Original article

Fruit quality and biochemical characteristics of some early ripening mandarin varieties

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Summary

Fruit quality and biochemical parameters, such as total phenolic content, sugars, organic acids, volatile compounds and antioxidant capacity, of six early ripening mandarin varieties ('L.A. Early', 'Okitsuwase', 'Primasol', 'Mihowase', 'Dobashi Beni' and 'Early St. Ann') were determined under Cukurova ecological conditions, Turkey. Three organic acids (citric acid, L-ascorbic and succinic acids) and three sugars (sucrose, glucose and fructose) were determined using high-performance liquid chromatography (HPLC). According to the results, citric acid was detected as the main organic acid. Sucrose was the major sugar. The highest sucrose level was found in 'Primasol' (4.54%) and the lowest in 'Okitsuwase' (3.65%). Similarly, a significant amount of L-ascorbic acid was detected in all varieties studied. Citrus juices are abundant in ascorbic acid content, which is an important antioxidant. For the total phenolic content, the amounts varied between 74.68 mg gallic acid equivalents per 100 gram (mg GAE 100 g^{-1}) for 'Okitsuwase' and 83.18 mg GAE 100 g^{-1} for 'Dobashi Beni'. Antioxidant activity was measured using the DPPH* assay and ranged from 68.51 to 91.01% in experimental mandarin juices.

Keywords
biochemical compounds, citrus, early ripening, fruit quality, mandarin

Significance of this study

What is already known on this subject?

- Mandarins remain the most consumed and demanded *Citrus* species due to some important advantages, such as smaller fruit, thinner skins, and easy peeling. *Citrus* species are important for the human diet because of their high nutrient content. They are also an important source of sugars and organic acids. In addition, citrus fruits and juices have important bioactive components, such as ascorbic acid, flavonoids, and phenolic compounds that are important for human nutrition. However, most of the information available on the quality characteristics and biochemical components of citrus fruit is from research focused on oranges and grapefruits considering juice industry.

What are the new findings?

- We characterized biochemical properties; such as the antioxidant capacity, the total phenolic compounds, organic acids, sugar contents, and aroma in the fruit juice of the early-ripening mandarin varieties which are economically important in citrus industry in terms of earliness.

What is the expected impact on horticulture?

- The consumers of citrus fruits and their derivatives demand high sensory, , and health-related qualities in recent years, increasing the value of citrus fruits for human health. The results of the present study suggest producers to choose the right variety for early citrus production considering fruit quality.

Introduction

Turkey is among the top-three citrus producing countries in the Mediterranean Basin and is ranked ninth in the world, accounting for approximately 3.2% of the 152 million tons of citrus produced globally in 2018 (FAO, 2020). The total citrus production of Turkey was 4,902,052 t in 2018. Mandarins are among the most popular citrus fruits in Turkey, accounting for approximately 33% of the total production (1,650,000 t) (FAO, 2020). Mandarins and their hybrids are currently the preferred citrus crop for new plantations. Although all citrus production is increasing, the proportion of mandarins is increasing more rapidly. Sixty-three percent of the mandarins are Satsuma cultivars, such as 'Owari', 'Okitsu Wase', 'Mihowase' and 'Dobashi Beni', and the remaining plantings include 'Clementine', 'Nova', 'Robinson', 'Fremont', 'Minneola', 'W. Murcott', 'Tango', 'Ortanique', and 'Primasol' (Yesiloglu *et al.*, 2017).

Currently, mandarins remain the most consumed and demanded *Citrus* species due to some important advantages, such as smaller fruit, thinner skins and easy peeling. The Satsuma mandarin is an important product in both domestic and foreign markets due to its early ripening and high quality during the early period. At present, early-season mandarin varieties are very popular in Turkey and in some Western markets because of their high quality, easy peeling, seedlessness, special taste, and being the first citrus of the season.

Citrus spp. has great importance among fruit species because of their high usage in both the fresh and processed markets (Canan *et al.*, 2016). Consumers now demand highly nutritional and healthy fruits and their products (Sdiri et al., 2012). Citrus fruit and its products beneficially affect human health because their consumption is associated with several benefits, such as the regulation of cardiovascular disease and the reduced risk of certain cancers (Cano and Bermejo, 2011). Citrus fruits have delicious flavors and antioxidant capacity, in addition to their health benefits (Legua *et al.*, 2014).

