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ANTIOXIDANT, PHYSICAL AND CHEMICAL CHARACTERISTICS OF CORNELIAN CHERRY FRUITS (*Cornus mas* L.) AT DIFFERENT STAGES OF RIPENESS

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Abstract. There has been growing interest for less utilized fruit species lately. Cornelian cherry is one of these species with significant antioxidant characteristics. In this study, several chemical properties and antioxidant characteristics of cornelian cherry fruits at four ripeness stages were evaluated. The stages were light yellow, blush, light red and dark red. Several fruit characteristics (fruit width, length, weight), total soluble solids (TSS), total titratable acidity (TA), sugar/acid ratio (TSS/TA), pH, fruit external color, total phenolic compounds (TPC), total antioxidant capacity (AOC) by trolox equivalent antioxidant capacity (TEAC), total monomeric anthocyanins (TMA) and tannins were evaluated. The stages were found to be significantly different for all factors evaluated. There was an overall increase in fruit width, length, weight, TSS, and SSC/TA over time, while TA averages decreased as the fruit matured. Fruit color progressively turned to dark red as anthocyanin accumulates. TPC and TEAC averages were similar at light yellow (8033 µg gallic acid equivalent GAE · g^{-1} fresh weight (fw) and 55.0 µmol trolox equivalent (TE) · g^{-1} fw) and reduced at the dark red stages (4162 µg GAE · g^{-1} fw and 7.8 µmol TE · g^{-1} fw). Tannin content decreased from 0.45 to 0.19% from light yellow to dark red stages.

Key words: anthocyanin, FRAP, phenolics, ripening, quality, TEAC

INTRODUCTION

Cornelian cherry is one of those less common but nevertheless interesting fruit species found in temperate zones of Turkey. In the family Cornaceae, the genus *Cornus* includes about 50 species of shrubs and trees, most of which are used for decorative purposes. Only a few species of this genus produce edible fruits. Among these, the most important one is cornelian cherry (*Cornus mas* L.), which is also suitable for use as

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a horticultural species due to its great abundance of flowers and rich foliage. Cornelian cherry is particularly appealing in park end gardens due to their attractive red fruits [Bijelic et al. 2011].

Turkey has a rich gene pool of cornelian cherry genotypes adapted to different local conditions in different regions of country. Most trees are open pollinated seedlings of wild genotypes which vary widely in terms of productivity and fruit characteristics. Seed propagation and long-term human selection has also given rise to a great diversity of trees [Yilmaz et al. 2009].

Recent interest in food phenolics has greatly increased on account of the antioxidant and free radical-scavenging abilities associated with some phenolics and their potential effects on human health [Hamid et al. 2011]. In recent decades, agronomic research has set priorities to obtain high yield, better resistance to disease and transportation and longer shelf-life of fruit. Thus, fruit breeding programs have been aimed at improving yield and fruit size, resistance to disease and pests, adaptation to particular growing systems and rate of ripening. Most recently, research has been focused on the quality of fruit [Capocasa et al. 2008]. Red fruits constitute a good source of natural antioxidant substances Özgen et al. [2009a]. The antioxidant properties of cornelian cherry have been documented in the literature and cornelian cherry has some of the highest ascorbic acid, anthocyanin, phenolic and antioxidant activity among the many other fruits [Pantelidis et al. 2007, Pawlowska et al. 2010].

Fruits are a good source of natural antioxidants, containing many different radical scavenger components that provide protection against harmful-free radicals and the result are associated with lower incidence of cancer and heart diseases in addition to number of other health benefits [Shui and Leong 2006, Hamid et al. 2011]. Antioxidants reduce oxidative damage to cells that can lead to cancer, heart disease, and other degenerative diseases. Especially, anthocyanin compounds that give cornelian cherry their red color, are powerful antioxidants [Prior 2003, Zafra-Stone et al. 2007]. Among natural compounds, phenolics and in particular flavonoids have been found to be an important part of human diet and are considered to be the active component in many medicinal plants [Cooper-Driver 2001]. Genotype-variety is the major factor in determining fruit nutritional quality. Nutritional quality is also affected by crop conditions, ripening stage, pre-harvest and post-harvest conditions, shelf-life and processing [Connar et al. 2002, Capocasa et al. 2008, Özgen et al. 2009b]. Recent research suggests that cornelian cherry contains significant phytochemical properties and antioxidant capacity [Yilmaz et al. 2009, Rop et al. 2010, Pawlowska et al. 2010, Hamid et al. 2011, Popovic´ et al. 2012]. Furthermore, their fruits are rich in sugar, antocyanins, organic acids and tannins [Seeram et al. 2002].

