Impact of harvest time on chemical composition and antioxidant activity of fresh and dried plum fruits

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Abstract

The aim of this study is to establish the impact of harvest time on the chemical composition and antioxidant activity of fruits. A two-year research (2011 and 2012) was conducted on fruits of the 'Čačanska Rodna' cultivar, collected by selective picking at three 7-day intervals during the season and dried in the experimental dryer at air temperature of 90°C. Chemical composition was determined in both fresh and dried fruits using standard methods. The antioxidant activity was established using the ABTS method, while the content of total phenols and flavonoids was determined by the spectrophotometric methods with Folin-Ciocalteu reagent and aluminium-chloride, respectively. Using high-performance liquid chromatography (HPLC), it was revealed that the major phenolic compound in fresh plums and prunes is neochlorogenic acid, followed by caffeic acid. The obtained results reveal that prunes possess a higher antioxidant activity than fresh plum fruits, in all harvest times, which is determined by the phenol and flavonoids contents. Despite the fact that fruits were picked selectively for the drying purposes, later harvest caused an increase in the dry matter content and a decrease in total acids, accompanied by a decrease in the content of caffeic acid on the other side. Changes in the content of all the other parameters in the fresh plum fruits and prunes show no regular patterns in correlation with the harvest time.

Keywords: plum, prune, 'Čačanska Rodna', phenolics, anthocyanins, HPLC-DAD

INTRODUCTION

In addition to its outstanding energy values, prune is also a high-value food, possessing a special dietetic and physiological significance. Besides its protective and therapeutic effect, prune is classified as a functional type of food (Piga et al., 2003; López et al., 2013), owing to high anti-oxidant activity (Donovan et al., 1998; Pellegrini et al., 2006).

Serbia has a long tradition of producing dried plums, and in some years prunes represent an important exporting product. Prune producers favour cultivars with combined characteristics, which reach technological maturity in the climatic conditions of Serbia in the period from mid-August to the final decade of September (Mitrović et al., 2006). Considering the limited duration of the drying season, it is necessary to provide sufficient quantities of the raw materials, which is achieved in a number of different ways: by using cultivars with different ripening times, using fruits from diverse locations, as well as by selective harvesting of fruits of the same plum cultivar (Mitrović et al., 2007).

There are numerous studies that confirm that the fruits of the 'Čačanska Rodna' cultivar represent a very good raw material for drying. Combined with the adequate drying technology, it produces prunes of the highest quality (Mitrović et al., 2001, 2013a, b).

The aim of this study was to determine the impact of different harvest time of plum fruits on chemical composition and antioxidant activity in both fresh and dried fruits of the 'Čačanska Rodna' cultivar.

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MATERIALS AND METHODS

The investigation was conducted over a two-year period (2011-2012). Plums of the cultivar 'Čačanska rodna' (*Prunus domestica* L.) were collected from an experimental orchard in Preljina (43°55'26"N; 20°26'52.11"E), a village situated in the plum-growing region of Čačak, Serbia. During the seasons under consideration, the fruits were picked selectively in the phase of technological maturity for drying (soluble solids content >18%), at three different periods separated by 7-day intervals. Fresh fruits for analysis were frozen immediately upon harvesting and preserved at temperature of -18°C.

The drying was conducted in a laboratory convective air dryer (Kandić et al., 2006). Fresh fruits of uniform size and maturity were placed on a tray. The air dryer can accommodate 6 trays, with pre-heated air of defined characteristics streaming through the trays. Fruits of the above-mentioned plum cultivars were placed on two symmetrically laid trays, ensuring uniform drying conditions, as the direction of the vertical air flow was alternated within 60 min intervals. The predetermined air drying temperature in our trials was 90°C and it was maintained throughout the drying process along with air velocity of 1 m s^{-1}. During the drying process, moisture losses from plum fruits were recorded at 60-min intervals by a digital balance. Drying was terminated when the dry matter content of the samples was about 75%. The initial dry matter of fresh plum fruits and final dry matter of prunes were determined by standard method, by drying at 105°C until the constant mass was reached. Considering the fact that the prunes are not put to use immediately upon drying, but have to be conditioned at room temperature in order to level the moisture content of the fruit (Zlatković, 2003), dried fruits were packed in a plastic bag and maintained at a room temperature for two months until the analyses.

