



Available on line at

Association of the Chemical Engineers of Serbia AChE

www.ache.org.rs/CICEQ

Chemical Industry & Chemical Engineering Quarterly 18 (1) 53–62 (2012)

CI&CEQ

MILAN N. MITIĆ
MIRJANA V. OBRADOVIĆ
DANIJELA A. KOSTIĆ
RUŽICA J. MICIĆ
EMILIJA T. PECEV

Faculty of Science and Mathematics,
Department of Chemistry,
University of Niš, Niš, Serbia

SCIENTIFIC PAPER

UDC 58(497.11):547.9

DOI 10.2298/CICEQ110701046M

POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF SOUR CHERRIES FROM SERBIA

The aim of this study was to evaluate the content of phenolics: the total phenols (TP), flavonoids (TF), anthocyanins (TA), as well as the total antioxidant capacity (TAC) in three sour cherry cultivars (Prunus cerasus L.) introduced to the southeast Serbia climate conditions. Among the investigated sour cherries, „Oblačinska“ cultivar contained the highest amounts of all groups of phenolics, followed by „Cigančica“ > „Marela“. A significant difference were observed in the phenolic content among different cultivars and growing seasons ($p < 0.05$), and the phenolic compounds were significantly higher in the growing season 2009. The examined cultivars possess a high antioxidant capacity, and all phenolics of high correlation with TAC. The following compounds were identified and quantified using HPLC-DAD: 4 anthocyanins, the most abundant of which was cyanidin-3-glucoside in „Marela“ and „Oblačinska“, and cyanidin-3-glucosylrutinoside in „Cigančica“, and 4 hydroxycinnamic acids, the most abundant of which was neochlorogenic acid in all sour cherry cultivars. The growing and ripening process on the tree of sour cherry cv. „Oblačinska“ was evaluated also. The results showed significant increases in total phenols during the ripening, the total anthocyanins and total antioxidant capacity and 4 quantified anthocyanins, however the neochlorogenic acid decreased during the ripening. The study indicated that the growing and climate conditions in southeast Serbia are convenient for introducing sour cherry cultivars.

Keywords: sour cherries; phenolic composition; antioxidant capacity; anthocyanins; growing season; ripening.

Because of the health benefits attributed to various fruits, numerous studies have been conducted in recent years to evaluate their properties in terms of quality and bioactivity [1-3]. Fruits are considered a natural source of antioxidants, including anthocyanins and polyphenols [4] compounds that can reduce the risk of degenerative diseases caused by oxidative stress, such as cancer and cardiovascular diseases [5]. Red fruits, including sour cherries, are rich in these types of compounds. Sour cherries (*Prunus cerasus* L.) are considered to be one of the richest sources of phenolic compounds [6,7], and they contain significant levels of anthocyanins. Anthocyanins from

sour cherries have been shown to possess strong antioxidant and anti-inflammatory activities [8].

There are many factors that influence the phenolic content and antioxidant capacity of sour cherries. The results obtained from several studies suggest that the composition and content of phenolic compounds in cherries are influenced by the cultivar, the growing season and the growing location [9,10]. Also, fruit ripening is associated with important biochemical changes, while the color change is mainly influenced by the concentration and distribution of different anthocyanins in the skin [11]. The total anthocyanins contents in red fruits increase during ripening [12,13]. The studies of ripening are of special interest because they allow the identification of the optimum point of maturity for harvesting and enable the delivery of fruit to consumers in its best condition in terms of nutritional, sensory and functional properties [14-16].

Corresponding author: M.N. Mitić, Faculty of Science and Mathematics, Department of Chemistry, University of Niš, Višegradska 33, P.O.Box 224, 18000 Niš, Serbia.

E-mail: milanmitic83@yahoo.com

Paper received: 1 July, 2011

Paper revised: 6 September, 2011

Paper accepted: 7 October, 2011

Sour cherry is a highly profitable fruit variety. Owing to its rapid coming to bearing, relatively easy growing and a good demand on market, there is a huge interest in speeding this fruit variety, so in Serbian fruit production it is in third place [17]. The main region for its growing in our country is south and south-eastern Serbia. Owing to the quality of it Serbia has become a significant exporter of sour cherry. The sour cherry „Oblačinska“ is the most demanded variety because of its high quality. It is characterized by a great uniformity of fruit size, simultaneous ripening and easy picking. The color is resistant to darkening and degradation. The large sour cherry production in Serbia is realized via constant creation of new sour cherry plantations on individual owners' property.

The sour cherry „Cigančica“ is a domestic spontaneously created species, which is spread in almost all parts of Serbia. The fruit is of dark red colour, semi-sweet, tasteful and of pleasant aroma and different size. Its fruit bearing are regular and abundant. It dominates in so-called “free market” in unorganised production.

The sour cherry cv. „Marela“ is a hybrid of sweet cherry and sour cherry. It is similar in aroma to cherry. Marela is very appreciated for its consumption as fresh fruit, as well as for industrial processing.

There are no studies on chemical profiles of sour cherry cultivars growing in Serbian climate conditions. Therefore, in this study the three introduced sour cherry cultivars, „Marela“ (early season ripening), „Oblačinska“ and „Cigančica“ (late season ripening) were evaluated under the southern Serbian growing conditions. The objective on the present study is to evaluate the content of phenolics (total phenols, flavonoids and anthocyanins) as quality markers as well as the antioxidant capacity in the mentioned sour cherries, depending on the cultivars and growing seasons (2008, 2009 and 2010). Also, the objective of this work was to study the evolution of several physicochemical parameters, anthocyanins, phenols and antioxidant capacity during the ripening of „Oblačinska“ sour cherry cultivar.

MATERIALS AND METHODS

Chemicals

All chemicals used for the high-performance liquid chromatography (HPLC) analysis were of analytical grade and were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO). All solvents and reagents were of HPLC grade and purchased from Merck (Darmstadt, Germany).

