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ORIGINAL ARTICLE / ORIGINALBEITRAG

Training System Plays a Key Role on Fruit Quality and Phenolic Acids of Sweet Cherry

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Abstract

In the study, the effects of different training systems (Steep Leader: SL, Spanish Bush: SB and Vogel Central Leader: VCL) on quality properties and bioactive components of "0900 Ziraat" sweet cherry fruit (*Prunus avium* L.) were investigated. The size, color and firmness values of the fruit varied depending on training system. The largest fruit was obtained in the SB training system. The fruit on trees trained VCL and SB had higher firmness than the fruit of trees trained SL. The color values of the fruit of VCL were higher than the other systems, while the vitamin C content was lower. The lowest acidity and soluble solids content (SSC) were measured in fruit trees trained SL. The highest values for bioactive compounds as phenolics, flavonoids were measured in fruit of SL training system. In the sweet cherry fruit, the major phenolic acid was catechin. The catechin, rutin, caffeic acid, 4-hydroxybenzoic acid, 4-aminobenzoic acid and transferulic acid content of the fruit in the SL training system were higher than those of SB and VCL. As a result, it was revealed that there is an effect of the training system on fruit quality; SB training system had higher values in terms of fruit size, whereas in terms of bioactive compound content, SL training system had higher values.

Keywords Training systems · Antioxidant · Catechin · Firmness · Flavonoids · Phenolics · Vitamin C · Sweet cherry · Prunus avium

Erziehungssysteme spielen eine Schlüsselrolle für die Fruchtqualität und den Gehalt an Phenolsäuren bei Süßkirschen

Schlüsselwörter Erziehungssysteme · Antioxidantien · Catechin · Festigkeit · Flavonoide · Phenole · Vitamin C · Süßkirsche · Prunus avium

Introduction

Sweet cherry forms high trees which grow vigorous and have narrow angle branches. The fruit yield and quality of such trees are low, and the labor cost of harvesting is high. Therefore, in sweet cherry, pruning and training system is highly significant. Some negative characteristics of sweet cherry trees can be changed by using dwarf or semi-dwarf rootstocks. However, it is difficult to obtain the desired yield from these trees without proper pruning and training system (Long 2003). Previous research (Peterson et al. 2003; Whiting et al. 2005; Blazkova et al. 2002; Radunic et al. 2011; Ağlar et al. 2016) determined that the vegetative and the generative development of the tree varied depending on the training system applied in sweet cherry. Therefore, choosing the proper training system in sweet cherry breed-

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Fig. 1 Pictures of training systems in 0900 Ziraat sweet cherry. **a** Step Leader (SL), **b** Spanish Bush (SB), **c** Vogel Central Leader (VCL)

ing is as significant as choosing the rootstock and cultivar. The previous studies revealed that there were differences in the yield and fruit quality of the pomegranate (Gill et al. 2011), sweet cherry (Musacchi et al. 2015), apple (Ozkan et al. 2012), peach and nectarine (Zec et al. 2016) depending on the training system.

The training system does not only affect the yield and precocity of the tree, but also can affect fruit quality and biochemical properties (Long 2003). In sweet cherry, there are many biochemical components which define the value of the fruit by playing role in aroma, color, taste and thus ripening (Ballistreri et al. 2013). Sweet cherry contains a higher proportion of biochemical compounds compared to other fruit species, but the content and concentrations of the biochemical compounds differs from depending upon factors such as climate, soil, maturity stage and cultural practices (Veberic et al. 2005). Previous research (Usenik et al. 2008; Jakobek et al. 2009) determined that the tree vigor has a significant effect on content and concentrations of the biochemical compounds of sweet cherry. The pruning and training treatments affecting tree size can be effective on chemical composition and concentration. The fact that Reynolds and Heuvel (2009) reported that the training system applied in grapes may change the sensory properties and biochemical composition in the fruit. However, there is no study on the effects of the training system on fruit quality and bioactive components of sweet cherry. The main aim of the research was to determine the role of the training systems on bioactive components, physical and mechanical properties of "0900 Ziraat" fruit.

Materials and Methods

Plant Materials

5-year old uniform "0900 Ziraat" sweet cherry trees (*Prunus avium*) grafted on SL 64 Saint Lucie GF 64 (SL 64) rootstock (*Prunus mahaleb* L.) in Suşehri, Sivas Province, Turkey (40° 10′ 10.63″N latitude, 38° 06′ 35.23″E longitude and 949 m altitude), with average annual precipitation and temperature, respectively, 21 kg m^{-2} and 11 °C (pH: 7.9, clay-loam soil), were used in the experiment.