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Citrus fruits are classified as acid fruits, because their soluble solids mainly consist of organic acid and sugars. Organic acids in citrus juice (citric, malic, and succinic acid) are important components because their concentration largely affects the taste characteristics and organoleptic quality (Rouseff and Martin, 1985; Ribeiro and Ribeiro, 2008). *Citrus* species are important for the human diet because of their high nutrient content. They are also an important source of sugars and organic acids. In addition, citrus fruits and juices have important bioactive components, such as ascorbic acid, flavonoids, and phenolic compounds that are important for human nutrition (Fernandez-Lopez *et al*., 2005; Ghasemi *et al*., 2009). Phenolic compounds can be found nearly in all types of fruit and vegetable species at different concentrations. Citrus fruits also have high levels of phenolic compounds with potential health-promoting properties (Sdiri *et al*., 2012). However, most of the information available on the quality characteristics and biochemical components of citrus fruit is from research focused on oranges and grapefruits. There are a limited number of previously reported papers on the nutritional values and biochemical components of mandarin varieties, to our knowledge. Previous studies revealed that some biochemical characteristics of the mandarins may vary depending on the variety used. For instance, Kelebek and Selli (2014) evaluated phenolic compositions and antioxidant capacities of mandarin juices in Turkey and reported that the phenolic compounds ranged from 36.6 to 132.6 mg L^{-1} among the investigated mandarin varieties. Similarly, Cimen (2019) evaluated various clementine mandarin varieties in terms of fruit quality traits and biochemical components under Adana ecological conditions. Researcher reported that ascorbic acid content, the total phenolic compounds, and antioxidant activities of the clementine mandarins significantly varied among varieties.

Therefore, this study was conducted in order to determine the fruit quality and fruit biochemical characteristics, such as antioxidant capacity, the total phenolic compounds, organic acids, sugar contents and aroma profiles in the fruit juice of the early-ripening mandarin varieties, including 'L.A. Early', 'Okitsuwase', 'Mihowase', 'Dobashi Beni', 'Early St. Ann', and 'Primasol', grown in Turkey.

Materials and methods

Plant material

Fruits of 'Okitsuwase' (*Citrus unshiu* Marc.), 'Mihowase' (*Citrus unshiu* Marc.), 'L.A. Early' (*Citrus unshiu* Marc.), 'Dobashi Beni' (*Citrus unshiu* Marc.), 'Early St. Ann' (*Citrus unshiu* Marc.), and 'Primasol' (*Citrus reticulata* Blanco) mandarin were used as material. Fruit samples were collected from 8-year-old trees located on the production lands of the Incesulu Company (lat. 36°48'39.96"N; long. 35°12'33.69"E; alt. 7 m). The mandarin varieties were harvested at optimum maturity and randomly selected (30 fruits for each cultivar) from nine trees. The fruit weight (g), height (mm), diameter (mm), fruit shape index (fruit height/diameter ratio), rind thickness (mm), juice content (%), total soluble solids (%), titratable acidity (%), and ripening index (RI) were determined. The yield per tree (kg $tree^{-1}$) was obtained by weighing the harvested fruit. Mature fruits of six mandarin varieties randomly selected (30 fruits for each cultivar) were immediately transferred to the physiology laboratory for quality analysis at the Department of Horticulture, Faculty of Agriculture, University of Cukurova.

Fruit external and internal characteristics

Fruits were randomly selected from nine trees and were weighed to determine the average fruit size. The fruit was sized at the equatorial diameter and height with a digital caliper (CD-15CPX, Mitutoyo, Japan). The fruits were cut in half and the thickness of the rind was measured with a digital caliper. Using a standard juicer, 30 fruits were weighed and juiced. The juice was weighed and expressed as a percentage of the total fruit weight. The total soluble solids (TSS) content was determined with a portable refractometer (FG-103/113, Soif Ltd., China) using a few drops of juice. The total acidity (TA) of the juice was determined by titration with 0.1 N sodium hydroxide (NaOH), using phenolphthalein as the indicator. The ripening indexes (RI) of mandarin varieties were calculated and presented as TSS/TA ratio (Iglesias *et al*., 2007).

