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ORIGINAL ARTICLE / ORIGINALBEITRAG

The Influence of Fruit Size on Quality Attributes and Bioactive Compounds of Sweet Cherry Fruit

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Abstract

The study was carried out to determine the effect of different fruit sizes on the quality and bioactive compounds of sweet cherry (*Prunus avium* L. cv. '0900 Ziraat'). The fruit was harvested on the basis of Centre Technique Interprofessionnel des Fruits et Légumes (CTIFL) color category 5, mahogany. The fruit was separated for analysis according to the CTIFL scale: S21 (21 mm), S22 (22 mm), S24 (24 mm), S25 (25 mm), and S26 (26 mm). As the size of the fruit increased, the firmness decreased. The fruit color values varied depending on fruit size. It was determined that the increase in size of the fruit reduced the color values. The soluble solids content and vitamin C values occurred the differences with effect of fruit size. The increasing of fruit size occurred the decreasing in vitamin C content and the increase in the amount of soluble solids. The effect of fruit size on total phenolics, flavonoids, and antioxidant activity was significant. While the highest value of total phenolic content was obtained in S25, the lowest value was determined in S26. In both DPPH and ferric reducing antioxidant power assays, the lowest antioxidant activity was obtained in S26-sized fruit. The individual phenol with the highest concentration in sweet cherry was catechin, and *p*-coumaric was the individual phenolic with the lowest concentration. There were differences in the concentrations of individual phenolics between fruit sizes. However, it cannot be stated that fruit size had an effect on individual phenolic concentration because this effect was inconsistent.

Keywords Antioxidant · Catechin · Firmness · Flavonoid · Vitamin C

Introduction

Sweet cherry is a fruit species preferred by consumers because of its taste, aroma, and positive effects on human health due to its high antioxidant activity, and consumption is steadily increasing. However, the color, size, and firmness of the fruit are significant quality characteristics affecting the market value. Large fruit, one of the main objectives of modern cherry cultivation (Shomura et al. 2008; Zhang and Whiting 2011; Wang et al. 2012; Chakrabarti et al. 2013), significantly affects the economic value and yield of the sweet cherry (Whiting et al. 2006; Zhang et al. 2010). Large fruits are preferred by consumers because of their visual appeal, taste, and high nutritional content (Looney et al. 1996; Blazkova et al. 2002). Phenolic compounds, flavonoids, and anthocyanins are the bioactive compounds that contribute significantly to the formation of color, taste, and aroma in sweet cherry (Ozturk et al. 2019).

Sweet cherry is very rich in terms of bioactive compounds. The concentration and composition of the bioactive compounds in fruit vary depending on the maturity of the fruit, cultural practices, and genetic and environmental factors (Serra et al. 2011). Serrano et al. (2005), Serradilla et al. (2012), and Usenik et al. (2014) reported differences in the concentration of bioactive compounds due to the maturity level of sweet cherry. Although fruit size, which is an indicator of maturity in the fruit, affects the market value of fruit because of its positive effects on appearance, taste, and aroma, it is very important to know the relationship between fruit size and the bioactive content of the fruit. This

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study was conducted to investigate the effects of fruit size on fruit quality attributes and bioactive compounds of the '0900 Ziraat' sweet cherry cultivar.

Materials and Methods

Plant Material

The experiment used 5-year-old uniform sweet cherry trees (*Prunus avium* cv. '0900 Ziraat') grafted on SL 64 rootstock (selection of *Prunus mahaleb* L.: Saint Lucie GF 64 [SL 64]) in Suşehri, Sivas province, Turkey (40° 10′ 09.67″N latitude, 38° 06′ 37.14″E longitude and 952 m altitude), which has average annual precipitation of 21 kg m^{-2}, average annual temperature of 11 °C, and clay–loam soil (pH 7.9). The trees were planted in an east–west direction with 4.0-m row spacing and 3.5 on-row tree spacing and were trained according to the Spanish bush system. Standard cultural practices (irrigation, fertilization, pruning, disease control) were regularly applied during the experimental period. Irrigation was applied by drip irrigation. Macronutrients and micronutrients were supplied in three aliquots on 1 March, 1 April, and 1 May in 2017. A total of 12 g of nitrogen (N), 20 g of 60% potassium oxide (K_2O), 5 g of monoammonium phosphate ($NH_4H_2PO_4$), and 20 g of potassium sulfate (K_2SO_4) were supplied to the trees. Additionally, 5 g of calcium nitrate (Ca [NO_3]) was supplied once on 15 May. No symptoms of nutritional deficiency were observed in the leaf or fruit during the growing season.

