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Effect of Maturity and Geographical Region on Anthocyanin Content of Sour Cherries (*Prunus cerasus* var. marasca)

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Summary

The influence of different stages of maturity on the anthocyanin content and colour parameters in three sour cherry Marasca ecotypes grown in two Dalmatian geographical regions has been studied. Anthocyanins were determined by HPLC-UV/VIS PDA analysis and the colour of fruit flesh and skin was measured by tristimulus colourimeter (CIELAB system). The major anthocyanins in all ecotypes at all stages of maturity were cyanidin 3-glucosylrutinoside and cyanidin 3-rutinoside, whereas pelargonidin glycosides were determined in lower concentrations. During ripening, anthocyanins did not change uniformly, but in most ecotypes they were determined in higher concentrations at the last stage of maturity (3.18 to 19.75 g per kg of dry matter). The formation of dark red, almost black colour in ripe Marasca cherries decreased redness (a*), brightness (L*) and colour intensity (C*). The results of two-way ANOVA test indicated that the growing region significantly influenced the accumulation of individual anthocyanins and L* value during ripening, while ecotype and the interaction between the growing region and the ecotype significantly affected total anthocyanin content of sour cherry.

Key words: sour cherry, Marasca variety, anthocyanins, colour, ripening

Introduction

Sour cherry var. Marasca (*Prunus cerasus* var. marasca) is one of the most important autochthonous Croatian varieties that achieve the best quality in Dalmatia, on arid, dry, karst land, terra rossa macadam and in clay soil. Although a unique botanical classification does not exist, Marasca cherries belong to the genera *Prunus* and *Cerasus* Mill., of the family Rosaceae. Known ecotypes of Marasca are Sokoluša, Recta, Brač 2, Brač 6, Vodice 1, Duguljasta and Poljička, and they vary in morphological traits, biological and agronomic properties (*1*). Sour cherry var. Marasca and its products (dry, frozen cherries, juice concentrates, jams, soft and alcoholic drinks) are very popular within Croatian food industry because of specific aroma and high dry matter and total acidity. The quality of the above-mentioned products significantly depends on the stage of maturity of sour cherries and this is closely related with anthocyanin and other bioactive compounds changing during ripening. Due to the presence of anthocyanins, Marasca fruit is of dark red colour and anthocyanins are distributed throughout the fruit's flesh and skin. The anthocyanins are often used as the main indicators of ripening in cherries and other fruits (*2*).

The composition and concentration of anthocyanins are significantly influenced by cultivars and environmental factors (*3*). The stage of maturity is an important factor that influences total and individual anthocyanin content, which increases at the end of maturation (*4–6*). Sour cherries have various anthocyanins such as cyanidin 3-glucosylrutinoside, cyanidin 3-rutinoside, cyanidin 3-glucoside, cyanidin 3-sophoroside, cyanidin 3-arabinosylrutinoside, pelargonidin 3-glucoside, peonidin 3-glucoside and peonidin 3-rutinoside (*7–11*). Furthermore, these anthocyanins may have significant roles in pro-

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moting good health and reducing the risks of chronic diseases (*12*). In particular, cherry consumption has been reported to alleviate arthritis and gout-related pain (*13,14*). Because of their possible beneficial effects on health, there is an intense interest in the composition and accumulation of these compounds in ripe fruits. There are few reports documenting anthocyanin concentration in Marasca sour cherries (*15,16*). Therefore, the aim of this study is to determine the influence of ripeness on anthocyanin concentrations, as well as colour changes (CIELAB colour measurement system) in sour cherry var. Marasca fruit ecotypes harvested in two growing regions (Zadar and Split, Croatia).

Materials and Methods

Standards and chemicals

Kuromanin chloride (cyanidin 3-glucoside chloride) and callistephin chloride (pelargonidin 3-glucoside chloride) were obtained from Fluka (Neu-Ulm, Germany). HPLC-grade acetonitrile, acetic and phosphoric acids were obtained from Merck (Darmstad, Germany).

Samples

Three ecotypes of sour cherry var. Marasca (Recta, Sokoluša and Brač 2) grown in two regions in Dalmatia (orchard Škabrnja in Zadar and orchard Kaštel Stari in Split) were harvested (during June 2005) at four stages of maturity. At the first stage of maturity cherries had light red colour (partially green), at the second the cherries were red, at the third they were dark red, and at the fourth stage of maturity they were also dark red but almost blackish. After harvesting, samples (approx. 1 kg of sour cherries) were packed in polyethylene bags, frozen and kept at –18 °C until analysis. Immediately before analysis, the samples were thawed and pitted. Flesh and skin were homogenized in purees with a blender (Mixy, Zepter International) and the purees were used for determination of anthocyanins.

