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Determination of antioxidant, total phenolic, total carotenoid, lycopene, ascorbic acid, and sugar contents of *Citrus* species and mandarin hybrids

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Abstract: This study aimed to determine the antioxidant capacity; the total phenolic, total carotenoid, sugar, and ascorbic acid contents; and some pomological characteristics of *Citrus* species (*Citrus reticulata*, *Citrus sinensis*, and *Citrus paradisi*) and mandarin (*Citrus reticulata*) and their new hybrids developed for yield at the Alata Horticultural Research Institute in Mersin, Turkey. With respect to antioxidant capacities, the highest value (45.28 µmol TE/100 g) was determined in Cocktail (*Citrus paradisi*) cultivar fruits among all examined cultivars and hybrids. We found that 7-19 (Clementine × Kara) mandarin hybrids had the highest total phenolic content (386.81 mg gallic acid equivalent/g) and the highest total carotenoid content (39.03 mg/kg). Considering the sugar contents of mandarin fruits, sucrose was identified to be the predominant sugar and the highest value (8.80 g/100 g) was identified in the Clementine cultivar. It was determined that the *Citrus* species and mandarin hybrids had lycopene contents ranging from 6.52 to 1.68 mg/kg. Overall, this study provides supporting evidence for the superiority of Cocktail fruit as an excellent source of antioxidant capacities.

Key words: Antioxidant, biochemical, *Citrus*, fruit

1. Introduction

Citrus fruits have important potential among fruit species because they are commonly used in both the fresh and processing markets. *Citrus* fruits have been known to be good sources of antioxidant species. For many years, the nutritional relevance of these foods resided almost exclusively in the fact that they were acknowledged as a good source of ascorbic acid and carotenoids (Stuetz et al., 2010). Some researchers have focused on the quantification of phenolic compounds and the antioxidant capacity of citrus fruits such as limes, grapefruits, sweet oranges, lemons, and tangerines (Kelebek et al., 2008; Ozgen et al., 2009; Abad-Garcia et al., 2012; Goulas and Manganaris, 2012; Zhang et al., 2014). *Citrus* fruits and juices are an important source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids, phenolic compounds, and pectins that are important to human nutrition (Ebrahimzadeh et al., 2004; Fernandez-Lopez et al., 2005; Jayaprakasha and Patil, 2007; Ghasemi et al., 2009). Furthermore, other compounds, such as the limonoids (triterpene derivatives), some flavones such as sinensetin and nobiletin, and phenylpropanoids such as the hydroxycinnamates, have high antioxidant potential and health-promoting capacities (Kaur and Kapoor, 2001). Additionally, catalyzing effects of polyphenol oxidase (PPO) enzymes cause browning reactions in fruits and vegetables. Phenolic compounds also result in blurring and sedimentation in drinks such as fruit juices and wines. Phenolic compounds are present in almost all fruits and vegetables at varying levels. Enzymatic browning does not occur in intact plant cells since phenolic compounds in cell vacuoles are separated from the PPO enzyme in the cytoplasm. Once tissues are damaged by slicing, cutting, or pulping, brown pigments are generated due to the reaction of phenolic compounds and PPO enzyme. For example, some fruits and vegetables such as apples, bananas, and potatoes immediately turn brown after slicing (Cemeroğlu et al., 2004; Gundogdu et al., 2011).

Previous studies indicated that phytochemical profiles and antiradical scavenging activity may significantly vary among *Citrus* species, among cultivars within the same species, and even within the same cultivar grown in diverse

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climatic conditions or under different cultural practices (Green et al., 2007; Cano et al., 2008; Jayaprakasha et al., 2008; Xu et al., 2008; Goulas and Manganaris, 2012). Recent studies have demonstrated the important role of citrus fruits in human health and nutrition. Accordingly, citrus fruits are becoming more popular and their consumption has been increasing recently. The *Citrus* species used in this study are abundantly grown in Turkey. The study aimed to determine the antioxidant capacity; the total phenolic, total carotenoid, sugar, and ascorbic acid contents; and some pomological characteristics of *Citrus* species and mandarins and their new hybrids developed for yield in the Mersin region of Turkey. These parameters are important for the determination of quality of citrus fruits. The study is important for exploring biochemical properties in mandarin hybrids, about which only limited research is available.