Plant material

The samples of sour cherries used in this study were obtained from seven-years-old cherry trees of „Marela“, „Oblačinska“ and „Cigančica“ cultivars from an orchard in Southern Serbia. The fruits of the cultivars were collected at random from multiple trees. The fruit samples were harvested during the 2008, 2009 and 2010 growing seasons. Also, the fruits of the cultivar „Oblačinska“ were collected into 7 ripening stages, ripening stage 7 corresponding to commercial maturity. After the harvesting the samples of each cultivar were picked in polyethylene bags and stored at -20 °C. Before the analysis, a portion of fresh fruits was partially defrosted and homogenized in a house blender.

Sample preparation

The polyphenolic constituents from sour cherry samples were extracted using conventional solvent extraction procedure. Ten grams of homogenized sour cherries (1 min in blender) were extracted in an ultrasound bath with 30 ml of methanol solution containing 0.1% HCl. Contact time was 60 min. After the extraction, the samples were filtered with Whatman No. 1 filter paper and the residual tissue was washed with 2×20 ml of solvent. The filtrates were combined in a total extract. Finally, the obtained sour cherry extracts were collected in graduated vessels of the same volume at 100 ml. The obtained extracts were used for spectrophotometric and HPLC measurements. The extraction was done in triplicate for each sour cherry cultivar.

Determination of total phenolics (TP)

The Folin-Ciocalteu reagent was used to determine the total phenolic compounds [18]. A volume 1 ml of sour cherry extract, diluted 5-6 times with methanol (to obtain absorbance within the range of the prepared calibration curve), was mixed with 0.5 ml of Folin-Ciocalteu reagent, previously diluted with distilled water (1:2). A volume of 2 ml of 20% sodium carbonate solution was added to the mixture, shaken thoroughly and diluted to 10 ml by adding distilled water. The mixture was to stand for 120 min and the blue color formed was measured at 760 nm using the Agilent UV-Vis spectrophotometer. Gallic acid was used as a standard for the calibration curve. The concentrations of gallic acid in the solution from which the curve was prepared were 0, 50, 100, 150, 250 and 500 mg/L ($R^2 = 0.998$) The content of TP was expressed as mg of gallic acid equivalent (GAE) per 100g of fresh mass of edible part of fruits. The result of each assay was obtained from three parallel determinations.

Determination of total flavonoids (TF)

The total flavonoids (TF) assay was done as previously described by Zhuang *et al.* [19] with minor modifications. A volume of 1 ml of diluted extract or standard solution of catechin (50-500 mg/L) was placed in a 10 ml volumetric flask, then 4 ml of dd H₂O, and after 5 min 0.3 ml of NaNO₂ (5%) and 1.5 ml of AlCl₃ (2%) were added. The mixture was shaken, and 5 min later, 2 ml of 1 M solution of NaOH were added, again well shaken. The absorbance was measured at 510 nm against the blank. The results were calculated according to the calibration curve for catechin ($R^2 = 0.999$). The content of TF was expressed as mg of catechin equivalent (CE) per 100 g of fm of edible part of fruits. All samples were analyzed in triplicate.

Determination of total antioxidant capacity of DPPH method

The free radical scavenging capacity of fruit extracts was analyzed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [20-22]. The antioxidant assay is based on the measurement of the color loss of DPPH solution by the change of absorbance of 515 nm caused by the reaction of DPPH with the tested sample. The reaction was monitored using UV-Vis Agilent spectrophotometer. Sour cherry extracts 0.2 ml or methanolic solution of Trolox (10-30 mM) and 1.8 ml of freshly prepared DPPH in methanol (20 mg L⁻¹) were put into a cuvette at room temperature. After 30 min of incubation period at room temperature, the absorbance was read against a blank at 515 nm. The determinations were performed in triplicate. The total antioxidant capacity was calculated as Trolox (mmol/100g f.w. of edible part of fruit) using the equation based on the calibration curve ($R^2 = 0.996$).

Determination of total anthocyanins (TA)

The total anthocyanins in the sour cherry extracts were determined using the pH-differential method previously described by Guisti and Wrolstad [23]. Anthocyanins demonstrate maxima of absorbance at 520 nm at pH 1.0. The colored oxonium form predominates at pH 1.0, and the colorless hemiacetal form at 4.5. The difference in absorbance is proportional to the anthocyanin content. The total anthocyanin content was expressed as cyanidin-3-glucoside equivalent and calculated via the following equation:

$$c(\text{mg}/100\text{gf.w.}) = \frac{A \times MW \times DF \times 1000}{\varepsilon l} \quad (1)$$

where $A = (A(520 \text{ nm, pH } 1.0) - A(700 \text{ nm, pH } 1.0)) - (A(520 \text{ nm, pH } 4.5) - A(700 \text{ nm, pH } 4.0))$; MW = cyanidin-3-glucoside molecular weight (449.2); DF =

= dilution factor; ε = cyanidin-3-glucoside molar absorptivity (26,000); l = cell pathlength (usually 1 cm).

HPLC Analysis

The anthocyanins and hydroxycinnamic acids were analyzed by a direct injection of the extracts, previously filtered through a 0.45 μm pore size membrane filter, in an Agilent Technologies 1200 chromatographic system equipped with an Agilent photodiode array detector (DAD) 1200 with RFID tracking technology for flow cells and a UV lamp, an automatic injector and Chemstation software. The separation of anthocyanins and hydroxycinnamic acid was performed on an Agilent-Eclipse XDBC-18 4.6 \times 150 mm column. Two solvents were used for the gradient elution: A - H₂O + 5% HCOOH and B - 80% ACN + 5% HCOOH + H₂O. The elution program used was as follows: from 0 to 28 min, 0.0% B, from 28 to 35 min, 25% B, from 35 to 40 min, 50% B, from 40 to 45 min, 80% B and finally for the last 10 min again 0% B. The flow rate was 0.8 ml min⁻¹ and the injection volume was 5 μl . The detection wavelengths were 320 and 520 nm. Identification [24] and quantification of the various phenolic compounds were made by means of calibration curves obtained with the standard solution of chlorogenic acid, *p*-coumaric acid, ferulic acid, cyanidin-3-glucoside and cyanidin-3-rutinoside. The results are expressed as mg/100g of sample.