Experimental Design

Three trees (one tree from each replicate) were selected on the basis of the trunk cross-sectional area. The fruit was hand-harvested on 25 June 2017 on the basis of Centre Technique Interprofessionnel des Fruits et Légumes (CTIFL) color category 5, mahogany. Fruit was immediately transported at 10 ± 1.0 °C and 85 ± 5.0 relative humidity for 2 h by frigorific vehicles to the postharvest physiology laboratory of the Horticulture Department of Ordu University. The fruit (500 g per size in each tree) was separated for analysis according to the CTIFL scale: S21 (21 mm), S22 (22 mm), S24 (24 mm), S25 (25 mm), and S26 (26 mm). The following analyses and measurements were performed on the fruit.

Fruit Weight, Width, Length, and Firmness

Fruit weight was measured using a digital scale accurate to ±0.01 g (Radwag PS 4500/C/1, Radom, Poland). Fruit length and width were determined with a digital caliper accurate to ±0.01 mm (model CD-6CSX, Mitutoyo, Tokyo, Japan). Fruit firmness was measured with a digital portable durometer (nondestructive device, Agrosta® 100 Field, Agrotechnologie, Paris, France), and the results were expressed as Durofel units (%). In Durofel units, 0 indicates that the fruit is too soft, and 100 indicates that the fruit is too firm. Twenty fruits of each replicate were used to determine fruit weight and firmness.

Color Characteristics

Color measurements were performed with a color meter (Konica Minolta, CR–400, Tokyo, Japan). Color data of jujube fruit were presented according to the Commission Internationale de l'Eclairage (CIE) system. Color characteristics were measured in the equatorial part of 10 fruits randomly selected from each replicate. L *, a *, and b * values defined the three-dimensional color space. Chroma (C^*) and hue angle (h°) were calculated using the following equations (McGuire 1992):

$$C^* = (a^{*2} + b^{*2})^{1/2},$$
$$h^\circ = \tan^{-1} b^*/a^*.$$

Soluble Solids Content, Titratable Acidity, and Vitamin C

Twenty fruits were initially selected from each replicate for soluble solids content (SSC), titratable acidity, and vitamin C analyses. Fruit stones were removed, and juice was extracted with the aid of an extractor (HR1855/70, Philips, Turkey). The SSC percentage was measured using a digital refractometer (PAL-1, McCormick, USA). About 10 ml of extract was diluted with 10 ml distilled water for titratable analyses. The amount of 0.1 N sodium hydroxide used for titrating the resultant solution to a pH of 8.2 was expressed in milligrams of malic acid (mg 100 g^{-1}). The rate between SSC and titratable acidity was used to determine SSC/titratable acidity. About 0.5 ml of extract was completed to 5 ml with 0.5% oxalic acid for vitamin C analyses (Ozturk and Ozer, 2019). An ascorbic acid test strip (catalog number 116981, Merck, Darmstadt, Germany) was immersed in the resultant solution for 2 s, and excess liquid on the test strip was removed. Readings were performed in a reflectometer (Merck RQflex plus 10), expressed as milligrams per 100 g (mg 100 g^{-1}).