Harvest maturity and postharvest conditions are some of the factors that may lead to changes in sensory and nutritional qualities of cornelian cherries. Cornelian cherries are frequently harvested at dark red stages, when their flavor is most desirable. Consumers do not usually eat cornelian cherry at any of the other maturation stages. Therefore, the effect of ripening on antioxidant and quality is a major issue. Most of the current literature is focused on antioxidant properties and photochemical properties of mature dark-red cornelian cherries. The objective of this study was to investigate the effects of harvest maturity on the physical and chemical characteristics of cornelian cherries.

MATERIALS AND METHODS

Plant material. Cornelian cherry fruits were harvested from a commercial orchard in Tecde, Malatya, Turkey. The altitude of the orchard is 960 m above the sea level. Fruits were hand harvested at approximately 15 day intervals at four different maturity stages (light yellow, blush, light-red and dark red) from the beginning of August to end of September 2010. The fruits were selected according to uniformity of shape and color and then transferred to laboratory for physical and phytochemical analysis. Samples were divided into two groups. The first group was used for fruit width, length, weight, total soluble solids (TSS), total acidity (TA), TSS/TA, pH and fruit external color. Fruit width and length of the fruit were measured by using caliper with a sensitivity of 0.01 mm. The fruit color was measured using a Minolta portable chromameter (Minolta, Model CR-400) which provided CIE L^*, a^* and b^* values. Slurries were used to determine TSS contents by refractometery (Atago, Pal-1) and for levels of TA using standard methodology. Then, about 200 g fruits with 4 replications for each of the maturity stages were frozen at -20°C. Cornelian cherry fruit from each maturity stage was thawed at room temperature, seeds were removedand then homogenized in a food processor.

Analytical procedures

Determination of total phenolic content (TPC). TPC was measured according to the procedure of Singleton and Rossi [1965]. Fruit slurries were extracted with buffer containing acetone, water, and acetic acid (70:29.5:0.5 v/v) for one h. in the dark. Each of the four maturity stages was replicated four times. Then, the extract, Folin-Ciocalteu's phenol reagent and water was incubated for 8 min followed by adding 7% sodium carbonate. After two hours, the absorbance was measured using an automated UV-VIS spectrophotometer at 750 nm. GA was used as standard. The results were expressed as μg GAE in g fresh weight basis (fw) basis.

Total monomeric anthocyanins (TMA). TMA was estimated by a pH differential method [Giusti et al. 1999] using a UV-VIS spectrophotometer (model T60U, PG Instruments). Absorbance was measured at 535 nm and 700 nm in buffers at pH 1.0 and 4.5 using A = (A535-A700) pH 1.0 – (A535-A700) pH 4.5 with a molar extinction coefficient of 30.900. Results were expressed as milligrams of cyanidin-3-galactoside equivalents in g fw basis.

The total antioxidant capacity (AOC). AOC was estimated by TEAC assays as suggested by Özgen et al. [2006]. For the standard TEAC assay, ABTS+ was dissolved in acetate buffer and prepared with potassium persulfate as described in Özgen et al. [2006]. The mixture was diluted in an acidic medium of 20 mM sodium acetate buffer (pH 4.5) to an absorbance of 0.700 ±0.01 at 734 nm for longer stability. For the spectrophotometric assay, 3 mL of the ABTS+ solution and 20 μL of fruit extract were mixed and incubated in 10 min and the absorbance was determined at 734 nm.

Tannins contents. The soluble tannins were measured by the Folin–Denis method [Taira 1996] and expressed as tannic acid on a fw basis. Tannin content was determined spectrophotometrically using a UV1208 spectrophotometer (Shimadzu, Japan).

Statistical analysis. Data was analyzed using SAS software and procedures [SAS 2005]. Means and standard deviations were calculated using PROC TABULATE. The analysis of variance (ANOVA) tables were constructed using PROC GLM procedures. The means of the stages were compared using Least Significant Difference (LSD) method at 5% significance level.

RESULT AND DISCUSSION

The maturity stages were found to be significantly different for all factors evaluated. This indicates that the visual differences selected in this experiment did represent different stages of ripeness. The means and significant separations are given in Table 1. The average fruit dimensions were 17.4 mm, 23.5 mm for width and length while the average fruit weight was 4.9 g. There was an overall increase over time in fruit width, length, weight, TSS, TSS/TA, while TA averages decreased as the fruits matured. The TSS reached to 16.0% and 16.5% at the light red and dark red stages. Although the stages tested were found to be significantly different for pH as well, pH was the only variable not showing a regular progression in value during maturity.

