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Full Length Article

Determination of Organics, Phenolics, Sugars and Vitamin C Contents of some Cherry Cultivars (*Prunus avium*)

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ABSTRACT

Organic acid, phenolic compound, sugar and vitamin C (L-ascorbic acid) contents of the standard Turkish cherry cultivar "0900 Ziraat" as well as the local cherry cultivars of "Beyrudi", "kısa sap" and "uzun sap" fruits were determined. Concerning organic acid contents in the examined cultivars, malic acid content was identified to be higher (13,914 g/kg in "kısa sap" local cultivar) than other organic acids. Gallic acid content was identified to be higher than the contents of other phenolic compounds and the highest amount was detected in "0900 Ziraat" cultivar (95,512 mg/100 g). Among the sugar types of fructose, glucose and sucrose, glucose was identified to be the dominant sugar in the examined cultivars and the highest amount was detected in local cultivar (215,907 g/kg). In terms of vitamin C content, the highest amount was detected in "kısa sap" local cultivar (11.448 mg/100 g). İn addition, it was found statistically important differences among organic acid contents, phenolic contents, sugar and vitamin C in cherry cultivars. © 2012 Friends Science Publishers

Key Words: Cherry; Organic acids; Phenolic compounds; Sugars; Vitamin C

INTRODUCTION

Cherry (*Prunus avium*) belongs to *Rosales* order, *Rosasea* family, *Prunoidea* subfamily and *Prunus* genus. This fruit, which can conveniently be grown in mild climate zones, is widely used in a variety of areas such as fruit juice industry, table consumption, cake and jam production. Hence, it has a high market value and demand potential (Özçağıran *et al.*, 2003).

The fruit has an ancient history and the motherland is South Caucasia, Caspian Sea and North-East Anatolia. Cherry has a wide geographical distribution in the world, which extended from the mentioned gene centers to east and west. Wild cherry trees grow in great abundance in North Anatolia Mountains, Taurus Mountains and East Taurus Mountains (Özbek, 1978). Annual world cherry production is 2,150,107 tonnes in which Turkey ranks first with an annual cherry production of 417,694 tonnes followed by USA (390,000 tonnes) and Italy (125,900 tonnes), respectively (Anonymous, 2011).

Its appetitive appearance and high value in human nutrition due to its content of organic acids, phenolic compounds and vitamins enhance the importance of this fruit. Furthermore, research findings indicate the positive impacts of the fruit on human health due to the antioxidant, antiallergic, anticancergenic and antimicrobial effects of its phenolic compound content (Murakami *et al.*, 2000; Yoshimura *et al.*, 2003; Dae-Ok *et al.*, 2005). While the organic acid content of the cherry fruits vary depending on the cultivar, their amount has also a determinant role on the taste of the fruit by affecting acid-sugar equilibrium. In fruits and vegetables, organic acids are mostly reported to exist free or in the form of other compounds such as salt, ester and glycoside (Cemeroğlu *et al.*, 1986).

The "0900 Ziraat" utilized in this study is a standard Turkish cultivar widely grown in our country and has superior fruit quality. "Beyrudi", "kısa sap" and "uzun sap" local cultivars, on the other hand, are local cultivars widely grown in Mardin region, which have high importance in local economy. The determination of organic acid, phenolic compound, sugar and vitamin C contents in the standard cultivar "0900 Ziraat" and local cultivars, "Beyrudi", "Kısa sap" and "Uzun sap" cultivars and the determination of the biochemical characteristics of the local cultivars by comparison with the standard cultivar will contribute to the improvement of these local cultivars for their utilization as standard cultivars in the forthcoming studies as well as to the conservation of genetic sources and biological diversity.