The trees were located with 4.0 m row spacing and 3.5 m on-row tree spacing in an east-west direction, and were trained by Spanish Bush (SB), Steep leader (SL) and Vogel Central leader (VCL) systems (Fig. 1). Irrigation, pruning, fertilization and disease control were regularly applied during study period. Irrigations were made by drip irrigation. According to nutrient levels and pH of the orchards's soil, nutrient elements were provided in three parts on March 1, April 1 and May 1. A total of 12 g nitrogen, 20 g potassium oxide (60%), 5 g monoammonium phosphate and 20 g potassium sulfate were provided per plants. Additionally, 5 g calcium nitrate was given once in May 15.

Nine trees (three trees in each replicate) were determined on the basis of the trunk cross-sectional area for each training system. The fruit (500 g for each tree) in each training system was hand-harvested at commercial maturity on the basis of CTIFL (Centre Technique Interprofessionneldes Fruit et Legumes, France) color category: 5-Mahogany. Fruit was immediately transferred at 10 ± 1.0 °C and 85 ± 5.0% relative humidity for 2 h by cooling vehicle to postharvest physiology laboratory of Ordu University, where they were seperated with no mechanical damage, disease-free, uniform size and greenish stems. The following analyses and measurements were performed on fruit.

Fruit Weight, Width, Length, Thickness and Firmness

The weight of fruit was measured with digital scale (±0.01 g) (Desis THB, Turkey). Fruit sizes (width, length, thickness) were determined by digital caliper (±0.01 mm) (Absolute-1103, Insize, Germany). The firmness was determined through digital portable durometer (Agrosta® 100 Field, 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 fruit in each tree per replicate were used to determine the fruit weight, width, length, thickness and firmness.

Color Characteristics

In each fruit, L*, chroma and hue angles were measured using a colorimeter (Konica-Minolta, CR-400, Japan). CIE (Commission Internationale de l'Eclairage system) was used in chromatic analyses. L*, chroma and hue angles values were determined in 20 fruit randomly taken from each replicate. 3-D color space was defined with the aid of L*, a^* and b^* values. The equations with $C^* = (a^{*2} + b^{*2})^{1/2}$ for chroma and and $h^\circ = \tan^{-1}$ b*/a* for hue angle were used.

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. In the fruit removed stones, juice was exracted by using an extractor (K 1583, Arcelik, Turkey). A digital refractometer (HI 96801, Hanna, Italy) was used to measured SSC (%). The juice of fruit was stirred with distilled water at the ratio of 1:1 for titratable acidity. For titrating, the prepared solution was used the amount of 0.1 N NaOH (sodium hydroxide). Obtained results were expressed in g malic acid 100 g^{-1}. 0.5 ml of prepared extract was stirred with 0.5% oxalic acid (5 ml) for vitamin C measurement. The ascorbic acid test strip (116981, Merck, Germany) was dipped in the prepared solution for 2 s, and settled in test adaptor of reflectometer (Merck RQflex plus 10, Germany). Obtained results were stated in mg 100 g^{-1} fresh weight (fw).

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 were separated from the pulp via centrifuging the slurry at 10.000 × g at 4 °C 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.

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 × 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}.

Statistical Analysis

Kolmogorov-Smirnov test was used to determined 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.

Table 1 Effects of training system on fruit weight, fruit sizes and firmness of "0900 Ziraat" sweet cherry fruit

Training System	Quality characteristics				
	Weight (g)	Width (mm)	Thickness (mm)	Length (mm)	Firmness (N)[a]
SL	9.21 b	20.49 b	22.60 b	21.70 b	45.73 b
SB	9.46 a	21.85 ab	24.35 a	22.96 a	48.00 a
VCL	9.05 b	23.02 a	20.60 c	21.54 b	47.53 a

SL Steep leader, *SB* Spanish bush, *VCL* Vogel central leader
[a]The scale ranges from 0 to 100 for very soft to very firm surfaces. $n = 180$ for the firmness (three replicate × three trees × twenty fruit). $n = 180$ for the weight and fruit sizes (three replicate × three trees × twenty fruit). Means in columns with the same letter do not differ according to Tukey's test at $P < 0.05$