Total Phenolics, Total Flavonoids, and Antioxidant Activity

Initially, 20 fruits were selected from each replicate for total phenolics, total flavonoids, and antioxidant activity. The stones were removed, and pulps were homogenized

in a blender. The resulting homogenates were then stored in three tubes at –20 °C until the analyses. The frozen homogenates were thawed at room temperature (≈ 21 °C) and rehomogenized with a blender. The fruit juice was separated from the pulp through centrifuging the slurry at 12,000 × g at 4 °C for 30 min. The resulting juice was then diluted with distilled water and refrozen at –20 °C in multiple aliquots to be used later for phenolic, flavonoid, and antioxidant analyses.

Total phenolics were determined with the aid of an automated ultraviolet-visible spectrophotometer (Shimadzu, Kyoto, Japan) in accordance with principles specified by Beyhan et al. (2010). Gallic acid was used as the standard. The results were expressed as microgram (µg) of gallic acid equivalent (GAE) per 100 g of fresh weight (FW): µg GAE g^{-1} FW.

Total flavonoid contents were determined in accordance with the principles specified by Chang et al. (2002). Quercetin was used as the test standard, and results were expressed as micrograms of quercetin equivalent (QE) on the FW basis (µg QE g^{-1} FW).

Two different procedures, DPPH radical scavenging activity (Blois 1958) and the ferric reducing antioxidant power assay (FRAP) (Benzie and Strain 1996), were employed to assess antioxidant activity of the sweet cherry. Results were expressed as micromoles per gram of Trolox equivalent (TE) on the FW basis (µmol TE g^{-1}) in both assays.

Individual Phenolics

Catechin, chlorogenic acid, rutin, caffeic acid, protocatechuic acid, 4-hydroxybenzoic acid, 4-aminobenzoic acid, and *p*-coumaric acid were measured. In the separation of phenolic acids with ultrahigh performance liquid chromatography (UHPLC; Ultimate 3000, Thermo Scientific, CA, USA), the method described by Ozturk et al. (2019) was used. The samples were distilled with distilled water at a ratio of 1:1 and were then centrifuged at 15,000 × g for 15 min. The supernatant was filtered with 0.45-µm millipore filters and then injected for UHPLC. The chromatographic separation was performed using a DAD detector (DAD-3000, CA, USA) in the UHPLC system. The analytes were separated by a 250 × 3.0 mm, 5-µm Hypersil GD phenyl column (Thermo Scientific, CA, USA) with temperature set at 30 °C. The elution solvents were aqueous 2.5% formic acid (solvent A) and 100% methanol (solvent B). The separation was conducted at 274 nm, and the total run time took 40 min. The injection volume was 20 µl, and the mobile phase flow rate was 1 ml min^{-1}. The results were expressed in milligrams per kilogram (mg kg^{-1}).

Statistical Analysis

The normality of the data was confirmed by the Kolmogorov–Smirnov test and the homogeneity of variances by Levene's test. The results obtained in each analysis were analyzed using SAS software version 9.1 (SAS Institute, Cary, NC, USA). Data were analyzed by one-way analysis of variance followed by Tukey's test. All analyses were performed with a 95% confidence level ($p < 0.05$).

Result and Discussion

Fruit Weight and Firmness

In sweet cherry, the fruit size, color, taste, aroma, and firmness are the quality characteristics affecting market value and consumer preferences (Chakrabarti et al. 2013; Shomura et al. 2008; Zhang and Whiting 2011; Wang et al. 2012). In this study, which we carried out to determine the effect of fruit size on quality and biochemical content, the effect of fruit size on fruit weight was statistically significant. It was determined that with an increase in fruit size, the fruit weight increased. This is an expected result. The weight of the fruit varied from 7.86 g (S21) to 9.60 g (S26) (Table 1).

