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**Research Article**

# The relationship between fruit color and fruit quality of sweet cherry (*Prunus avium* L. cv. '0900 Ziraat')

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**ABSTRACT**

The study was carried out to determine the relationship between fruit color on the fruit quality and bioactive compounds of sweet cherry. The highest mass, width and length of fruit were obtained when harvested in CTIFL 2, whereas the lowest values were recorded in CTIFL 6. With the increase in color intensity, the softening in fruit occurred. The firmness of CTIFL 6 was about half of the CTIFL 2. With the darkening of the color, SSC significantly increased in fruit. The highest SSC value was determined in CTIFL 6, but the lowest SSC was recorded in CTIFL 2. The fruit of CTIFL 3, had significantly higher vitamin C than the other fruit. The lowest vitamin C was measured in CTIFL 2. The fruit of CTIFL 4-6 had higher total phenolics and total flavonoids content than other color levels. In both DPPH and FRAP assays, the highest antioxidant activity was measured in CTIFL 6, whereas the lowest was determined in CTIFL 2. The effect on color levels of phenolic compounds was significant. Catechin and chlorogenic acid were major phenolic acids in fruit. As a result, it was revealed that color levels had significant effect on bioactive compounds of sweet cherry.

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## 1. Introduction

Sweet cherry, a healthy dietary product, has significant phenolic compounds such as anthocyanin, quercetin, hydroxycinnamates, vitamin C, carotenoids and melatonin in addition to relatively low calorie content, glycemic action, high antioxidant activity, sugars and organic acids (Whiting et al., 2005).

In sweet cherry, there is a relationship between the fruit ripening and the bioactive content of the fruit. Sweet cherry fruit with optimal maturity have the highest quality and richest nutrient content (Mahmood et al., 2013). Phenolic compounds contribute to fruit quality characteristics such as color, aroma, flavor, etc. (Tomas-Barberan et al., 2001). In sweet cherry, not only genetic factors, but also environmental factors and cultural management practices are effective in the composition of biochemical compounds. Especially the temperature difference between the day and night before harvest, light intensity and fruit maturity stage can affect the content of bioactive compounds. Serra et al. (2011) reported that the phenolics and anthocyanin content and antioxidant activities of the fruit may vary depending on the factors preharvest and postharvest, on the climatic factors and on the maturity stage when the fruit is harvested. In the previous studies (Crisosto et al., 2003; Serrano et al., 2005; Usenik et al., 2013; Erbas et al., 2018), it was determined that the fruit maturity stage in sweet cherry has a significant effect on fruit quality and biochemical content.

In sweet cherry, the fruit quality characteristics such as fruit color, size, firmness, taste and flavor are main factors that determine consumer preference (Esti et al., 2002). According to the researchers (Wermund et al., 2005; Kappel et al., 1996), an 'ideal' sweet cherry should be large, dark-red and sweet. However, does the fruit color level have an effect on the quality characteristics and bioactive compounds of sweet cherry? The aim of this study was to determine the effect of the fruit color on fruit quality characteristics and bioactive compounds of '0900 Ziraat' sweet cherry.

## 2. Materials and methods

### *2.1. Plant material*

The study was conducted in 2017 in Suşehri, Sivas Province, Turkey. 5-year-old sweet cherry trees (*Prunus avium* cv. '0900 Ziraat') grafted on SL 64 clonal rootstock were selected for the experiment. The trees were planted in an east-west direction with 4.0 m row spacing and 3.5 on-row tree spacing and trained by Spanish Bush system. Standard cultural practices (disease control, pruning, irrigation and fertilization) were regularly applied during the study.

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Irrigations were applied by drip irrigation. On March 1, April 1 and May 1, 3 times fertilizer (a total of 12 g nitrogen, 20 g potassium oxide, 5 g monoammonium phosphate and 20 g potassium sulphate) were supplied to trees. Additionally, 5 g calcium nitrate [Ca (NO3)] was supplied once in May 15.

