

## Phenolic content and antioxidant capacity of fruits of plum cv. 'Stanley' (*Prunus domestica* L.) as influenced by maturity stage and on-tree ripening

Nemanja Miletić\*, Branko Popović, Olga Mitrović, Miodrag Kandić

Department for Fruit Processing Technology, Fruit Research Institute, Kralja Petra I/9, 32000 Čačak, Serbia

\*Corresponding author: n.m.miletic@gmail.com

### Abstract

Fruits of plum 'Stanley' were analysed for total anthocyanins, total phenolics and antioxidant capacity over three successive harvest years. Fresh fruits were harvested five times per year. The total anthocyanins content ranged from 5 to 57 mg/100 g, expressed as cyanidin-3-glucoside equivalents, on a fresh-weight basis. The anthocyanin concentrations were higher in fruits of successive harvesting dates, meaning that anthocyanin accumulation seemed to occur constantly during fruit development and ripening. The total phenolic content was within the range of 70 to 214 mg gallic acid equivalents/100 g fresh weight. The highest concentrations of anthocyanins and phenolics were observed in plum fruits harvested in 2009, which is most likely due to the favourable weather conditions. The free radical galvinoxyl was used to evaluate antioxidant capacity of plum fruits. The total antioxidant capacity of fresh fruits, expressed as the rate constant  $k_1'$ , ranged from 0.950 to 3.010 s<sup>-1</sup>. Antioxidant capacity also strongly depended on ripening stage, showing completely opposite trend compared to that of total phenolics. Since total phenolics and antioxidant capacity performed nearly 'object and its reflection in the mirror' trend, consequently the lowest antioxidant capacity was observed in plum fruits harvested in 2009.

**Keywords:** Antioxidant capacity, plum, ripening, 'Stanley', total anthocyanins, total phenolics

**Abbreviations:** HS – harvest stage; CYN-3-GLU – cyanidin-3-glucoside; FW – fresh weight; GAE – gallic acid; SD – standard deviation; ANOVA – analysis of variance.

### Introduction

Phenolic compounds are secondary metabolites, widely distributed in plants. They are important components of many fruits and vegetables not only for their major influence on sensory qualities of the fruit (colour, flavour, taste), but also for their antioxidant, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory properties (Cao & Cao, 1999; Eberhardt et al., 2000; Joshipura et al., 2001; Juranic et al., 2005; Duthie, 2007; Alesiani et al., 2010). High consumption of fruits and vegetables has been considered to reduce the risk of a number of major diseases. Even though the data on health effects of phenolic compounds cannot be considered comprehensive, it is strongly suggested that phenolic compounds are associated with our health, although the mechanisms have not yet fully understood (Scalbert et al., 2005). The role of fruits and vegetables in disease prevention is partly associated with the antioxidant properties of their constituent polyphenolics (Sahidi & Wanasundara, 1992; Kinsella et al., 1993; Scalbert & Williamson, 2000). Fruit polyphenols include a wide range of compounds such as hydroxycinnamic acids, flavan-3-ols, gallic acid derivatives, flavonols, and anthocyanins. The phenolic composition of fruits varies greatly, and therefore it is very important to determine accurately the overall total content of phenolics. The composition, content and distribution of phenolic phytochemicals depend on fruit ripeness, cultivar specificities, cultural practices, geographic origin, growing season and postharvest storage conditions

(Kim et al., 2001; Kayano et al., 2003; Bureau et al., 2009; Deshmukh et al., 2011). Plum fruits contain copious amounts of natural phenolic phytochemicals, such as flavonoids, phenolic acids, anthocyanins, and other phenolics, which may function as effective natural antioxidants in our daily diet (Weinert et al., 1990; Gil et al., 2002; Cevallos-Casals et al., 2006; Vizzotto et al., 2007; Kristl et al. 2011). It was proved that plum fruits have several times higher total antioxidant capacity than apples, the latter being one of the most commonly consumed fruits in our diet (Wang et al., 1996). Murcia et al. (2001) showed that plum fruits demonstrated very good scavenger activity against oxygen-derived free radicals, such as hydroxyl and peroxy radicals. Measuring the total antioxidant activity may lead to a misleading conclusion due to the frequently observed antagonistic or synergistic interactions among various components of food. Various kinds of antioxidant components in plum fruits may play the essential role in the combinative or synergistic contribution to the total antioxidant activity.

There are numerous references in literature to the antioxidant activity of plum cultivars, but little information exists regarding the antioxidant activity during plum fruit ripening and harvesting. There have been a few reports on the antioxidant activity of plum cultivars during ripening (Diaz-Mula et al., 2008; Kristl et al., 2011). Diaz-Mula et al. (2008) experimented with eight different plum cultivars and found that it was in the skin that anthocyanins accumulated first,

then in flesh to increasingly grow all through to the last sampling date. Total phenolics content showed a similar pattern in all cultivars, progressively increasing throughout the ripening period, although important differences were found among cultivars. Moreover, antioxidative capacity increased throughout the ripening process on-tree in both skin and flesh tissues for all plum cultivars, suggesting that anthocyanins might contribute to the antioxidant activity more than other phenolic compounds. The objective of our study was to determine the effect of maturity stage on the phenolic composition of plum 'Stanley' in order to establish the optimal harvest date to reach high phytochemicals content related to antioxidant activity.

