CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF FRUITS OF THREE PLUM CULTIVARS GRAFTED ON FOUR ROOTSTOCKS

Mirjana RADOVIĆ[1*], Dragan MILATOVIĆ[2], Živoslav TEŠIĆ[3], Dragana DABIĆ ZAGORAC[4], Gordan ZEC[2], Đorđe BOŠKOV[2]

[1]Faculty of Agriculture, University of East Sarajevo, Bosnia and Herzegovina
[2]Faculty of Agriculture, University of Belgrade, Serbia
[3]University of Belgrade, Faculty of Chemistry, University of Belgrade, Serbia
[4]Innovative Centre, Faculty of Chemistry, University of Belgrade, Serbia
*Corresponding author: rmirjana26@yahoo.com

Abstract

Chemical and antioxidant properties of fruits of three plum cultivars ('Čacanska Rana', 'Čacanska Lepotica' and 'Čacanska Najbolja') grafted on four rootstocks (Myrobalan, 'Pixy', 'Fereley' and 'St. Julien A') were studied in the region of Belgrade (Serbia). Chemical composition of plum fruits was found to be more cultivar- then rootstock-dependent. The average values for soluble solids, total sugars, inverted sugars, sucrose, and total acids content in cultivar/rootstocks combinations ranged from 11.7 to 14.2%, from 9.5 to 11.5%, from 6.6 to 7.6%, from 2.7 to 3.9%, and from 0.71 to1.11%, respectively. The contents of soluble solids and total sugars were the highest in fruits of 'Čacanska Najbolja', while the lowest contents were found in fruits of 'Čacanska Rana' cultivar. Total phenolic content (TPC) in the skin and flesh of plums was in the range from 4.44 to 15.93 mg GAE g^{-1}, and from 0.38 to 0.86 mg GAE g^{-1} respectively. TPC in the skin was 6–20 times higher than in the flesh of the same cultivar/rootstock combinations. Differences in TPC among rootstocks were not statistically significant, but significant differences among cultivars were found. Radical-scavenging activity (RSA) in the skin and flesh ranged from 39.08 to 78.49 μmol TE g^{-1}, and from 10.40 to 16.97 μmol TE g^{-1} respectively. Significant differences in RSA were found among cultivars and cultivar/rootstock combinations, whereas differences between rootstocks were not significant. The highest RSA was found in fruits of 'Čacanska Najbolja' cultivar.

Keywords: *Prunus domestica, cultivar/rootstock combinations, total phenolic content, radical-scavenging activity.*

Introduction

One of the most important commodities, consumed worldwide, owing to its degree of acceptance by consumers is plum fruit. Plums and their products have many beneficial effects on human health: improving bone health, antioxidant and anti-inflammatory activity, improving memory, reducing the risk of cardiovascular diseases, possess laxative effects, anti-allergy, and antimicrobial activity (Igwe and Charlton, 2016). This fruits are considered as a functional food because of the high content of bioactive compounds such as dietary fiber, sorbitol, phenolic compounds, and minerals (Stacewicz-Sapuntzakis et al., 2001). According Bohačenko et al. (2010), in addition to economic and physical parameters, such as yield, weight of fruit, shape, color of skin, stone mass, sensitivity of the fruit to biotic and abiotic factors, in the commercial use of the fruit, the chemical parameters also have an important role.

Thanks to the high sugar content, fruits of fresh plums, and especially dried plums (prunes), are an excellent source of energy. Although the main sugars in the plum fruit are monosaccharides, they do not affect the rapid increase in blood sugar levels due to the high