Fruit firmness, which is a significant quality characteristic, directly affects the fruit quality and postharvest life of sweet cherry. The fruit firmness varies depending on genetic factors and variety. However, the maturity stage of the fruit can affect firmness (Blazkova et al. 2002). When the fruit firmness values were reviewed, it was determined that the effect of fruit size on firmness was statistically significant and that the firmness decreased as the size of fruit increased. It was observed that the smallest fruit (S21) had the greatest firmness value (41.27%), but the lowest firmness value (32.67%) was obtained from the largest (S26)

Table 1 Effect of size on fruit weight and firmness of sweet cherry fruit

Size levels[a]	Quality characteristics	
	Weight (g)	Firmness (%)[b]
S21	7.86 c	41.27 a
S22	8.22 bc	36.60 b
S24	8.75 b	35.13 b
S25	9.24 ab	33.93 c
S26	9.60 a	32.67 c

[a]Size levels were determined according to the Centre Technique Interprofessionnel des Fruits et Légumes (CTIFL)
[b]The scale ranges from 0 (very soft) to 100 (very firm)
$n = 120$ for firmness (three replicates × 20 fruits × two different measurements for each fruit), and $n = 60$ for weight (three replicates × 20 fruits). Means in columns with the same letter do not differ according to Tukey's test at $P < 0.05$

Table 2 Effects of size level on color characteristics of sweet cherry fruit

Size levels	Color characteristics		
	L*	Chroma	Hue angle
S21	40.14 a	43.98 a	28.29 a
S22	36.37 b	38.45 b	25.20 b
S24	34.97 bc	36.86 b	21.49 c
S25	33.72 c	33.57 c	20.59 c
S26	33.08 c	31.70 c	19.51 c

$n = 120$ for the color characteristics (three replicates × 20 fruits × two different measurements for each fruit). Means in columns with the same letter do not differ according to Tukey's test at $P < 0.05$

fruit. However, it was also determined that the differences between S22 and S24 and between S25 and S26 were not statistically significant (Table 2).

Fruit size is determined as a result of cell division and cell expansion. As cells expand, the bonds between cells become weaker, and the cell wall components are degraded. This results in a reduction in fruit firmness (Harker et al. 1997). Johnston et al. (2002) reported that in apple, bigger fruit was less firm. However, Usenik et al. (2014) stated that the fruit size in sweet cherry had no effect on fruit firmness.

Color Characteristics

The attractive color is one of the main characteristics of cherry quality (Esti et al. 2002; Usenik et al. 2005; Gabriele et al. 2013). The results of this study showed that the effect of fruit size on color values was statistically significant. The fruit color values decreased as fruit size increased. It was determined that the L * value, representing brightness in the fruit, ranged from 33.08 to 40.14 and that the brightness decreased as the size of the fruit increased; that is, the color intensity increased as the size of the fruit increased. Chroma value expresses vividness in fruit; the higher the chroma value, the more vivid the fruit color. In this study, the chroma value decreased as the size of the fruit increased. The highest chroma value was measured in the S21 fruit (43.98), while the lowest value (31.71) was obtained from S26 fruit (Table 2). The red intensity increases as maturity increases in sweet cherry, and a reduction of hue angle value indicates increasing intensity of red color in the fruit. The results of the study confirm this. It was observed that as the size of the fruit increased, the hue angle decreased, and the red color intensity increased. Considering that 25% of fruit size occurs 1 week before harvest (Blazkova et al. 2002), it can be stated that the size and color intensity increase as fruit maturity fruit progresses. Usenik et al. (2014), whose findings were similar to ours, observed that the chroma and hue angle values decreased with an increase in the size of sweet cherry fruit.

SSC, Titratable Acidity, SSC/Acidity, and Vitamin C

The SSC/titratable acidity ratio in sweet cherry is closely related to the formation of flavor in the fruit. This rate increases in proportion to maturity (Crisosto et al. 2002). Blazkova et al. (2002) reported that in 'Karešova' cherry cultivar, there was a significant increase in the SSC ratio with the progression of maturity but no significant change in acidity. They also determined that there was a positive correlation between the size of the fruit and SSC. The results of our study confirm those of these researchers. In the study, it was determined that an increase in SSC and SSC/acidity ratio dependent on fruit size occurred; however, there was no significant change in acidity ratio. The lowest SSC value (11.30%) was measured in the S21fruit, whereas the highest value (13.70%) was recorded in S26 fruit (Table 3). In addition, it was determined that the acidity ranged from 0.43% to 0.47%, but the difference between sizes was not statistically significant. Usenik et al. (2014), whose findings were similar to ours, reported that the SSC/acidity ratio increased in proportion to the size of the fruit.

