Color Parameters and Total Anthocyanins of Sour Cherries (*Prunus cerasus* L.) During Ripening

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Summary

Color is very important indicator of quality of fresh fruit. It also serves for estimating the stage of maturity of fruit. Plant pigments responsible for the color of some kind of fruits are anthocyanins. Anthocyanins are the flavonoids which are present in high amounts in sour cherries. The aim of this study was to determine total anthocyanins and color parameters of sour cherries 'Cigančica' and 'Keleris' collected in Osijek and Zadar (Croatia) in 2005 during ripening. Color parameters of skin and flesh of sour cherries were determined with colorimetric CIE LAB method and total anthocyanins were determined by means of high performance liquid chromatography (HPLC) using UV/VIS PDA detector. Total anthocyanin was higher in sour cherries cv. Keleris grown in Zadar than in cv. Cigančica grown in Osijek during ripening although cv. Keleris is light colored genotype. Obtained results suggested that warm Mediterranean climate could have influence on high anthocyanin synthesis during ripening. Analysis of variance showed that stage of ripening did not influence total anthocyanin concentrations, but influenced almost all color parameters. Parameter H° was good indicator of color variation during ripening in both sour cherry cultivars.

Key words

CIE LAB color, ripening, sour cherries, total anthocyanins

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Introduction

Sour cherries (*Prunus cerasus* L.) are popular as fruit crop and in fruit industry. They are rich source of anthocyanins, pigments that are responsible for red color of fruit. Changes in cherries upon ripening are easily apparent by looking at their color change. Fruit ripening is associated with important biochemical changes and color change is mainly influenced by the concentration and distribution of different anthocyanins in the skin (Gao & Mazza, 1995). Total anthocyanins in red fruit increases during ripening (Marinova et al., 2005; Pedisić et al., 2007). Color of red fruit is indicator of maturity (Mazza & Minniatti, 1993; Esti et al., 2002). Number anthocyanins were determined in sour cherries, but the main anthocyanins are 3-glucosylrutinoside and rutinoside of cyanidin (Šimunić et al., 2005). The colorimetric CIE system is widely used in the assessment of color quality and color changes during ripening (Heredia et al., 1998). According to CIE concepts, the human eye has three color receptors -red, green and blue- and all colors are combinations of those (Abbot, 1999). For sour cherry which is a non-climateric stone fruit, color is important parameter for assessment of commercial harvest date. Numerous studies have been done on anthocyanins and color change in sweet cherries (Barrett et al., 1994; Esti et al., 2002; Mozetič et al., 2004; Gonçalves et al., 2007), while studies on sour cherries are rare. Usually used parameters for color variation of sweet cherries during ripening are a* values and hue angle (H°) (Barrett et al., 1994). According to Mozetič et al. (2004), the better parameters for anthocyanin accumulation are L* and C*. A confounding phenomena regarding C*, is that it will increase with pigment concentration to a maximum, and then decrease as the color darkens. Thus a pink and a dark red color can have identical values for C* (Wrolstad et al., 2005). According to Gonçalves et al. (2007) the L*, C* and H° of ripe sweet cherries were lower then those in partially ripe cherries and decrease in chroma means an increase in the tonality of the fruit color. As expected, the total anthocyanins were higher in ripe cherries then in partially ripe one (Gonçalves et al., 2004).

In sour cherries 'Petrovaradinska', 'Cigančica', 'Erdy jubileum', 'Oblačinska' grown in Croatia the total anthocyanin concentrations were from 2.7 to 28.0 mg of cyanidin-3-glucosylrutinoside (Cy-3-GR)/100 g of fresh weight (Šimunić et al., 2005). Kim et al. (2005) reported that in sour cherries 'Balaton', 'Danube', 'Schattenmorelle' and 'Sumadinka' total anthocyanins were in range from 49.1 to 109.2 mg cyanidin-3-glucoside equivalents (CGE)/100 g of fresh fruit. Total anthocyanin concentrations in sweet cherries ranged from 82 to 297 mg/100 g for dark cherries and from 2 to 41 mg/100 g for the light colored cherries (Gao & Mazza, 1995). Because of possible health benefits of anthocyanins and due to their color and taste they have generated a great deal of interest (Wang et al., 1999). The aim of this study was to determine total anthocyanins and color change of sour cherries 'Keleris' and 'Cigančica' grown in Dalmatian (Zadar) and continental (Osijek) regions of Croatia during ripening. Cv. Keleris is light and cv. Cigančica is dark colored sour cherry genotype.