*2.2. Experimental design*

The study was designed as three replicate and there was one tree on each replicate. The trees were selected on the basis of the trunk cross-sectional area. The fruit were hand-harvested at commercial maturity (21 June 2017). Fruit was immediately transported at 10±1.0 °C and 85±5.0 for 2 h by frigorific vehicles to postharvest physiology laboratory of Horticulture Department of Ordu University. The fruit (500 g for per color level in each tree) were separated for analysis on the basis of fruit color using the CTIFL (Centre Technique Interprofessionneldes Fruit et Legumes, France) card: CTIFL 2, CTIFL 3, CTIFL 4, CTIFL 5 and CTIFL 6. The following analyses and measurements were performed on fruit.

*2.3. Fruit weight, width, length and firmness*

Fruit weight was measured using a digital scale (±0.01 g) (Desis THB, Turkey). Fruit length and width were determined with a digital caliper (± 0.01 mm) (Absolute-1103, Insize, Germany). Fruit firmness was measured with a digital portable durometer (nondestructive device, Agrosta® 100 Field, Agrotechnologie, France) and results were expressed as DurofelUnits (%). In Durofel Units, 0 indicates that the fruit is very soft and 100 indicates that the fruit is very firm. Twenty fruit each replicate were used to determine the fruit weight, length, width and firmness.

*2.4. Soluble solids content (SSC), titratable acidity and vitamin C*

For chemical analyses (SSC, vitamin C, acidity), twenty fruit (each tree) were taken from each tree. The stones of the fruit have been removed. Juice was extracted by using an extractor (HR1855/70, Philips, Turkey). Soluble solids content (SSC) was measured by using the digital refractrometer (PAL-1, McCormic, USA) and Vitamin C was measured by using 0.4 % oxalic acid solution described by Ruck (1963). Titratable acidity was measured by using 0.1 N NaOH solution.

*2.5. Total phenolics, total flavonoids and antioxidant activity*

Twenty fruit (each tree) were taken from each tree for bioactive compounds. In the fruit removed stones, pulps were homogenized through a blender. 2 tubes (homogenates) were kept at -22 °C until the analyses. For biochemical analyses, frozen samples were resolved at 21 °C. Then, fruit juice of samples was separated from the pulp via centrifuging the slurry at 10.000 × g at 4 ℃ for 35 min. Prepared juice was diluted with distilled water. Samples were refrozen at -22 °C for use in analyses of bioactive compounds.

Total phenolics were defined according to the principles specified by Meda et al. (2005). An automated UV-Vis spectrophotometer (Thermo Scientific, Genesys 180, USA) was used to measured total phenolics. As the standard, gallic acid was used. The results were stated in µg gallic acid equivalents (GAE) g-1 fw (fresh weight).

Total flavonoids were measured according to method reported by Meda et al. (2005). As the standard, quercetin was used. The results were stated in µg quercetin equivalents (QE) $g^{-1}$ fw.

For antioxidant activity, DPPH (2.2-diphenyl-1-picryl-hydrazyl-hydrate) (Blois, 1958) and FRAP (Ferric ions (Fe+3) reducing antioxidant power) (Benzie and Strain, 1996) assays were used. The results were stated in µmol Trolox equivalent (TE) $g^{-1}$ fw in both assays.

*2.6. Individual phenolics*

In the study, catechin, chlorogenic acid, 4-aminobenzoic acid, caffeic acid, protocatechuic acid, transferulic acid, rutin, 4-hydroxybenzoic acid and p-coumaric acid were measured. The chromatographic separation was performed by using a DAD detector (DAD-3000, USA) in ultra-high performance liquid chromatography (UHPLC, Thermo Scientific, Ultimate 3000, USA), the method defined by Ozturk et al. (2015).

The samples were distilled with distilled water at the ratio of 1:1 and after they were centrifuged at 15 000 × g for 15 min. The supernatant was separated out with 0.45 µm millipore filters and then injected to UHPLC. The analytes were separated by 250 x 3.0 mm, 5 µm Hypersil GD phenyl column (Thermo Scientific, 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. Total run time took 40 min. Injection volume was 20 µL and the mobile phase flow rate was 1 ml $min^{-1}$. The obtained results were expressed in mg $kg^{-1}$.

*2.7. Statistical analysis*

Kolmogorov-Smirnov test was used to determine the normality of the data and the homogeneity of variances by the Levene's test. The results for each analysis were analyzed with SAS (SAS Institute Inc., version 9.1, USA) software. Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test. All analyses were performed with a 95% confidence level.