Vitamin C is considered to be one of the most significant antioxidants required for plant growth and defense (Foyer and Noctor 2011); it is found in cell organelles such as mitochondria, plastids, peroxisomes, and apoplasts (Smirnoff 2000; Tijero et al. 2016). Sweet cherry is a very rich fruit species in terms of vitamin C (Seeram et al. 2002) and contains twice as much ascorbic acid (a form of vitamin C) as oranges (Yilmaz et al. 2009). In a study conducted by Usenik et al. (2014), it was determined that in 'Kordia' cultivar, vitamin C ranged from 4.39 to 5.26 mg 100 g^{-1}. In our

Table 3 Effects of size level on soluble solids content (SSC), titratable acidity, SSC/acidity, and vitamin C of sweet cherry fruit

Size levels	SSC (%)	Titratable acidity (malic acid g 100 g^{-1})	SSC/acidity	Vitamin C (mg 100 g^{-1})
S21	11.30 c	0.44 a	25.68 d	6.27 a
S22	11.40 c	0.43 a	26.51 c	6.18 a
S24	12.20 b	0.44 a	27.73 b	5.40 b
S25	13.00 ab	0.47 a	27.65 b	5.27 b
S26	13.70 a	0.44 a	31.13 a	5.33 b

$n = 9$ for the SSC, titratable acidity, SSC/acidity, and vitamin C (three replicates × three different measurements for each replicate). Means in columns with the same letter do not differ according to Tukey's test at $P < 0.05$

Table 4 Effects of size level on total phenolics, total flavonoids, and antioxidant activity of sweet cherry fruit

Size levels	Bioactive compounds			
	Total phenolics (μg GAE g^{-1})	Total flavonoids (μg QE g^{-1})	DPPH (μmol TE g^{-1})	FRAP (μmol TE g^{-1})
S21	288.02 b	128.39 a	1.76 b	9.61 c
S22	234.48 d	122.50 a	1.58 c	8.43 c
S24	254.13 c	155.89 a	1.63 c	12.18 b
S25	303.73 a	146.07 a	1.90 a	15.28 a
S26	192.24 e	105.80 b	1.44 d	8.85 c

$n = 9$ for the bioactive compounds (three replicates × three different measurements for each replicate). Means in columns with the same letter do not differ according to Tukey's test at $P < 0.05$

study, vitamin C content was found to be relatively higher, between 5.33 (S26) and 6.27 (S21) mg 100 g^{-1} (Table 3). In addition, it was observed that the vitamin C content decreased as the size of the fruit increased. However, Tijero et al. (2016) reported that vitamin C content was highest in the period when the fruit reached full maturity in terms of fruit color and size. In contrast to our findings, Usenik et al. (2014) reported that fruit size did not have a significant effect on vitamin C content.