2. Materials and methods

Robinson (*Citrus reticulata*), Clementine (*Citrus reticulata*), Cocktail (*Citrus paradisi*), Valencia (*Citrus sinensis*), Kara (*Citrus reticulata*), 10-18 hybrid (Clementine × Kara), 39-9 hybrid (Clementine × Cocktail), 38-13 hybrid (Clementine × Cocktail), 20-2 hybrid (Clementine × Valencia), and 7-19 hybrid (Clementine × Kara) fruits were used in the study, which were grown at the Alata Horticultural Research Institute (Mersin Province). About 30 fruits were homogeneously collected from each selected mandarin tree during the harvest period of November and December. The samples were placed in cloth bags and then transferred to the laboratory for analyses. Immediately after picking, fruits were stored at 80 °C for subsequent analysis. Total soluble solid (TSS) content was determined with a digital refractometer (Atago, model ATC-1E, Kyoto, Japan). The total acidity (TA) was measured by titration with 0.1 N NaOH. The fruit color was measured using a Minolta portable chroma meter (Minolta, model CR-400, Tokyo, Japan), which provided CIE L^*, a^*, and b^* values. Chroma and hue° values were calculated from these values. For each fruit sample, three replicates were thawed at room temperature, seeds were removed and homogenized in a standard food blender, and excess fruits (20–30 individual fruits) were used to minimize naturally occurring fruit-to-fruit variations. Slurries were assayed for TA using standard methodology.

Total phenolic (TP) content was measured according to the procedure of Singleton and Rossi (1965). Briefly, fruit slurries were extracted with buffer containing acetone, water, and acetic acid (70:29.5:0.5 v/v) for 2 h in darkness. Samples were replicated three times. Extracts were combined with Folin–Ciocalteu phenol reagent and water and incubated for 8 min, followed by the addition of 7% sodium carbonate. After 2 h, the absorbance at 750 nm was measured with an automated UV-Vis spectrophotometer (PG Instruments, model T60U, Lutterworth, UK). Gallic acid was used as the standard.

Sugars were extracted following a modified version of the method described by Bartolome et al. (1995). Samples were homogenized in a mixer and then vortexed at 4000 rpm for 10 min. Exactly 5 g of sample was diluted with deionized distilled water to a total volume of 10 m:. After vortexing for 1 min, 2 mL of sample was injected directly into the HPLC instrument after filtration through a 0.45-µm filter (Millipore, Bedford, MA, USA). HPLC analysis of sugars was performed on LC-20A equipment consisting of LC-20AD pumps, an inline degasser, a CTO-20A column oven, an SCL-10A system controller, and a refractive index detector, operated by LC solution software (Shimadzu, Kyoto, Japan). Sugars were separated on an INTERSIL NH2 column (5.0 µm, 4.6 × 250 mm) (GL Sciences, Tokyo, Japan) at 30 °C. The mobile phase was acetonitrile:water (80: 20, v/v) at a flow rate of 1.3 mL min^{-1}. The quantification was performed according to external standard solution calibrations. The results were expressed as g 100 g^{-1} fresh weight.

The antioxidant activity of the cultivars was evaluated by DPPH free radical-scavenging method. Samples of 5 g of 5 mL of 80% methanol solution were taken and the mixture was stirred sufficiently and then vortexed 4 °C at 4000 rpm for 20 min in a Hettich Mikro 220R centrifuge (Tuttlingen, German). A sample of 100 µL was centrifuged with 2460 µL of juice and 1.1-diphenyl-2-picrylhydrazyl (DPPH*, 80% methanol, 0.025 g/L) was added. Distilled water (100 µL) was used as the control sample. The absorbance of the samples was measured as the time to loss of 80% methanol for 0, 20, 30, 45, and 60 min. A spectrophotometer set at 515 nm measured the values (BioTek PowerWave, Winooski, VT, USA) and the measurement data set at 5 min were used (Klimczak et al. 2007).

Pigment extraction from juices and saponification procedures were carried out according to the previously reported method of Meléndez-Martínez et al. (2007). HPLC analyses were carried out with a Shimadzu LC-20AD system, consisting of a quaternary pump, a column temperature control oven (CTO-10AS), an autosampler unit (SIL-20A), a degasser module (DGU-20A5), and a UV detector (SPD-20A). Supernatant (50 µL) was injected into the INTERSIL ODS-2 column (5.0 µm, 4.6 × 250 mm). The column was kept at 20 °C and the flow rate was 1 mL/min. The UV detector was set to 450 nm. Methanol (A), methyl-tert-butyl ether (B), and ultrapure water (C) were used as mobile phases. According to the preliminary experiments, the best gradient elution determined was as follows: 0 min: 90% A + 5% B + 5% C; 5 min: 95% A + 5% B; 40 min: 75% A + 25% B; 55 min: 55% A + 45% B; 60 min: 90% A + 5% B + 5% C; 65 min: 90% A + 5% B +