The soluble solid content (SSC) of the fruit was determined by a manual refractometer (3828, Carl Zeiss, Germany). Titratable acidity (TA) was determined by neutralizing the fruit extract with 0.1 N NaOH to pH 8.2, using phenolphthalein as indicator. Sucrose, inverted sugars, and total sugars content were determined by Luff-Schoorl method (Tanner and Brunner, 1979).

The monomeric anthocyanin pigment content of the aqueous extracts was determined using the previously described pH-differential method (Liu et al., 2002). Pigment content was calculated as mg of cyanidin-3-glucoside equivalents 100 g^{-1} of dry matter (mg C3GE 100 g^{-1} DM). Total flavonoid content was determined by a colorimetric method described previously (Zhishen et al., 1999; Liu et al., 2002). The results are expressed as mg of catechin equivalents 100 g^{-1} of dry matter (mg CE 100 g^{-1} DM). The total phenolic content was determined using a modified Folin-Ciocalteu colorimetric method (Singleton et al., 1999; Liu et al., 2002), with results expressed as milligrams of gallic acid equivalents 100 g^{-1} of dry matter (mg GAE 100 g^{-1} DM). Antioxidant activity was determined by the ABTS assay. $ABTS^{\bullet+}$ radical cation scavenging activity was determined according to the method described by Re et al. (1999). Results were expressed as Trolox equivalent antioxidant activity (mmol TE 100 g^{-1} DM).

The extracts of fruit samples were prepared according to the method of Hertog et al. (1992). They were analyzed using an Agilent 1260 series HPLC (Agilent Technologies, CA, USA) linked to a ChemStation software, using a ZORBAX Eclipse Plus C18 column (4.6×150 mm, 3.5 µm particles). Injection volume was 5 µL and the temperature was set at 30°C. Solvent A was 1% formic acid and solvent B was acetonitrile. The gradient used was as follows: 0-10 min, 15% of B in A; 10-25 min, 15-50% of B in A; 25-30 min, 50-80% of B in A; 30-32 min, 10% of B in A. By using this gradient (flow rate 0.5 mL min^{-1}), separation was achieved in fruit extracts. The HPLC equipment was used with a diode array detector (DAD). Phenolic compounds were detected at 260 nm (protocatechuic acid), 280 nm (gallic acid), 329 nm (neochlorogenic acid, chlorogenic acid, and caffeic acid), 360 nm (rutin) and 520 nm (cyanidin). Phenolic compounds were identified according to peak retention time and UV/Vis spectra, by comparing them with those of the standards. The quantities of the different phenolic compounds were based on peak areas, and expressed as mg 100 g^{-1} DM.

The study presents the mean values of the two-year research, obtained by analysis of variance (ANOVA)using Statistica 7 software (StatSoft, Inc., OK, USA). The pair-wise

comparisons between different parameters were performed using Duncan's test ($P<0.05$).

RESULTS AND DISCUSSION

Table 1 shows the values of the chemical composition of fresh and dried fruits of the cultivar 'Čačanska Rodna' selectively harvested in three periods during the season. Based on the content of the total dry matter and soluble solids, it can be concluded that there is a significant difference depending on the harvest time, despite the fact that all of the fruits were picked selectively at the phase of technological ripeness for drying.

Table 1. Chemical analysis of fresh and dried plum fruits from three different harvest times.

Parameter	Fresh plums				Dried plums			
	I	II	III	ANOVA[1]	I	II	III	ANOVA
Total dry matter (%)	20.49c	22.08b	23.10a	***	-	-	-	-
Soluble solids (%)	18.69b	20.56a	21.33a	***	-	-	-	-
pH value	3.42	3.56	3.74	NS	-	-	-	-
Titr. acidity (g 100 g^{-1} DM)	3.74a	3.29a	2.55b	**	3.44a	2.86b	2.61b	***
Total sugar (g 100 g^{-1} DM)	64.93	64.13	62.55	NS	57.40	56.03	56.95	NS
Inv. sugar (g 100 g^{-1} DM)	37.84	38.09	37.06	NS	51.65	49.61	51.05	NS
Sucrose (g 100 g^{-1} DM)	25.73	24.74	24.21	NS	5.46	6.10	5.61	NS
Sugar-acid ratio	17.81b	19.62b	25.62a	*	16.79b	19.77a	21.91a	**

[1]NS: non-significant, * significant at $P<0.05$, ** significant at $P<0.01$, *** significant at $P<0.001$.