Table 2 Effects of training system on color characteristics of "0900 Ziraat" sweet cherry fruit

Training System	Color characteristics			
	L*	a*	Chroma	Hue angle
SL	31.69 b	31.54 b	33.37 b	18.47 b
SB	31.99 b	32.50 ab	34.49 b	18.75 b
VCL	34.56 a	33.36 a	36.29 a	21.59 a

$n = 360$ for the color characteristics (three replicate × three trees × twenty fruit × two different measurements for each fruit). Means in columns with the same letter do not differ according to Tukey's test at $P < 0.05$

Results and Discussion

Weight, Size, Firmness and Color Characteristics

In fruit of sweet cherry trees, firmness, color and sizes are significant quality characteristics that affect consumer preferences and market value (Shomura et al. 2008; Zhang and Whiting 2011). These quality characteristics may vary depending on genetic (Gonçalves et al. 2005; Whiting et al. 2005; Sitarek and Bartosiewicz 2012), ecological factors and cultural practices (Serra et al. 2011).

Due to their effect on tree vigor, pruning and training are very effective treatments in terms of quality in sweet cherry. The training system applied in sweet cherry can affect quality characteristics such as fruit size, color and firmness (Peterson et al. 2003). In our study, it was determined that fruit size, color and firmness values differs from depending on the training system (Table 1). It was observed that the fruit of the SB training system was larger. Whiting et al. (2005) stated that the training system had an important effect on fruit quality, and that the fruit of the SB training system were 16% heavier than other training systems, and this may be due to the trees of SB system are the vigorous and have lower yields.

However, Blazkova et al. (2002) and Radunic et al. (2011) stated that there was no difference between the training systems in terms of fruit weight. In our study, there was no difference between the SB and VCL training systems in terms of firmness values. However, compared to the other training systems, the fruit firmnes in SL system was lower (Table 1). Similarly, Marini (2009) reported that the firmness values varied depending on the training system, whereas Ağlar et al. (2016) determined that the training systems have no effect on fruit firmness. a* color value represents red color. The increase in a* value in fruit indicates the increase in red color intensity. In the present study, it was observed that there were differences between the training systems in terms of red coloration, and a* value indicating red coloration was higher in VCL training system (Table 2). Similarly, Peterson et al. (2003), Cantin et al. (2010) and Ağlar et al. (2016) stated that the training system can be effective on fruit color.

Changes in fruit quality characteristics depending on the training system can be explained by the effect of pruning and training system on the tree vigor (Tareen and Tareen 2004; Sitarek and Bartosiewicz 2012), product load (Whiting et al. 2005; Blazkova et al. 2002) and light intensity on the tree (Robinson 1997; Hampson et al. 2002). Also, Bennewitz et al. (2011) reported that there was a decrease in yield per tree due to pruning and training applications, therefore, the competition between fruit for assimilation substances reduced, as a result the fruit quality as sizes and firmness increased, and there was a positive relation between the fruit quality and pruning severity.

SSC, Titratable Acidity and Vitamin C

In the study, it was determined that the biochemical properties such as SSC, acidity and vitamin C varied depending on the training system. The SSC and the titratable acidity content in the fruit of the SB and VCL were similar, but the highest values were obtained from the fruit of SL training system. Although there is no difference between SB and SL training systems in terms of vitamin C, the highest values were determined in the fruit of VCL training system (Table 3). Similarly, Veberic et al. (2005) reported that

Table 3 Effects of training system on SSC, titratable acidity and vitamin C of "0900 Ziraat" sweet cherry fruit

Training system	Biochemical characteristics		
	SSC (%)	Titratable acidity (g malic acid 100 g^{-1})	Vitamin C (mg 100 g^{-1} fw)
SL	11.87 b	0.44 b	6.53 a
SB	12.30 a	0.49 a	6.85 a
VCL	12.37 a	0.47 a	5.17 b

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

Table 4 Effects of training system on bioactive compounds of "0900 Ziraat" sweet cherry fruit

Training system	Bioactive compounds			
	Total phenolics (µg GAE g^{-1} fw)	Total flavonoids (µg QE g^{-1} fw)	DPPH (µmol TE g^{-1} fw)	FRAP (µmol TE g^{-1} fw)
SL	298.82 a	229.57 a	1.95 a	15.34 a
SB	245.78 b	152.95 b	1.76 b	9.46 b
VCL	254.62 b	134.28 b	1.65 b	9.50 b