Material and methods

Plant material

Sour cherries 'Keleris' were harvested from Zadar - orchard Škabrnja and sour cherries 'Cigančica' from orchard Osijek, Croatia in June 2005. Cherries harvested at three different stages of maturity and at different time of ripening were evaluated sensorially. Fruits were picked around the tree at eye level. Cherries were evaluated for skin and flesh color. All samples (within 2 h of harvest) were packed in polyethylene bags and kept at - 18 °C for two weeks until analysis.

Methods

Color analysis

Color change of sour cherries (skin and flesh) was measured with colorimetric CIE LAB method using a tristimulus colorimeter (Colortec PCM). Instrument measured L* as measure of lightness and two coordinates a* and b*. L* is a measure of lightness, where values range from completely opaque (0) to completely transparent (100), a* is a measure of redness (or –a* of greenness) and b* of yellowness (or –b* of blueness) on the hue-circle. The hue angle [H° = arctan (b*/ a*)] describes the relative amounts of redness and yellowness where 0 °/360 ° is defined for red/magenta, 90 ° for yellow, 180 ° for green and 270° for blue color. Chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) gives further information on the saturation or intensity of color (McGuire, 1992; Voss, 1992). Outer (skin) and inner (flesh) color was measured on 10 samples. Color of sour cherry fruit was presented as L*, a*, C*, and H° values.

Anthocyanin analysis

Phenolics were extracted according to Chaovanalikit & Wrolstad (2004) with certain modifications. The extract was made up to 25 ml with acidified water (0.01% aqueous HCl, v/v), blown with nitrogen gas and stored at -18 °C until further analyzed. Cherry phenolic extracts were filtered through preconditioned C-18 Sep-Pack cartridges to separate anthocyanins from nonanthocyanin phenolics. The anthocyanins were separated from the phenolics using acidified methanol which was evaporated under vacuum at 40 °C. HPLC analysis of cherry phenolics was performed using a reversed-phase HPLC system (Varian Prostar system) with a ProStar Solvent Delivery Modul 230, injector Rheodyne 7125 and detector ProStar 330 UV/VIS – Photodiode Array Detector (PDA). Anthocyanins were separated using a Pinaclle C-18 column (5 µm) 250 mm × 4.6 mm inner dia (Restek, Bellefonte, USA). For HPLC separation of anthocyanins as solvent A was used 100% HPLC-grade acetonitrile and as solvent B 1% phosphoric acid, 3% acetic acid (glacial) and 5% acetonitrile (v:v:v) in water. 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. The flow rate was 1 ml/min. Anthocyanins were identified by matching the UV-VIS spectra and retention time with authentic standards (cyanidine-3-glucoside chloride, pelargonidine-3-glucoside chloride and peonidine-3-O-glucoside chloride). Quantification was made by the external standard method using calibration

Table 1. Color parameters and total anthocyanins of sour cherry cv. Keleris grown in Zadar (Zd) area and sour cherry cv. Cigančica grown in Osijek (Os) area[a]

Stage of maturity	a* o.c.	L* o.c	C* o.c.	H° o.c	a* i.c.	L* i.c.	C*i.c.	H° i.c.	Tot. anthocy.[b]
				Keleris, Zadar					
I stage of maturity	11.75	33.21	16.13	43.28	12.11	38.54	16.52	40.91	3.53
II stage of maturity	10.57	23.94	13.30	34.93	23.11	21.50	25.56	31.78	3.22
III stage of maturity	7.26	27.98	8.90	31.74	13.72	28.90	16.42	30.25	4.29
				Cigančica, Osijek					
I stage of maturity	13.59	30.71	16.92	33.47	12.19	28.84	16.13	39.59	2.49
II stage of maturity	8.87	18.60	9.24	6.22	10.10	13.77	10.32	5.98	1.25
III stage of maturity	9.08	16.99	9.9	314.86	10.91	14.17	11.01	300.89	1.97

a o.c. - outer color (skin); i.c.-inner color (fruit flesh); b - total anthocyanins expressed as sum of individual anthocyanins (g/kg of dry matter)

of standards as a reference and was based on peak area from HPLC analyses and from mass concentration of compound. Obtained mass concentration of compounds (mg mL-1) was calculated on the mass of edible part of fruit and mass of dry matter (mg kg-1fresh and mg kg-1dry matter). Total anthocyanins were expressed as sum of individual anthocyanins. The samples were prepared in three replications.

Statistical analysis

Statistical analysis of variance and linear regression were performed using STATISTICA, version 7.1, StatSoft, Inc. (2005).