Preparation of juice sample

Representative samples (30 fruits per cultivar) at the maturity stage were collected from three trees per cultivars, and 30 homogeneous fruits (based on color, size, and absence of defects) were selected for each variety for analytical determinations. The selected fruits were squeezed with a kitchen juicer. The juice from the mandarin varieties was centrifuged at 4,000 rpm for 20 min (Eppendorf 3810 R; Hamburg, Germany) and then filtered through 0.45 µm pore size membrane filters and stored at -18 °C until HPLC analysis. All reagents and solvents were purchased from Sigma Chemical Co. (St. Louis, MO).

Extraction of sugars and acids

For sugars and acids, the same samples were used, triplicate analysis were applied and for each replicate, approximately 500 mL of fruit juice was used. Ethanolic (80%, v/v) extraction was performed according to the Miron and Schaffer (1991) HPLC technique. For the organic acids, meta-phosphoric acid (3%) was used with a method developed by Bozan *et al*. (1997).

Liquid chromatographic analysis of sugars and organic acids

The sugars and organic acids were identified and quantified by high-performance liquid chromatography (LC-10A, Shimadzu, Japan) consisting of a RID and UV detector. Individual sugars were detected using a Nucleosil NH_2 analytical column (150 mm × 4.6 mm *i.d.*, 5 µm) (Shimadzu, Japan) at room temperature with a flow rate of 1 mL min^{-1}, using 75% aqueous acetonitrile. In order to determine the organic acids, a 250 mm × 4.6 mm *i.d.*, 5 µm, reverse-phase Ultrasphere ODS analytical column (Beckman), operating at room temperature with a flow rate of 1 mL min^{-1}, was used with 0.5% aqueous meta-phosphoric acid as the eluent.

Analysis of total phenols

The total phenolic content of the fruit juices was determined using Folin–Ciocalteu reagent with the modified method of Spanos and Wrolstad (1990). Total phenols were calculated as gallic acid equivalents (GAE). The data are presented as the average of triplicate analyses.

Total antioxidant activity

The total antioxidant activity of the mandarin juices was calculated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging method reported by Brand Williams *et al*. (1995) with modifications (Sanchez-Moreno *et* *al.*, 1998). The absorbance was measured at 515 nm with a Multiskan GO microplate spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA).

Analysis of volatiles

The analysis of volatiles was performed using fruit juice treated with 5 M $CaCl_2$. Volatile compounds were analyzed using HS/GC/MS apparatus (Clarus 600, PerkinElmer, USA) equipped with an HP-5 MS (30 m × 0.25 mm × 0.25 µm) fused-silica capillary column. Helium (1 mL min^{-1}) was used as a carrier gas. The GC conditions were as follows: injector temperature, 280 °C; splitless, interface temperature, 300 °C; carrier gas flow, 0.6 mL min^{-1}; initial column heating program of 40 °C for 2 min, then increased to 250 °C at a rate of 5 °C min^{-1} and remaining at this temperature for 20 min for column purge. The extraction temperature was 40 °C at 20 min for headspace.

Identification of phenolic compounds

Approximately 500 mL samples were used for each replicate for the extraction of phenolics, and three replicates were used separately. Fruit juice samples were treated with liquid nitrogen in a mortar. Three mL of this homogenate were collected, and 10 mL of acetone/water (1:4, v/v) mixture and 0.1 mL of trifluoroacetic acid were added into the flask and refluxed for 1 h. Then, the mixture was filtered and injected to the HPLC. The liquid chromatographic apparatus (Hewlett-Packard HP1100) consisted of an in-line degasser, pump, and controller coupled to a photodiode array detector equipped with an automatic injector (20 µL injection volume) interfaced to a PC running ChemStation chromatography manager software (Hewlett-Packard). Separations were performed on a 150 mm × 4.6 mm *i.d.*, 5 µm, reverse-phase Nucleosil C18 analytical column (Supelco, PA) with a flow rate of 1 mL min^{-1}. Detection was carried out with a sensitivity of 0.1 a.u.f.s. between the wavelengths of 200 and 600 nm. Elution was performed using a solvent mixture of 2.5% HCOOH in water (solvent A) and 2.5% HCOOH in acetonitrile (solvent B). The composition of B was increased from 5 to 13% in 15 min, increased to 15% in 5 min and to 30% in an additional 5 min and held for 3 min, increased to 45% in 4 min and held for 3 min, increased to 90% in 5 min and held for 5 min, and then returned to the initial conditions for 5 min. Components were identified and quantified by comparison of their retention times to those of authentic standards.