Ripe cornelian cherry flavor is partially conditioned by the sugars and acids ratio. Sugar- acid ratio ranged from 3.7 to 8.4, from light yellow to dark red stage. Demir and Kalyoncu [2003] also reported a significant variation among full ripe cornelian cherry genotypes for sugar-acid ratio.

Table 1. Mean and mean separations for several pomological parameters of cornelian cherries fruits sampled from four maturation stages.

Stage	Width (mm)	Length (mm)	Weight (g)	TSS (%)	TA (%)	TSS/TA (%)	pH
Light yellow	15.9 d	22.3 d	3.9 d	11.3 c	3.0 a	3.7 c	2.9 ab
Blush	16.8 c	22.8 c	4.2 c	12.2 b	2.4 b	5.1 b	2.7 b
Light red	17.4 b	23.8 b	5.0 b	16.0 a	2.2 c	7.4 a	2.8 bc
Dark red	19.4 a	25.0 a	6.4 a	16.5 a	2.0 d	8.4 a	3.0 a
Mean	17.4	23.5	4.9	14	2.4	5.9	2.8
$LSD_{5\%}$	0.4	0.6	0.4	0.6	0.1	1.7	0.1

Among the color variables, *L* exhibited a continuous decrease as the fruits matured. *b* and *hue* demonstrated similar patterns, while the *a* and *chroma* variables displayed more complex pattern. The highest values for the color parameters were obtained from the light yellow to dark red stages. For *a*, the lowest value was measured at the light yellow stage, while the lowest value for *chroma* was at the dark red stage. Changes in *b* values indicate a color change from light yellow to blue; changes in *a* values indicate a turn in color from green to red. Similar patterns were also observed for *h*°. Changes *h*° values indicate a turn in color from green to red (tab. 2). Similar results for changes in color during fruit maturity have been reported by Celik et al. [2008] in cranberry and Özgen et al. [2009b] in arbutus andrachne fruits.

Table 2. Mean and mean separations for color parameters of cornelian cherries fruits sampled from four maturation stages.

Stage	*L*	*a*	*b*	*Chroma*	*Hue*
Light yellow	69.0 a	7.4 d	39.4 a	40.5 c	79.3 a
Blush	59.3 b	24.9 c	37.9 a	45.5 b	57.0 b
Light red	36.4 c	43.2 a	27.7 b	51.5 a	32.4 c
Dark red	29.8 d	36.6 b	14.6 c	39.7 d	20.7 d
Mean	48.6	28	29.9	44.3	47.4
$LSD_{5\%}$	2.7	2.8	4.2	3.2	5.1

Table 3. Mean and mean separations for total phenolic content (TPC), antioxidant capacity (TEAC), anthocyanin (TMA) and tannins of cornelian cherries fruits sampled from four maturation stages.

Stage	TPC[a] (µg GAE · g^{-1} fw)	TEAC[b] (µmol TE · g^{-1} fw)	TMA[c] (µg cy-3-glu · g^{-1} fw)	Tannins[d] (%)
Light yellow	8033 a	55.0 a	4.9 c	0.45 a
Blush	8206 a	55.3 a	16.5 b	0.38 b
Light red	7110 b	27.1 b	65.8 a	0.37 b
Dark red	4162 c	7.8 c	65.0 a	0.16 c
Mean	6878	36.3	38	0.34
$LSD_{5\%}$	350	1.9	2.2	0.02

[a]TPC contents were estimated by the Folin-Ciocalteu assay of Singleton and Rossi [1965]. Values are expressed as µg gallic acid equivalents (GAE) · g^{-1} fresh weight (fw)
[b]TEAC values were determined by the method of Özgen et al. [2006]. Values are expressed as µmol of trolox equivalents (TE) · g^{-1} g fw
[c]TMA were determined by the pH-differential method of Giusti et al. [1999]. Values are expressed as µg cyanidin 3-glucoside equivalents · g^{-1} fw
[d]Tannin values were determined by the method of Taira [1996]. Values are expressed as % fresh weight (fw)

The overall averages for the TPC, TEAC, TMA and tannins were 6878 µg GAE · g^{-1} fw, 36.3 µmol TE · g^{-1} fw, 38.0 µg cy-3-glu · g^{-1} fw, and 0.34%. These average values confirm that cornelian cherry is a good source of phenols, anthocyanin, tannins and therefore is a fruit with high antioxidant capacity. TPC and TEAC averages displayed similar patterns: the highest averages were recovered from the light yellow (8033 μg GAE · g^{-1} fw) and 55.0 μmol TE · g^{-1} fw) and blush (8206 μg GAE · g^{-1} fw and 55.3 μmol TE · g^{-1} fw) stages; and the averages were reduced at the light red (7110 μg GAE · g^{-1} fw and 27.1 μmol TE · g^{-1} fw) and dark red stages (4162 μg GAE · g^{-1} fw and 7.8 μmol TE · g^{-1} fw). A reverse pattern was observed for TMA and tannins. TMA was lowest at the light yellow stage and reached to 65.0 and 65.8 μg cy-3-glu · g^{-1} fw at the dark red and light red stages. Tannin content was reduced from 0.45 to 0.16% from the light yellow to dark red stages (tab. 3).