## Results and discussion

### Total anthocyanin content

Fruit ripening is associated with important biochemical changes that modify colour, texture, taste and other quality traits. In our study, maturity at harvest had a marked effect on the total anthocyanins content in plum fruits. The changes in total anthocyanin content during ripening are presented in Fig 1. Our results show that in fruits of 'Stanley' the total anthocyanins content significantly changes during ripening ( $p < 0.001$ ) within the same year (Table 3). At the harvest stages in the present study, anthocyanin concentrations were higher in fruits of successive picking dates. These results suggest that anthocyanins accumulation seemed to occur constantly during the fruit development and ripening. Furthermore, a significant correlation between total anthocyanins content and harvest stage was observed ( $R = 0.815, 0.955, \text{ and } 0.966$  for 2008, 2009 and 2010, respectively,  $p < 0.001$ ). It is well known that anthocyanin content in the fruit increases with maturity (Hui & Nip, 2006). The anthocyanin concentrations are within the range reported for other red/purple plum cultivars (Franke et al., 2004; Cevallos-Casals et al., 2006; Wu et al., 2006; Vizzotto et al., 2007). Compared to the reports of other researchers, anthocyanin concentration in this study is close or slightly lower than that of fourteen red-fleshed plums *Prunus salicina* Erhr. and hybrids (33 to 173 mg/100 g) (Cevallos-Cavals et al., 2006), which is also similar to the results of Wu et al. (2006) (12.0 to 82.2 mg/100 g). On the other hand, Franke et al. (2004) obtained quite lower anthocyanin content in *Prunus domestica* L. (4.5 to 11.3 mg/100 g). From the maturity stages HS1 to HS4 total anthocyanins increased by average 4.75 times.

During this period values increased from 5.10 to 34.65 mg/100 g FW (harvest year 2008), from 10.65 to 44.88 mg/100 g FW (harvest year 2009), and from 9.81 to 29.01 mg/100 g FW (harvest year 2010). From the maturity stages HS4 to HS5 total anthocyanins continued the rising trend in seasons 2009 and 2010, while they decreased in season 2008. The differences in the present results may be due to the different climate conditions during the observed harvest years. Similar results were obtained by other researches (Usenik et al., 2009; Bureau et al., 2009). Usenik et al. (2009) investigated the accumulation of anthocyanins and the development of fruit colour during ripening of plum fruits of 'Jojo', 'Valor', 'Čačanska Rodna', and 'Čačanska Najbolja'. With the exception of 'Jojo', the study reveals that, all the cultivars had the highest amount of total anthocyanins either at the last but one or at the last sampling date. Bureau et al. (2009) investigated ripening of apricots, and concluded that anthocyanin concentrations increased, reached a maximum

and decreased towards the maturity stage or remained at their maximum level without subsequent decrease. Thus, different conditions (year, location, climate) appear to affect the anthocyanin accumulation in apricot.