Analysis of anthocyanins

Extraction

Anthocyanins were extracted according to the modified Chaovanalikit and Wrolstad method (*10*). Each sour cherry fruit puree (10 g) was mixed with 20 mL of acetone and then extracted using ultrasound bath for 10 min and filtered on a Büchner funnel using Watmann No. 1 paper. The filter cake was re-extracted twice with 10 mL of 70 % acetone (70 % acetone/30 % water, by volume). Filtrates were combined and mixed with 80 mL of chloroform, then centrifuged at 40×*g* for 10 min by Hettich Centrifuge (Model Rotofix 32, Germany). The aqueous phase was collected and evaporated in vacuum at 40 °C until an acetone residue was removed. The fraction was made up to 25 mL with acidified water (0.01 % aqueous HCl, by volume), blown with nitrogen gas and stored at –18 °C until further analysis. The samples were prepared in three replications.

Purification

The anthocyanins in the examined samples were purified using modified Chaovanalikit and Wrolstad procedure (*10*). Simple fractionation of phenolic cherry extracts was performed using preconditioned C-18 Sep-Pak cartridges (Supelco, Bellefonte, PA, USA) in order to separate anthocyanins from nonanthocyanin phenolics. C-18 Sep-Pak cartridge was activated with methanol followed by 0.01 % aqueous HCl (by volume). A volume of 2 mL of aqueous extract (previously centrifuged) was adsorbed onto a minicolumn. Sugars, acids and other water-soluble compounds were removed by washing the minicolumn with 10 mL of 0.01 % aqueous HCl (by volume). Polyphenolics were eluted with 5 mL of ethyl acetate and anthocyanins with 5 mL of acidified methanol (0.01 % HCl in MeOH). The methanol fraction containing anthocyanins was concentrated to dryness using a Büchi rotavapor R-215 (Büchi, Switzerland) at 40 °C. Anthocyanins were redissolved in acidified water (0.01 % aqueous HCl), and injected into high-performance liquid chromatography (HPLC) apparatus.

HPLC analysis

Chromatographic separation was performed using HPLC analysis with a Varian ProStar system equipped with a ProStar Solvent Delivery Module 230, Injector Rheodyne 7125, ProStar 330 UV/VIS Photodiode Array detector. Separation of anthocyanins was performed on a Pinnacle II C-18 (250×4.6 mm i.d., 5 μm) including Pinnacle C-18 guard column (10×4 mm i.d., 5 μm) (Restek, Bellefonte, PA, USA). The applied content of solvent and gradient elution conditions were adopted from Chaovanalikit and Wrolstad (*10*) with few modifications. For HPLC separation of anthocyanins, 100 % HPLC-grade acetonitrile was used as solvent A, and phosphoric acid, acetic acid (glacial), acetonitrile and water (1:3:5:91, by volume) as solvent B. The program was isocratic at 0 % A for 5 min, a linear gradient from 0 to 20 % A for 15 min, and a linear gradient from 20 to 40 % A for 5 min. Operating conditions were: column temperature 20 °C, injection volume 20 μL, flow rate 1 mL/min, UV/VIS photo diode array detection at 520 nm. Identification was made by matching the retention time of the separated peaks and the retention time of authentic standards. Additionally, identification was confirmed using characteristic UV/VIS spectra. Quantification was made by the external standard method using calibration of standards as a reference. Standard solutions of cyanidin 3-glucoside chloride and pelargonidin 3-glucoside chloride were prepared in a range from 130.23 to 415.72 mg/L. Concentrations of cyanidin 3-glucosylrutinoside and cyanidin 3-rutinoside were expressed as the equivalent of cyanidin 3-glucoside, and the concentration of pelargonidin 3-rutinoside as the equivalent of pelargonidin 3-glucoside. Quantitative determination was based on peak area from HPLC analyses and from mass concentration of the compound. The obtained mass concentration of compounds (mg/mL) was calculated based on the mass of the edible part of the fruit and the mass of dry matter (mg/kg and g/kg, respectively). Dry matter (dm) was determined by drying at 105 °C to constant mass.

Analytical quality control

Recovery was measured by the addition of known amounts of each standard to sour cherry puree prior to extraction. In the calculation of the final results, no correction for recovery was applied to the data.

CIELAB colour measurement system

In all samples the colour of the external and internal part of Marasca cherry fruit during ripening was determined with Hunter colourimeter (Colourtec PCM, USA) (*17*). The colour of the fruit was determined with a*, C*, L* and H° parameters. The procedure of determination involved previous standardization of the instrument with standard white and black plate delivered with the instrument. L^* indicates lightness read from 0 (completely opaque or black) to 100 (completely transparent or white). A positive $+a^*$ value indicates redness and negative –a* value indicates greenness on the hue circle. The hue angle (H°), determined as arctan b*/a*, expresses the colour nuance and the values are defined as follows: 0°/360° – red/magenta, 90° – yellow, 180° – bluish-green and 270° – blue. The chroma, obtained as $(a^{*2}+b^{*2})^{1/2}$, is the measure of chromaticity (C*), which denotes the purity or saturation of the colour and colour intensity (*17*). The obtained values represent an average of 10 measurements of each sample.