Statistical analyses

The data obtained in this study were subjected to an analysis of variance (ANOVA) to determine significant differences. Means were separated using LSD test at $P \leq 0.05$ and $P \leq 0.01$. All statistical analyses were performed by using SAS v 9.00 statistics.

Results and discussion

Fruit yield per tree (kg tree^{-1})

The fruit yield per tree significantly differed among the varieties investigated. The highest fruit yield was recorded for the 'Okitsu' variety, whereas it was lowest in 'L.A. Early' and 'Early St. Ann' mandarin varieties (Table 1). Previous researchers reported that the 'Okitsu' Satsuma mandarin is a selection of Satsuma with a good yield and quality for the fresh market, with outstanding features, such as early ripening and seedless characteristics (Cantuarias-Aviles *et al.*, 2010; Tazima *et al.*, 2013).

Fruit external and internal quality

Significant differences were found between mandarin varieties in terms of fruit weight, fruit height and diameter, total acid (TA) and ripening index (RI) but not for the fruit shape index, rind thickness, TSS, or fruit juice content (Table 1). The highest fruit weight, fruit height and fruit diameter were observed in the 'Primasol' mandarin variety, whereas the fruit weight and diameter were lowest in 'Early St. Ann' mandarin variety (Table 1). The results obtained in the present study are consistent with those of Goldenberg *et al.* (2018), who demonstrated that Satsumas are regarded as medium-small to medium. TSS varied from 8.30% to 9.07%, and the juice content ranged from 43.37% to 50.97% (Table 1). Total acid values ranged from 1.16% to 2.19%. 'Okitsu', 'L.A. Early' and 'Early St. Ann' mandarin varieties had the lowest total acid contents, whereas 'Primasol' and 'Dobashi Beni' had the highest contents. The fruit ripening index is a widely used indicator to determine the citrus fruit maturity level. High values of the RI (TSS:TA) ratio were confirmed in fruits of 'L.A. Early', 'Okitsu', and 'Early St. Ann', whereas 'Primasol' and 'Dobashi Beni' had the lowest values. Fruit internal and external properties are the most important quality parameters and are affected by many factors, such as genetic variability, climate and environment, and rootstock. Internal quality and taste of fruits are primarily feature of sugars and acids during fruit maturation. The results obtained in the present study are consistent with those described in previous reports (Ebel *et al.*, 2004; Tazima *et al.*, 2013).

Table 1. External and internal fruit quality and fruit yield of the mandarin varieties.

Variety	Fruit weight (g)	Fruit height (mm)	Fruit diameter (mm)	TSS (%)	TA (%)	RI (TSS/TA)	Juice content (%)	Fruit yield (kg tree^{-1})
Dobashi Beni	130.33 bc	56.50 b	65.90 bc	8.67	2.07 a	4.20 bc	50.97	160 c
L.A. Early	132.00 bc	55.88 b	64.94 bc	9.00	1.21 c	7.64 a	48.44	120 d
Mihowase	140.96 b	58.24 ab	67.76 b	9.07	1.70 b	5.36 b	44.64	170 b
Okitsuwase	127.80 bc	60.58 a	63.26 cd	8.30	1.16 c	7.24 a	45.70	190 a
Primasol	171.66 a	62.00 a	71.76 a	8.50	2.19 a	3.88 c	43.37	180 ab
Early St. Ann	114.13 c	56.15 b	61.49 d	8.50	1.25 c	6.83 a	47.35	130 d
Prob > f	*0.0020*	*0.0256*	*0.0001*	*0.0859*	*0.0001*	*0.0002*	*0.1201*	*0.0001*
$LSD_{0.05}$	*21.699*	*4.0001*	*3.018*	*NS*	*0.286*	*1.419*	*NS*	*10.072*

Means with different letters for each parameter in a column are significantly different at $P<0.05$ by LSD test.
NS: not significant.