## 3. Results

*3.1. Fruit size and firmness*

The size values of the fruits harvested with different color level according to CTIFL scale are showed in Table 1. There were the significant differences in the fruit size values between the color stages. Fruit weight, fruit width and fruit length was ranged from 8.44 g to 9.25 g, 24.02 mm to 24.88 mm, and 22.64 mm to 23.06 mm; respectively. The highest values were measured with CTIFL 2, but there were no statistically significant between the values of CTIFL 2, CTIFL 4 and CTIFL 5. Although there was no significant difference in fruit size values between CTIFL 3 and CTIFL 6, the lowest values were measured in fruit of CTIFL 6. Fruit firmness values were determined between 25.93 N and 47.93 N. As the color intensity increased in fruit, fruit firmness decreased. The highest firmness value was recorded with CTIFL 2, while the lowest was measured with CTIFL 6.

**Table 1**. Effects of coloring level on fruit weight, fruit sizes and firmness of sweet cherry fruit

| Color level | Quality characteristics | | | |
|---|---|---|---|---|
| | Weight (g) | Width (mm) | Length (mm) | Firmness * |
| CTIFL 2 | 9.25 a | 24.88 a | 23.06 a | 47.93 a |
| CTIFL 3 | 8.62 b | 24.53 b | 22.59 b | 46.07 a |
| CTIFL 4 | 9.05 a | 24.79 a | 22.68 b | 34.87 b |
| CTIFL 5 | 9.19 a | 24.78 a | 22.89 a | 33.80 b |
| CTIFL 6 | 8.44 b | 24.02 c | 22.64 b | 25.93 c |

CTIFL (1–7 scale): Exocap color [Centre Techique Interprofessionnel des Le´gumes (CTIFL)]. * The scale ranges from 0 to 100 for very soft to very firm surfaces. n= 60 for the firmness (three replications × ten fruits × two different measurements for each fruit). n= 60 for the weight and fruit sizes (three replications × twenty fruits).

*3.2. SSC, titratable acidity and vitamin C*

SSC, titratable acidity and vitamin C values were showed in Table 2. It was determined that the SSC ratio was increased depending on increase in fruit color intensity. The SSC ratio in CTIFL 2 was 11.90%, while in CTIFL 6 it was recorded as 18.27%. However, the difference between CTIFL 3, CTIFL 4 and CTIFL 5 SSC ratios was not significant. In the study, the effect of fruit color on titratable acidity was not significant and the acidity rate was 0.27 %. In the study, it can be said that the fruit color has a significant effect on vitamin C content. However, this effect showed inconsistency. The lowest vitamin C content (7.33 mg 100 $g^{-1}$) was obtained from fruit, which belong to CTIFL 2, whereas the highest value (9.28 mg 100 $g^{-1}$) in terms of vitamin C was recorded with CTIFL 3. However, there were no significant differences between CTIFL 4 and CTIFL 5 vitamin C values (Table 2).

**Table 2**. Effects of coloring level on SSC, SSC/acidity, acidity and vitamin C of sweet cherry fruit

| Color level | Biochemical characteristics | | | |
|---|---|---|---|---|
| | SSC (%) | Titratable acidity (mg malic acid 100 $g^{-1}$) | SSC/Acidity | Vitamin C (mg 100 $g^{-1}$) |
| CTIFL 2 | 11.90 c | 0.28 a | 42.50 c | 7.33 d |
| CTIFL 3 | 13.90 b | 0.27 a | 51.48 b | 9.28 a |
| CTIFL 4 | 13.80 b | 0.27 a | 51.11 b | 8.56 b |
| CTIFL 5 | 14.39 b | 0.27 a | 53.30 b | 8.40 b |
| CTIFL 6 | 18.27 a | 0.27 a | 67.67 a | 8.02 c |

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

*3.3. Bioactive compounds*

Total phenolic, total flavonoid and antioxidant activity of the fruit were determined and showed in Table 3. There were differences in total phenolic and total flavonoid content between the color intensity. The total phenolic content and the total flavonoid content ranged from 196.17 to 256.63 µg GAE $g^{-1}$ fw and 115.62 to 65.29 µg QE $g^{-1}$ fw respectively. In terms of total phenolic and total flavonoid content, the highest values were recorded with CTIFL 6, whereas the lowest value was obtained with CTIFL 2. There was no significant difference between the CTIFL 4, CTIFL 5 and CTIFL 6 color intensity in terms of total flavonoid and total phenolic content. Again, the difference between CTIFL 2 and CTIFL 3 was not significant.