Results and disscusion

Quantitative differences of anthocyanin concentrations were observed between the two studied cultivars ('Keleris' and 'Cigančica') during ripening from different growing areas (Zadar and Osijek) (Table 1). Sour cherry 'Keleris' from Zadar growing area contained 1.4 to 2.58 fold higher total anthocyanin concentration than 'Cigančica' grown in Osijek area at all stages of maturity, even sour cherry 'Keleris' is light and 'Cigančica' dark colored genotype. They also differed in the color of fruit flesh. It is interesting that in other study, in sweet cherries anthocyanin concentrations were lower in light colored cultivars (Gao & Mazza, 1995). In both sour cherry cultivars during ripening, concentration of total anthocyanins decreased in 2nd stage of maturity, and in case of cv. Cigančica total anthocyanins decreased for 50%. In last (3rd) stage of maturity total anthocyanins increased and in case of cv. Keleris they were higher than in 1st stage of maturity. Total anthocyanins of cv. Keleris in last stage of maturity were 4.29 g/kg of dry matter and in cv. Cigančica were 1.97 g/kg of dry matter. Obtained results are much higher then previously reported in the literature (Kim et al., 2005; Šimunić et al., 2005). The reasons for these differences in total anthocyanin concentrations could be due to differences in the growing areas. Zadar is located in the Dalmatian region where high temperature and many sunshine hours characterize the area. Many authors reported that cultivar, climatic conditions, environmental factors, harvest and maturity stage can affect the anthocyanin concentrations in food. The difference between day and night temperatures can influence on anthocyanin concentrations also (Macheix et al., 1990; Tomas-Barberan & Espin, 2001). According to Awad et al. (2000) the sun-exposed apples had higher anthocyanin concentration. Color variation of skin and fruit flesh in 'Keleris' and 'Cigančica' were presented with parameters a*, L*, C*, H° of skin (o.c.) and fruit flesh (i.c.) (Table 1). All skin color parameters of cv. Keleris during ripening decreased uniformly and almost all color parameters were lower in ripe cherries as has been previously reported for sweet cherries (Goncalves et al., 2007). Color parameters of fruit flesh changed unevenly, except H° values (color nuance) which indicate red color of skin and flesh of sour cherries cv. Keleris (Table 1). $a^*_{i.c.}$ (redness) and $C^*_{i.c.}$ (color intensity) values of cv. Keleris increased in 2nd and then decreased in last stage of maturity and were similar to the values of 1st stage of maturity. $L^*_{i.c.}$ (lightness) value decreased in 2nd and then increased in last stage of maturity, but was considerable lower then in 1st stage of maturity. In sour cherries cv. Cigančica almost all color parameters of skin and flesh decreased during ripening, although values of a*, L*, C* were very similar in 2nd and 3rd stage of maturity (Table 1). Lower C* value indicates an increase in tonality of the fruit color (Goncalves et al., 2007). H° values of fruit flesh and skin of cv. Cigančica comported uniformly, decreased in 2nd stage of maturity and increased in 3rd stage of maturity and expressed more blue/magenta then red color nuance.

Table 2. Influence of stage of maturity on color parameters of skin and fruit flesh in sour cherries cv. Keleris grown in Zadar and cv. Cigančica grown in Osijek area by ANOVA statistical analysis

Color parameter	Stage of maturity		Skin/fruit flesh	
	F	p	F	p
a*	1.195	0.455	3.369	0.208
L*	11.461	0.080	0.319	0.629
C*	1.287	0.437	3.799	0.191
H°	198.306*	≤ 0.01	23.748*	≤ 0.05
		Cigančica, Osijek		
a*	4.570	0.180	0.311195	0.633058
L*	112.827*	≤ 0.01	13.227	0.068
C*	46.594*	≤ 0.05	0.552	0.535
H°	1047.297*	≤ 0.001	0.207	0.694

* Statistically significant

The statistical analyses of linear regression showed that parameter a* of fruit flesh was in correlation with total anthocyanins during ripening only in sour cherries 'Cigančica'. In sweet cherries strong correlation coefficient between a* and cyanidin-3-rutinoside was determined (Goncalves et al., 2007). ANOVA showed that stage of maturity significantly influenced on parameter H° in sour cherries cv. Keleris, while in cv. Cigančica influenced on color parameters H°, L* and C* (Table 2). Barret & Gonzales (1994) reported that color of sweet cherry during ripening can be followed with parameters a* and H°. According to Mozetič et al. (2004) better indicators of anthocyanin accumulation are L* and C*. Skin color of ripe, dark colored cv. Cigančica had lower L* values then light colored cv. Keleris, and great differences where in H° values. Similar results were obtained in another study on sweet cherries 'Sciazza' and 'Ferrovia' (Esti et al., 2002).