TPC and antioxidant averages detemined by TEAC decreased during maturation. Similar results were reported by Celik et al. [2008], who studies four maturation stages in cranberry fruit. The phenolic level also decreased during the maturation process in apple fruit [Burda et al. 1990], acerola fruits [Lima et al. 2005] and strawberries [Pineli et al. 2011]. TMA was very low in light yellow fruit and then gradually increased in the mature red fruits. The change is due to the degradation of chlorophylls and synthesis of anthocyanins [Özgen et al. 2009a]. Tannin content was reduced from the light yellow to dark red stages. Similar phenomena were observed in persimmon fruits by Candir et al. [2009]. Tannin content in cornelian cherry depending on genotype was also reported from 0.65% to 1.31% [Bijelić et al. 2012].

CONCLUSIONS

Cornelian cherry fruits are a significant source of phenolic compounds, anthocyanins and total flavonoids. Antioxidant activity was high in fruits and varied greatly among the maturity stages. Therefore cornelian cherry could be considered a good source of natural antioxidants [Yilmaz et al. 2009, Hamid et al. 2011]. Our results clearly demonstrated that some of the chemical properties and antioxidant capacities of cornelian cherry fruits are affected by maturation stages. Significant variability was found for overall pomological parameters, fruit colors and photochemical properties of four maturation stages. The light yellow stage of cornelian fruits had the highest total phenolics, antioxidant capacity and tannins content. The level of the potent antioxidants in cornelian cherry (total phenolics, anthocyanins and tannins) were likely affected the levels of antioxidant capacity. Although ripe berries are, in general, more edible, greater antioxidant contents and activities were observed at the light yellow and blush stages mostly due to high tannin content. More detailed studies about the phenolic profiles during the ripening of different genotypes need to be pursued and their relation to color, flavor, tannins and other attributes should be carried out. Further research could elucidate the main sources of quality of light yellow and ripe cornelian cherries, which could help in genetic improvement programs.

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ANTYOKSYDACYJNE, FIZYCZNE I CHEMICZNE CECHY OWOCÓW DERENIA JADALNEGO NA RÓŻNYCH ETAPACH DOJRZAŁOŚCI

Streszczenie. Ostatnio obserwuje się rosnące zainteresowanie mniej używanymi owocami. Dereń jadalny jest jednym z gatunków o istotnych cechach antyoksydacyjnych. W niniejszym badaniu oceniono pewne właściwości chemiczne oraz cechy antyoksydacyjne derenia jadalnego w czterech etapach dojrzałości, mianowicie w fazie jasnożółtej, różowej, jasnoczerwonej i ciemnoczerwonej. Oszacowano kilka cech owoców (szerokość, długość, masa), *całkowitą* ilość rozpuszczonych substancji stałych (TSS), ogólną kwasowość potencjalną (TA), stosunek cukier/kwas (TSS/TA), pH, zewnętrzny barwa owocu, całkowitą zawartości *związków* fenolowych (TPC), całkowitą *zdolność* antyoksydacyjną (TEAC), całkowitą zawartość monomerycznych antocyjanin (TMA) oraz tanin. Okazało się, że poszczególne etapy były zupełnie inne dla wszystkich ocenianych czynników. Zaobserwowano ogólny wzrost szerokości owocu, długości, masy, TSS oraz SSC/TA w czasie, natomiast średnie TA zmniejszały się wraz z dojrzewaniem owocu. Barwa owocu stopniowo przechodziła w ciemnoczerwoną wraz z gromadzeniem się antocyjanin. Średnie TPC i TEAC były podobne w fazie jasnożółtej (8033 µg ekwiwalentu kwasu galusowego GAE · g^{-1} św.m. oraz 55,0 µmol ekwiwalentu Trolox (TE · g^{-1} św.m.), natomiast zmniejszony w fazach ciemnoczerwonych (4162 µg GAE · g^{-1} św.m. oraz 7,8 µmol TE · g^{-1} św.m.). Zawartość tanin zmniejszyła się z 0,45 do 0,19% między fazą jasnożółtą a ciemnoczerwoną.

Słowa kluczowe: antocyjaniny, FRAP, fenole, dojrzewanie, jakość, TEAC

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