Statistical analysis

Analysis of variance (ANOVA) and Duncan's multiple range method were used to compare any significant difference between the samples. The values are expressed as mean \pm standard deviations. The differences are considered significant at $p < 0.05$. All the analyses were carried out in triplicates.

RESULTS AND DISCUSSION

Total phenolics, flavonoids, anthocyanins and total antioxidant capacity

The contents of total phenolics (TP), total flavonoids (TF), total anthocyanins (TA) and total antioxidant capacity (TAC) in three sour cherry cultivars („Marela“, „Oblaćinska“ and „Cigančica“) introduced in the southeast Serbian climate conditions during three growing seasons (2008, 2009 and 2010) are given in Table 1. TP content in the sour cherries harvested in 2008 determined with Folin-Ciocalteu assay ranged from 72.05 mg of gallic acid equivalents (GAE) per 100g of f.w. in early season ripening cultivar „Marela“ to 140.76 mg of GAE per 100g f.w. in late-season ripening cultivar „Oblaćinska“. In the same cultivar harvested in 2009, TP content was higher and ranged

Table 1. Total phenols (TP), flavonoids (TF), anthocyanins (TA) and antioxidant capacity (TAC) in sour cherry cultivars in growing seasons 2008, 2009 and 2010 (data are reported as mean \pm standard deviations with three replication; mean in a column followed by different letters are significantly different among sour cherry cultivars in one growing season; different letters denote a significant difference of one cultivar between the growing seasons)

Parameter	Cultivar	2008	2009	2010
TP ^a , mg GAE/100g f.w.	Marela	72.05 \pm 0.45c ¹ ,c ²	87.83 \pm 1.82c,a	78.98 \pm 1.70c,b
	Oblačinska	140.76 \pm 3.04a,b	159.03 \pm 3.97a,a	146.12 \pm 2.80a,b
	Cigančica	91.02 \pm 0.52b,c	122.10 \pm 3.60b,a	112.83 \pm 4.62b,b
TF ^a , mg CE/100g f.w.	Marela	65.51 \pm 1.64b,a	65.99 \pm 2.86c,a	64.38 \pm 2.15c,a
	Oblačinska	75.58 \pm 0.79a,c	124.51 \pm 0.82a,a	110.87 \pm 6.55a,b
	Cigančica	75.86 \pm 1.13a,b	87.82 \pm 1.84b,a	93.70 \pm 5.40b,a
TAA ^a , mg C3GE/100g f.w.	Marela	39.02 \pm 1.28b,b	48.24 \pm 1.67b,a	45.24 \pm 1.11c,a
	Oblačinska	54.14 \pm 2.06a,c	64.43 \pm 0.57a,b	81.55 \pm 1.78a,a
	Cigančica	55.76 \pm 1.24a,c	62.30 \pm 0.98a,b	66.72 \pm 1.55b,a
TAC ^a , mmol TE/100g f.w.	Marela	3.12 \pm 0.08b,ab	3.36 \pm 0.11b,a	2.87 \pm 0.10b,b
	Oblačinska	3.47 \pm 0.07a,a	3.80 \pm 0.18ac,a	3.40 \pm 0.04a,a
	Cigančica	3.49 \pm 0.13a,a	3.51 \pm 0.08cb,a	3.00 \pm 0.16b,b

from 87.83 to 159.03 mg of GAE/100g f.w., and also ranged from 78.98 to 146.12 mg GAE/100g f.w. in 2010.

„Oblačinska“ had the highest total phenolic content, followed by „Cigančica“ and „Marela“. According to the results obtained in our research, it is observed that in 2008, 2009 and 2010 growing season, late-season ripening cultivars „Oblačinska“ and „Cigančica“ contained higher amounts of total phenolics. The average content of total phenolics of sour cherry „Oblačinska“ was about two times higher than that of sour cherry „Marela“. Cultivar with the lowest amount of phenolics was „Marela“, and this was because it is an early season ripening cultivar, when lower temperatures decrease the biosynthesis of phenolics.

Significant differences were found in the total phenolic content in one growing season when comparing „Marela“, „Oblačinska“ and „Cigančica“ ($p < 0.05$). Significant differences were found in the total phenolic content in „Marela“ and „Cigančica“ when comparing 2008, 2009 and 2010 growing seasons ($p < 0.05$); however, no significant differences were found in the total phenolic content in „Oblačinska“ when comparing 2008 and 2010 growing seasons ($p < 0.05$). It is well known that genetic and agronomic or environmental factors play an important role in the phenolic composition and thus the nutritional quality of fruits [25-27]. TP Values of the Serbian sour cherry cultivars analysed in this study were in the range obtained for sour cherry cultivars grown from other countries such as Croatian (78.02-144.79 mg GAE/100g f.w.) [6], Italy (141.9 mg GAE/100g f.w.) [28] and the USA (146.1-312.4 mg GAE/100mg f.w.) [9].