MATERIALS AND METHODS

Plant material: This study was performed in the experimental area of Mardin province from South-East of

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Turkey. The fruit samples of the examined cultivars were taken from 0900 Ziraat cultivar grafted onto seedling rootstock (*Prunus avium* L.) rootstock, planted at 6 x 5 m spacing and local cultivars grafted onto seedling rootstock, planted at 5 x 4 m spacing. 2 kg of fruit samples were collected homogeneously from the standard cultivar "0900 Ziraat" and local cultivars, "Beyrudi", "Kısa ap" and "Uzun sap" when they were fully mature. The samples were stored for a short duration at -20°C and analyses were immediately started against the risk of decay and loss of Vitamin C content.

Analysis of organic acids: The standards used in organic acid analyses (succinic acid, malic acid, oxalic acid, fumaric acid & citric acid) were obtained from Sigma company (St. Louis, MO, USA) and c*hromatographically pure* H_2SO_4 was supplied from Merck company (Darmstadt, Germany). Milli-Q (Bedford, MA, USA) was employed for the preparation of standards and samples.

The method of Bevilacqua and Califano (1989) was modified and used in the extraction of organic acids. İn briefly; 5 g obtained cherry fruits were taken in centrifuge tubes. The samples were added 20 ml 0.009 N H_2SO_4 and homogenized (Heidolph Silent Crusher M, Almanya). Afterwards, the samples were blended by a shaker (Heidolph Unimax 1010, Germany) for 1 h and were centrifuged for 15 min at 15000 rpm. The supernatant was then filtered first through a filter paper, then filtered twice through a 0.45 µm membrane filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA) and lastly passed through SEP-PAK C_{18} cartridge.

Organic acids were subjected to analysis in HPLC instrument based on the method of Bevilacqua and Califano (1989) (Agilent HPLC 1100 series G 1322 A, Germany). Aminex HPX - 87 H, 300 mm x 7.8 mm column (Bio-Rad Laboratories, Richmond, CA, USA) was used in HPLC system and the instrument was controlled by a PC with Agilent software. The DAD detector in the system (Agilent, USA) was set at wavelengths of 214 and 280 nm. 0.009 N H_2SO_4 filtered through a 0.45 µm membrane filter was employed for the mobile phase.

Analysis of phenolic compounds: The phenolic compounds of gallic acid, protocatechuic acid, catechin, chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, 0-coumaric acid, phloridzin, vanillic acid, rutin, syringic acid and quercetin were determined.

Phenolic compounds were separated by HPLC based on the method of Rodriguez-Delgado *et al*. (2001). Firstly 5 g cherry fruit was homogenized. Second the obtained cherry juice were diluted with 1:1 distilled water and centrifuged for 15 min at 15000 rpm. The supernatant was then filtered through 0.45 µm Millipore filters and injected into HPLC instrument. Chromatographic separation was performed in Agilent 1100 (Agilent, USA) HPLC system by using DAD detector (Agilent. USA) and 250*4.6 mm, 4 µm ODS column (HiChrom, USA). For mobile phase, Dissolvent A Methanol-acetic acid-water (10:2:88) and Dissolvent B Methanol-acetic acid-water (90:2:8) were employed for mobile phase and gradient elusion program was applied as presented in Table I. Separation was achieved at 254 and 280 nm; flow rate and injection volume were determined as 1 mL/min. and 20 µL, respectively.

Analysis of sugars: The analyses were based on the modified method of Melgarejo *et al*. (2000). Fructose, glucose, sucrose and maltose standards in the fruits were used for the analyses of sugar. İn analyze 5 g cherry fruit was homogenized, then was centrifuged for 2 min at 12000 rpm and passed through SEP-PAK C_{18} cartridge. The supernatant was stored at -20°C until analysis. The sugar levels in the filtered sample were quantitated by µbondapak-NH_2 column with the help of 85% acetonitrile liquid mobile phase in HPLC instrument with refractive index detector. The concentrations were calculated based on the externally provided standards.