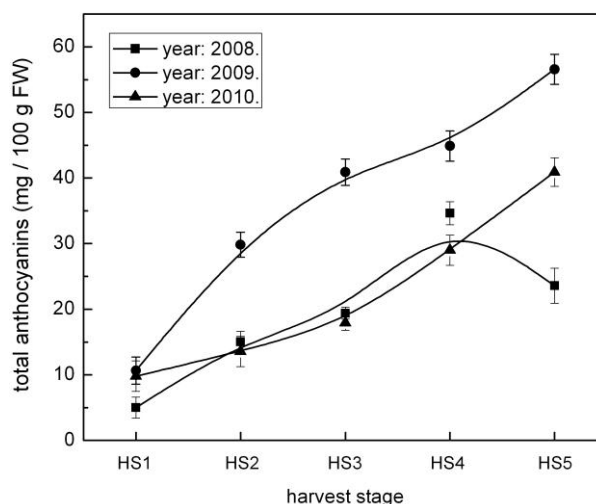
### Total phenolic content

Important changes occurred in physicochemical parameters of plum cultivars during development and on-tree ripening. Maturity stage dramatically affects the total phenolic content in the fruit. The changes in total phenolic content during maturation are presented in Fig 2 (curve 1). Total phenolic content was significantly different between the studied ripening stages ( $p < 0.001$ ) within the same year (Table 3). In our study, the total phenolic content ranged from 73 to 111 mg/100 g FW (harvest year 2008), 149 to 208 mg/100 g FW (harvest year 2009), and 102 to 190 mg/100 g FW (harvest year 2010). Such differences are most likely owing to the different outside air temperature and rainfall rate (Table 1), given the well known inverse correlation between phenolic concentrations and air temperature (Salgado et al., 2008; Xu et al., 2011). The total phenolics content in fruits of 'Stanley' in the study carried out in our laboratory was within the range reported in other plum cultivars (Gil et al., 2002; Cevallos-Cavals et al., 2006). In our study, the total phenolic content in fruits of 'Stanley' was lower than that previously reported for *Prunus domestica* (160-300 mg/100 g) (Los et al., 2000) and *Prunus salicina* cultivars (298 to 563 mg/100 g FW) (Cevallos-Cavals et al., 2006), and was considerably higher than that obtained in the study of commercial cultivars (14–109 mg/100 g) (Gil et al., 2002). Kim et al. (2003) obtained 174.0 mg of total phenolics in fresh fruits of 'Stanley' per 100 g, while Chun et al. (2004) obtained 236.7 mg GAE. In seasons 2009 and 2010 (Fig 2), total phenolic content primarily decreased from HS1 to HS3, and subsequently increased, whereas in 2008, the total phenolics had quite the opposite trend, i.e. they first increased from HS1 to HS4 to subsequently decrease. Differences in the results presented may be attributed to the different climate conditions. Similar results were obtained by other researches. Amiot et al. (1995) investigate the influence of maturity stage on phenolic content of pear fruits. In pear cultivars 'Williams', 'Harrow Sweet' and 'Guyot', fruits were picked at three different times a year. In the cultivars examined, the total phenolics showed three different trends. Namely, the total phenolics content in fruits of 'Williams' tended to increase and subsequently decrease, whereas in 'Harrow Sweet' phenolic content decreased with the delayed harvest time. In contrast, in fruits of 'Guyot', total phenolics increased with ripening. The fluctuations in total phenolic content in all the three harvest years, as shown by our results, appear to be difficult to account for. In most fruits, ripeness degree markedly affected both quality and quantity of the various phenols. Generally, it is well known that phenolic acid concentrations decrease during ripening, whereas flavonoid concentrations increase (Macheix et al., 1990; Manach et al., 2004). The lack of a clear trend concerning total phenolics content may be due to the variations in composition of compounds that fall within phenols during ripening, as reported by Buta & Spaulding (1997) and Raffo et al. (2002) in their studies of tomato fruits. In other words, degradation of some phenolics was faster or slower than biosynthesis of some other phenolics.

**Table 1.** Monthly and summer season (June–September) air temperature and rainfall rate for the Premeča region in 2008–2010.

Month	Air temperature (°C)				Rainfall rate (mm)			
	2008	2009	2010	L-TA*	2008	2009	2010	L-TA*
January	1.7	0.7	0.9	0.3	26.0	50.0	33.0	36.6
February	5.5	2.6	3.0	2.3	8.0	32.0	52.0	30.7
March	8.5	8.1	7.9	6.8	53.5	42.5	54.5	50.2
April	13.7	14.8	13.3	11.5	35.5	12.5	52.0	33.3
May	19.4	20.2	17.9	16.8	36.0	43.0	98.8	59.3
June	23.3	21.4	21.3	20.0	79.0	98.4	81.0	86.1
July	23.5	24.0	23.5	21.5	95.6	41.0	90.0	75.5
August	25.3	24.7	23.7	21.2	36.0	35.5	78.5	50.0
September	15.9	19.2	17.3	16.7	73.0	30.0	25.0	42.7
October	14.0	11.6	10.0	11.4	30.5	91.5	63.0	61.7
November	8.2	8.7	10.3	6.0	32.0	72.0	54.6	52.9
December	4.9	3.7	1.8	1.4	36.0	97.0	37.0	56.7
Mean or total for year	13.7	13.3	12.6	11.3	541.1	645.4	719.4	635.7
Mean or total for summer season	22.0	22.3	21.5	19.8	283.6	204.9	274.5	254.3
Number of warm days (T>30 °C) in summer season	43	41	38					

\* long-term average for 1965–2010 period in the Premeča region.

**Fig 1.** Changes of anthocyanin content during fruit ripening in the plum cv ‘Stanley’ over three successive harvest years.

### Antioxidant capacity

Many free radicals, that are generated in organisms, are extremely reactive and are known to be a biological product of partial reduction of molecular oxygen (Williams & Jeffrey, 2000). The damage mediated by free radicals is manifested in the disruption of membrane fluidity, protein denaturation, lipid peroxidation, oxidative DNA and alteration of platelet functions, which has been generally considered to be linked with many chronic health problems such as cancers, inflammation, aging and atherosclerosis (Fridovich, 1978; Kinsella et al., 1993). An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may, therefore, have health-promoting effects in the prevention of degenerative diseases. The galvinoxyl free radical quenching procedure, a decolorization assay

using free purple galvinoxyl radicals, was shown to be a very useful tool for expeditious measuring of antioxidant activity in individual chemical compounds or complex fruit extracts (Smith *et al.*, 2000). As presented in Fig 2, antioxidant capacity was also significantly affected by ripening stage (curve 2) ( $p < 0.001$ ). Namely, antioxidant capacity, expressed as the rate constant  $k_1$ , showed completely opposite trend compared to that of the total phenolic content. These two parameters, thus represented in the same graph for the same harvest year, performed nearly ‘*object and its reflection in the mirror*’ trend. The results obtained showed that plum fruits containing the highest total phenolics do not necessarily exhibit the highest antioxidant capacity.