Statistical analyses

The differences in the anthocyanin composition and concentration as well as in colour parameters of three ecotypes of sour cherry var. Marasca between four stages of maturity and two growing regions were tested by two-way analysis of variance at 5 % significance level. The least significant differences were obtained using the LSD test ($p \leq 0.05$). The similarity of three ecotypes according to individual and total anthocyanins, and also colour parameters was tested by cluster analysis using Single linkage method and Euclidian distances. All statistical analyses were performed using the software package Statistica v. 7.1 (StatSoft Ltd., Tulsa, OK, USA).

Results and Discussion

Anthocyanins of sour cherry Marasca during ripening

For better description of autochthonous Croatian variety and understanding of metabolic changes in anthocyanins during ripening, the profiles of individual anthocyanin compounds and the variations in anthocyanin concentrations in sour cherry Marasca ecotypes were studied.

In the present study, 6 anthocyanins, namely cyanidin 3-glucosylrutinoside (Cy 3-GR), cyanidin 3-rutinoside (Cy 3-R), pelargonidin 3-glucoside (Pel 3-G), pelargonidin 3-rutinoside (Pel 3-R), peonidin 3-glucoside (Peo 3-G) and peonidin 3-rutinoside (Peo 3-R) were identified in the ecotypes of sour cherry var. Marasca (Table 1). Cy 3-GR and Cy 3-R were the two major anthocyanins determined at all stages of maturity, while Pel 3-G and Pel 3-R were found at low concentrations and Peo 3-G and Peo 3-R only in traces (not presented in Table 1). Other results confirmed that different sour cherry cultivars (Montmorency, Danube, Balaton (Újfehértói fürtös), Schattenmorelle, Sumadinka, Petrovaradinska, Cigančica (Cigány meggy), Érdy jubileum and Oblačinska) contained Cy 3-GR and Cy 3-R as the main pigments (*10–12*). In the analyzed ecotypes, concentration changes of anthocyanins during ripening did not show characteristic pattern or uniformity, and were relatively stable near the harvest. At the last stages of maturity, nearly all identified anthocyanins had the highest concentration, whereas at the beginning of ripening in most ecotypes Cy 3-GR, Cy 3-R, Pel 3-G and Pel 3-R were found at low concentrations. The same accumulation trend of anthocyanins during ripening was observed in ecotypes harvested in Zadar and Split regions. Similar accumulation trend of anthocyanins during the ripening period was documented in other fruits. For example, anthocyanin (cyanidin 3-galactoside) concentration in apples was relatively high early in the season and gradually decreased to a very low level during growth, but started to increase again near maturation (*18*). Pirie and Mullins (*19*) reported about anthocyanins increasing in the skin of grape berries Shiraz throughout berry ripening and they became relatively stable near the harvest. Most investigations reported an increase in the anthocyanin accumulation in the skin of grape berries from the veraison until harvest (*20–22*).

In the studied ecotypes (Brač, Sokoluša, Recta) concentration of Cy 3-GR increased 1- to 4.8-fold during ripening, and at the 4th stage of maturity the highest Cy 3-GR concentrations were determined in ecotype Recta followed by Sokoluša and Brač 2. In relation to others, ecotype Recta, from both regions, had the highest concentration of Cy 3-GR almost at all stages of maturity. Cy 3-GR was the most abundant anthocyanin in all ecotypes and at the 4th stage of maturity accounted for 44.50 to 68.40 % of the total amount of all anthocyanins. It was followed by Cy 3-R, which accounted for 14.75 to 51.54 % of total anthocyanins. In ripe Marasca cherries, Cy 3-GR ranged from 1.69 to 8.86 g per kg of dm and Cy 3-R ranged from 0.76 to 10.18 g per kg of dm. Concentration of Cy 3-R was the highest in ecotype Recta grown in Split region in all stages of maturity, which is probably influenced by numerous factors, *e.g.* microclimatic conditions. For better understanding of anthocyanin biosynthesis, it should be studied at a cell level, which was not the objective of this study. Anthocyanin biosynthesis is influenced by many enzymes and it is poorly understood. The pelargonidin glycosides contributed only from 1.01 to 17.55 % of the total amount of all identified sour cherry anthocyanins. Pel 3-G in ripe Marasca cherries ranged from 0.21 to 1.13 g per kg of dm and Pel 3-R ranged from 0.20 to 0.79 g per kg of dm, while ecotype Sokoluša grown in Split region contained Pel 3-R in traces. In literature, in ripe sour cherry cultivars, Cy 3-GR concentration ranged from 88.95 to 227.6 mg per 100 g of fresh mass (fm) and Cy 3-R concentration ranged from 15.45 to 21.97 mg per 100 g of fm (*12*). In Montmorency cherry, Cy 3-R (10.47 mg per 100 g of fm expressed as Cy 3-glucoside) was determined only in fruit skin, similarly to Pel 3-G (*10*).

