Physicochemical Composition, Phenolic Content and Antioxidant Activity of Sour Cherry cv. Marasca During Ripening

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Summary

Sour cherry cv. Marasca is Dalmatian cultivar from XVI century. Cultivation is limited on the north and central part of Dalmatia and on the part of the islands, where it achieves the best quality of fruit, high content of dry matter and sugar respectively, agreeable aroma and intense color. Sour cherry cv. Marasca is source of biologically active ingredients, organic and inorganic compounds, dietary fibers, aromatic compounds and high content of phenolic compounds, particularly anthocyanins and hormone melatonin. Many epidemiological studies showed that phenolic compounds have antioxidant and anti-inflammatory properties, and they have beneficial effect on human health. Marasca is rich with mentioned compounds.

Physicochemical composition (total and soluble dry matter, pH value, total acidity) during ripening of cv. Marasca ecotype Recta (*Cerasus marasca recta*) grown in Zadar and Split area were determined. Total and soluble dry matter increased and pH value and total acidity showed little change during ripening. The content of phenolic compounds increased during ripening as well as content of anthocyanins, while antioxidant activity decreased with ripening. That indicates there was no correlation between antioxidant activity and content of total phenolic compounds.

Key words

sour cherry Marasca, anthocyanins, total phenolics, antioxidant activity

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Introduction

Sour cherry Marasca (*Prunus Cerasus* or *Cerasus Marasca*) is an important and well-known Croatian cultivar (Medin, 1989). There are assumptions about origin of sour cherry Marasca, that it is autochthonous Dalmatian cultivar, but also that Marasca has been growing in the Central Asia as wild species and was transferred to the Adriatic coast (Medin, 1971). Due to cultivar characteristics, specific and agreeable aroma and intense color Marasca has significant position among sour cherries. The most popular product of sour cherry Marasca is liqueur Maraschino. According to botanical classification Marasca belongs to family *Rosaceae*, genus *Prunus*, species *Prunus cerasus* L. cv. Marasca (Host) Vis. and genus *Cerasus,* species *Cerasus Vulgaris* Mill cv. Marasca (Host) Savulescu (Host, 1831; Savulescu, 1956).

According to pomology classification, Marasca is cherry with small leaf, colored juice and soft flesh (Medin, 1971). Eco- type, climatic conditions and geographical area of growing influence chemical composition of sour cherry Marasca. The best quality of fruit is achieved in the north and central part of Dalmatia (from Zadar to Makarska) and part of the islands, in the regions of warm and dry climate, mainly near 200 m above sea level. Sour cherry Marasca is one genotype, but mixture of several types (Mišić, 1989). Types vary in morphological, biological and agronomic properties. Growers prefer types *Cerasus marasca recta,* Sokoluša, Brač 2, Brač 6, and Vodice 1, because they are self-reproductive. It is very important property, especially, when are unfavorable climate conditions in the time of blossom. Sour cherry Marasca is source of biologically active ingredients, organic and inorganic compounds. It is important to excerpt content of carbohydrates, proteins and elements like Ca, P, Fe, K, Mg, Se, vitamins A, C, B – complex, beta – carotene, folic acid, dietary fibers, aromatic compounds and high content of phenolic compounds, particularly anthocyanins, pigments which are responsible for dark red color of fruit (Dekazos, 1970; Chandra et al., 1992). Environmental factors like temperature and light are very important for development of red pigments in fruit (Saure, 1990). The degree of ripeness considerably affects the content and proportion of the various phenolic compounds. Ripening involves series of biochemical complex reactions. During the later stages of ripening of berries, anthocyanins increased and became the major flavonoids (Vveokuskaja and Vorsa, 2004). Scientific researches showed that sour cherry contains high level of hormone melatonin (3.5 ng g^{-1}) which has antioxidant properties, but also can regulate process of sleeping, stunted process of cell aging and have anti-inflammatory effect (Burkhardt et al., 2001). Sour cherry (*Prunus cerasus*) is very interesting for scientists and consumers because of good sensory properties and phenolic compounds. We did not find published papers about sour cherry cv. Marasca until now. Many epidemiological studies showed that phenolic compounds have antioxidant and anti-inflammatory properties, prevent various human degenerative diseases, and they have beneficial effect on human health (Mazza and Miniati, 1993).