In the total phenolic content, flavonoids were predominant, and their amount varied from 65.51 mg CE per 100g of f.w. in „Marela“ to 75.86 mg CE/100g

f.w. in „Cigančica“ in 2008, from 65.99 in ‘Marela’ to 124.51 mg CE/100g f.w. in „Oblačinska“ in 2009, whereas in 2010 their amounts ranged from 64.38 to 110.87 mg CE/100g f.w. An approximately 1.89-fold differences in the total flavonoid content was found between the highest and lowest ranked cultivars, „Oblačinska“ and „Marela“ in 2009 ($p < 0.05$). It was calculated that percentages of TF in TP varied between 49 and 83% in sour cherry fruits grown in 2008, between 72 and 78% in sour cherry fruits grown in 2009, and between 79 and 88% in sour cherry fruits grown in 2010. Comparing the level of TF in TP between 2008, 2009 and 2010 growing seasons, it was observed that sour cherries harvested in 2010 contained a higher percentage of TF in TP. Also, significant differences were found in the total flavonoid content in comparison among cultivars „Oblačinska“, „Marela“ and „Cigančica“ in some years (except for 2008). No significant differences in the total flavonoid content were found in comparisons among growing seasons 2008, 2009 and 2010 from „Marela“.

The amount anthocyanins in the three sour cherry cultivars introduced to southern Serbian climate conditions are given in Table 1, also. It is evident that „Oblačinska“ is the richest source of anthocyanins among the examined cultivars. The amount of anthocyanins in „Oblačinska“ varied between 54.14 (year 2008) and 81.55 mg C3GE per 100g f.w. (year 2010). In other cultivars as follows: „Cigančica“ > „Marela“. The anthocyanins content of „Marela“ was significantly different from the others ($p < 0.05$). Comparing the growing seasons 2008, 2009 and 2010, the environmental growing conditions showed some differences (Table 2). The Meteorological Service Knja-

Table 2. Mean air temperatures, data from Meteorological Service, Krnjaževac, Serbia

Growing season	April		May		June	
	I Decade	II Decade	I Decade	II Decade	I Decade	II Decade
2008	10.4	13.7	12.8	17.3	18.5	20.7
2009	12.9	12.0	17.6	14.3	20.7	21.6
2010	12.9	12.0	17.1	13.9	19.0	24.5

ževac data confirmed that, in 2009, the mean daily air temperature in June was perfect for the biosynthesis of phenolic compounds in fruit, while the second decade of June 2010 was perfect for the increase of anthocyanin content.

Studies have shown that numerous factors such as harvest season, variety, stage of harvesting, climatic conditions and growing season can affect the composition and concentration of individual as well as total anthocyanins [3,29]. In most species, fruit anthocyanins concentrations increase with ripening, as their biosynthesis proceeds faster than the fruit growth [14,30]. Anthocyanins are synthesized *via* the phenyl propanoid pathway and for anthocyanins biosynthesis the structural and regulatory enzymes are required, but environmental influence is also very important [31]. Light stimulated the synthesis of flavonoids, especially anthocyanins [32].

The total antioxidant capacity (TAC) in three sour cherry cultivars introduced in southeast Serbian climate conditions are given in Table 1. The TAC of

„Marela“ was lower than in other cultivars. The highest TAC was observed in „Oblačinska“ sour sherry fruits, a cultivar which contained significantly higher amounts of all examined groups of phenolics. The differences in values for different grown seasons for sour cherry „Oblačinska“ were not statistically significant ($p < 0.05$) (Table 1).

HPLC Analysis of phenolics

Using HPLC-DAD techniques, we performed a comparative analysis of the levels of individual anthocyanins. A typical HPLC chromatogram of the methanol extracts from sour cherry recorded at 520 nm is shown in Figure 1. The sample was the anthocyanin fingerprint of sour cherries, consisting of four compounds. Peaks were identified by comparison of their HPLC retention times and UV-Vis absorption spectra with the authentic compound as cyanidin derivative (peak 1), cyanidin-3-glucosyl rutinoside (peak 2), cyanidin-3-glucoside (peak 3) and cyanidin-3-rutinoside (peak 4). These compounds have previously been

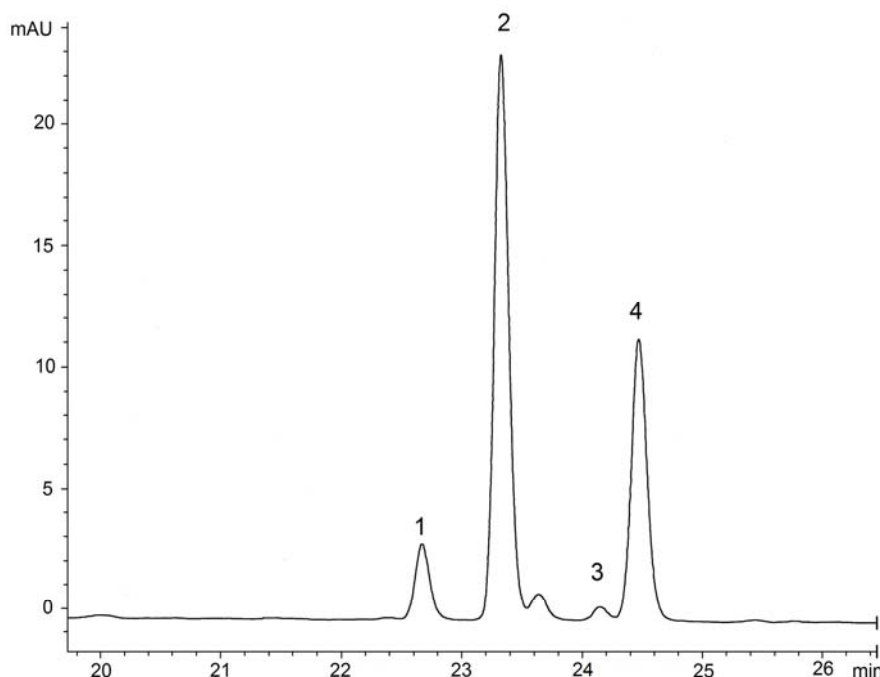


Figure 1. HPLC chromatogram (520 nm) of anthocyanins in sour cherry „Oblačinska“: 1) cyanidin derivate, 2) cyanidin 3-glucosyl-rutinoside, 3) cyanidin 3-glucoside, 4) cyanidin 3-rutinoside.

characterised in the cultivars of sour cherries [9,10,33,34] and sour cherry juice [35].