$n = 27$ for the bioactive compounds (three replicate × three trees × three different measurements for each replicate). Means in columns with the same letter do not differ according to Tukey's test at $P < 0.05$

the chemical content of fruit may vary depending on the cultural practices such as pruning and training. However, Gonkiewicz (2011) reported that the pruning and training system had no effect on biochemical properties such as SSC and acidity. The sufficient light diffusion on the tree canopy is highly significant to obtain quality fruit (Jackson 1980; Robinson et al. 1983). Heinicke (1964) emphasized that tree size and tree structure were the most significant factors affecting the light intensity of the tree. In the present study, the differences occurred to depending on training systems in terms of biochemical content can be explained by the differences in photosynthesis and transpiration rate as a result of the effect of the training system on tree size, light intake and distribution (Robinson 1997; Hampson et al. 2002; Ferree and Warrington 2003).

Bioactive Compounds

Polyphenol compounds such as phenolic compounds, anthocyanins and flavonoids as secondary metabolites in fruit, due to their positive effects on human health (Ferretti et al. 2010) and fruit quality (color, flavor and taste) (Tomas-Barberan and Espín 2001), are significant for consumers and researchers. However, these compounds are the subject of curiosity in their growers due to the effects of polyphenols on plant resistance to pathogens (Ruhmann et al. 2002). The concentration of these compounds varies depending on the genetic and ecological factors, the rootstock and cultivar and cultural practices such as irrigation, fertilization and pruning (Facteau et al. 1996; Serra et al. 2011; Ozturk et al. 2017; Aglar and Saracoglu 2018). As a matter of fact, in our study, it was determined that there were differences between the bioactive compound values of the fruit depending on the training system. In particular, the fruit of SL had higher total flavonoids, total phenolics and antioxidant activity (both DPPH and FRAP assay) than those of the trees trained by SB and VCL (Table 4). However, there was no difference in bioactive compounds between SB and VCL. It was determined that there were differences between the training systems in terms of individual phenolic compounds. It was seen that in the sweet cherry fruit, the major phenolic acid was catechin. It was observed that the fruit of the SL training system had a higher content in terms of individual phenolic compounds. Especially, catechin, rutin, caffeic acid, 4-hydroxybenzoic acid, 4-aminobenzoic acid and transferulic acid content in the fruit of the SL system were significantly higher than those of SB and VCL. In this study, *p*-coumaric acid in SB and VCL training system; and transferulic acid in SB training system could not be determined (Table 5). Previous research revealed that there were differences in the yield and fruit quality of pomegranate (Gill et al. 2011), cherry (Lauri and Claverie 2005; Musacchi et al. 2015), apple (Ozkan et al. 2012; Dallabetta et al. 2014), peach and nectar (Zec et al. 2016) depending on the training system. Again, in the previous research (Usenik et al. 2008; Jakobek et al. 2009), it has been determined that the tree vigor has a significant effect on content and concentrations of the biochemical compounds of sweet cherry. In addition, Reynolds and Heuvel (2009) suggested that the training system applied in grapes may change the sensory properties and biochemical composition in the fruit.

Conclusion

As a result, it can be concluded that the training system had an important effects on fruit quality and bioactive compounds in sweet cherry fruit. The SL training system had

Table 5 Effects of training system on individual phenolics compounds of "0900 Ziraat" sweet cherry fruit

Individual phenolics	Training system		
Compounds (mg kg^{-1} fw)	SL	SB	VCL
Catechin	1244.5 a	823.7 b	741.0 b
Chlorogenic acid	30.90 a	31.37 a	30.97 a
Rutin	23.07 a	13.09 b	9.88 b
Caffeic acid	5.70 a	3.13 b	2.71 b
Protocatechuic acid	3.08 a	2.89 b	3.05 a
4-hydroxybenzoic acid	4.04 a	3.14 b	3.20 b
4-aminobenzoic acid	1.10 a	0.93 b	0.79 c
p-coumaric acid	2.43	Nd	Nd
Transferulic acid	4.60 a	Nd	1.85 b

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

higher values in terms of both fruit quality characteristics and bioactive compounds than SB and VCL training system. Therefore, it can be stated that SL training system is the most suitable training system for "0900 Ziraat" sweet cherry cultivar.

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

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