TABLE 2. Sugar compositions (%) of the mandarin juices.

Varieties	Sucrose	Glucose	Fructose	Total sugars
L.A. Early	4.23±0.06 b	3.08±0.29 cd	3.35±0.18 ab	10.66±0.52 bc
Okitsuwase	3.65±0.05 c	3.35±0.01 abc	3.54±0.01 a	10.54±0.05 c
Primasol	4.54±0.16 a	2.83±0.23 d	2.89±0.21 c	10.25±0.58 c
Mihowase	4.14±0.27 b	3.20±0.11 bc	3.28±0.08 b	10.62±0.22 bc
Dobashi Beni	4.02±0.11 b	3.57±0.18 a	3.59±0.14 a	11.18±0.25 ab
Early St. Ann	4.27±0.13 b	3.43±0.04 ab	3.60±0.08 a	11.29±0.15 a
Prob > F	*0.0003*	*0.0033*	*0.0003*	*0.0269*
$LSD_{0.05}$	*0.267*	*0.316*	*0.247*	*0.628*

Means with different letters for each parameter in a column are significantly different at $P<0.05$ by LSD test.

Sugar and organic acid composition of mandarin juices

Results of the sugar composition of mandarin juices are presented in Table 2. The levels of mean fructose, glucose, sucrose and total sugars in the mandarin varieties were significantly different ($p<0.05$). The total amounts of sugar were 10.66%, 10.54%, 10.25%, 10.62%, 11.18% and 11.29% in the 'L.A. Early', 'Okitsuwase', 'Primasol', 'Mihowase', 'Dobashi Beni', and 'Early St. Ann', respectively. According to the literature, sucrose is the main sugar in nearly all mandarin varieties (Lee and Coates, 2000; Roussos, 2011; Sdiri *et al.*, 2012). Sucrose (3.65–4.54%) levels were higher than fructose (2.89–3.60%) and glucose (2.83–3.57) levels. Yun *et al.* (2010) reported that the glucose, sucrose, and fructose concentrations were 44.3, 38.4, and 40.5 mg g^{-1}, respectively. In our study, the highest sucrose level was found in 'Primasol' juices (4.54%) and the lowest was in 'Okitsuwase' juices (3.65%). Fructose and glucose were the highest in 'Dobashi Beni' and 'Early St. Ann' fruit samples, whereas they were the lowest in 'Primasol'. The sugar profile of mandarin juice is an important component of the chemical composition and is suggested as an indicator for determining the authenticity of juice samples (Lee and Coates, 2000; Castellari *et al.*, 2009).

The organic acid composition of fruits is interesting because the flavor of the fruit flesh is highly dependent on the balance between organic acids and soluble sugars (Karadeniz, 2004). The organic acid profile was determined, and citric, L-ascorbic acid and succinic acid were quantified in mandarin juices (Table 3). The differences among varieties for succinic acid levels were significant ($p<0.05$). The organic acid concentration may vary depending on the origin, climate, rootstock, variety, and degree of maturity (Marsh *et al.*, 2003). The major organic acid detected in mandarin variety samples was citric acid. Citric acid was detected at concentrations between 2.06 and 1.40% ('Dobashi Beni' and 'L.A. Early', respectively). Additionally, succinic acid was also detected in the present study, and the amount ranged from 0.26 to 0.14% ('L.A. Early' and 'Primasol', respectively). The results of the present study are similar to the citric acid levels previously reported in citrus fruits (Legua *et al.*, 2014; Sdiri *et al.*, 2012; Kelebek and Selli, 2011). In our study, the highest level of L-ascorbic acid was detected in 'Early St. Ann' (49.58 mg 100 g^{-1}), and the lowest was in 'Dobashi Beni' Satsuma juices (41.75 mg 100 g^{-1}). Sdiri *et al.* (2012) reported 21.19–29.80 mg 100 mL^{-1} ascorbic acid in mandarin varieties, slightly lower than our present results. These slightly lower amounts may be related to the varieties, rootstocks, environmental conditions or analytical methods were used. However, the results obtained in the present study are similar to the L-ascorbic acid levels previously reported in citrus (Fattahi *et al.*, 2011; Kelebek and Selli, 2011; Legua *et al.*, 2014).