**Table 3**. Effects of coloring level on bioactive compounds of sweet cherry fruit

| Color level | Bioactive compounds | | | |
|---|---|---|---|---|
| | Total phenolics | Total flavonoids | DPPH | FRAP |
| CTIFL 2 | 201.08 b | 115.62 b | 1.59 c | 7.95 d |
| CTIFL 3 | 196.17 b | 116.23 b | 1.69 b | 10.42 c |
| CTIFL 4 | 246.27 a | 152.14 a | 1.70 b | 10.21 c |
| CTIFL 5 | 243.50 a | 159.82 a | 1.73 b | 11.44 b |
| CTIFL 6 | 256.63 a | 165.29 a | 1.89 a | 13.69 a |

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

It was determined that the fruit color has a significant effect on antioxidant activity. According to both antioxidant assays (DPPH and FRAP), the highest antioxidant activity was recorded from CTIFL 6, while the lowest antioxidant activity was measured in CTIFL 2.

In the study, catechin and chlorogenic acid were the main phenolic acids. It was seen that the fruit color had a significant effect on individual phenolic compounds. The

highest catechin value (1057.7 mg $kg^{-1}$) was measured in CTIFL 3, whereas the lowest value (518.0 mg $kg^{-1}$) was recorded the fruit, which belong to CTIFL 6. The highest chlorogenic acid and rutin concentration was determined from CTIFL 4 and CTIFL 5, respectively, but the lowest values were obtained from the fruit of CTIFL 2. It was determined that the CTIFL 4, CTIFL 5, and CTIFL 6 have significantly higher caffeic acid content than the others. The highest 4-aminobenzoic acid and *p*-coumaric acid were measured in the fruit of the CTIFL 2 and CTIFL 3 (Table 4).

**Table 4**. Effects of color levels on individual phenolic compounds of '0900 Ziraat' sweet cherry fruit

| Individual Phenolics | Color levels | | | | |
|---|---|---|---|---|---|
| Compounds (mg $kg^{-1}$) | CTIFL 2 | CTIFL 3 | CTIFL 4 | CTIFL 5 | CTIFL 6 |
| Catechin | 922.2 b | 1057.7 a | 785.0 c | 644.5 d | 518.0 e |
| Chlorogenic acid | 15.92 d | 18.50 c | 26.92 a | 28.43 a | 24.75 b |
| Rutin | 6.70 d | 7.47 c | 8.24 b | 9.48 a | 7.54 c |
| Caffeic acid | 2.11 b | 2.43 b | 3.61 a | 3.65 a | 3.82 a |
| Protocatechuic acid | 2.16 a | 2.05 a | 2.24 a | 2.08 a | 2.09 a |
| 4-hydroxybenzoic acid | 3.83 b | 4.21 a | 3.79 b | 4.12 a | 2.87 c |
| 4-aminobenzoic acid | 1.25 a | 1.31 a | nd. | nd. | 0.96 b |
| *p*-coumaric acid | 2.08 a | 1.95 a | 1.77 b | 1.75 b | 1.71 b |

nd: not determine. n= 9 for the individual phenolic compounds (three replications × three different measurements for each replication). Means in same line with the same letter do not differ according to Tukey's test at P<0.05.

## 4. Discussion

Although there are differences between the fruit size values depending on the fruit color, it cannot be said that fruit color has an effect on fruit weight. Usenik et al. (2014) reported that fruit in CTIFL 6 had higher fruit mass and sizes than those in CTIFL 3. The main determinant of fruit size is the number of cells formed in the cell division phase of development. The greater number of cells in this phase, the larger the fruit size (Bohner and Bangerth, 1988). However, considering the fact that the growth in fruit continues until the ripening period, it is not an unexpected result that there is no linear relationship between the color levels and the fruit size or the bigger fruits have been obtained from the CTIFL 2. As a matter of fact, Beever and Hopkirk (1990) reported that nearly 2/3 of the increase in fruit size and weight occurs in the first 10 weeks after the fruit set and then a relatively slow development continues until harvest.