Although all of the fruits under consideration were of an adequate level of ripeness for drying, containing over 18% of soluble solids (Mitrović et al., 2013a), it can be observed that the fruits harvested at period I showed the lowest content of soluble solids and total dry matter (18.69%, 20.49%). These contents were increased with each further harvesting, so the fruits collected at period III had the highest content of soluble solids and total dry matter (21.33%, 23.10%). It is further concluded that there is a decrease of the total acids contents in the fruits (from 3.74 to 2.55 g 100 g^{-1} DM) with every subsequent harvest. These data indicate that as the drying season progresses, there is a progressive increase of the maturity level in fruits, despite the fact that fruits are picked selectively. By the analysis of the total and inverted sugars contents, it can be concluded that the harvest time has no impact on these values, and this is observed both in the fresh and dried fruits. While fresh fruits contain on average 62-64 g 100 g^{-1} DM of total sugars, dried fruits have been found to contain 56-57 g 100 g^{-1} DM of total sugars, which is in agreement with the results obtained by Miletić et al. (2013). Reduced sugar contents in prunes compared to fresh fruits may have occurred as a consequence of sugar participation in forming the products of the Millard reaction due to loss of moisture in the fruit, combined with the impact of drying temperature exceeding 60°C (Wilford et al., 1997).

The content of total anthocyanins, which in the fresh fruit fell within the range from 32.09 to 43.50 mg C3GE 100 g^{-1} DM, in the fruits collected at harvest period III recorded the highest values of this parameter (Table 2). Examining the degree of ripeness of the cultivar 'Stanley' plum fruits, Miletić et al. (2012) point to the continual flow of the biosynthesis of anthocyanins during the ripening of the fruits, causing the fruits harvested at a later period developed a darker colour. Considering the fact that fruits in our experiment were harvested selectively in the phase of technological ripeness for drying (at all three harvest times), the differences in the anthocyanins contents were statistically insignificant. Antocyanins represent highly unstable compounds when exposed to high temperatures, and tend to disappear almost completely after drying at 90°C, so that their contents in the dried fruit fall within the range from 3.92 to 4.17 mg C3GE 100 g^{-1} DM.

Table 2. Total anthocyanins, total flavonoids and total phenolics content (mg 100 g^{-1} DM), and the antioxidant activity (mmol TE 100 g^{-1} DM) in fresh and dried plum fruits from three different harvest times.

Parameter	Fresh plums				Dried plums			
	I	II	III	ANOVA[1]	I	II	III	ANOVA
Total anthocyanins	32.09	42.73	43.50	NS	4.17	3.92	3.89	NS
Total flavonoids	299.1	170.3	157.5	NS	321.8a	225.9ab	149.4b	*
Total phenolics	480.8	322.3	347.3	NS	639.9	575.6	483.6	NS
ABTS	2834	1321	1739	NS	3006	2766	2244	NS

[1]NS: non-significant, * significant at $P<0.05$.

The content of total flavonoids in the fresh plum fruits ranged between 157.5 and 299.1 mg CE 100 g^{-1} DM, whereas the total phenolics content was in the range from 347.3 to 480.8 mg GAE 100 g^{-1} DM. The total antioxidant activity, as determined using ABTS method, fell in the range between 1321 and 2834 mmol TE 100 g^{-1} DM. The harvest time made no impact on the contents of the parameters under consideration, which is in accordance with the method of fruit harvesting. Drying of fresh plum fruits induced an increase in the total phenolics content (from 483.6 to 639.9 mg GAE 100 g^{-1} DM), which in turn resulted in an increase of the anti-oxidant activity of the dried plum fruits (from 2244 to 3006 mmol TE 100 g^{-1} DM). This actually means that the antioxidant activity is highly dependent on the total phenolics content. Apart from reduction in the anthocyanins content, there is a concurrent change in total phenols during the drying process which develops along a practically unpredictable pattern involving highly complex mechanisms dependent on the drying temperature applied (Piga et al., 2003; López et al., 2013; Miletić et al., 2013).