content of fibers and sorbitol (Eskin and Snait, 2006). Dominant sugar in plum fruit is glucose, followed by sorbitol, sucrose and fructose (Stacewicz-Sapuntzakis et al., 2001).
Phenolic compounds are biologically active substances having antioxidant properties and positive effects on human health (Walkowiak-Tomczak, 2008). The main phenolic compounds in the plum fruits are caffeic acid derivatives: neochlorogenic acid (3-*O*-caffeoylquinic acid), and chlorogenic acid (5-*O*-caffeoylquinic acid) (Stacewicz-Sapuntzakis et al., 2001). The skin contains about five times more phenolic substances than the flesh (Stacewicz-Sapuntzakis et al., 2001).
Antioxidant activity of plum fruits is higher in comparison to other pome and stone fruits, with the exception of sour cherries. In relation to the apple it is two to four times higher (Wang et al., 1996; Halvorsen et al., 2002; Kim et al., 2003; Cho et al., 2007). The antioxidant activity values are significantly higher in the skin than in the flesh of the fruits, which can be explained by the higher content of total phenolic compounds and anthocyanins (Stacewicz-Sapuntzakis et al., 2001).
Rootstocks can affect not only the vegetative growth and yield, but also the fruit quality. Although fruit quality is mostly cultivar-associated trait, the significant effects of rootstocks on fruit quality in different fruit crops have been reported (Usenik et al., 2010; Orazem et al., 2011; Bartolini et al., 2014; Reig et al., 2016; Font i Forcada et al., 2019; Iglesias et al., 2019; Milošević et al., 2020).
The influence of rootstocks on plum fruit quality attributes have been poorly studied, especially in the European plum. Most of the work has been related to their influence on the content of soluble solids and titratable acids in the fruit (Daza et al., 2008; Rato et al., 2008). The effects of the rootstocks on the composition of sugars and phenolic compounds in plum fruits have not thus far been investigated in detail. Therefore, the aim of this study was to determine the influence of three cultivars and four rootstocks on plum fruit chemical composition and antioxidant activity.

Material and methods

The fruits were taken from the plum orchard at the Experimental Station 'Radmilovac' of the Faculty of Agriculture in Belgrade (Serbia). Four rootstocks were included: one seedling rootstock (Myrobalan) and three clonal rootstocks ('Pixy', 'Fereley' and 'St. Julien A'). Three table plums cultivars ('Cacanska Rana', 'Cacanska Lepotica' and 'Cacanska Najbolja') were grafted on each rootstock. Every combination cultivar/rootstock was represented by six trees. The orchard was planted in 2010. Planting distance was 4 m between rows, and in the row different distances were applied depending on the rootstock vigor: 2.3 m for Myrobalan seedling, 2.0 m for 'Fereley' and 'St. Julien A' and 1.7 m for 'Pixy'. Training system was the Spindle. Standard cultural practices were applied, including drip irrigation. From every combination cultivar/rootstock, 50 fruits were picked at commercial maturity and used for chemical analyses. Chemical composition of fruits of three plum cultivars grafted on four rootstocks was studied over three years (2013-2015), while the total phenolic content and radical-scavenging activity in the skin and flesh were studied for only one year (2014).
The soluble solids were determined using a refractometer (Pocket PAL-1, Atago, Japan). Total sugar content was determined using Luff - Schoorl method. Total acids were determined by titration with NaOH and expressed as malic acid.
The extracts of plum skin and flesh were prepared according the previously described method (Pantelić et al., 2016). Frozen plums were used to separate skin from the flesh. The skin (approximately 3 g) and flesh (approximately 5 g) were extracted with 50 mL methanol containing 0.1 % HCl and stirred for 1 h on a magnetic agitator at room temperature. The extracts were left for 24 h in the dark at 4ºC, filtered, and the clear supernatants were

collected. The extraction step was repeated two more times and all supernatants were collected and evaporated to dryness under reduced pressure at 40ºC. The residue after evaporation was dissolved in a mixture of methanol/water (60:40, v/v) to 50 mL. All the extractions were performed in triplicate and the extracts were filtered through 0.45 µm membrane filters (Syringe Filter, PTFE, Supelco) before analysis.
The total phenolic content (TPC) in skin and flesh extracts was determined according the slightly modified method described by Singleton and Rossi (1965). Gallic acid was used as the standard in the concentration range of 20–100 mg L^{-1}. TPC values were expressed as milligrams of gallic acid equivalent (GAE) per gram of frozen weight (FW). $DPPH^{\cdot}$-scavenging activity of all extracts was assayed according to the method previously described by Pavlović et al. (2013). The Trolox calibration curve (100–600 µmol L^{-1}) was plotted as a function of the percentage inhibition of the $DPPH^{\cdot}$. The results are expressed as micromoles of Trolox equivalents per gram of sample (µmol TE g^{-1}).
Data of all measurements presented in the tables are the mean of three replicates ± standard deviation. Tukey's test was used to detect the significance of differences ($P \leq 0.05$) between mean values.