5% C; 65% solvent A plus 30% solvent B and 5% solvent C plus 30 min, gradient switched to 25% solvent A, 75% solvent B; final gradient conditions were 20 min gradient of 60% solvent A, 35% solvent B, 55% solvent C, then held for 10 min. The mobile phases were returned to initial conditions over 5 min. Injection volumes of 50 µL were used for samples and standards.

Ascorbic acid determination was performed using the HPLC method and extraction procedure according to Lee and Coates (1999). The HPLC column was maintained at 25 °C and the flow rate was 0.5 mL/min. Supernatant (10 µL) was injected into the INTERSIL ODS-3 column (5.0 µm, 4.6 × 250 mm). The photodiode array detector was set at 244 nm, and 2% KH_2PO_4 (pH 2.4) was used as the mobile phase.

Total carotenoid and carotenoid components were extracted following a modified version of the method described by Meléndez-Martínez et al. (2007). Samples were homogenized using a mixer, and 5 g of puree was weighed into centrifuge tube and extracted with HPLC grade solvents of 25 mL of extraction solution (hexane/acetone/methanol, 50/25/25, with 0.1% butylated hydroxytoluene). The mixture were mixed and then centrifuged for 10 min at 4000 rpm and 4 °C. The supernatant phase was used for absorbance measured at 450 nm by spectrophotometer (BioTek PowerWave). Total carotenoids were calculated using the extinction coefficient of β-carotene (E1/2 = 2505).

3. Results and discussion

Citrus species and mandarins and their new hybrids were used in this study. The study aimed to determine antioxidant capacity; total carotenoid, lycopene, β-carotene, total phenolic, sugar, and ascorbic acid contents; and some pomological characteristics of *Citrus* species and mandarin hybrids developed at the Alata Horticultural Research Institute of General Directorate of Agricultural Research and Policies (Turkish acronym: TAGEM) in Mersin, Turkey. There were statistically significant differences among the *Citrus* species and mandarin hybrids in terms of biochemical and color values (Tables 1–3).

The antioxidant capacities of the examined cultivars and hybrids were determined by DPPH (1,1-diphenyl-2-picrylhydrazyl radical) method in our study. The findings indicated that the Cocktail cultivar and the 20-2 (Clementine × Valencia) hybrid have the highest antioxidant capacity (45.28–44.23 µmol TE/100 g) among the studied cultivars and hybrids. On the other hand, the 38-13 (Clementine × Cocktail) hybrid has the lowest antioxidant capacity (12.61 µmol TE/100 g). Except for the Robinson cultivar and 20-2 (Clementine × Valencia) hybrid, the antioxidant capacities of other standard cultivars were higher than those of the hybrids. In this study, total phenolic contents ranged between 200.60 (38-13) and 386.81 mg gallic acid equivalent/g (7-19), total carotenoid contents between 17.01 (39-9) and 39.03 mg/kg (7-19), β-carotene contents between 13.78 (39-9) and 32.01 mg/kg (7-19), and lycopene contents between 1.68 (Kara) and 6.52 mg/kg (10-18) (Table 1). Goulas and Manganaris (2012) explored the phytochemical content and antioxidant potential of citrus fruits (pulp) grown in Cyprus. In their study, total phenolics, total flavonoids, and β-carotene content of the Mandora cultivar (*Citrus reticulata* × *Citrus sinensis*) were reported as 57.7 mg/g,

Table 1. Antioxidant capacity, total phenolic, total carotenoid, β-carotene, and lycopene contents of examined *Citrus* species and mandarin hybrids (fresh weight).