**Table 2.** Selected harvest stages for sample collection of fruits of cv ‘Stanley’.

Denomination	Abbreviation	Maturity stage	Harvesting date
Harvest stage 1	HS1	ripe violet	14. Aug (2008) 18. Aug (2009) 20. Aug (2010)
Harvest stage 2	HS2	ripe purple	21. Aug (2008) 25. Aug (2009) 27. Aug (2010)
Harvest stage 3	HS3	ripe deep purple	28. Aug (2008) 01. Sep (2009) 03. Sep (2010)
Harvest stage 4	HS4	ripe blue	04. Sep (2008) 08. Sep (2009) 10. Sep (2010)
Harvest stage 5	HS5	ripe deep blue	12. Sep (2008) 15. Sep (2009) 17. Sep (2010)

Moreover, an increase in antioxidant capacity corresponds to the decrease in the total phenolic content. Fernandez-Orozco et al. (2011) studied the antioxidant capacity of phenolic compounds during growth and ripening of olive fruits *Olea europaea* cv ‘Arbequina’, and also observed the ‘*object and its reflection in the mirror*’ trend. In all the three successive harvest years, the fluctuations in antioxidant capacity were observed, though without a particular trend. Tlili et al. (2011) studied changes in bioactive compounds and antioxidant activities of five watermelon cultivars at four different fruit ripening stages. They also observed the lack of a clear trend, and attributed these results to the complexity of the composition of foods as well as to the dependency of fruit antioxidant power on the synergistic effects and redox interactions between the different nutrient and “non nutrient” molecules, which together contribute to the possible health benefits. Kristl et al. (2011) investigated the contribution of extractable antioxidants and non-extractable phenolics to the total antioxidant activity of four plum cultivars (‘Valor’, ‘Stanley’, ‘Hanita’, and ‘Tophit’), as well as to the changes in total antioxidant activity during the final week of ripening. They also observed the variation in the total antioxidant capacity of ‘Stanley’ and ‘Hanita’ plum fruits over the ripening period. After the determination on the first sampling date, the total antioxidant activity of fruits of ‘Stanley’ slightly decreased and subsequently increased by 38%. Many authors have studied the correlation between bioactive compounds and antioxidant capacity in various fruits (Raffo et al., 2002; Fernandez-Orozco et al., 2011; Tlili et al., 2011). In our study, after considering data from all harvest years, no significant correlation between antioxidant capacity and total anthocyanin content was evidenced. The antioxidant capacity is more closely related to the total phenolic content ( $R = -0.891$  and  $-0.909$  for 2009 and 2010, respectively; unexpectedly,  $R = 0.018$  was obtained for 2008, most likely due to the different climate conditions over the observed harvest year).

## Materials and methods

### Plant material

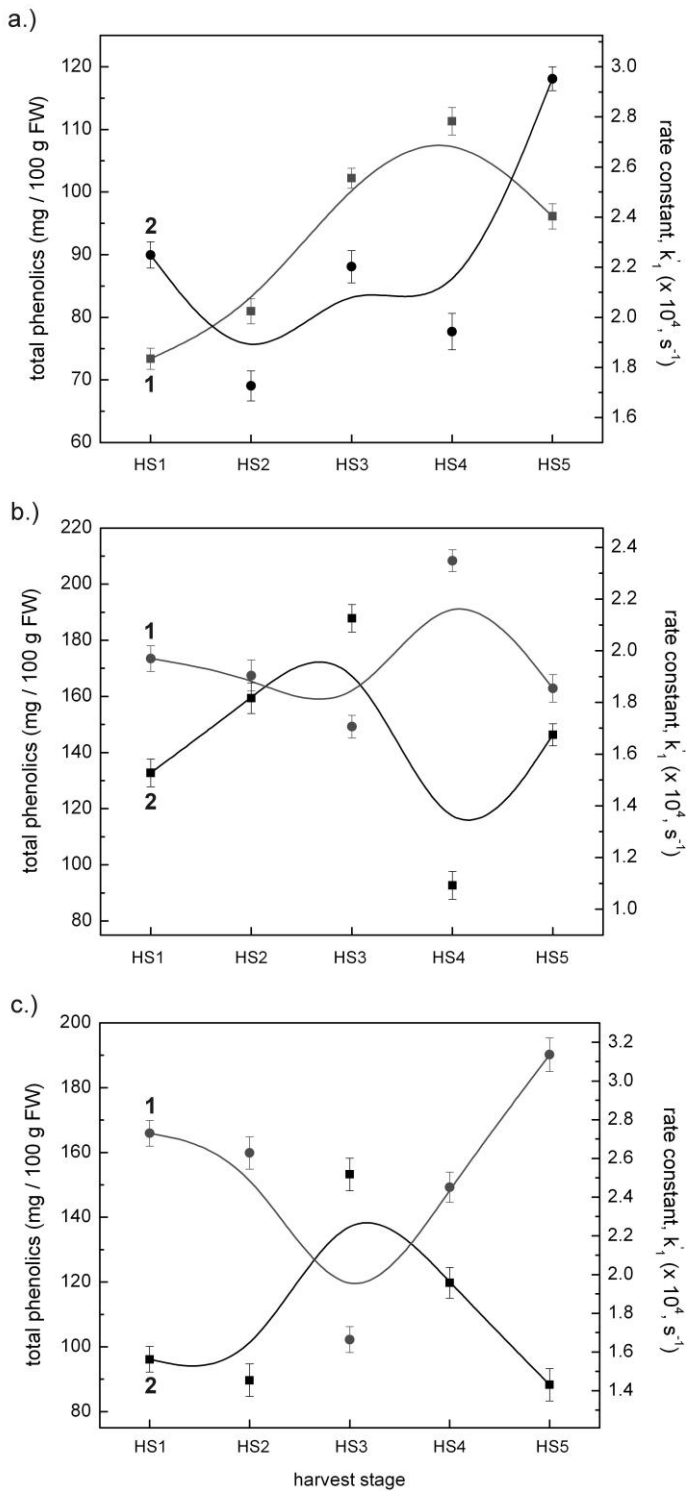
Fruits of ‘Stanley’ (*Prunus domestica* L.) grafted on rootstock *Prunus cerasifera* L. were collected from a commercial orchard established in 1999 in Premeća