Total phenolic contents of mandarin juices

The total amount of phenolic compounds in the mandarin juices was detected in this study (Table 4). The differences among mandarin varieties for total phenolic contents was not statistically significant in the one-way ANOVA. The amount of the total phenolic content ranged between 74.68 mg GAE 100 mL^{-1} for 'Okitsuwase' and 83.18 mg GAE 100 mL^{-1} for 'Dobashi Beni' (Table 3). The highest total phenolics among the six mandarin varieties was found in 'Dobashi Beni' samples, whereas 'Okitsuwase' had the lowest. Gil-Izquierdo *et al.* (2001) reported that navel orange juice contains 839 mg of phenolic per liter. Another study reported that there was 54.12–73.88 mg 100 mL^{-1} total phenolic content in mandarin varieties, which is slightly lower than our present results (Sdiri *et al.*, 2012).

TABLE 3. Organic acid compositions of the mandarin juices.

Varieties	Citric acid (%)	L-ascorbic acid (mg 100 mL^{-1})	Succinic acid (%)
L.A. Early	1.40±0.03	44.67±1.77	0.26±0.04 a
Okitsuwase	1.72±0.35	44.08±7.65	0.24±0.07 a
Primasol	1.69±0.27	44.58±5.02	0.14±0.02 b
Mihowase	1.94±0.22	45.25±2.00	0.25±0.02 a
Dobashi Beni	2.06±0.28	41.75±2.13	0.23±0.01 a
Early St. Ann	1.48±0.21	49.58±4.63	0.25±0.03 a
Prob > F	*0.0510*	*0.8024*	*0.0239*
$LSD_{0.05}$	*NS*	*NS*	*0.061*

Means with different letters for each parameter in a column are significantly different at $P<0.05$ by LSD test.
NS: not significant.

Table 4. Total phenolic content (mg GAE 100 mL^{-1}) and antioxidant activity (%DPPH) of the mandarin juices.

Variety	Total phenolic content (mg GAE 100 mL^{-1})	DPPH (%)
L.A. Early	80.68±0.85	91.01±0.97
Okitsuwase	74.68±6.57	77.61±8.71
Primasol	80.57±12.29	77.05±4.65
Mihowase	78.52±3.52	86.13±5.36
Dobashi Beni	83.18±4.21	84.53±1.01
Early St. Ann	75.44±3.62	68.51±6.34
Prob > F	*0.5594*	*0.0030*
$LSD_{0.05}$	*NS*	*9.432*

Means with different letters for each parameter in a column are significantly different at $P<0.05$ by LSD test.
NS: not significant.

Total antioxidant activity of mandarin juices

The DPPH free radical-scavenging procedure for determining antioxidant activities was used in several studies (Sanchez-Moreno *et al*., 1998; Klimczak *et al*., 2007). It is a simple, rapid, and stable assay for detecting the antioxidant activity in citrus juice samples. The antioxidant activity of mandarin juices is presented in Table 4. The antioxidant activity ranged from 68.51 to 91.01% for the mandarin juices. 'L.A. Early' showed higher antioxidant activity than the other mandarin varieties, and the lowest activity was in 'Early St. Ann' juices. Xu *et al*. (2008) stated that the DPPH content in a 'Satsuma' cultivar was 133.65%. In the study of Ghasemi *et al*. (2009), DPPH (radical scavenging activity) content in Clementine fruit was reported as 3.2 mg mL^{-1} and in Washington navel (tissues) as 2.8 mg mL^{-1}. Other studies also showed that the DPPH content ranged from 12.61 to 45.28 µmol TE 100 g^{-1} in *Citrus* species and mandarin hybrids (Canan *et al*., 2016). Kelebek and Selli (2014) indicated that the 'Satsuma' mandarin showed higher antioxidant activity compared to the 'Fremont' and 'Robinson' varieties. Our results are consistent with the results of these researchers. These differences could be attributed to cultivar-specific characteristics. Additionally, the stage of citrus fruit ripening may have effects on the bioactivity component and antioxidant capacity. Fattahi *et al*. (2011) reported that the different effects of ripening stage played an important role on the antioxidant capacity of citrus fruits.