Citrus species and mandarin hybrids	Antioxidant capacity (µmol TE/100 g)	Total phenolics (mg gallic acid equivalent/g)	Total carotenoids (mg/kg)	β-carotene (mg/kg)	Lycopene (mg/kg)
Robinson (*Citrus reticulata*)	20.45 ± 0.98 cd*	209.37 ± 1.37 d	26.67 ± 0.67 bc	22.67 ± 0.54 cd	4.19 ± 0.12 bc
Clementine (*Citrus reticulata*)	33.10 ± 0.68 ac	302.38 ± 0.91 bc	27.23 ± 0.12 bc	22.33 ± 0.13 cd	3.27 ± 0.20 cd
Cocktail (*Citrus paradisi*)	45.28 ± 0.76 a	214.88 ± 0.87 d	37.40 ± 0.33 a	31.79 ± 0.93 a	3.20 ± 0.07 cd
Valencia (*Citrus sinensis*)	40.32 ± 1.01 ab	270.56 ± 0.67 c	29.87 ± 0.98 b	25.89 ± 0.36 bc	2.09 ± 0.24 de
Kara (*Citrus reticulata*)	33.78 ± 0.51 ac	218.55 ± 1.10 d	36.92 ± 0.73 a	32.00 ± 0.94 a	1.68 ± 0.06 e
10-18 (Clementine × Kara)	24.99 ± 0.42 bd	339.50 ± 1.17 ab	34.90 ± 0.53 a	28.62 ± 0.70 ab	6.52 ± 0.19 a
39-9 (Clementine × Cocktail)	31.19 ± 0.46 ac	312.37 ± 0.78 bc	17.01± 0.11 d	13.78 ± 0.17 e	4.68 ± 0.37 b
38-13 (Clementine × Cocktail)	12.61 ± 0.24 d	200.60 ± 1.01 d	24.64 ± 0.18 c	19.71 ± 0.31 d	4.49 ± 0.13 b
20-2 (Clementine × Valencia)	44.23 ± 0.73 a	284.02 ± 0.74 c	38.35 ± 0.43 a	29.92 ± 0.61 a	2.93 ± 0.08 d
7-19 (Clementine × Kara)	33.10 ±0.37 ac	386.81 ± 0.93 a	39.03 ± 0.85 a	32.01 ± 0.42 a	2.38 ± 0.15 de

*There are significant ($P < 0.01$) differences between values with different letters in the same lines.

1.15 mg/g, and 94.2 µg/g, respectively. In another study, Xu et al. (2008) stated that total carotenoid, total phenolics, and DPPH contents in the Satsuma cultivar were 9.14 mg/L, 1109.23 mg/L, and 133.65%, respectively. In the study of Ghasemi et al. (2009), phenol, flavonoids, and DPPH (radical scavenging activity) content in Clementine fruits (tissues) was reported as 396.8 mg/g, 17.1 mg/g, and 3.2 mg/mL, respectively. The same researchers found phenol, flavonoids, and DPPH (radical scavenging activity) content in Washington Navel (tissues) to be 232.5 mg/g, 1.2 mg/g, and 2.8 mg/mL, respectively. Our findings in the present study are in line with the results of these mentioned researchers. Significant differences were determined among the cultivars and hybrids with respect to biochemical distribution. These differences could be attributed to cultivar-specific characteristics. Flavonol glycosides, which are one of the phenolic compounds, are light yellow in color and exist in almost all plants. As light is required for their synthesis in plants, they are more abundantly present in the skins of fruits. Since they affect color formation, climatic factors of temperature and light are particularly important determinants (Cemeroğlu et al., 2004). Additionally, phenolic compounds also generate a sour taste in fruit products and blurred appearance in fruit juices (Cemeroğlu et al., 2004). Hence, phenolic compounds are highly important in the fruit juice processing industry.

The glucose, fructose, sucrose, ascorbic acid, total acidity, and color (*L, a* and *b*) values and TSS contents of mandarin cultivars and hybrids were also investigated. There were statistically significant differences among cultivars and hybrids in terms of sugar, ascorbic acid, total acidity, color values, and TSS contents ($P < 0.05$) (Tables 2 and 3). The 10-18 (Clementine × Kara) mandarin hybrid had the highest fructose (4.90 g/100 g) and glucose (5.30 g/100 g) contents. The lowest fructose (1.62 g/100 g) and glucose (1.50 g/100 g) content was determined in the Valencia cultivar, and Clementine had the highest (8.80 sucrose content). Additionally, the Kara cultivar had the lowest sucrose (2.64 g/100 g) content. The Clementine cultivar had the highest ascorbic acid (656.43 mg/kg) and the 7-19 hybrid had the lowest ascorbic acid (244.41 mg/kg) content. Sucrose contents were generally found to be higher than glucose and fructose contents in this study. Soluble sugars and their metabolites are major contributors to fruit quality. Citrus fruit juice sacs obtain their sugar supply via the phloem and through nonvascular cell-to-cell apoplastic transport during fruit development. However, no sugar is transported into juice sacs after harvest, and the main sugar resource comes from either starch catabolism or gluconeogenesis. Sucrose is the major carbohydrate and photoassimilate stored in the fruit (Yun et al., 2010). In the same study, the average concentrations of fructose, glucose, and sucrose were determined as 40.5, 38.4, and 44.3 mg/g, respectively (Yun et al., 2010). In another study, fructose, glucose, and sucrose contents of Clementine (rootstock: Carizzo) fruit juice were determined as 18.5 g/L, 18.1 g/L, and 89.5 g/L, respectively (Navarro et al., 2010). Our findings in the present study are in line with the results of these mentioned researchers.