The concentrations of Cy 3-GR and especially Cy 3-R in Marasca cherries are in agreement with the data reported by Kim *et al.* (*12*) and Chaovanalikit and Wrolstad (*10*). Compared to Montmorency cherry cultivar, all ecotypes of Marasca variety contained higher concentration of Pel 3-G. The highest levels of individual and total anthocyanins were determined in ecotype Recta (total anthocyanins were 19.75 g per kg of dm). Total anthocyanins of Recta ecotype grown in Split region were 1- to 3-fold higher at all stages of maturity than in the

Table 1. The composition and concentration of anthocyanins in three ecotypes of sour cherry var. Marasca grown in Zadar and Split regions and harvested at four stages of maturity

Ecotype/ growing region	Stage of maturity	*w*(anthocyanin)/(g/kg of dm)				
		Cy 3-GR	Cy 3-R	Pel 3-G	Pel 3-R	Σ
Recta Zadar	1st	3.66±1.21	(0.61±0.02)^{a}	(0.89±0.04)^{c}	0.23±0.01	5.39
	2nd	3.44±1.32	(0.51±0.03)^{a}	(0.89±0.03)^{c}	0.67±0.02	5.51
	3rd	2.75±1.05	(1.38±0.01)^{a}	(0.76±0.05)^{c}	0.58±0.03	5.47
	4th	3.83±1.34	(2.00±0.12)^{a}	(1.03±0.08)^{c}	0.40±0.01	7.26
Recta Split	1st	1.85±0.09	(6.37±0.12)^{b}	tr	tr	8.22
	2nd	0.53±0.03	(6.01±0.20)^{b}	tr	tr	6.54
	3rd	1.39±0.05	(7.72±0.41)^{b}	(0.56±0.02)^{d}	0.66±0.01	10.33
	4th	8.86±0.23	(10.18±0.65)^{b}	(0.51±0.01)^{d}	0.20±0.01	19.75
Sokoluša Zadar	1st	1.68±0.04	0.88±0.04	tr	tr	2.56
	2nd	3.80±0.09	1.09±0.05	1.17±0.04	tr	6.06
	3rd	0.91±0.04	0.92±0.03	1.03±0.05	0.99±0.06	3.85
	4th	4.02±0.06	0.95±0.02	1.13±0.07	0.34±0.01	6.44
Sokoluša Split	1st	1.04±0.04	2.09±0.17	0.20±0.01	tr	3.33
	2nd	0.63±0.02	3.48±0.21	0.06±0.02	0.40±0.02	4.57
	3rd	2.44±0.11	0.46±0.01	0.98±0.03	0.28±0.01	4.16
	4th	3.42±0.15	0.76±0.03	0.82±0.05	tr	5.00
Brač 2 Zadar	1st	0.82±0.06	(2.36±0.07)^{e}	(0.88±0.01)^{g}	0.17±0.001	(4.23)^{h}
	2nd	2.08±0.01	(1.90±0.09)^{e}	(1.35±0.03)^{g}	0.68±0.02	(6.01)^{h}
	3rd	2.22±0.05	(2.41±0.03)^{e}	(0.94±0.02)^{g}	0.74±0.01	(6.31)^{h}
	4th	3.28±0.13	(2.50±0.06)^{e}	(0.80±0.01)^{g}	0.79±0.03	(7.37)^{h}
Brač 2 Split	1st	0.92±0.02	(0.15±0.001)^{f}	(0.13±0.001)^{g}	0.45±0.02	(1.65)^{i}
	2nd	0.21±0.01	(0.42±0.02)^{f}	(0.11±0.001)^{g}	tr	(0.74)^{i}
	3rd	0.92±0.05	(0.42±0.01)^{f}	(0.12±0.001)^{g}	tr	(1.46)^{i}
	4th	1.69±0.02	(0.82±0.03)^{f}	(0.21±0.001)^{g}	0.46±0.01	(3.18)^{i}

Results are shown as means±S.D. with three replications; tr – trace value<0.05 mg/L; Cy 3-GR – cyanidin 3-glucosylrutinoside, Cy 3-R – cyanidin 3-rutinoside, Pel 3-G – pelargonidin 3-glucoside, Pel 3-R – pelargonidin 3-rutinoside
Different letters in superscripts denote a significant difference between growing regions (a–d for Recta, e–i for Brač 2). Differences among the stages of maturity were not significant