Results and discussion

According to many authors, the key parameters that determine the quality and the acceptance of the fruit by consumers are the content of soluble solids and total acids, as well as ration between them. (Nergiz and Yildiz, 1997; Crisosto et al., 2004). Soluble solids content of three plum cultivars grafted on four rootstocks ranged from 11.7 % to 14.2 % (Table 1). Highest values for soluble solids were found in 'Čačanska Najbolja', while the lowest values were found in 'Čačanska Rana' cultivar. However, differences among rootstocks were not significant. On the other hand, significant differences among cultivars were found. Also, significant differences for soluble solids were found between cultivar/rootstock combinations.

Table 1. Chemical composition of fruits of three plum cultivars grafted on four rootstocks (average 2013-2015).

Cultivar/rootstock combination	Soluble solids (%)	Total sugars (%)	Inverted sugars (%)	Sucrose (%)	Total acids (%)	Sugar/ acid ratio
Č. Lepotica/Fereley	13.0±0.3 de	10.6±0.6 c	7.6±0.6 a	2.9±0.4 bc	1.06±0.10 ab	10.0
Č. Lepotica/Myrobalan	13.8±0.6 abc	10.7±1.1 bc	7.4±1.0 ab	3.1±0.6 bc	0.98±0.03 bc	10.9
Č. Lepotica/Pixy	13.5±1.1 bcd	10.6±1.3 c	7.4±1.0 ab	3.0±0.6 bc	1.05±0.08 ab	10.1
Č. Lepotica/St. Julien A	13.3±1.1 cde	10.5±1.3 cd	7.5±1.2 ab	2.8±0.8 bc	1.06±0.14 ab	9.9
Č. Najbolja/Fereley	13.9±0.4 abc	11.5±0.6 a	7.4±0.9 ab	3.9±0.5 a	0.73±0.10c	15.6
Č. Najbolja/Myrobalan	14.2±0.6 a	11.4±1.1 a	7.6±1.0 a	3.6±0.4 a	0.75±0.06 c	15.2
Č. Najbolja/Pixy	14.1±0.7 ab	11.3±0.8 ab	7.5±0.9 ab	3.6±0.4 a	0.73±0.08 c	15.4
Č. Najbolja/St. Julien A	14.0±0.5 ab	11.3±0.7 ab	7.3±1.1 ab	3.8±0.4 a	0.71±0.09 c	15.9
Č. Rana/Fereley	11.7±1.2 f	9.5±1.2 e	6.6±1.2 c	2.7±0.3 c	1.06±0.07 ab	8.9
Č. Rana/Myrobalan	13.1±1.3 de	10.1±1.4 cde	7.1±1.6 abc	3.1±0.6 bc	1.11±0.07 a	9.1
Č. Rana/Pixy	12.8±1.2 de	10.4±0.9 cd	7.4±0.8 ab	2.8±0.3 bc	1.11±0.03 a	9.4
Č. Rana/St. Julien A	12.7±1.5 e	9.9±1.6 de	7.0±1.3 bc	2.7±0.6 c	1.06±0.05 ab	9.4
Rootstocks						
Fereley	12.9±0.3 b	10.5±0.7 a	7.2±0.9 a	3.2±0.4 a	0.95±0.08 a	11.0
Myrobalan	13.7±0.3 a	10.7±1.1 a	7.4±1.0 a	3.3±0.5 a	0.95±0.04 a	11.3
Pixy	13.5±0.7 a	10.8±1.0 a	7.4±0.9 a	3.1±0.4 a	0.96±0.05 a	11.2
St. Julien A	13.3±1.1 ab	10.6±1.3 a	7.3±1.2 a	3.1±0.5 a	0.94±0.08 a	11.2

Cultivars						
Čacanska Lepotica	13.4±0.7 b	10.6±1.3 b	7.5±1.0 a	3.0±0.7 b	1.04±0.09 a	10.2
Čacanska Najbolja	14.1±0.5 a	11.4±0.9 a	7.4±0.8 ab	3.7±0.4 b	0.73±0.08 b	15.5
Čacanska Rana	12.6±1.2 c	10.0±1.5 c	7.0±1.3 b	2.9±0.4 b	1.08±0.05 a	9.2

Means followed by the same letter in a column are not significantly different (Tukey's test, $p \leq 0.05$).