Among the cherries analyzed, the sour cherry „Oblaćinska“ showed the highest amount of anthocyanins, cyanidin-3-glucosyl rutinoside, at 62.09 mg/100g f.w. (Table 3). Two other sour cherries, „Cigančica“ where next to „Marela“ having cyanidin-3-glucosyl rutinoside at 38.42 and 24.73 mg/100g f.w., respectively. The published reports suggest that cyanidin-3-glucosyl rutinoside is always the predominant anthocyanin in sour cherries, although the total anthocyanin content varied significantly [9,34,36].

Sour cherries commonly contain neochlorogenic acid, *p*-coumaric acid derivative, *p*-coumaric acid and ferulic acid (Figure 2, Table 3). Neochlorogenic acid

was found to be the major hydroxycinnamic acid in sweet and sour cherries, the amount of which was relatively higher in sour cultivars than sweet cultivars [9]. Among the hydroxycinnamic acids, neochlorogenic acid composed 73-79%. Chaovanalikit and Wrulstad [7] showed that the level of hydroxycinnamic acid of cherries ranged from 30.0 to 87.0 mg/100g, which is higher than that of the cherries studied here (11.82-17.23 mg/100g f.w.)

Changes during ripening

Fruit ripening is associated with important biochemical changes that modify color, texture, taste and other quality traits. For a better description of autochthonous Serbian variety and understanding of chan-

Table 3. Individual anthocyanins and hydroxycinnamic acids in sour cherries in growing season 2010 (the data are presented with means \pm standard deviations, $n = 3$)

Compound	Marela	Oblaćinska	Cigančica
Anthocyanins			
Cyanidin-derivate ^a	4.93 \pm 0.22	2.96 \pm 0.18	8.13 \pm 0.36
Cyanidin-3-gluc.-rutin.	24.73 \pm 0.72	62.09 \pm 1.95	38.42 \pm 1.35
Cyanidin-3-glucoside	0.88 \pm 0.09	1.31 \pm 0.10	1.24 \pm 0.11
Cyanidin-3-rutinoside	9.49 \pm 0.52	12.95 \pm 0.78	17.13 \pm 1.02
Hydroxycinnamic acid			
Neochlorogenic acid ^b	10.51 \pm 0.83	12.64 \pm 0.65	9.37 \pm 0.88
<i>p</i> -Coumaric acid derivate ^c	1.05 \pm 0.12	1.73 \pm 0.08	1.54 \pm 0.20
<i>p</i> -Coumaric acid	1.22 \pm 0.09	2.08 \pm 0.05	0.58 \pm 0.11
Ferulic acid	0.84 \pm 0.05	0.78 \pm 0.02	0.33 \pm 0.02

^aThe concentration of cyanidin derivate is expressed as cyanidin-3-glucoside equivalents; ^bthe level of neochlorogenic acid is expressed as chlorogenic acid equivalents; ^cthe level of *p*-coumaric acid derivate is expressed as *p*-coumaric acid equivalents

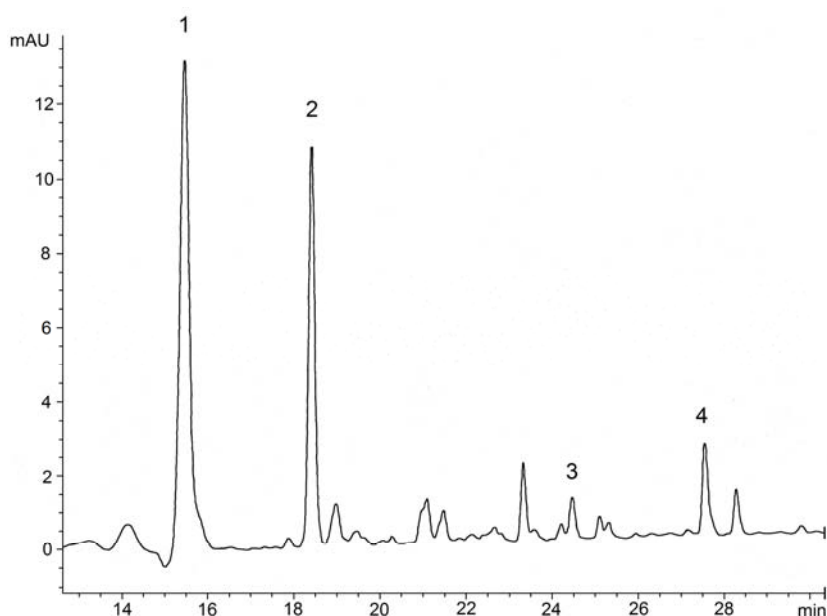


Figure 2. HPLC chromatogram (320 nm) of hydroxycinnamic acids in sour cherry „Oblaćinska“: 1) neochlorogenic acid, 2) *p*-coumaric acid derivate, 3) *p*-coumaric acid, 4) ferulic acid.

ges of quality during ripening, the total phenolics, total anthocyanins and total antioxidant capacity, as well as individual phenolic compounds in sour cherry „Oblačinska“ were studied (Figures 3-5).