There is a negative correlation between fruit firmness and ripening (Whiting et al., 2005). Understanding the biological mechanism underlying fruit softening is essential to manipulate it without affecting other desirable aspects of ripening, such as color, flavor, aroma, or nutritional value (Hagermann et al., 2000). With the progress of cherry fruit development, color intensity increases and, concomitantly, fruit softens. The reason for this is that pectin's break down and the cell wall resistance decreases, during the last period of fruit development. Also in '0900 Ziraat' fruit, color intensity increased while firmness decreased with progressing of development. In the study, it was seen that with the progression of the maturation in the fruit, color intensity increased and firmness decreased. Yoo et al. (2010) and Faniadis et al. (2010) reported that fruit firmness naturally decreased during ripening. In contrast to our study, Usenik et al. (2014), reported that the fruit color had not any effect fruit firmness values.

The SSC/acidity ratio in cherry fruit is one of the significant criteria that determines taste formation, and it is indicator of the fruit ripening. It is desired that this ratio is high. Fruit color does not have any effect on acidity. However, significant increases in SSC and SSC/acidity was observed due to increase color intensity in the fruit. SSC and SSC/acidity ratio in CTIFL 2 was 11.90% and 42.50%, respectively, whereas they were determined as 18.27% and 67.67%, respectively in CTIFL 6 (Table 2). This is an expected result. Because, as the fruit ripens, sugar concentration increases, while acids remain relatively constant (Looney et al., 1996). Indeed, Usenik et al. (2014) reported that SSC/acidity ratio increased with maturity, this rate was 16.3 in CTIFL 4 and it increased to 20.2 in CTIFL 6. The differences in values may be due to the cultivar in the study. However, our results confirm the findings of the researcher.

In the study, it was determined that the fruit color level has a significant effect on vitamin C content. In sweet cherry, the fruit color is darkened depending upon the increase of the fruit ripening. Usenik et al. (2014) reported that fruit color and fruit size had no significant effect on vitamin C content. At the occurrence of this difference, climate change, cultural applications and genetic factors may have an effect.

Bioactive compounds such as phenolic compounds, flavonoids and anthocyanins, which determine the antioxidant capacity of fruits, play a therapeutic and restorative role in human health in addition to the effect on the quality, taste, aroma and flavor in fruit (Mikulic-Petkovsek et al., 2012; Sen et al., 2014). In our study, total phenolic and flavonoids contents and antioxidant capacity of fruit changed in parallel. The fruit, which have high total flavonoids and total phenolic content, have higher antioxidant activity. With increasing fruit color intensity, increases in bioactive compounds were observed. The concentration of bioactive compounds varies depending on cultivar, environmental and post-harvest factors, climatic characteristics and maturity stage (Serra et al., 2011). In our study, it was determined that as maturity progressed, the bioactive content increased. Usenik et al. (2014) reported that with increasing in color intensity on sweet cherry, phenolic compounds increased, but antioxidant activity decreased in contrast to our study. And also, researchers (Serrano et al., 2005; Serradilla et al., 2012), reported that the bioactive compounds in sweet cherry may vary

depending on the stage of maturity.

In the study, catechin and chlorogenic acid were the main phenolic acids. It was seen that the fruit color had a significant effect on individual phenolic compounds. Similar to the results of our study, Usenik et al. (2014) reported that fruit color has a significant effect on the concentration of individual phenolic compounds. The researcher reported that with increasing color intensity, rutin concentration increased, but *p*-coumaroylquinic acid content decreased. As a matter of fact, the nutrient content loss occurs in fruits with optimal maturity.

As a result, it was revealed that physical, mechanical and biochemical properties of the fruit may vary depending on the fruit color of sweet cherry. In considering the marketing, processing, transfer and storage of the cherry, the harvesting of the fruit in the optimal maturity is a critical factor. In this context, it is thought that it would be more appropriate in CTIFL 4 and CTIFL 5 to the harvest on '0900 Ziraat' cultivar.

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**Disclosure statement**

No potential conflict of interest was reported by the author

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