According to Milenković et al. (2006) early maturing plum cultivars developed at the Institute of Fruit Growing in Čačak, contain from 12.5 % to 14.8 % of soluble solids, while the medium and late maturing plum cultivars have from 16.8 % to 32.0 %. In the literature, the content of soluble solids in cultivars of European plum usually ranges from 16-18% (Miletić and Petrović, 1996; Oparnica and Jovanović, 2000; Nenadović-Mratinić et al., 2007). The content of soluble solids in a large number of cultivars in the conditions of the Belgrade region was in the interval from 12.3 % to 21.9 %, and these values were correlated with maturation time (Nenadović-Mratinić et al., 2007; Milatović et al., 2011, 2016, 2017, 2018).
One of the important parameters of quality of fruit is the content of total acids. The highest values of content of total acids were found in combinations 'Čačanska Rana'/Myrobalan and 'Čačanska Rana'/'St. Julien A' (1.11 %), while the lowest values were obtained in combination 'Čačanska Najbolja'/'St. Julien A' (0.71%) (Table 1). Differences in content of total acids among rootstocks were not significant, while the cultivar 'Čačanska Najbolja' had a statistically significantly lower value of content of total acids in relation to the other two cultivars. In the study of Dobričević et al. (2014), the content of total acids was lower, while the slightly higher value was observed by Miletić et al. (2005). The average content of total acids in the conditions of Belgrade for European plums was 0.9% (with variation from 0.5 to 1.9%) (Milatović, 2019). The acid content decreases rapidly after harvest, during storage of fruit (Milatović, 2019).
Total sugar content in plum fruits ranged between 9.5% ('Čačanska Rana'/'Fereley') to 11.5 % ('Čačanska Najbolja'/'Fereley') (Table 1). Analysis of data were not showed statistically significant differences in total sugar content among rootstocks. The highest average total sugar content was found in fruits from trees grafted on 'Pixy' rootstock, and the lowest were found in fruits from trees grafted on 'Fereley' rootstock. Cultivars manifested significant differences in total sugar content. The highest values of total sugar content (for all four rootstocks) were found in 'Čačanska Najbolja' cultivar, then in 'Čačanska Lepotica', while they were the lowest in 'Čačanska Rana' cultivar.
According Milenković et al. (2006) the total sugar content in the fruit of plum cultivars developed at the Institute of Fruit Growing in Čačak varied from 8.30% to 12.33%, while Milošević and Milošević (2012) established the interval of total sugar content 10.31-11.78 %. The results of our research of total sugar content were lower than that reported by Dugalić et al. (2014). The content of sugar in the fruit of plum depends of the cultivar (Nenadović-Mratinić et. al., 2007; Milošević and Milošević, 2011a; 2011b), temperature and the amount of rainfall in the period of maturation (Vangdal et al., 2007). In our study, total sugar content was the lowest in the earliest maturing cultivar, 'Čačanska Rana', and the highest in latest maturing cultivar 'Čačanska Najbolja'. All these findings confirmed the statements, that early maturing plum cultivars tend to have lower total sugar content in comparison with later maturing ones (Crisosto et al., 2007; Neumüller, 2010; Sahamishirazi et al., 2017).
The content of inverted sugars in the tested cultivars grafted on different rootstocks ranged between 6.6 to 7.6 % (Table 1). Significant differences of inverted sugars contents among cultivar/rootstock combinations were found. However, differences among rootstocks were not statistically significant. Cultivar 'Čačanska Lepotica' showed the highest amount of inverted sugars (7.5 %), followed by 'Čačanska Najbolja' (7.4 %) and 'Čačanska Rana' (7.0 %). According to Nergiz and Yildiz (1997) the content of inverted sugars varied between 37.6 % and 75.0 % in relation to the content of total sugars.