same ecotype grown in Zadar region and about 1- to 9-fold higher than in the other analyzed sour cherries (Table 1). The lowest total anthocyanin content in ripe Marasca cherries grown in Split and Zadar regions had ecotypes Brač 2 (3.18 g per kg of dm) and Sokoluša (6.44 g per kg of dm). In Montmorency cherries, total anthocyanins were 36.5 mg per 100 g of fm (*10*) and in Danube, Balaton (Újfehértói fürtös), Schattenmorelle and Sumadinka cultivars, they ranged from 45.57 to 98.6 mg per 100 g of fm (*12*). Generally, Marasca variety contained higher total anthocyanins than previously mentioned cherries. According to Šimunić *et al.* (*11*) total anthocyanins of sour cherry cultivars Petrovaradinska, Cigančica (Cigány meggy), Érdy jubileum and Oblačinska grown in the continental part of Croatia varied from 2.7 to 28 mg per 100 g of fm expressed as Cy 3-GR. Total anthocyanins of Balaton (Újfehértói fürtös) sour cherries were 50.1 mg per 100 g (*9*), and in sour cherry cultivars grown in Hungary, total anthocyanins were in a range from 40 to 200 mg per l00 g (*23*). Differences in anthocyanin concentrations between Montmorency, Balaton (Újfehértói fürtös) and Marasca cherry cultivars could be due to the distribution of anthocyanins through the fruit. Montmorency cherry has anthocyanins only in the skin, while Balaton (Újfehértói fürtös) and Marasca cherries have anthocyanins in the flesh and skin.

The concentration of anthocyanins has been recommended as the index of quality of cherries (*24*). Dark coloured sour cherries had the highest concentrations of anthocyanins. Significant differences in anthocyanin content were found among the analyzed ecotypes ($p \leq 0.05$). The concentrations of individual as well as total anthocyanins during ripening were also remarkably different depending on the ecotype as well as the growing region. Statistical analysis showed that growing region had significant influence on individual anthocyanins (Cy 3-R, Pel 3-G, Pel 3-R) in ecotypes Brač 2 and Recta ($p \leq 0.05$). Comparing the concentrations of anthocyanins during ripening in the same ecotypes from different growing regions, the highest Cy 3-GR concentrations were determined in all ecotypes from Zadar growing region except in ecotype Recta. The same findings were observed for pelargonidin glycosides. However, Cy 3-R was determined at higher concentration in sour cherries grown in Split region. Anthocyanin profile in all Marasca ecotypes was the same, independent from the growing region or

ecotypes, but microclimate conditions probably influenced anthocyanins, especially Cy 3-R content. Variation in the concentration of individual or total anthocyanins in the sour cherry fruits during ripening could be influenced by seasonal conditions, particularly temperature during cherry growth and development.

Studies have shown that numerous factors such as harvest season, variety, stage of harvesting, climatic conditions and growing season can affect the composition and concentration of individual as well as total anthocyanins (*3*,*23*,*25*). In most species, fruit anthocyanin concentrations increase with ripening, as their biosynthesis proceeds faster than the fruit growth (*2*,*4*). Anthocyanins are synthesized *via* the phenylpropanoid pathway and for anthocyanin biosynthesis the structural and regulatory enzymes are required, but environmental influence is also very important (*26*). Light stimulates the synthesis of flavonoids, especially anthocyanins (*3*), which protects plants from harmful UV radiation acting as a protective filter (*27*). This clearly indicates why the sour cherries growing in the region of Dalmatia where Split and Zadar are located have higher anthocyanin content. This region has a lot of sunny days during the year. According to Tomás-Barberán and Espín (*25*), temperature and particularly differences between day and night temperatures can affect the anthocyanin accumulation in some fruits.

The stage of maturity did not significantly affect the individual and total anthocyanin concentrations of sour cherries (Table 2). Cluster analysis of individual and total anthocyanins from Recta, Šokoluša and Brač 2 ecotypes grown in Split and Zadar regions is shown in Fig. 1. The obtained results confirmed that according to individual and total anthocyanins, it is possible to distinguish Brač 2 and Recta from Split and Zadar growing regions.

Colour of sour cherry Marasca during ripening

Colour parameters in ecotypes of sour cherry var. Marasca from Zadar and Split growing regions are shown in Table 3. External and internal colour of sour cherries was measured with Hunter colourimeter and parameters a*, C*, L* and H° were determined. During fruit ripening important biochemical changes modify the colour,

Table 2. Two-way analysis of variance: F-values and significance level of the combined effect of growing region and ecotype, and stage of maturity and ecotype on individual and total anthocyanins of sour cherry Marasca

Anthocyanins	F-value			F-value		
	Growing region	Ecotype	Growing region × ecotype	Stage of maturity	Ecotype	Stage of maturity × ecotype
Cy 3-GR	0.904ns	1.866ns	0.120ns	1.005ns	2.309ns	1.446ns
Cy 3-R	18.622***	23.442***	35.185***	0.020ns	2.812ns	0.129ns
Pel 3-G	21.110***	0.353ns	1.369ns	0.634ns	0.138ns	0.399ns
Pel 3-R	4.521*	0.567ns	0.232ns	1.575ns	0.490ns	0.521ns
Total anthocyanins	0.041ns	7.543**	7.016**	0.117ns	4.41*	0.740ns