Table 3 shows the polyphenolic components of the fresh and dried plum fruits.

Table 3. Contents of selected phenolics (mg GAE 100 g^{-1} DM) in fresh and dried plum fruits from three different harvest times.

Parameter	Fresh plums				Dried plums			
	I	II	III	ANOVA[1]	I	II	III	ANOVA
Rutin	2.86	4.14	3.93	NS	1.96a	1.07b	1.29b	*
Neochlorogenic acid	25.54	20.23	27.21	NS	21.93a	14.77ab	9.60b	*
Chlorogenic acid	5.51a	2.53c	4.88b	**	2.60a	1.61ab	1.20b	*
Caffeic acid	10.72	9.49	11.29	NS	14.93a	9.21b	7.31b	*
Protocatechuic acid	1.72	1.71	1.97	NS	1.26a	0.98b	1.29a	**
Galic acid	3.11	2.91	3.28	NS	10.97b	11.10b	14.16a	*
Cyanidin	4.35b	5.90ab	7.65a	**	-	-	-	-

[1]NS: non-significant, * significant at $P<0.05$, ** significant at $P<0.01$.

The most abundant hydroxycinnamic acid is neochlorogenic acid, reaching the values of 20.23-27.21 mg GAE 100 g^{-1} DM in the fresh fruits and 9.60-21.93 mg GAE 100 g^{-1} DM in the dried fruits. It is followed by free caffeic acid (9.49-11.29 mg GAE 100 g^{-1} DM in the fresh fruits and 7.31-14.93 mg GAE 100 g^{-1} DM in the dried fruits). In the study of four plum cultivars, Usenik et al. (2008) found that the contents of the neochlorogenic acid ranged between 19.3 and 120 mg GAE 100 g^{-1} FW, stating that these high values are a characteristic of the plum as a fruit species. Oxy-cinnamic acids (neochlorogenic acid and caffeic acid) were found to be the most abundant of all polyphenolic compounds in the fruits of 'Čačanska Rodna' plum cultivar. It is in accordance with previous findings with plum cultivars 'Valjevka' (Miletić et al., 2013), 'Sugar' and 'President' (Piga et al., 2003), as well as 'Agen' (Raynal et al., 1989). Other polyphenolic compounds (rutin, gallic acid and protocatechuic acid) were detected both in the fresh and dried fruits, whereas cyanidin was found only in the fresh fruits. It is noteworthy that drying at the temperature of 90°C induces an increase in the content of gallic acid (from 2.91-3.28 mg GAE 100 g^{-1} DM in the fresh fruits to 10.97-14.16

mg GAE 100 g^{-1} DM in the dried fruits), which is in accordance with the results obtained by Miletić et al. (2013) in plum cultivars 'Valjevka' and 'Mildora'. This is most probably occurring as a consequence of gallic acid release from the bonded forms in which it is present in procyanidnes. The harvest time had no impact on the contents of the examined polyphenolic components in the samples of fresh plums. On the other hand, a statistically significant difference in the content of these components was observed in the dried fruits. The content of gallic acid was the highest in the fruits collected in harvest period III, whereas the contents of other polyphenolic components were higher in the dried fruits obtained using the fresh fruits collected in harvest period I.

CONSLUSIONS

Plum fruits selectively harvested in different harvest times in the phase of technological ripeness for drying showed differences in the levels of soluble solids and total dry matter. The values of these parameters tend to increase with each further harvest time, while at the same time the content of total acids gradually decreases. The harvest time made no significant impact on the contents of total anthocyanins, phenolics or the total antioxidant activity in both fresh and dried fruits. Drying of fruits progressively increases the contents of the total phenolics, eventually resulting in the increase of the antioxidant activity. The impact of the harvest time on the content of individual phenolic components was significantly more prominent in the dried fruits, compared to fresh ones. In both fresh and dried fruits the most abundant phenolic components were the neochlorogenic and caffeic acids, whereas significant levels of gallic acid were detected in the dried fruits.

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