The total phenolic content showed an increase from 26.2 (year 2010) to 42.0% (year 2009). A similar accumulation of total phenols during the ripening of the sour cherry „Maraska“ has been described by Pedisic *et al.* [12]. Stages 1-3 were significantly different from stage 5-7. Total antioxidant capacity varied between 2.56 mmol trolox equivalents/100g fresh weight (stage 1) and 3.28 mmol TE/100g f.w. (stage

7) in growing season 2008; 2.58-3.80 in grown season 2009 and 2.42-3.40 in grown season in 2010. A similar behavior was found for both total antioxidant activity and total phenolic compounds, for which little decreases at early stages of development followed by the increases from at late stages were observed. TAC reached the maximum activity at stage 7 which coincided with the maximum concentration of total polyphenolic compounds. When linear regression was performed, a highly positive correlation ($R^2_{2008} = 0.986$; $R^2_{2009} = 0.847$; $R^2_{2010} = 0.907$) was found between TAC and the total phenolic compounds. Thereafter,

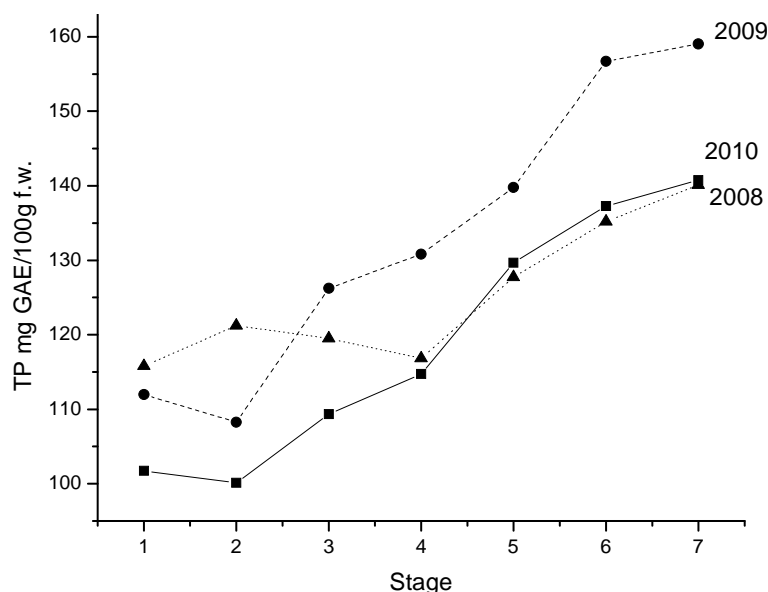


Figure 3. Changes of total phenolic content (mg/100g) during cv. „Oblačinska“ ripening in the three grown seasons, ▲ : 2008; ● : 2009; ■ : 2010.

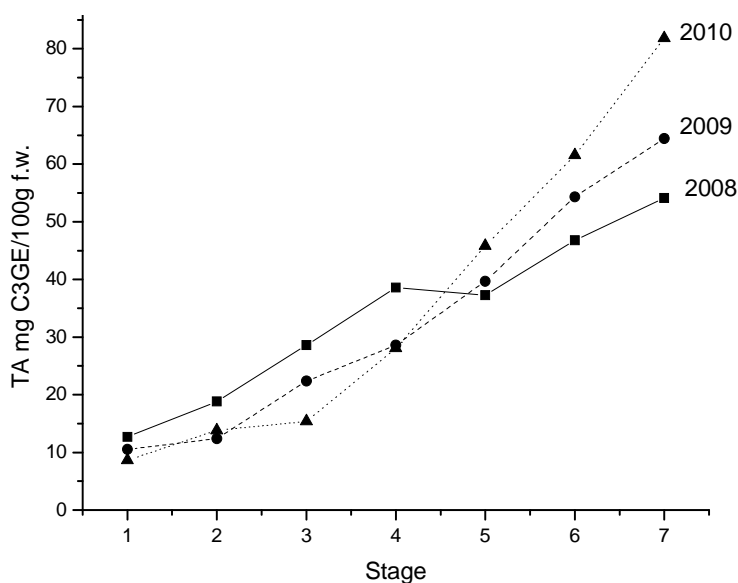


Figure 4. Changes of anthocyanin content (mg/100g) during cv. „Oblačinska“ ripening in the three grown seasons, ■ : 2008; ● : 2009; ▲ : 2010.

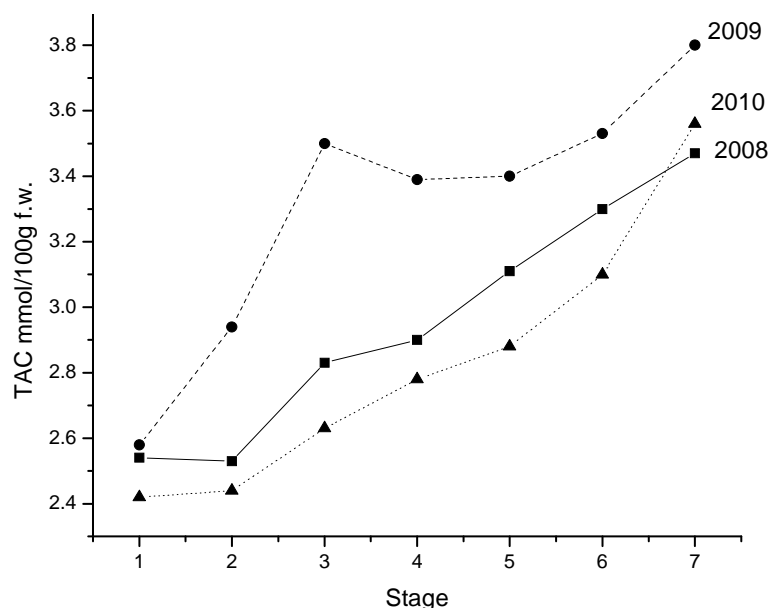


Figure 5. Changes of total antioxidant capacity (mmol/100g) during cv. „Oblačinska” ripening in the three grown seasons, ■: 2008; ●: 2009; ▲: 2010.

from late stages (4-7) and coinciding with the greatest accumulation of anthocyanins, a highly positive correlation ($R^2_{2008} = 0.968$; $R^2_{2009} = 0.835$; $R^2_{2010} = 0.978$) was also found between TAC and the anthocyanin concentration. They represent an accumulation of anthocyanins that is reflected in the change of color of the fruit during the ripening [15]. The correlation between TAC and anthocyanins compounds during ripening has been found in several fruits [1,37,38].