Analysis of vitamin C: 5 g cherry fruit was sampled, the sample was taken in a test tube and 5 mL 2.5% M-phosphoric acid solution was added. The mixture was centrifuged at + 4 °C 6500 x g for 10 min. 0.5 mL of clear supernatant was taken from centrifuge tube and supplemented with 2.5% M-phosphoric acid solution to obtain a 10 mL mixture. This mixture was filtered through a teflon filter and injected into HPLC instrument. HPLC analyses of vitamin C were performed in C_{18} column (Phenomenex Luna C_{18}, 250 x 4.60 mm, 5 µ). Column oven temperature was set at 25ºC. For the mobile phase, ultra distilled water with a set pH of 2.2 by H_2SO_4 was used at 1 mL/min. flow rate. Quantitations were performed at DAD detector in a wavelength of 254 nm. For the identification of peak and amount of vitamin C, different concentrations (50, 100, 500, 1000, 2000 ppm) of L–ascorbic acid (Sigma A5960) were employed (Cemeroğlu, 2007).

Statistical analysis: SPSS 11.0 (www.spss.com) statistical package software was used in the analysis of the data set. One-way analysis of variance was applied to the data and "Duncan's Multiple-Range Test" was used for the comparison of averages.

RESULTS AND DISCUSSION

Organic acids: The oxalic acid, citric acid, malic acid, succinic acid and fumaric acid contents of the standard cultivar "0900 Ziraat" and local cultivars, "Beyrudi", "kısa sap" and "uzun sap" were determined in the study. The findings indicated that oxalic acid content ranged between 0.06-0.33 g/kg, citric acid content between 0.69-3.08 g/kg, malic acid content between 9.52-13.91 g/kg, succinic acid content between 3.69-7.89 g/kg and fumaric acid content between 1.82-3.63 mg/kg (Tables II & III). Considering the organic acid contents of the examined cultivars, the content of malic acid was identified to be

Table I: Gradient elusion program

Time (min)	Dissolvent A(%)	Dissolvent B (%)
0	100	0
15	85	15
25	50	50
35	15	85
45	0	100

Table II: Organic acid contents of cherry cultivars

Cultivars	Oxalic acid (g/kg)	Citric acid (g/kg)
0900 Ziraat	0.08±0.05 c*	1.72±0.03 b
Beyrudi	0.17±0.01 b	3.08±0.11 a
Kısa sap	0.06±0.01 c	1.16±0.02 c
Uzun Sap	0.33±0.06 a	0.69±0.06 d

Table III: Organic acid contents of cherry cultivars

Cultivars	Malic acid (g/kg)	Succinic acid (g/kg)	Fumaric acid (mg/kg)
0900 Ziraat	10.92 ± 0.05 c*	3.69 ± 0.16 c	2.51 ± 0.05 b
Beyrudi	11.10 ± 0.05 b	6.43 ± 0.09 b	3.63 ± 0.06 a
Kısa sap	13.91 ± 0.02 a	6.16 ± 0.02 b	2.04 ± 0.03 c
Uzun Sap	9.52 ± 0.02 d	7.89 ± 0.33 a	1.82 ± 0.01 d

Table IV: Phenolic contents of cherry cultivars (mg/100 g)

Cultivars	Gallic acid	Catechin	Chlorogenic acid	Caffeic acid
0900 Ziraat	95.51±0.49 a*	2.42±0.45 b	1.14±0.20 bc	29.02±0.23 a
Beyrudi	67.64 ±0.99 b	1.76±0.18 b	1.50±0.01 ab	9.73±0.20 c
Kısa sap	50.33 ±0.74 c	1.87±0.35 b	0.98±0.04 c	12.40±0.39 b
Uzun Sap	42.33 ±0.60 c	5.02±0.41 a	1.59±0.08 a	11.51±0.31 b