The aim of this study was to examine influence of different harvest dates and growing area (Split and Zadar) on physicochemical and phenolic composition and antioxidant activity of cv. Marasca ecotype Recta (*Cerasus marasca recta).*

Materials and methods

Plant material

Cultivar Marasca ecotype Recta (*Cerasus marasca recta)* grown in Škabrnja (Zadar area) and in experimental field of Institute for Adriatic Crops and Karst Reclamation in Kaštel Stari (Split area) were harvested in June of 2005. Ripening was monitored through four harvest dates. After harvesting all samples were packed in dark polyethylene bags and kept at -18 °C for six weeks before analysis were carried out. Samples were thawed, pitted and juiced; flesh and skin homogenized in pulp and then analyzed.

Methods

In all samples soluble and total dry matter, pH value and total acidity were determined (Regulation, 1983).

Total phenolics, nonflavonoids and flavonoids were determined using the Folin-Ciocalteu colorimetric method described by Amerine and Ough (1980) and Singleton and Rossi (1965) with some modification. Phenolics of the fruits were extracted from 10 g of fresh samples using 40 ml of 80% aqueous ethanol. The mixture was extracted for 20 minute in inert atmosphere, filtered through Whatman filter paper using a Buchner funnel. Extraction of the residue was repeated using the same conditions. The filtrates were combined and diluted to 100 ml in volumetric flask with 80% aqueous ethanol. Obtained extract was used for determination of total phenolics, nonflavonoids and flavonoids. The formaldehyde precipitation was used to determine flavonoids in fruit samples (Kramling and Singleton, 1969). The content of total phenolics and nonflavonoids was measured as follow: 0.5 ml diluted extracts or standard solution of gallic acid (20-500 mg L^{-1}) added to a 50 ml volumetric flask containing 30 ml of ddH_2O, then 2.5 milliliter of Folin/Ciocalteu's reagent was added to the mixture and shaken. After 5 min, 7.5 mL of 7% Na_2CO_3 solution was added with mixing and solution was immediately diluted to 50 mL with ddH_2O. After incubation at room temperature for two hours, the optical density of the solution at 760 nm was measured. The content of flavonoids was calculated as difference between total

Table 1. The results of total and soluble dry matter, pH value and total acidity for cv. Marasca, ecotype Recta.[a]

Ecotype	Growing area	Harvest date	Total dry matter (%)	Soluble dry matter (%)	pH value	Total acidity (%)
Rekta	Zadar	I	18.26±0.90	17.00±0.8	3.24±0.78	2.28±0.77
		II	18.48±0.89	18.00±0.78	3.26±0.86	2.72±0.87
		III	21.90±1.02	21.25±0.92	3.26±0.91	2.39±0.91
		IV	27.27±1.12	25.00±0.98	3.37±0.94	2.11±0.67
Rekta	Split	I	16.77±0.75	18.25±0.56	3.33±0.88	1.88±0.54
		II	26.15±0.77	25.00±0.65	3.25±0.59	2.66±0.74
		III	23.17±0.81	24.25±0.77	3.42±0.99	2.32±0.69
		IV	24.70±0.98	26.50±0.87	3.39±0.89	2.13±0.78