Conclusion

Color of sour cherries is important quality attribute. Measuring color parameters can be used to monitor pigment evolution and anthocyanins and can provide an objective judgment of food quality. Good parameter for color variation in sour cherries during ripening is hue angle (H°). Great differences in total anthocyanin concentrations in two different cultivars were determined. On anthocyanin concentrations significantly influenced climatic conditions.

Reference

Abbot, J.A. (1999). Quality measurement of fruits and vegetables. Postharv Biol Technol 15: 207-225

Awad M.A., de Jager, A., van Westing L. A. (2000). Flavonoid and chlorogenic acid levels in apple fruit: characterisation of variation. Sci Hortic 83: 249-263

Barrett, D. M., Gonzales, C. (1994). Activity of softening enzymes during cherry maturation. J Food Sci 59(3): 574–577

Chaovanalikit, A., Wrolstad, R.E. (2004). Anthocyanin and polyphenolic composition of fresh and processed cherries. J. Food Sci 69: 107-117

Esti, M., Cinquanta, L., Sinesio, F., Moneta, E., Di Matteo, M. (2002). Physicochemical and sensory fruit characteristic of two sweet cherry cultivars after cool storage. Food Chem 76: 399-405

Gao, L., Mazza, G. (1995). Characterization, quantification and distribution of anthocyanins and colourless phenolics in sweet cherry. J Agric Food Chem 43: 343–346

Girard, B., Kopp, T. G. (1998). Physicochemical characteristics of selected sweet cherry cultivar. J Agric Food Chem 46: 471–476

Gonçalves, B., Silva, A.P., Moutinho-Pereira, J., Bacelar, E., Rosa, E., & Meyer, S.A. (2007). Effect of ripeness and postharvest storage on the evolution of colour and anthocyanins in cherries (*Prunus avium L.*). Food Chem103: 976-984

Heredia, F. J., Francia-Aricha, E. M., Rivas-Gonzalo, J. C., Vicario, I. M., Santos- Buelga C (1998). Chromatic characterization of anthocyanins from red grapes-I. pH effect. Food Chem 4:491-498

Kim, D.O., Heo, H.J., Kim, Y.J., Yang, H.S., Lee, C.Y. (2005). Sweet and sour cherry phenolics and their protective effects on neuronal cells. J Agric Food Chem 53: 9921–9927

Macheix, J.J., Fleuriet, A., Billot, J. (1990). Fruit phenolics. CRC Press: Boca Raton, FL.

Marinova, D., Ribarova, F., Atanassova, M. (2005). Total phenolics and total flavonoids in Bulgarian fruits and vegetables. J University Chem Technol Metallurgy, 40 (3): 255-260

Mazza, G., & Miniatti, E. (1993). Anthocyanins in fruits, vegetables and grains. CRC Press: Boca Raton, FL.

McGuire, R.G. (1992). Reporting of objective colour measurements. Hort Sci 27 (12): 1254-1255

Mozetič, B., Trebše, P., Simčič, M., & Hribar, J. (2004). Changes of anthocyanins and hydroxycinnamic acids affecting the skin colour during maturation of sweet cherries (*Prunus avium* L.). Lebensm.-Wiss. u.-Technol. 37: 123–128

Pedisić S., Levaj B., Dragović-Uzelac V., Kos K. (2007). Physicochemical composition, phenolic content and antioxidant activity of sour cherry cv. Marasca during ripening. Agric Cons Sci 72(4): 295-300

Šimunić V., Kovač S., Gašo-Sokač D., Pfannhauser W., & Murković M., (2005). Determination of anthocyanins in four Croatian cultivars of sour cherries (*Prunus cerasus*). Eur Food Res Technol 220: 575-578

Tomás-Barberán, F. A., & Espín, J. C. (2001). Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. J Sci Food Agric 81: 853-876

Voss, D.H. (1992). Relating colourimeter measurement of plant colour to the royal horticultural society colour chart. Hort Sci 27 (12): 1256–1260

Wrolstad R.E., Durst R. W., Lee J. (2005). Tracking color and pigment changes in anthocyanin products. Trends Food Sci Technol 16: 423–428

H. Wang, M.G. Nair, G.M. Strasburg, Y.C. Chang, A.M. Booren and J.I. Gray (1999). Antioxidant and anti-inflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries. J Nat Prod 62: 294–296

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