*: Different letters in columns indicate significantly different values at $p \leq 0.05$

higher than the contents of the other organic acids and malic acid was detected as the dominant organic acid of all cultivars. On the other hand, the content of fumaric acid was identified to be lower than the contents of the other organic acids. In this study conducted in Mardin region, statistically important differences were identified among the organic acid contents of the cultivars and these differences are possibly attributed to cultivar specific characteristics. Usenik *et al.* (2008) examined 13 cherry cultivars and reported that their malic acid contents ranged between 3.53-8.12 g/kg, citric acid contents between 0.11-0.54 g/kg and fumaric acid contents between 0.97-7.56 mg/kg. The same researchers identified that malic acid was the dominant organic acid of all examined cherry cultivars. Similar findings were obtained by other researchers (Girard & Kopp, 1998; Usenik *et al*., 2010; Serradilla *et al.,* 2011) and the findings of our study are identified to be in agreement with the findings of these researchers.

Phenolic compounds: Considering the phenolic compound contents of the cherry cultivars grown in Mardin region, it was identified that gallic acid content ranged between 42.33-95.51 mg/100 g, catechin content between 1.76-5.02 mg/kg, chlorogenic acid content between 1.59-0.98 mg/100 g, caffeic acid content between 9.73-29.02 mg/100 g, cyringic acid content between 4.54-11.72 mg/100 g, p-coumaric acid content between 2.37-11.77 mg/100 g, ferulic acid content between 13.42-20.96 mg/100 g, o-coumaric acid content between 10.42-21.69 mg/100 g, phloridzin content between 4.55-10.45 mg/100 g, protocatechuic acid content between 1.26-5.32 mg/100 g, vanillic acid content between 17.06-32.49 mg/100 g, rutin content between 5.45-26.76 mg/100 g and quercetin content between 8.46-14.32 mg/100 g. Gallic acid content of cultivars was identified to be higher than the content of other phenolic compounds. Statistically important differences were recorded among the phenolic compound contents of cultivars (Tables IV, V & VI). In the study of Usenik *et al.* (2008), chlorogenic acid content of different cherry cultivars ranged between 0.60-2.61 mg/100 g and rutin content ranged between 2.06-5.78 mg/100 g. In another study conducted on different cherry cultivars, catechin content was found to range between 2.7-8.6 mg/100 g, chlorogenic acid content between 1.9-8.9 mg/100 g, rutin content between 7.8-34.2 mg/100 g and quercetin content between 0.6-5.3 mg/100 g (Serra *et al*., 2011). Similar findings were obtained in different studies on phenolic compound content of cherry fruit (Mattila *et al*., 2006; Jakobek *et al*., 2009; Gonzalez-Gomez *et al*., 2010; Usenik *et al*., 2010; Serradilla *et al.,* 2011).

Sugars and Vitamin C: With respect to the sugar contents of the examined cherry cultivars, glucose content (119.84-215.90 g/kg) was found to be higher than the contents of fructose (25.24-61.44 g/kg) and sucrose (1.73-7.60 g/kg). the dominant sugar was found glucose and the high glucose was found kısa sap (215.90 g/kg) local cultivars. Vitamin C content of the cultivars ranged between 6.01-11.448 mg/100 g (Table VII). Local cultivars were identified to have higher glucose contents compared to that of "0900 Ziraat" standard cherry cultivar. Vitamin C content was the highest in the local cultivar "kısa sap" compared to those of other cultivars. Statistically important differences were recorded among the sugar contents of the cultivars. In the study of Usenik *et al.* (2008), glucose content of different cherry cultivars was found to range between 61.8-123 g/kg, fructose content between 47.6-101.5 g/kg and sucrose content between 3.57-12.5 g/kg. In the study of Girard and Kopp (1998) on different cherry cultivars, glucose content ranged between 5.5-8.8 g/100 g, fructose content between 4.4-6.4 g/100 g and ascorbic acid content between 8.4-17.6 mg/100 g. The findings of our study conducted in Mardin region are generally in agreement with the findings of the other researchers and are within the limit values. However, some values were recorded to be higher, while some were lower. These differences are possibly attributed to the specific characteristics of the cultivars utilized in the study.