[a] values are means of two replications

phenolic content and nonflavonoid content. Total phenolics, nonflavonoids and flavonoids were expressed as mg of gallic acid equivalents (GAE) per kilogram of fresh weight of edible part of fruit (Amerine and Ough, 1980). The extract of total phenolics was used for determination of antioxidant activity using 2,2-diphenil-1-picrylhydrazyl radical method (DPPH) (Brand-Williams, 1995). The total anthocyanin content in extract from selected fruits was determined using bisulphite bleaching method (Riberéau-Gayon and Stonestreet, 1965). Anthocyanins of the fruits were extracted from 2 g of fresh samples using 2 ml of 0.1% HCl (v/v) in 96% ethanol and 40 mL of 2% aqueous HCl (v/v). The mixture was centrifugated at 5500 rpm for 10 min. The obtained supernatant was used for determination of the total anthocyanin. The content of the total anthocyanin was measured as follow: 10 ml of extract was pipet into two test tubes, and then 4 mL of 15 % sodium bisulfite was added to one tube and 4 mL of ddH_2O to the other. After 15 minutes of incubation at room temperature the absorbance of each mixture at 520 nm was measured. The molar absorbance value for cyanidin-3,5-diglucoside was used as a standard value. Results were expressed as mg cyanidin-3,5-diglucoside equivalents per kg of fresh weight of edible part of fruit.

Results and discussion

The results of physicochemical composition for cv. Marasca, ecotype Recta from Zadar and Split area during ripening (I, II, III, IV harvest date) are present in Table 1.

Percentage of total and soluble dry matter of Recta from Zadar increased with ripening except in II harvest date. Recta from Split area had higher value of dry matter than in III harvest date, but values for II, III and IV harvest dates were about 1.5% within interval and that anomaly ascribed to the fact that fruit from sunny side of tree contains higher portion of dry matter than fruit from shade side of tree. Dry matter of Recta from Zadar and Split areas was very similar in IV harvest date. Recta from Zadar area had higher total dry matter for 3%, and Recta from Split area soluble dry matter for 1.5%. Total acidity and pH value, opposed to dry matter, showed smaller characteristic sequence. In Recta from Zadar area pH value increased, while Recta from Split area showed insignificant increase of pH value, with some oscillation, during ripening. Total acidity and pH have unique sequence. Total acidity of Recta from Zadar area in II harvest date increased and then decreased over III stage until IV harvest date and total acidity value on the end was lower than in I harvest date. The same trend was observed in Recta from Split area, even final values were similar. Difference was that Recta from Split area in I harvest date had total acidity lower than Recta from Zadar area, but Recta from Split in IV harvest date had higher value than Recta from Zadar area, there was insignificant increase of pH value through ripening respectively. Total and soluble dry matter and pH value and total acidity in other sour cherries are lower (Money and Christian, 1958; Medin, 1971).

Quantity change of total phenolics during ripening (I, II, III, IV harvest date) for cv. Marasca, ecotype Recta from Zadar and Split are shown in Figure 1.

Total phenolics increased during ripening in Recta from Zadar and Split area. There was noticeable increase of flavonoids and nonflavonoids in sour cherry Marasca.

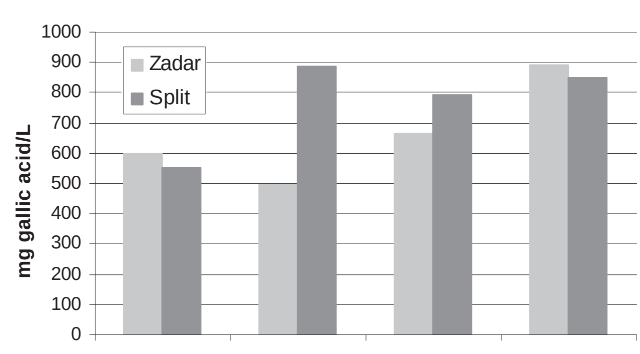

Figure 1.
Quantity change of total phenolics during ripening for cv. Marasca, ecotype Recta. Values are means of two replications