Aroma-volatile contents of mandarin juices

The volatile composition of mandarin variety juices is shown in Table 5. A total of 24 volatile compounds were identified in mandarin variety juices, including 15 terpenes, 5 alcohols, 3 aldehydes and 1 acid. There were differences between the mandarin varieties in terms of total volatile

Table 5. Aroma-volatile composition of the mandarin juices.

Compound name	Mihowase	Primasol	Okitsuwase	L.A. Early	Early St. Ann	Dobashi Beni
Terpenes						
α-terpinene	3.21	2.55	1.42	1.78	2.45	8.01
β-myrcene	0.03	-	-	0.04	-	8.33
a-pinene	0.42	3.06	0.35	0.13	0.58	1.11
a-thujene	0.52	0.31	0.27	0.67	0.51	2.75
β-ocimene	0.11	0.14	0.19	0.28	0.15	0.34
Limonene	87.92	87.78	90.2	90.24	89.06	73.69
α-gurjunene	0.12	-	0.07	0.08	0.07	0.34
Elemene	0.11	0.05	-	-	0.06	0.89
a-cubebene	0.03	-	-	-	-	0.25
Coapene	0.09	0.05	0.05	0.06	0.05	0.12
Caryophyllene	0.39	-	0.16	0.12	0.21	0.38
Patchoulene	0.17	0.11	0.24	0.17	0.18	-
a-murolene	0.1	-	0.14	0.12	0.09	-
a-cadiene	0.2	0.10	0.14	0.07	0.12	0.76
a-calacorene	0.03	-	-	-	-	-
Total terpenes	93.45	94.15	93.23	93.76	93.53	96.97
Alcohols						
1-octanol	0.24	0.59	0.75	0.64	0.73	-
Linalool	0.04	-	-	0.06	0.09	0.21
o-cumenol	0.30	0.1	0.23	0.5	-	0.73
Terpineol	3.70	1.09	1.56	1.7	2.37	0.85
3-nonen-1-ol	0.05	0.05	-	0.06	-	-
Total alcohols	4.33	1.83	2.54	2.96	3.19	1.79
Aldehydes						
Capraldehyde	0.21	0.47	0.15	0.13	0.13	0.82
Nonadienal	0.09	-	0.07	0.05	0.05	0.09
Benyldehyde	1.89	3.55	3.93	3.08	3.09	-
Total aldehydes	2.19	4.02	4.15	3.26	3.27	0.91
Acetic acid	0.03	-	0.06	0.06	0.04	0.26

Table 6. Phenolic compounds of the mandarin juices detected by HPLC (mg 100 mL^{-1}).

Compounds	Mihowase	Primasol	Okitsuwase	L.A. Early	Early St. Ann	Dobashi Beni
Hydroxycinnamic acids						
Caffeic acid	0.62	0.72	0.69	0.60	0.22	0.55
p-coumaric acid	10.03	54.13	9.70	12.20	13.89	14.16
Ferulic acid	0.78	1.18	0.53	0.82	0.58	0.82
Flavonol						
Kaempferol	3.33	1.35	2.08	3.41	2.27	4.05
Myricetin	34.45	34.00	38.79	6.03	7.67	36.94
Quercetin	1.08	1.45	2.25	60.44	32.62	53.27