(43°46’33”N, 20°25’34”E), the village situated in a renowned plum growing region of Čačak, Serbia. Fruits were collected from mid-August to mid-September in 2008, 2009, and 2010. The climate data involving all the three periods were obtained from local meteorological station of the Republic Hydrometeorological Service of Serbia (Table 1).

The data on all the three years observed show that the average air temperatures during both the calendar year and the summer season were higher than the long-term average (Table 1). In 2008, the average air temperature was higher than in seasons 2009 and 2010. Furthermore, the greatest number of warm days ( $T > 30$  °C) was also observed in 2008. Nevertheless, in 2009 the average temperature in summer season (June-September) was higher than in 2008 and 2010. The total annual rainfall rate was rather low in 2008. It was higher in 2009 and notably higher in 2010, compared to the long-term period. Anyhow, the total rainfall rate over summer season was the highest in 2008. All the environmental parameters influence the content of the polyphenolic compounds in fruits. Since fruits developed cultivar-typical coloration, it is assumed, that the weather conditions in the ripening period were favourable for the development of the colour.

### Plum harvesting and sampling

Over the seasons examined, fruits were picked at five different terms at seven-day intervals during maturation stage. Plum sampling involved five harvest stages (HS1, HS2, HS3, HS4, and HS5) as described in Table 2. Fruits were collected at appropriate maturity stages, based on a predetermined fruit classification. In terms of fruit colour and edibility, samples varied among harvesting stages from violet to deep blue shades. Harvesting time depended on form of fruit utilization (Childers, 1949). The commercial plum orchard included 150 trees distributed in 10 rows with 15 trees each (5 x 4 m tree spacing). The sampling addressed the following parameters: position of fruit on the branch, position of the branch on the tree, position of the tree in the orchard and sun exposure to assess representative sample in the orchard. Samples were collected as follows: fifty samples of fruits (with no mechanical injuries or disease indications) were selected from five trees on each sampling date (ten fruits per tree). Plum trees located in the middle of the row were used for the experiment. Each harvesting stage included



**Fig 2.** Changes of total phenolic content (curve 1) and antioxidant capacity (curve 2) during plum maturity period in the plum cv ‘Stanley’ over three successive harvest years (a) 2008, (b) 2009, and (c) 2010.

random selection of a plum tree in the middle of each even/odd row. Each subsequent sampling was conducted on the adjacent tree in the same row. After harvesting, fruits were immediately transported to the laboratory of the

Department for Fruit Processing Technology where they were stored at  $-20\text{ }^{\circ}\text{C}$  until the analysis. For each picking term, three replicates of ten plums were randomly selected amongst 50 fruits to determine total anthocyanins, total phenolics and antioxidant capacity. The whole edible part of the fruit was used in the study. Plum fruits were carefully cut in halves and pits removed by hand. Mesocarp and exocarp were frozen by pouring into liquid nitrogen and homogenized using a stainless steel blender.

#### *Determination of anthocyanin content*

The monomeric anthocyanin pigment content of the aqueous extracts was determined using the previously described pH-differential method (Torre & Barritt, 1977; Prior et al., 1998; Boyles & Wrolstad, 1993; Liu et al., 2002). Briefly, 20 g of grinded fruit was blended with 40 mL of extracting solvent (95% ethanol/1.5 N HCl, 85:15). The extract was collected by filtration with an additional 30 ml of solvent washing. The residue was soaked with 70 mL of extracting solvent, and the extract was collected after 2h. The total extracts were pooled and brought up to 200 mL. A UV/VIS spectrophotometer (PU 8740 UV/VIS, England) and a 1-cm path length disposable cell were used for spectral measurements at 510 and 700 nm. Pigment content was calculated as milligrams cyanidin-3-glucoside (cyn-3-glu) per 100g fresh weight (mg cyn-3-glu / 100 g FW) using an extinction coefficient of 26900 L/cm/mol and molecular weight of 449.2 g/mol.