The change of quantities of cyanidin-3-glucosyl rutinoside, cyanidin-3-rutinoside and neochlorogenic acid during ripening for the sour cherry „Oblačinska“

in growing season 2010 are shown in Figure 6. In the studied sour cherry cultivar, the concentration of cyanidin-3-glucosyl rutinoside increased 3.2-fold during the ripening, cyanidin-3-rutinoside increased 4.9-fold during the ripening, however, concentration of neochlorogenic acid decreased 2.2-fold during the ripening. Hydroxycinnamic acids concentration are generally high in young fruits, falling first rapidly and then more slowly during maturation and postharvest storage [39].

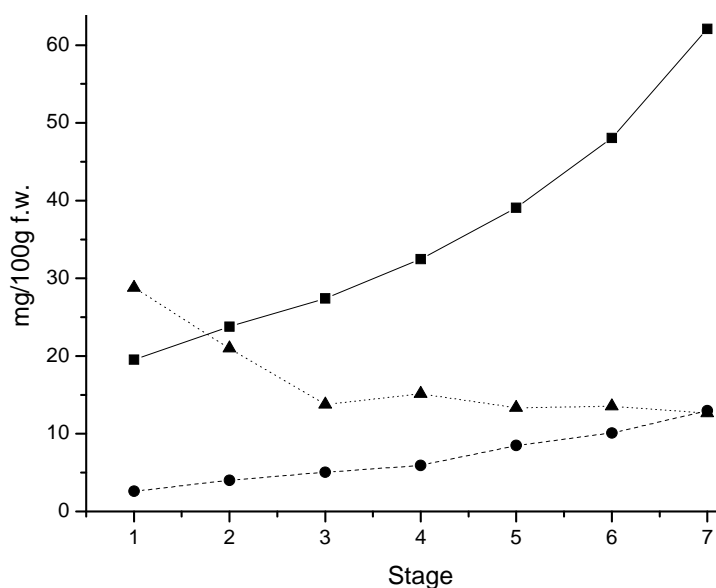


Figure 6. Changes of individual anthocyanins (mg/ 100 g) during cv. „Oblačinska” ripening in 2010 growing season: cyanidin-3-glucosyl rutinoside (■), cyaniding 3-rutinoside (▲) and neochlorogenic acid (●).

CONCLUSION

Generally, growing and climate conditions in Serbia are convenient for introducing sour cherry cultivars („Oblačinska“, „Marela“ and „Cigančica“). This was proven by the good quality of sour cherry fruit, by the contained high mass fractions of different groups of phenolics (particularly important flavonoids, especially anthocyanins) during three consecutive growing seasons. The richest source of TP, TF and TA among cultivars was „Oblačinska“, followed „Cigančica“ and „Marela“. Antioxidant capacity reached the highest levels in „Oblačinska“ and „Cigančica“ sour cherry fruits. Total antioxidant capacity, total phenolic compounds and anthocyanins decreased or slightly increased in the early stages of sour cherry developments but exponentially increased from stage 4, which coincided with the anthocyanin accumulation and fruit darkening.

Acknowledgements

This work was supported by the European Union, FP7-REG-POT-2007-3-01, KBBE: food, agriculture and biotechnology, project “CHROMOLAB-ANTIOXIDANT”, No. 204756, and by the Serbian Ministry of Education and Science (grant number 142015).