ns – not significant, *p≤0.05, **p≤0.01, ***p≤0.001

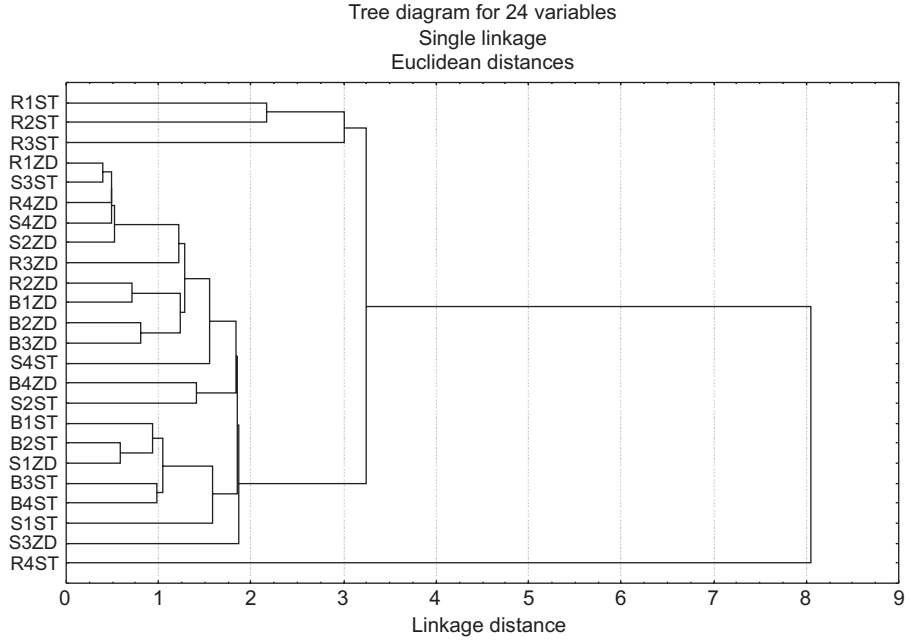

Fig. 1. Cluster analysis of individual and total anthocyanins in three ecotypes of sour cherry var. Marasca at four stages of maturity from two growing regions (Zadar and Split)

Table 3. Colour parameters in ecotypes of sour cherry var. Marasca from two growing regions (Zadar and Split) at four stages of maturity

Cultivar/ growing region	Stage of maturity	L* e.c.	L* i.c.	C* e.c.	C* i.c.	H° e.c.	H° i.c.	a* e.c.	a* i.c.
Recta Zadar	1st	30.6±4.26	(35.1±7.93)^{a}	13.2±4.6	16.5±5.57	(47.4±10.30)^{d}	28.8±13.89	9.0±3.6	13.7±3.97
	2nd	29.2±1.84	(36.2±6.31)^{ac}	13.7±4.17	17.1±2.86	(15.1±12.27)^{d}	23.3±18.63	13.1±4.07	15.0±3.31
	3rd	27.0±2.89	(27.2±1.26)^{bc}	7.0±2.54	9.6±2.42	(310.2±3.14)^{e}	310.2±10.08	4.8±1.26	9.2±2.42
	4th	23.3±3.79	(21.8±2.87)^{b}	5.7±3.13	7.3±3.10	(279.5±39.68)^{e}	300.3±36.48	2.1±3.13	5.8±2.69
Recta Split	1st	31.3±2.33	(38.6±2.54)^{a}	13.2±3.44	21.7±2.41	(36.5±6.49)^{d}	40.8±12.69	10.7±3.14	16.3±4.23
	2nd	25.0±2.79	(29.0±2.53)^{ac}	6.9±3.38	12.9±5.54	(44.1±21.96)^{d}	53.7±25.63	4.0±1.04	6.0±3.02
	3rd	17.4±3.64	(23.4±3.37)^{bc}	6.4±2.55	9.7±2.07	(309.3±12.1)^{e}	50.2±25.36	4.3±1.50	5.4±3.03
	4th	18.1±3.05	(22.5±3.97)^{b}	6.8±2.35	10.0±3.45	(309.0±11.63)^{e}	313.3±10.29	5.2±2.27	8.9±3.34
Sokoluša Zadar	1st	30.2±5.83	37.3±8.28	(16.3±4.11)^{f}	20.1±5.53	34.5±11.92	51.9±21.38	(12.8±2.01)^{h}	10.7±4.69
	2nd	34.5±1.81	40.4±5.42	(9.9±3.40)^{g}	17.8±4.08	20.7±12.12	29.2±15.29	(9.0±2.78)^{i}	14.7±2.38
	3rd	27.9±1.93	30.2±3.83	(6.4±2.55)^{g}	10.7±3.10	29.1±10.80	27.7±16.14	(5.5±2.34)^{i}	9.2±3.21
	4th	25.8±2.89	25.2±4.88	(6.4±2.47)^{g}	10.5±5.27	310.0±8.01	44.0±13.01	(5.7±2.26)^{i}	7.0±2.77
Sokoluša Split	1st	23.4±3.79	31.4±4.63	(14.4±4.21)^{f}	21.1±4.32	29.7±9.77	35.7±9.20	(12.4±3.88)^{h}	17.1±4.16
	2nd	18.2±3.89	27.9±1.95	(6.0±2.52)^{g}	11.1±3.89	39.8±29.67	54.5±29.2	(4.3±2.85)^{i}	5.0±3.10
	3rd	30.8±2.0	30.6±1.93	(4.7±1.10)^{g}	5.6±1.60	312.0±9.04	30.3±18.21	(4.1±1.26)^{i}	4.4±1.26
	4th	23.3±2.77	25.3±1.58	(4.8±3.46)^{g}	11.1±5.31	313.5±9.65	317.2±11.01	(3.4±2.18)^{i}	7.6±2.23
Brač 2 Zadar	1st	29.2±2.97	36.2±6.16	14.5±4.87	17.6±5.03	30.0±7.56	45.5±17.88	12.3±3.81	11.1±3.52
	2nd	28.1±4.35	35.3±8.05	11.9±5.95	19.6±2.97	26.3±14.02	43.5±14.82	10.0±4.30	13.9±4.39
	3rd	24.6±3.08	26.6±2.99	6.0±3.3	12.8±5.10	27.6±22.93	35.8±28.81	5.0±3.35	9.2±6.05
	4th	15.2±1.51	23.6±2.66	6.9±1.86	8.0±1.35	20.8±10.96	22.2±14.12	6.4±2.02	7.2±0.96
Brač 2 Split	1st	12.8±6.08	18.6±5.44	28.3±9.76	28.7±11.68	12.1±7.44	46.2±7.21	27.3±8.83	19.2±6.85
	2nd	23.0±3.19	26.6±2.84	6.1±2.50	17.6±3.51	39.8±29.39	56.7±15.1	3.9±2.36	9.6±5.03
	3rd	22.0±1.52	22.9±4.39	5.3±1.20	9.0±3.41	318.2±6.98	321.9±3.51	4.3±1.14	7.6±3.22
	4th	18.1±2.42	16.5±3.75	9.9±3.69	10.8±4.32	310.8±12.93	40.6±26.23	6.2±2.38	8.1±5.65