In the recent studies conducted on the phenolic compound and organic acid contents of the cherry cultivars, this fruit is reported to have a significant role in

Table V: Phenolic contents of cherry cultivars

Cultivars	Phloridzin(mg/100 g)	Protocatechuic acid (mg/100 g)	Vanillic (mg/ 100 g)	Rutin (mg/100 g)	Quercetin (mg/100 g)
0900 Ziraat	10.45± 0.08 a*	1.26± 0.25 c	20.33± 0.21 c	5.45± 0.30 c	8.46± 0.45 c
Beyrudi	8.65± 0.03 b	3.53± 0.20 b	32.49± 0.67 a	6.33± 0.16 c	14.32± 0.28 a
Kısa sap	9.21± 0.08 b	3.99± 0.11 b	17.06± 0.12 d	26.76± 0.32 a	10.90± 0.14 b
Uzun Sap	4.55± 0.26 c	5.32± 0.25 a	28.85± 0.19 b	11.42± 0.25 b	8.58± 0.21 c

Table VI: Phenolic contents of cherry cultivars

Cultivars	Ferulic acid (mg/100 g)	*o*-Coumaric acid (mg/100 g)	Syringic acid (mg/100 g)	*p*-Qoumaric acid (mg/100 g)
0900 Ziraat	20.9 ± 0.07 a*	12.16 ± 0.03 c	11.72 ± 0.61 a	11.77 ± 0.09 a
Beyrudi	15.24 ± 0.08 b	21.69 ± 0.15 a	8.45 ± 0.19 ab	2.37 ± 0.36 d
Kısa sap	13.42 ± 0.21 c	15.54 ± 0.27 b	8.83 ± 0.60 ab	7.25 ± 0.21 b
Uzun Sap	13.85 ± 0.09 c	10.42 ± 0.28 d	4.54 ± 0.69 b	5.68 ± 0.11 c

Table VII: Sugar and vitamin C content of cherry cultivars

Cultivars	Vitamin C (mg/100 g)	Fructose (g/kg)	Glucose (g/kg)	Sucrose (g/kg)
0900 Ziraat	6.94 ±0.06 c*	50.47 ±0.21 c	119.84 ±0.35 d	7.60 ±0.04 a
Beyrudi	6.01 ±0.02 d	61.44 ±0.11 a	151.91 ±0.10 c	3.17 ±0.04 c
Kısa sap	11.44±0.11 a	25.24 ±0.16 d	215.90 ±0.95 a	1.73 ±0.01 d
Uzun Sap	10.87 ±0.08 b	58.70 ±0.26 b	173.99 ±0.54 b	4.29 ±0.05 b

*: Different letters in columns indicate significantly different values at $p \leq 0.05$

human health and nutrition (Kim *et al.*, 2005). Organic acids, while being effective in numerous physiological functions (taste formation, maturation etc.) in the fruits, are also highly important for human health (Cemeroğlu & Acar, 1986). Phenolic compounds, on the other hand, are also effective in numerous physiological functions in the fruits. For instance, they have a determinant role in taste formation, particularly resulting in sour taste in mouth. Furthermore, some phenolic compounds are highly important due to their antioxidant properties.

CONCLUSION

Concentrations of Biochemical characteristic (phenolic, organic acid, sugar & vitamin C) are known to be strongly influenced by some factors such as cultivars, genotypes and rootstocks (Goncales & Ark, 2006). Our data show that there are important differences between genotypes. Organic acid, Vitamin C and Sugar (expect Sucrose) contents were found high in local cultivars than 0900 Ziraat cultivars. The Phenolic component was found irregular between cultivars.

The results of this study could be significant for determination of biochemical characteristic of cherry cultivars and reference for forthcoming studies.

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