levels. Terpenes qualitatively represent the main group of the volatile component and comprised 93.45% of the total volatile compounds in mandarin juices. The most abundant terpenoids were detected as limonene (ranging from 73.69 to 90.24%, according to the variety) and α-terpinene (ranging from 1.42 to 8.01%, according to the variety) (Table 5). The results obtained in the present study are consistent with previous studies, that reported that the first components are a group of mostly terpenes in pomelo, mandarin and orange (Gonzalez-Mas *et al*., 2011), and tangerine hybrids (Miyazaki *et al*., 2010). Some other terpenes, such as a-pinene, a-thujene, a-cadiene, caryophyllene, β-ocimene, elemene, and β-myrcene were also detected. Such compounds were previously identified as volatile compounds of lemon and other citrus varieties (Lota *et al*., 2002; Qiao *et al*., 2008). Other terpenes (α-gurjunene, a-cubebene, coapene, patchoulene, a-murolene, and a-calacorene) were detected in small quantities in fruit juice. Alcohols were identified as one of the most important volatile components in citrus. Five alcohols (terpineol, o-cumenol, l-octanol, 3-nonen-1-ol, and linalool) were found in the fruit juice. The most pronounced alcohol was terpineol, which is a terpene alcohol, followed by o-cumenol and l-octanol. Similarly, alcohols play an important role in some *Citrus* species' flavor, including mandarin (Goldenberg *et al*., 2016), tangerine hybrids (Miyazaki *et al*., 2010), and different *Citrus* species (Gonzalez-Mas *et al*., 2011). Only a few volatile substances were identified in other chemical classes, such as aldehydes and acids, and no esters of aroma compounds were found in the mandarin varieties. The results obtained in the present study are consistent with previous studies that reported that satsumas and clementines had lower proportions of aldehyde volatiles and no esters (Goldenberg *et al*., 2016).

Phenolic compounds composition of mandarin juices

A total of six individual phenolic compounds were identified and quantified in mandarin juices, including hydroxycinnamic acids (3) and flavonol (3) (Table 6). Three hydroxybenzoic acids, caffeic, p-coumaric and ferulic acid, were identified in mandarin juice. P-coumaric acid was the most dominant extractable phenolic acid, followed by ferulic and caffeic acid. The amount of p-coumaric acid ranged from 9.70 mg 100 mL^{-1} in 'Okitsu' to 54.13 mg 100 mL^{-1} in 'Primasol'. The concentrations of ferulic acid ranged from 0.53 mg 100 mL^{-1} in 'Okitsu' to 1.18 mg 100 mL^{-1} in 'Primasol'. The amount of caffeic acid ranged from 0.22 mg 100 mL^{-1} in 'Early St. Ann' to 0.72 mg 100 mL^{-1} in 'Primasol'. The hydroxycinnamic acids obtained are similar to previous studies (Kelebek and Selli, 2014; Zhang *et al*., 2014), but the amounts and order of hydroxycinnamic acid obtained in our study was different as follows: p-coumaric acid>ferulic acid>caffeic acid. These differences may be attributed to the genetic backgrounds of the *Citrus* species, agricultural practices, and/or environmental factors. Three flavonol were identified, including myricetin, quercetin and kaempferol (Table 6). The contents of myricetin ranged from 6.03 mg 100 mL^{-1} in 'L.A. Early' to 38.79 mg 100 mL^{-1} in 'Okitsu'. Quercetin levels were from 1.08 mg 100 mL^{-1} in 'Mihowase' to 60.44 mg 100 mL^{-1} in 'L.A. Early'. The total amount of kaempferol ranged from 1.35 mg 100 mL^{-1} ('Primasol') to 4.05 mg 100 mL^{-1} ('Dobashi Beni'). Sdiri *et al*. (2012) reported that there was a small amount of quercetin flavonol (0.15–0.66 mg 100 mL^{-1}) in mandarin juices. Similarly, a small amount of quercetin and kaempfenol flavonol was reported in Chinese wild mandarin (Zhang *et al*., 2014), and these three flavonols were also reported in *Citrus hystrix* (Butryee *et al*., 2009). The variability in the content of flavonols may be genetic differences, ecological conditions, soil, rootstocks, and cultural treatments.

Conclusion

In the present study, we evaluated newly-introduced early season mandarin varieties ('Primasol', 'L.A. Early', and 'Early St. Ann') together with the most commonly produced mandarin varieties for early season markets ('Okitsuwase', 'Mihowase', and 'Dobashi Beni') in terms of fruit quality and biochemical properties. As a result, 'Okitsuwase' and 'Primasol' were found to be the most productive mandarins in terms of fruit yield, whereas 'Primasol' mandarin had stand out especially for the fruit size (both fruit weight and diameter). Considering all evaluated varieties, citric acid and sucrose were determined as the main organic acid and sugar, respectively. This study revealed differences significant between fruit quality of six different early ripening mandarin varieties. Thus, growers who want to produce for early-season market will benefit from these results in terms of fruit quality. Besides, knowing biochemical characteristics of the species will facilitate the work of breeders and citrus producers.

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