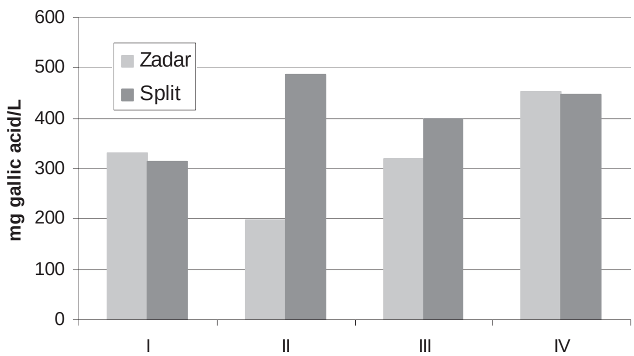

Figure 2.
Quantity change of flavonoids during ripening for cv. Marasca, ecotype Recta. Values are means of two replications

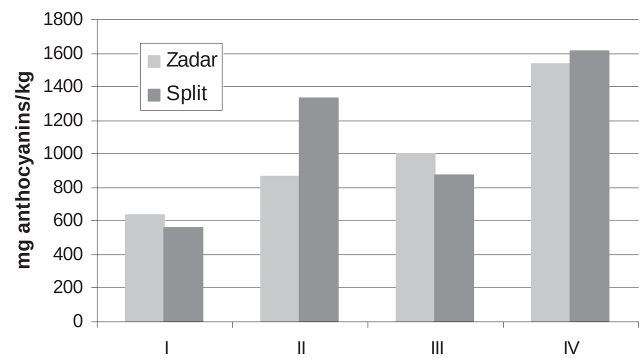

Figure 4.
Quantity change of anthocyanins during ripening for cv. Marasca, ecotype Recta. Values are means of two replications

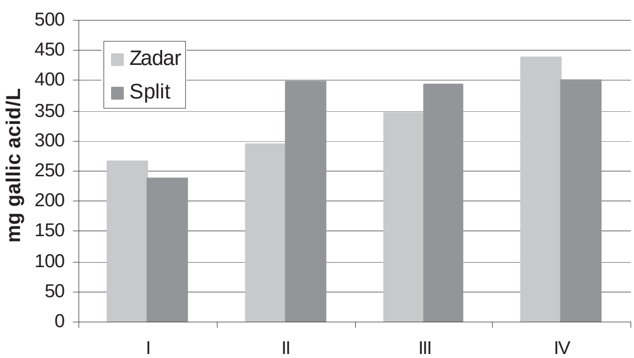

Figure 3.
Quantity change of nonflavonoids during ripening for cv. Marasca, ecotype Recta.· Values are means of two replications

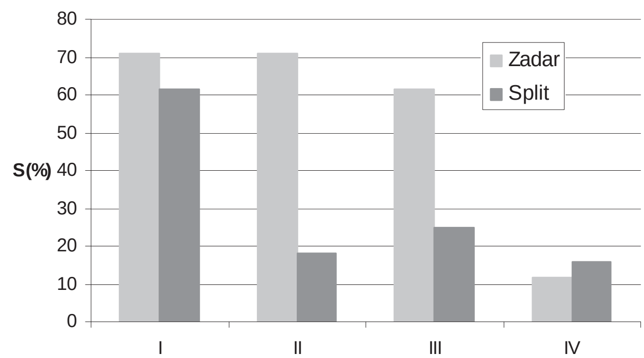

Figure 5.
Change of antioxidant activity of cv. Marasca, ecotype Recta, during ripening. Values are means of two replications

Considering geographical growth area, Recta from Zadar area in full maturity (IV harvest date) contained some more total phenolics, nonflavonoids and flavonoids, although during ripening (especially in II and III harvest date) higher quantity of those compounds contained Recta from Split.

Quantity change of flavonoids during ripening (I, II, III, IV harvest date) for cv. Marasca, ecotype Recta from Zadar and Split are shown in Figure 2.

Quantity change of nonflavonoids during ripening (I, II, III, IV harvest date) for cv. Marasca, ecotype Recta from Zadar and Split are shown in Figure 3.