e.c. – external colour, i.c. – internal colour, colourimeter measurements of an average of 10 measurement of each sample
L* – lightness, +a* – redness, H° – colour nuance, C* – chromaticity
Different letters in superscripts denote a significant difference among the stages of maturity (a–e for Recta, f–i for Sokoluša). Differences between the growing regions were not significant

texture, taste and other quality traits (*4*). According to Mazza and Miniati (*24*), colour is an important parameter for ripening and quality valuation of fresh and processed fruit. One of the characteristic aspects of the maturation of red fruits is the change of initial green colour to a red, violet or blackish colour, caused by the accumulation of anthocyanins and chlorophyll degradation (*28*). In general, full dark red cherries have higher consumer acceptance than full bright red cherries (*29*). Marasca sour cherries are dark coloured genotype which at a ripe stage develops a very dark red, almost blackish colour.

A general decrease in L*, C* and a* values of ripe Marasca cherries was observed, dominant was dark red colour, and hue angle (H°) expressed more red-blue/ magenta than red colour nuance. CIELAB parameters for Marasca sour cherries did not differ significantly between the skin and pulp of the fruit. Higher variation in H° values of external and internal cherry colour was in the case of Sokoluša from Zadar and Brač 2 from Split. Lower C* and L* values indicate the development of darker colour, while a* values were higher at the 1st stage of maturity than in the last, but still within the range of red colour (positive values). However, to use a* value as a measure of redness is wrong, because the samples with identical a* values may exhibit colours ranging from purple to red and orange (*30*). Parameters L*, C* and H° are related to the physiological attributes of visual response (*31*). Hue describes the visible colour and chroma describes the brightness or intensity of the hue. Indices of L*, C* and H° are usually useful for tracking colour changes (*30*). The decrease in chroma means an increase in the tonality of the fruit colour (*32*).

According to Mozetič *et al.* (*2*) good indicators of colour variation in sweet cherry Petrovka (dark coloured genotype) were C* and L*, because Petrovka formed a new, darker colour cast at the end of the ripening, which resulted in the decrease of redness and colour intensity. Generally, in sour cherry Marasca ecotypes, parameters L* and H° indicate a development of dark purple-red colour during ripening. ANOVA showed that the stage of maturity significantly influenced ($p \leq 0.05$) most of the colour parameters, while growing region significantly influenced ($p \leq 0.05$) only L* values (Table 4). Least signif-

icant difference (LSD) analysis showed that the stage of maturity influenced colour parameters in ecotype Recta (H° external colour (e.c.) and L* internal colour (i.c.)) and Sokoluša (C* e.c. and a* e.c.), while the growing region did not (Table 3). Esti *et al.* (*33*) investigated sweet cherries Sciazza (dark red genotype) and Ferrovia (light red genotype). Sciazza fruits with uniform dark red colour of the skin (lower L* values) were less red (lower a* values) with respect to Ferrovia, but the external hue varied markedly between the two varieties with values of about 350° for Sciazza (red-blue) and <10° for Ferrovia (red-yellow). Cluster analysis of sour cherry ecotypes Recta, Sokoluša and Brač 2 grown in Split and Zadar regions according to colour parameters showed that colour variation was not found in the samples of ecotype Brač 2 from Zadar growing region at the 1st and 2nd stage of maturity (Fig. 2). Generally, samples were more grouped based on the stage of maturity than on the growing region.