#### *Determination of total phenolic*

The total phenolic content was determined using a modified Folin-Ciocalteu colorimetric method (Singleton et al., 1999; Liu et al., 2002) and the results were expressed as milligrams of gallic acid equivalents per 100 g fresh weight (mg GAE/100 g FW). Grinded sample (4.0 g) was stirred vigorously with 40 mL of extraction solution consisting of methanol and distilled water (80% v/v) and was kept for 2 hours in the dark at room temperature. The mixture was centrifuged in two sequential times for 15 min at 3500 rpm, and supernatant was filtered through a 0.45  $\mu\text{m}$  Minisart filter before analysis. A 40  $\mu\text{L}$  of fruit extracts or gallic acid standard solution (Merck, Darmstadt Germany) was mixed with 3.16 mL of distilled water whereupon 200  $\mu\text{L}$  of Folin-Ciocalteu reagent (Merck) was added and allowed to stand for 8 min before 600  $\mu\text{L}$  of 20%  $\text{Na}_2\text{CO}_3$  solution was added. Solution was well mixed and absorbance at 765 nm against an appropriate blank was determined after 2 hours. Data are reported as means for at least three replications.

#### *Galvinoxyl free radical quenching assay*

The antioxidant capacity of the tested plum extracts by galvinoxyl free radical was carried out by following the decrease in absorbance of galvinoxyl in ethanol at 428 nm (Smith & Hargis, 1985; Shi & Niki, 1998; Smith et al., 2000). The concentration of galvinoxyl radical was always significantly lower than that of the tested extracts to obtain the pseudo-first-order rate constant,  $k_1$ . The extraction procedure was identical to the one employed for the determination of anthocyanin content. An aliquot (1.5 mL) of the plum extract was added to a galvinoxyl solution (0.6 mL) (TCI America, Portland, MO, USA) to ensure that the final concentration of the extract be 50  $\mu\text{g}/\text{mL}$ . The quenching of the galvinoxyl radical was recorded for five minutes at 30 sec intervals using a UV/VIS spectrophotometer (PU 8740

**Table 3.** Changes of anthocyanins content, total phenolic content and antioxidant capacity at different ripening stages over three successive harvest years.

Year	Harvest stage	Total anthocyanins	Total phenolics	Antioxidative capacity
2008	HS1	5.01 ± 0.42 e	72.42 ± 1.10 d	2.39 ± 0.43 b
	HS2	14.43 ± 1.12 d	78.76 ± 2.02 c	1.70 ± 0.10 d
	HS3	18.92 ± 1.06 c	99.10 ± 3.18 b	2.20 ± 0.09 bc
	HS4	34.17 ± 1.50 a	113.54 ± 2.02 a	1.95 ± 0.04 cd
	HS5	23.06 ± 2.09 b	96.22 ± 2.39 b	2.94 ± 0.07 a
ANOVA		***	***	***
2009	HS1	10.78 ± 1.48 d	171.97 ± 1.52 b	1.38 ± 0.11 c
	HS2	30.74 ± 1.55 c	166.92 ± 2.47 bc	1.71 ± 0.06 b
	HS3	41.70 ± 1.37 b	150.93 ± 6.71 d	2.09 ± 0.09 a
	HS4	43.99 ± 1.55 b	211.07 ± 2.63 a	1.02 ± 0.07 d
	HS5	54.72 ± 3.21 a	162.63 ± 4.19 c	1.67 ± 0.20b
ANOVA		***	***	***
2010	HS1	10.35 ± 0.95 e	165.56 ± 2.86 b	1.38 ± 0.11 c
	HS2	13.04 ± 0.94 d	156.34 ± 4.55 c	1.71 ± 0.06 b
	HS3	18.44 ± 1.19 c	100.68 ± 3.56 e	2.09 ± 0.09 a
	HS4	28.68 ± 0.62 b	148.71 ± 4.89 d	1.02 ± 0.07 d
	HS5	39.61 ± 2.40 a	193.68 ± 4.11 a	1.67 ± 0.20 b
ANOVA		***	***	***

Values marked with different letters within the same year and column denote statistically significant differences (Duncan's test,  $p < 0.05$ ). ns, \*, \*\*, \*\*\*: non significant or significant at  $p < 0.05$ , 0.01, 0.001, respectively.

UV/VIS, England) after which the data were plotted as a function of  $\ln [\text{Abs } t/\text{Abs } 0]$  vs time to obtain rate plots, the slope of which yielded  $k_1$ , the rate constant.

#### Statistical analysis

In all the experiments, three samples were analysed and all the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). Data were analysed by one-way analysis of variance (ANOVA) to examine differences among harvest dates, using Statistica 7 (StatSoft, Inc., Tulsa, OK, USA). The pairwise comparisons between different parameters were carried out using Duncan's test ( $p < 0.05$ ).