Sucrose content in plum fruits ranged between 2.7 % to 3.9 %. Differences among cultivars and rootstocks were not statistically significant. Among cultivars, the highest values of sucrose content were found in 'Čačanska Najbolja' (3.7 %), and the lowest values in 'Čačanska Rana' (2.9 %). Dugalić et al. (2014) reported that sucrose was the major sugar over the studied years in the tested cultivars, with a content of 4.52 %.
One of the good indicators of the overall quality of the fruit is the sugar/acid ratio, a common index for ripening and quality. The value of the sugar/acid ratio is higher in later maturing plum cultivars (Crisosto et al., 2004; Nenadović-Mratinić et al., 2007), but it also depends on the environmental conditions (Vitanova et al., 2007). The combination 'Čačanska Rana'/'Fereley' had the lowest value of sugar/acid ratio (8.9), while the combination 'Čačanska Najbolja'/'St. Julien A' had the highest value (15.9). Forni et al. (1992) reported sugar/acid ratio between 5.2 and 25.6.
Our study confirmed that soluble solids and total sugar contents are greatly affected by the cultivar. Namely, early maturing plum cultivars tend to have lower total sugar content in comparison with later maturing ones.
Total phenolic content (TPC) in the skin of plum fruits ranged from 4.44 to 15.93 mg gallic acid equivalent (GAE) g^{-1} FW, while in the flesh it varied between 0.38 and 1.52 mg GAE g^{-1} FW (Table 2). The highest average TPC values in the plum skin extracts were found in Myrobalan rootstock, and in the flesh in 'St. Julien A' rootstock. However, differences among rootstocks were not statistically significant. On the other hand, significant differences among cultivars were found.

Table 2. Total phenolic content (TPC, mg GAE g^{-1} FW), and radical-scavenging activity (RSA, μmol TE g^{-1} FW) in the skin and the flesh of three plum cultivars grafted on four rootstocks (2014).

Cultivar/rootstock combination	Skin		Flesh	
	TPC	RSA	TPC	RSA
Č. Lepotica/Fereley	4.44 ± 0.00 k	39.08 ± 0.79 i	0.72 ± 0.00 c	14.73 ± 0.03 c
Č. Lepotica/Myrobalan	4.89 ± 0.12 j	41.30 ± 0.29 h	0.57 ± 0.01 e	14.86 ± 0.02 c
Č. Lepotica/Pixy	6.30 ± 0.04 g	42.81 ± 2.67 gh	0.77 ± 0.02 b	14.73 ± 0.05 c
Č. Lepotica/St. Julien A	5.41 ± 0.05 i	43.89 ± 0.90 g	0.86 ± 0.01 a	16.97 ± 0.05 a
Č. Najbolja/Fereley	8.48 ± 0.03 e	57.60 ± 1.42 c	0.68 ± 0.01 d	14.89 ± 0.23 c
Č. Najbolja/Myrobalan	15.93 ± 0.11 a	78.49 ± 2.45 a	0.78 ± 0.01 b	13.49 ± 0.27 e
Č. Najbolja/Pixy	10.21 ± 0.10 c	52.54 ± 0.45 d	0.77 ± 0.03 b	15.32 ± 0.05 b
Č. Najbolja/St. Julien A	7.94 ± 0.05 f	50.77 ± 0.12 e	0.56 ± 0.01 e	13.41 ± 0.05 e
Č. Rana/Fereley	9.21 ± 0.10 d	47.70 ± 0.95 f	0.52 ± 0.03 f	10.40 ± 0.47 g
Č. Rana/Myrobalan	7.72 ± 0.09 f	57.39 ± 0.46 c	0.38 ± 0.02 h	11.00 ± 0.10 f
Č. Rana/Pixy	5.93 ± 0.09 h	44.38 ± 0.00 g	0.50 ± 0.01 g	13.13 ± 0.11 e
Č. Rana/St. Julien A	11.96 ± 0.29 b	62.70 ± 0.77 b	0.66 ± 0.00 f	14.26 ± 0.31 d
Rootstocks				
Fereley	7.38 ± 2.57 a	48.13 ± 9.26 a	0.64 ± 0.17 a	13.34 ± 2.55 a
Myrobalan	9.51 ± 5.73 a	59.06 ± 18.65 a	0.58 ± 0.21 a	13.12 ± 1.96 a
Pixy	7.48 ± 2.37 a	46.58 ± 5.22 a	0.68 ± 0.15 a	14.39 ± 1.13 a
St. Julien A	8.44 ± 3.30 a	52.43 ± 9.55 a	0.69 ± 0.15 a	14.88 ± 1.86 a
Cultivars				
Čacanska Lepotica	5.26 ± 0.80 b	41.76 ± 2.06 b	0.73 ±0.12 a	15.32±1.10 a
Čacanska Najbolja	10.64 ± 3.65 a	59.85 ± 12.76 a	0.70 ± 0.12 a	14.28 ±0.97 ab
Čacanska Rana	8.71 ± 2.55 ab	53.04 ± 8.48 a	0.52 ± 0.11 b	12.20±1.81 b

