP; mg/100g fw) and antioxidant capacity (AC; mmol/100g fw)

**Slika 3.** Korelacija između ukupnih fenola (TP; mg/100g fw) i antioksidacijskog kapaciteta (AC; mmol/100g fw) Maraske i Oblačinske  
 $p \leq 0.05$

Since sour cherry is abundant with polyphenols, especially anthocyanins, which are strong antioxidants, AC of samples was examined by DPPH method and obtained results are given in Table 1. AC values ranged from 0.563 – 1.077 mmol/100g fw, with 0.833 mmol/100g fw established as grand mean. Considerably variable AC values in Maraska and Oblačinska were reported previously (Pedisić et al., 2007; Repajić et al., 2015), but it is difficult to compare them with results of this study due to application of different extraction and determination methods. Equally as with TP, statistical analysis revealed a significant effect of cultivar and location on AC of sour cherry ( $p \leq 0.05$ ), while at the same time it was not significantly affected by the maturity



stage ( $p \geq 0.05$ ) (Table 2). Moreover, AC highly correlated with TP ( $r = 0.95441$ ,  $p \leq 0.05$ ) (Figure 3).



**Figure 4** Distribution of Maraska and Oblačinska samples in two dimensional coordinate system defined by the first two principal components (PC1 and PC2) according to the investigated influences: a) influence of cultivar; b) influence of location; c) influence of maturity stage  
 TP = total phenols (mg/100g fw), TAC = total monomeric anthocyanins (mg/100g fw), AC = antioxidant capacity (mmol/100g fw)

**Slika 4.** Raspodjela uzoraka Maraske i Oblačinske u dvodimenzionalnom koordinatnom sustavu definiranom s prve dvije glavne komponente (PC1 i PC2) prema ispitivanim utjecajima: a) utjecaj kultivara; b) utjecaj lokacije; c) utjecaj stupnja zrelosti

TP = ukupni fenoli (mg/100g fw), TAC = ukupni monomerni antocijani (mg/100g fw), AC = antioksidacijski kapacitet (mmol/100g fw)

Considering cultivar, Maraska showed greater AC (0.989 mmol/100g fw) compared to Oblačinska (0.677 mmol/100g fw). The differences among locations were also noticed, where cherries from Zadar area showed higher AC values (0.873 mmol/100g fw), opposed to cherries from Osijek area (0.793 mmol/100g fw). Maraska was characterized with higher AC values in both locations (Zadar = 1.013 mmol/100g fw, Osijek = 0.966 mmol/100g fw) in comparison to Oblačinska (Zadar = 0.732 mmol/100g fw, Osijek = 0.621 mmol/100g fw). As for ripening, cherries showed variations among maturity stages, but without significant differences ( $p \geq 0.05$ ) and regardless of harvest date, cherries retained seemingly respectable AC values. Observing the AC levels among cultivars in respect to maturity stage, same trend between Maraska and Oblačinska can be noticed, where both cultivars accomplished highest AC values in the 2<sup>nd</sup>

maturity stage (Table 2).

Lastly, PCA was conducted to investigate possible grouping of cherries depending on cultivar, climate of the locations and maturity stage. Obtained results are presented on Figure 4, where first two components (PC1, PC2) explained 98.52% of total variance. Continuous variables (TP, TAC, AC) highly correlated with PC1 (TP,  $r = -0.96$ ; TAC,  $r = -0.67$ ; AC,  $r = -0.95$ ). As for PC2, only TAC highly correlated with these component ( $r = -0.74$ ). As can be seen in Fig. 4a, apparent separation of cultivars can be observed, where Maraska samples distinguished from Oblačinska ones by the TP, TAC and AC, what is in accordance with previously discussed results. Despite the statistically significant differences for TP and AC between locations (Zadar > Osijek), grouping by the location was not noticeable (Fig.4b), which can be attributed to insignificant influence on TAC and numerically slight differences in determined parameters between locations. Certain separation among samples regarding to maturity stage can be observed (Fig.4c), especially among cherries from the 1<sup>st</sup> and 2<sup>nd</sup> maturity stage and ones from the 3<sup>rd</sup> maturity stage.

## Conclusion

Obtained results showed that both cultivars are abundant with phenolic compounds as well as by good antioxidant potential. However, Maraska cherry could be emphasized due to the higher bioactive properties. Mediterranean climate conditions positively affected the accumulation of sour cherry bioactive compounds, wherein their highest contents were achieved by the latest maturity stage.

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## Utjecaj klime i zrenja na bioaktivna svojstva višnje Maraska i Oblačinska

### Sažetak

Cilj ovog rada bio je ispitati utjecaj klimatskih uvjeta i zrenja na ukupne fenole (TP), ukupne monomerne anticijane (TAC) i antioksidacijski kapacitet (AC) višnji Maraska i Oblačinska. Iz tog su razloga u uzorcima višnje Maraska i Oblačinska ubranih u okolici Zadra te Osijeka 2014. godine u tri stupnja zrelosti određeni TP korištenjem Folin-Ciocalteu reagensa, TAC pH-diferencijalnom metodom i AC metodom DPPH. Unatoč tomu što je statistička analiza pokazala značajne razlike u TP i AC između kultivara i lokacija uzgoja, dobiveni rezultati pokazuju značajna funkcionalna svojstva oba kultivara. Ipak, Maraska je postigla veće TP, TAC i AC vrijednosti u odnosu na Oblačinsku. Nadalje, mediteranski klimatski uvjeti pozitivno su utjecali na akumulaciju bioaktivnih spojeva u oba kultivara, pri čemu je najviši sadržaj uglavnom svih ispitivanih bioaktivnih spojeva postignut u zadnjem terminu berbe.

**KLjučne riječi:** višnja, kultivar, klima, zrenje, biološki aktivni spojevi