#### Conclusions

In conclusion, fruits of plum cv 'Stanley' were harvested at five terms over three successive years. It was observed that maturity had a significant effect on total anthocyanins content and total phenolic content. Fruit anthocyanins content increased with maturity, which indicates that anthocyanins accumulation occurred constantly during fruit development. On the other hand, the lack of clear trend in changes of total phenolics during plum maturity was observed, most likely due to the variations in the different compounds that fall within phenols during maturation. The lack of clear trend in changes of antioxidant capacity during fruit development was also evidenced, nevertheless high correlation between antioxidant capacity and total phenolics was observed.

#### Acknowledgements

This work was subsidised by the Ministry of Science and Technological Development of the Republic of Serbia (Project TR-31093). The authors thank Mirjana Pajić for chemical analysis and Branislav Milinković for computer analysis.

#### References

- Alesiani D, Canini A, D'Abrosca B, DellaGreca M, Fiorentino A, Mastellone C, Monaco P, Pacifico S (2010) Antioxidant and antiproliferative activities of phytochemicals from Quince (*Cydonia vulgaris*) peels. *Food Chem.* 118:199–207
- Amiot MJ, Tacchini M, Aubert SY, Oleszek W (1995) Influence of cultivar, maturity stage, and storage conditions on phenolic composition and enzymatic browning of pear fruits. *J Agr Food Chem.* 43:1132–1137
- Boyles MJ, Wrolstad RE (1993) Anthocyanin composition of red raspberry juice: influences of cultivar, processing, and environmental factors. *J Food Sci.* 58:1135–1141
- Bureau S, Renard CMGC, Reich M, Ginies C, Audergon JM (2009) Change in anthocyanin concentrations in red apricot fruits during ripening. *LWT-Food Sci Technol.* 42:372–377
- Buta JG, Spaulding DW (1997) Endogenous levels of phenolics in tomato fruit during growth and maturation. *J Plant Growth Regul.* 16:43–46
- Cao Y, Cao R (1999) Angiogenesis inhibited by drinking tea. *Nature* 398:381–381
- Cevallos-Casals BA, Byrne D, Okie WR, Cisneros-Zevallos L (2006) Selecting new peach and plum genotypes rich in phenolic compounds and enhanced functional properties. *Food Chem.* 96:273–280
- Childers NF (1949) Culture of plums. In: Gregory RW (ed) *Fruit science: orchard and small fruit management*. J. B. Lippincott Company, Philadelphia, PA, USA
- Chun OK, Kim DO (2004) Consideration on equivalent chemicals in total phenolic assay of chlorogenic acid-rich plums. *Food Res Int.* 37:337–342
- Diaz-Mula HM, Zapata PJ, Guillén F, Castillo S, Martínez-Romero D, Valero D, Serrano M (2008) Changes in phytochemical and nutritive parameters and bioactive compounds during development and on-tree ripening of eight plum cultivars: A comparative study. *J Sci Food Agr.* 88:2499–2507