Total Phenolics, Total Flavonoids, and Antioxidant Activity

Sweet cherry is becoming more and more popular due to its bioactive compounds with antioxidant characteristics, generally including polyphenols, vitamins, anthocyanins, and carotenoids (Usenik et al. 2008; Serradilla et al. 2012). Although sweet cherry is rich in bioactive compounds, the concentration of these compounds in the fruit may vary depending on genetic factors (Ballistreri et al. 2013; Habib et al. 2015) and on the maturity stage of the fruit (Serrano et al. 2005; Serra et al. 2011; Serradilla et al. 2012; Usenik et al. 2014). In our study, there were statistically significant differences in bioactive compounds between fruit size levels. When we evaluated the total phenolic content, the highest value (303.73 mg) was recorded in size level S25 (303.73 mg), followed by S21 (288.02 μg GAE g^{-1}), S24 (254.13 μg GAE g^{-1}), S22 (234.48 μg GAE g^{-1}), and S26 (192.24 μg GAE g^{-1}), respectively. In terms of total flavonoid content, the highest value was 155.89 μg QE g^{-1}, found in S24, but the differences between S21, S22, S24, and S25 were not statistically significant. However, the lowest flavonoid content was recorded in the size level S26 fruit. There were significant differences in terms of antioxidant activity (DPPH and FRAP) between size levels. However, the effect of fruit size on these values can be said to be inconsistent. It was determined that DPPH values ranged from 1.44 μmol TE g^{-1} (S26) to 1.90 μmol TE g^{-1} (S25), while the difference between S22 (1.58 mol TE g^{-1}) and S24 (1.63 μmol TE g^{-1}) was not statistically significant. In S21 fruit, the DPPH value was 1.76 μmol TE g^{-1}. When the FRAP values were reviewed, the highest value was 15.28 μmol TE g^{-1} (S25), followed by 12.18 μmol TE g^{-1} (S24), 9.61 μmol TE g^{-1} (S21), 8.85 μmol TE g^{-1} (S26), and 8.43 μmol TE g^{-1} (S22; Table 4). Usenik et al. (2014) reported that in sweet cherry, fruit size had a significant effect on the concentration of bioactive compounds. However, the differences in composition of bioactive compounds in sweet cherry may be related to the ripening stage of the fruit (Serrano et al. 2009).

Table 5 Effects of size levels on individual phenolic compounds of '0900 Ziraat' sweet cherry fruit

Individual phenolic compound (mg kg^{-1})	Size levels				
	S21	S22	S24	S25	S26
Catechin	967.2 a	733.1 b	538.2 c	898.2 a	534.1 c
Chlorogenic acid	25.16 a	nd	nd	26.36 a	nd
Rutin	8.81 a	7.67 b	5.93 c	9.94 a	5.85 c
Caffeic acid	1.98 c	4.21 a	2.87 b	3.17 b	2.94 b
Protocatechuic acid	2.50 a	2.36 a	2.50 a	nd	2.68 a
4-hydroxybenzoic acid	3.06 b	3.28 b	3.69 a	3.18 b	3.76 a
Epicatechin	nd	nd	6.54 b	nd	6.83 a
4-aminobenzoic acid	1.98 a	2.03 a	1.67 b	2.18 a	1.54 b
p-coumaric acid	0.77 b	0.93 a	0.91 a	0.93 a	0.87 a

$n = 9$ for the individual phenolics compounds (three replicates × three different measurements for each replicate). Means in same line with the same letter do not differ according to Tukey's test at $P < 0.05$
nd not determined

Individual Phenolic Compounds

In the study, it was determined that the major phenolic acid in sweet cherry was catechin, followed by chlorogenic acid and rutin. Compared with other size levels, S21 and S25 fruits had significantly higher catechin and rutin contents. The highest caffeic acid content (4.21 mg kg^{-1}) was obtained from S22, whereas the lowest value (1.98 mg kg^{-1}) was recorded in S21. Compared with other size levels, S24 and S26 had significantly higher 4-hydroxybenzoic acid content. However, there was no difference statistically between S21, S22, and S25 fruits in terms of 4-aminobenzoic acid, and these sizes had more 4-aminobenzoic acid content than those of the other sizes. Compared with other sizes, the S21 fruit had significantly less *p*-coumaric acid (Table 5). In general, it was seen that the amounts of the individual phenolic compounds were significantly different depending on the fruit size. However, the effect of fruit size on individual phenolics cannot be said to be consistent. Usenik et al. (2014) reported that the influence of fruit size on individual phenolics was unstable. Also, Serradilla et al. (2011) reported that the effect of fruit ripening on epicatechin content is not clear. However, Usenik et al. (2014) determined that fruit size had an effect on the amount of rutin present.

Conclusions

In sweet cherry, the fruit size is an important factor affecting consumer preference, and it was found to have a significant effect on the bioactive compounds and the quality characteristics. Size levels S25 and S26 are ideal fruit sizes for the '0900 Ziraat' sweet cherry cultivar.

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Conflict of interest E. Aglar, O. Saracoglu, B. Ozturk, O. Karakaya, and U. Ateş declare that they have no competing interests.

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