Means followed by the same letter in a column are not significantly different (Tukey's test, $p \leq 0.05$).

As for the plum skin extracts, highest values for TPC were found in 'Cacanska Najbolja', and the lowest values in 'Cacanska Lepotica'. In the plum flesh extracts, the highest values for TPC were found in 'Cacanska Lepotica', then in 'Cacanska Najbolja', while the lowest were obtained for 'Cacanska Rana' cultivar. Significant differences for TPC were also found among individual cultivar/rootstock combinations. High variability in the range of TPC was reported in previous studies in European plums (Chun et al., 2003; Kim et al., 2003; Rupasinghe et al., 2006; Rop et al., 2009). Sahamishirazi et al. (2017) found that TPC in 178 European plum cultivars ranged between 38.4 and 841.5 mg gallic acid equivalent (GAE) 100 g^{-1} of fresh weight. However, the results of TPC reported in the literature are obtained analyzing the whole fruit, precisely edible part of fruit consisting of flesh and skin. In our research, flesh and skin were analyzed separately. Because of that, our results are not fully comparable with others. Our results confirmed the findings of Chun et al. (2003) that 'Cacanska Najbolja' is among cultivars with the highest TPC. According to Stacewicz-Sapuntzakis et al. (2001) the skin contains about five times more phenolic substances than the flesh. In our study, TPC values determined in the fruit skin extracts were 6-20 times higher than in the flesh extracts of the same cultivar/rootstock combinations.

The total antioxidant capacity determined by scavenging DPPH assay in the plum skin varied between 39.08 and 78.49 µmol Trolox equivalents (TE) g^{-1} FW, and in the flesh varied from 10.40 to 16.97 µmol TE g-1 FW. Significant differences in radical-scavenging activity (RSA) were found among cultivars and cultivar/rootstock combinations, whereas differences between rootstocks were not significant. The highest values for RSA in the fruit flesh and skin were found in 'Cacanska Lepotica' and the lowest in 'Cacanska Rana' cultivar.

Our results for RSA values were higher than the results obtained by Voća et al. (2009) for three European plum cultivars and for 14 red flesh Japanese plum genotypes reported by Cevallos-Casals et al. (2006). Values of RSA in the skin of the plum fruit were 3 to 6 times higher than in the flesh of the same cultivar/rootstock combinations. The reasons for this are higher values for TPC, and absence of anthocyanins in the flesh. Significant correlations were found between TPC and RSA both in the skin (r = 0.93) and the flesh of plum fruit (r = 0.80). Significant correlation between TPC and antioxidant activity of the plum fruit obtained in our study are in accordance with previous findings (Kim et al., 2003; Rupasinghe et al., 2006; Rop et al., 2009; Mihalache Arion et al., 2014).

Conclusion

Our study confirmed that chemical composition of plum fruits was more cultivar- then rootstock-dependent. The earliest maturing cultivar, 'Cacanska Rana' had the lowest contents of soluble solids and total sugars, whilst the latest maturing cultivar, 'Cacanska Najbolja' had the highest contents of these ingredients. Total phenolic content in the fruit skin was 6-20 times higher than in the flesh of the same cultivar/rootstock combinations. Similarly, radical-scavenging activity in the skin was 3 to 6 times higher than in the flesh. Significant correlations were found between total phenolic content and radical-scavenging activity both in the skin (r = 0.93) and the flesh of plum fruit (r = 0.80). These results are of interest, as the phenolic content of fruits can be related to their antioxidant activity and their health-promoting properties.

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