- Deshmukh SR, Wadegaonkar VP, Bhagat RP, Wadegaonkar PA (2011) Tissue specific expression of anthraquinones, flavonoids and phenolics in leaf, fruit and root suspension cultures of Indian mulberry (*Morinda citrifolia* L.). *Plant Omics J.* 4:6-13
- Duthie SJ (2007) Berry phytochemicals, genomic stability and cancer: Evidence for chemoprotection at several stages in the carcinogenic process. *Mol Nutr Food Res.* 51:665-674
- Eberhardt MV, Lee CY, Liu RH (2000) Antioxidant activity of fresh apples. *Nature* 405:903-904
- Fernandez-Orozco R, Roca M, Gandul-Rojas B, Gallardo-Guerrero L (2011) DPPH-scavenging capacity of chloroplastic pigments and phenolic compounds of olive fruits (cv. Arbequina) during ripening. *J Food Compos Anal.* 24:858-864
- Franke AA, Custer LJ, Arakaki C, Murphgy SP (2004) Vitamin C and flavonoid levels of fruits and vegetables consumed in Hawaii. *J Food Compos Anal.* 17:1-35
- Fridovich I (1978) The biology of oxygen radicals. *Science* 201:875-880
- Gil M, Tomas-Barberan F, Hess-Pierce B, Kader A (2002) Antioxidant capacities, phenolic compounds, carotenoids, and vitamin A contents of nectarine, peach, and plum cultivars from California. *J Agr Food Chem.* 50:4976-4982
- Hui YH, Nip WK (2006) Food biochemistry and food processing. Wiley-Blackwell, Hoboken, NJ, USA
- Joshiyura KJ, Hu FB, Manson JE, Stampfer MJ, Rimm EB, Speizer FE, Colditz G, Ascherio A, Rosner B, Spiegelman D, Willett WC (2001) The effect of the fruit and vegetable intake on risk for coronary disease. *Ann Intern Med.* 143:1106-1114
- Juranic Z, Zizak Z, Tasic S, Petrovic S, Nidzovic S, Lepasovic A, Stanojkovic T (2005) Antiproliferative action of water extracts of seeds or pulp of five different raspberry cultivars. *Food Chem.* 93:39-45
- Kayano S, Yamada NF, Suzuki T, Ikami T, Shioaki K, Kikuzaki H, Mitani T, Nakatani N (2003) Quantitative evaluation of antioxidative components in prunes (*Prunus domestica* L.). *J Agr Food Chem.* 51:1480-1485
- Kim YC, Koh KS, Koh JS (2001) Changes of flavonoids in the peel of Jeju native citrus fruits during maturation. *Food Sci Biotechnol.* 10:483-487
- Kim DO, Jeong SW, Lee CY (2003) Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chem.* 81:321-326
- Kinsella JE, Frankel E, German B, Kanner J (1993) Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technol.* 47:85-89
- Kristl J, Slekovec M, Tojnko S, Unuk T (2011) Extractable antioxidants and non-extractable phenolics in the total antioxidant activity of selected plum cultivars (*Prunus domestica* L.): Evolution during on-tree ripening. *Food Chem.* 125:29-34
- Liu M, Li XQ, Weber C, Lee CY, Brown J, Liu RH (2002) Antioxidant and antiproliferative activities of raspberries. *J Agr Food Chem.* 50:2926-2930
- Los J, Wilska JJ, Pawlak M (2000) Polyphenolic compounds of plums (*Prunus domestica*). *Pol J Food Nutr Sci.* 50:35-38
- Macheix JJ, Fleurient A, Billot J (1990) Phenolics compounds in fruit processing. In: Mitra S (ed) Fruit phenolic. CRC Press, Boca Raton, FL, USA
- Manach C, Scalbert A, Morand C, Remesy C, Jimenez L (2004) Polyphenols: food sources and bioavailability. *Am J Clin Nutr.* 79:727-747
- Murcia MA, Jiménez AM, Martínez-Tomé M (2001) Evaluation of the antioxidant properties of Mediterranean and tropical fruits compared with common food additives. *J Food Protect.* 64:2037-2046
- Prior RL, Cao GH, Martin A, Sofic E, McEwen J, O'Brien C, Lischner N, Ehlenfeldt M, Kalt W, Krewer G, Mainland CM (1998) Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *J Agr Food Chem.* 46:2686-2693
- Raffo A, Cherubino L, Vincenzo F, Ambrozino P, Salucci M, Gennaro L, Bugianesi R, Giuffrida F, Quaglia G (2002) Nutritional value of cherry tomatoes (*Lycopersicon esculentum* cv. Naomi F1) harvested at different ripening stages. *J Agr Food Chem.* 50:6550-6556
- Sahidi F, Wanasundara PK (1992) Phenolic antioxidants. *Crit Rev Food Sci.* 32:67-103
- Salgado PR, Favarin JL, Leandro RA, de Lima Filho OF (2008) Total phenol concentrations in coffee tree leaves during fruit development. *Sci Agric.* 65:354-359
- Scalbert A, Williamson G (2000) Dietary intake and bioavailability of polyphenols. *J Nutr.* 130:2073-2085
- Scalbert A, Manach C, Morand C, Rémésy C., Jiménez L (2005) Dietary polyphenols and the prevention of diseases. *Crit Rev Food Sci.* 45:287-306
- Shi H, Niki E (1998) Stoichiometric and kinetic studies on *Ginkgo biloba* extract and related antioxidants. *Lipids* 33:365-370
- Singleton VL, Orthofer R, Lamuela-Raventos RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Method Enzymol.* 299:152-178
- Smith RC, Hargis JH (1985) Reaction of urates and other antioxidants with galvinoxyl. *Microchem J.* 31:18-21
- Smith MAL, Marley KA, Seigler D, Singletary KW, Meline B (2000) Bioactive properties of wild blueberry fruits. *J Food Sci.* 65:352-356
- Tilli I, Hdidder C, Lenucci MS, Ilahy R, Jebari H, Dalessandro G (2011) Bioactive compounds and antioxidant activities during fruit ripening of watermelon cultivars. *J Food Compos Anal.* 24:923-928
- Torre LC, Barritt BH (1977) Quantitative evaluation of *Rubus* fruit anthocyanin pigments. *J Food Sci.* 42:488-490
- Usenik V, Štampar F, Veberič R (2009) Anthocyanins and fruit colour in plums (*Prunus domestica* L.) during ripening. *Food Chem.* 114:529-534
- Vizzotto M, Cisneros-Zevallos L, Byrne DH (2007) Large variation found in the phytochemical and antioxidant activity of peach and plum germplasm. *J Am Soc Hortic Sci.* 132:334-340
- Wang H, Cao G, Prior RL (1996) Total antioxidant capacity of fruits. *J Agr Food Chem.* 44:701-705
- Weinert I, Solms J, Escher F (1990) Diffusion of anthocyanins during processing and storage of canned plums. *Food Sci Technol-Leb.* 23:396-399
- Williams GM, Jeffrey AM (2000) Oxidative DNA damage: endogenous and chemically induced. *Regul Toxicol Pharm.* 32:283-292
- Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL (2006) Concentrations of anthocyanins in common fruits in the United States and estimation of normal consumptions. *J Agr Food Chem.* 54:4069-4075
- Xu C, Zhang Y, Zhu L, Huang Y, Lu J (2011) Influence of growing season on phenolic compounds and antioxidant properties of grape berries from vines grown in subtropical climate. *J Agr Food Chem.* 59:1078-1086