REFERENCES

- [1] M.J. Serradilla, M. Lozano, M.J. Bernalte, M.C. Ayuso, M. Lopez-Corrales, D. Gonzalez-Gomez, *LWT-Food Sci. Technol.* **44** (2011) 199-205
- [2] D. Gonzalez-Gomez, M. Lozano, M.I. Fernandez-Leon, M.J. Bernalte, M.C. Ayuso, A.B. Rodrigues, *J. Food Compos. Anal.* **23** (2010) 533-539
- [3] F.A. Tomas-Barberan, J.C. Espin, *J. Sci. Food Agric.* **81** (2001) 853-876
- [4] C. Kaur, H.C. Kapoor, *Int. J. Sci. Technol.* **36** (2001) 703-725
- [5] Q. Ma, K. Kinner, *J. Biol. Chem.* **227** (2002) 2477-2484
- [6] V. Dragovic-Uzelac, B. Levaj, D. Bursac, S. Pedisic, I. Radojčić, A. Bisko, *Agric. Cons. Sci.* **72** (2007) 279-284
- [7] A. Chaovanalikit, R.E. Wrolstad, *J. Food. Sci.* **69** (2004) 67-72
- [8] H. Wang, M.G. Noir, G.M. Strasburg, Y. Chang, A.M. Boren, I.J. Gray, D. L. DeWitt, *J. Nat. Prod.* **62** (1999) 294-296
- [9] D.O. Kim, H.J. Heo, Y.J. Kim, H.S. Yang, C.Y. Lee, *J. Agric. Food Chem.* **53** (2005) 9921-9927
- [10] A. Kirakosyan, E.M. Seymour, D.E. Urcuyo Leanes, P.B. Kaufman, S.F. Bolling, *Food. Chem.* **115** (2009) 20-25
- [11] L. Gao, G. Mazza, *J. Agric. Food Chem.* **43** (1995) 343-346
- [12] S. Pedisic, B. Levaj, V. Dragovic-Uzelac, K. Kos, *Agric. Cons. Sci.* **72** (2007) 295-300
- [13] S. Pedisic, B. Levaj, V. Dragovic-Uzelac, D. Skevin, M. Skendrovic Babojevic, *Agric. Cons. Sci.* **74** (2009) 259-262
- [14] B. Mozetic, P. Trebse, M. Simcic, J. Hribar, *Lebensm. Wiss. Technol.* **37** (2004) 123-124
- [15] M. Serrano, F. Guillen, D. Martinez-Romero, S. Castillo, D. Valero, *J. Agric. Food Chem.* **53** (2005) 2741-2745
- [16] M. Serrano, H.M. Diaz-Mula, P.J. Zapata, S. Castillo, F. Guillen, D. Martinez-Romero, M. Valverde Juan, D. Valero, *J. Agric. Food. Chem.* **57** (2009) 3240-3246
- [17] M. Fotiric, D. Nikolic, V. Rakonjac, *Genetica* **39** (2007) 297-304
- [18] V.L. Singleton, J.A. Rossi, *Am. J. Enol. Vitic.* **16** (1965) 144-158
- [19] X.P. Zhuang, Y.Y. Lu, G.S. Yang, *China Herb. Med.* **23** (1992) 122-124
- [20] J. Lachman, M. Sulc, M. Schila, *Food Chem.* **103** (2007) 802-807
- [21] A. Turkoglu, M. Duru, N. Mecan, I. Kivrak, K. Gezer, *Food Chem* **101** (2007) 267-273
- [22] D. Villano, M.S. Fernandez-Pachon, A.M. Troncoso, M.C. Garcia-Parrilla, *Food Chem.* **94** (2006) 394-404
- [23] M.M. Guisti, R.E. Wrolstad, In: R.E. Wrolstad, Ed., *John Wiley & Sons, New York*, 2001, p. F1.2.1-1.2.13
- [24] K.R. Maatta-Riihinen, A. Kamal-Eldin, A.R. Torronen, *J. Agric. Food Chem.* **52** (2004) 6178-6187
- [25] S. Pedesic, V. Dragovic-Uzelac, B. Levaj, D. Skevin, *Food Technol. Biotechnol.* **48** (2010) 86-93
- [26] L. Jakobek, M. Seruga, S. Voca, Z. Sindrak, N. Dobricevic, *Sci. Hortic.* **123** (2009) 23-28
- [27] S.H. Hakkinen, A.R. Torronen, *Food Res. Int.* **33** (2000) 517-527
- [28] S. Piccolella, A. Fiorentina, S. Pacifico, B. D'Abrosca, P. Uzzo, P. Monaco, *J. Agric. Food Chem.* **56** (2008) 1928-1935
- [29] A. Sass-Kiss, J. Kiss, P. Milotay, M.M. Kerek, M. Toth-Markus, *Food Res. Int.* **38** (2005) 1023-1029
- [30] S. Bureau, C.M.G.C. Renard, M. Reich, C. Ginies, J.M. Audergon, *LWT-Food Sci. Technol.* **42** (2009) 372-377
- [31] Y. Hamazu, *Steward Postharv. Rev.* **2** (2006) 1-7
- [32] A. Solovchenko, M. Merzlyak, *Phytochem. Protobiol. Sci.* **2** (2003) 861-866
- [33] V. Simunic, S. Kovac, D. Gaso-Sokac, W. Pfannhauser, M. Kurkovic, *Eur. Food Res. Technol.* **220** (2005) 575-578
- [34] F. Blando, C. Gerardi, I. Nicoletti, *J. Biomed. Biotechnol.* **5** (2004) 253-258
- [35] D. Bonerz, K. Wurth, H. Dietrich, F. Will, *Eur. Food Res. Technol.* **224** (2007) 355-364
- [36] V. Mulabagal, G.A. Lang, D.L. De Witt, S.S. Dalavoy, H.G. Noir, *J. Agric. Food Chem.* **57** (2009) 1239-1246
- [37] S. Bureau, C.M.G.C. Renard, M. Reich, C. Ginies, J.M. Audergon, *LWT-Food Sci. Technol.* **42** (2009) 372-377
- [38] H.M. Diaz-Mula, S. Castillo, D. Martinez-Romero, D. Valero, P.J. Zapata, F. Guillen, M. Serrano, *Food Sci. Tech. Int.* **15** (2009) 535-543
- [39] H-D. Mosel, K. Herrmann, Z. *Lebensm-Un.* **154** (1974) 324-327.

MILAN N. MITIĆ
MIRJANA V. OBRADOVIĆ
DANIJELA A. KOSTIĆ
RUŽICA J. MICIĆ
EMILIJA T. PECEV

*Prirodno-matematički fakultet,
Departman za hemiju, Univerzitet u
Nišu, Višegradska 33, p. pr. 224,
18000 Niš, Srbija*

NAUČNI RAD

POLIFENOLNI SASTAV I ANTIOKSIDATIVNA AKTIVNOST CRVENIH VIŠANJA IZ SRBIJE

Cilj ovog rada je određivanje fenolnog sastava: totalnih fenola (TP), totalnih flavonoida (TF), antocijana (TA) i totalnog antioksidativnog kapaciteta u tri uzoraka višnje sa jugoistočnog područja Srbije. Među ispitivanim uzorcima, Oblačinska višnja sadrži najviše fenolnih jedinjenja, praćena Cigančicom i Marelom. Značajna razlika je uočena u sadržaju fenolnih jedinjenja između različitih vrsta u različitim godinama zrenja. Ispitivane sorte imaju visok antioksidativni kapacitet koji je u visokoj korelaciji sa fenolnim jedinjenjima. Za određivanje individualnih komponenti koristili smo HPLC-DAD metodu i odredili sledeće komponente: 4 antocijana, od kojih je cijanidin-3-glukozid najzastupljeniji u Mareli i Oblačinskoj višnji, a cijanidin-3-glukozidrutinozid u Cigančici; 4 hidroksicimetnih kiselina, od kojih je neohlorogenska najzastupljenija u svim sortama. Takođe smo pratili rast i sazrevanje Oblačinske višnje. Rezultati su pokazali značajno povećanje ukupnih fenola (totalnih antocijana, antioksidativnog kapaciteta) tokom zrenja, međutim sadržaj neohlorogenske kiseline se smanjuje. Iz rezultata se može zaključiti da jugoistočni region Srbije ima povoljne uslove za gajenje i rast ove kulture.

Ključne reči: višnje, fenolni sastav, antioksidativni kapacitet, antocijani, rast, sazrevanje.