Conclusions

Changes of anthocyanin concentrations during ripening did not show a characteristic pattern; however, at the last stage of maturity all ecotypes of Marasca variety contained the highest concentration of anthocyanins. Results from this study indicate that ecotypes from Zadar growing region are a richer source of anthocyanins than the ecotypes from Split, especially when ripe. The most abundant anthocyanin in all investigated ecotypes was Cy 3-GR, independent from the ecotypes or growing region. Besides Cy 3-GR, other anthocyanins (Cy 3-R, Pel 3-G and Pel 3-R) were also determined, but at lower concentrations. Changes of colour parameters L* and H°

Table 4. Two-way analysis of variance: F-values and significance level of the combined effect of growing region and ecotype, and the stage of maturity and ecotype on colour parameters of sour cherry Marasca

Colour parameters	F-value			F-value		
	Growing region	Ecotype	Growing region × ecotype	Growing region	Ecotype	Stage of maturity × ecotype
L* e.c.	6.226*	2.136ns	0.024ns	1.336ns	1.715ns	0.580ns
C* e.c.	0.035ns	0.408ns	0.402ns	11.218***	1.089ns	0.782ns
H° e.c.	1.760ns	0.496ns	0.434ns	8.266**	0.956ns	0.497ns
a* e.c.	0.042ns	0.518ns	0.290ns	7.234**	1.078ns	0.845ns
L* i.c.	4.428*	1.569ns	0.807ns	4.974*	1.884ns	0.325ns
C* i.c.	0.003ns	0.349ns	0.298ns	16.110***	1.171ns	0.337ns
H° i.c.	0.484ns	0.823ns	0.785ns	2.701ns	1.190ns	1.482ns
a* i.c.	0.264ns	0.148ns	0.216ns	4.734*	0.208ns	0.020ns

ns – not significant, *p≤0.05, **p≤0.01, ***p≤0.001
L* – lightness, +a* – redness, H° – colour nuance, C* – chromaticity
e.c. – external colour, i.c. – internal colour

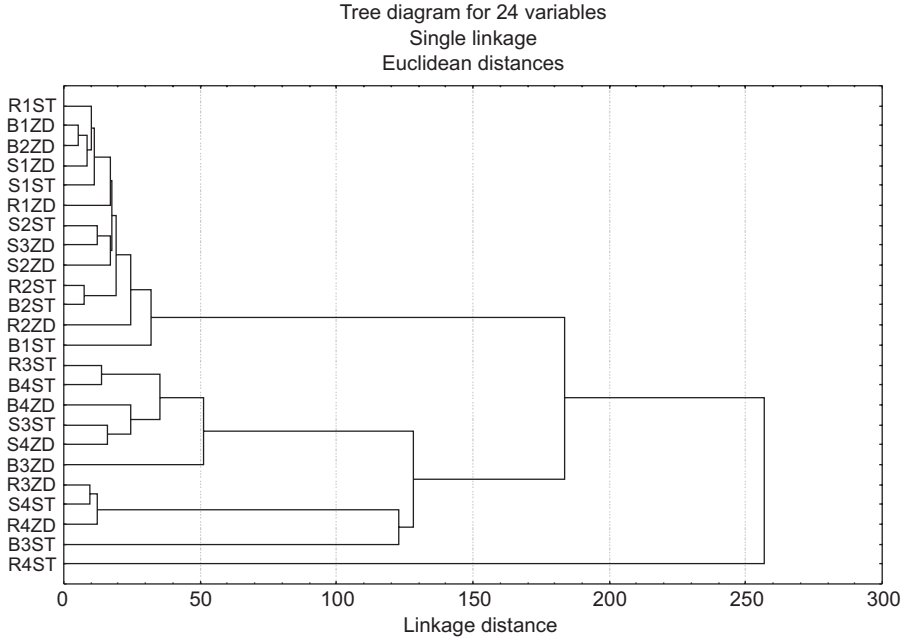

Fig. 2. Cluster analysis of colour parameters for three ecotypes of sour cherry var. Marasca at four stages of maturity from two growing regions (Zadar and Split)

indicate the development of dark purple-red colour during ripening in sour cherry Marasca ecotypes. Colour changes were in agreement with the accumulation of anthocyanins during ripening. Sour cherries from Marasca variety contained higher concentrations of anthocyanins compared to those from other cultivars reported in the literature.

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