Quantities of nonflavonoids and flavonoids showed anomaly similar to those, which showed total phenolics. Total phenolics constitute flavonoids with nonflavonoids and certain polyphenolic group. Also, Folin-Ciocalteu colorimetric method is not very specific method for total phenolics. Furthermore, many others compounds like sugar, chinonnes also react with Folin-Ciocalteu reagent and they can give apparently high results. Small differences in quantity of phenolic compounds between III and IV harvest dates indicate appearance of devour maturity already in III harvest date (Hrazdina, 1982). Phenolics are very complex compounds whose biosynthesis happens during whole plant life and large number of groups exists. During ripening, degradation of certain and synthesis of some other compounds happens (Murray et al., 1982.) Quantity change of anthocyanins during ripening (I, II, III, IV harvest date) for cv. Marasca, ecotype Recta from Zadar and Split are shown in Figure 4.

The content of anthocyanin pigments depends on cultivar and environmental factors. Sass – Kiss et al. (2005) reported that content of anthocyanins in different cultivars of sour cherries range between 400 – 2000 mg l^{-1}. In our study quantity of anthocyanins increased during ripening and in IV harvest date was the highest, which conforms the fact that anthocyanin is the main sour cherry's pigment (Kang et al., 2003). Constant and regular increase of anthocyanins in Recta from Zadar was noted, while II harvest date of Recta from Split, showed higher values than in I harvest date, but in III harvest date that value decreased little and increased again in IV harvest date. Anthocyanins, as one of numerous phenolic compounds, are subjects of constant biosynthesis during ripening and their quantity constantly increase. Position of fruit on the tree can cause anomaly (fruit at the sunny side of tree ripe faster) and climate conditions in period between harvesting (Murray et al., 1982).

Change of antioxidant activity of cv. Marasca ecotype Recta during ripening (I, II, III, IV harvest date) from Zadar and Split areas is shown in Figure 5.

Antioxidant activity decreased during ripening in investigated samples. It was higher in Recta from Split then in Recta from Zadar area. It was noticed that first three harvest dates of Recta from Zadar area showed similar values, but on IV harvest date antioxidant activity rapidly decreased. In our study, between antioxidant activity and content of total phenolic compounds was no correlation. During ripening different phenols biosynthesize and they have different antioxidant activity, thus percentage of antioxidant activity does not have to be equal with quantity of total phenolics, which case was here (Kalt et al., 1999). Antioxidant activity is related with specific phenolic compounds, for which is the highest antioxidant activity proved in particular substrate (Gao et al., 2005). In this study we did not achieve nor analyses nor identification of certain phenolic compounds. With DPPH method, we could not determine the individual phenols and their antioxidant activity. Besides, there is number of methods for determination of antioxidant activity and application of some other method would ratify these results before conclusion about link between phenolic quantity and antioxidant activity.

Scheme of experiment was that fruit would be harvested on four harvest dates during ripening in both growth area, but in Split area maturation appear quickly and faster than in Zadar area. Results of total and soluble dry matter presented in Table 1. Physical properties of harvested fruit, which were visible during harvesting, screening, washing and packing conformed that on IV harvest date samples were in full maturity.

Conclusion

Total and soluble dry matter of analyzed samples of Recta from Zadar and Split growing area increased during ripening, while pH value and total acidity showed little changes during maturation. Analyzed samples of Recta from both growing area showed similar values for total and soluble dry matter and for total acidity.

Anthocyanin quantity in sour cherry cv. Marasca, ecotype Recta increased during ripening and quantity of anthocyanins was slightly higher in Recta from Split area.

Quantity of total phenolics, flavonoids and nonflavonoids increased during ripening in both analyzed samples of sour cherry cv. Marasca, ecotype Recta, and their quantities were slightly higher in Recta from Split area. Fruit with high content of total phenolics is nutritionally very valuable. Antioxidant activity generally decreased during ripening in all investigated samples. Antioxidant activity of Recta from Zadar area was higher than antioxidant activity of Recta from Split area.

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