Soluble solid values ranged between 8.50% and 16.40%. The 38-13 mandarin hybrid had the highest soluble solid content while the Kara cultivar and 10-18 hybrid had the lowest. The accessions also varied in color measurements. The Cocktail cultivar had the highest L^* and b^* values while Kara had the lowest L^* and b^*. Additionally, the Robinson cultivar had the highest a^* and the 38-13 hybrid had the lowest a^*. In the literature, total acidity and TSS levels

Table 2. Sugars, ascorbic acid, and total acidity contents of examined *Citrus* species and mandarin hybrids (fresh weight).

Citrus species and mandarin hybrids	Fructose (g/100 g)	Glucose (g/100 g)	Sucrose (g/100 g)	Ascorbic acid (mg/kg)	Total acidity (%)
Robinson (*Citrus reticulata*)	3.42 ± 0.11 b*	3.54 ± 0.13 b	5.51 ± 0.34 bc	651.33 ± 0.93 a	1.03 ± 0.06 f
Clementine (*Citrus reticulata*)	2.49 ± 0.23 d	2.46 ± 0.17 c	8.80 ± 0.62 a	656.43 ± 1.03 a	1.18 ± 0.05 ce
Cocktail (*Citrus paradisi*)	2.30 ± 0.27 e	2.38 ± 0.20 c	4.57 ± 0.53 d	353.17 ± 0.77 d	1.28 ± 0.11 c
Valencia (*Citrus sinensis*)	1.62 ± 0.15 g	1.50 ± 0.06 e	3.51 ± 0.20 e	579.99 ± 1.10 b	1.13 ± 0.08 df
Kara (*Citrus reticulata*)	2.79 ± 0.13 c	2.29 ± 0.16 c	2.64 ± 0.16 f	305.85 ± 1.23 e	1.08 ± 0.13 ef
10-18 (Clementine × Kara)	4.90 ± 0.37 a	5.30 ± 0.41 a	6.00 ± 0.47 b	418.27 ± 0.90 c	0.85 ± 0.04 g
39-9 (Clementine × Cocktail)	1.74 ± 0.08 fg	1.72 ± 0.06 d	3.75 ± 0.05 e	323.96 ± 0.57 de	1.62 ± 0.16 b
38-13 (Clementine × Cocktail)	2.21 ± 0.23 e	2.25 ± 0.16 c	5.09 ± 0.14 c	361.98 ± 0.81 d	2.06 ± 0.20 a
20-2 (Clementine × Valencia)	1.83 ± 0.07 f	1.85 ± 0.10 d	5.34 ± 0.21 c	365.53 ± 0.74 d	1.22 ± 0.09 ce
7-19 (Clementine × Kara)	1.72 ± 0.07 fg	1.77 ± 0.16 d	5.95 ± 0.13 b	244.41 ± 0.50 f	1.27 ± 0.20 cd

*There are significant ($P < 0.01$) differences between values with different letters in the same lines.

Table 3. Color values and TSS contents of examined *Citrus* species and mandarin hybrids.

Citrus species and mandarin hybrids	*L**	*a**	*b**	TSS (%)
Robinson (*Citrus reticulata*)	65.76 ± 1.12 c*	35.04 ± 0.73 a	67.28 ± 1.51 a	11.60 ± 0.26 c
Clementine (*Citrus reticulata*)	65.44 ± 1.02 c	34.15 ± 0.46 a	64.25 ± 0.97 a	12.60 ± 0.49 b
Cocktail (*Citrus paradisi*)	76.02 ± 0.32 a	3.16 ± 0.11 c	75.98 ± 0.90 a	10.20 ±1.01 d
Valencia (*Citrus sinensis*)	65.86 ± 0.46 bc	24.49 ± 0.14 ab	39.94 ± 0.46 b	9.00 ± 0.36 e
Kara (*Citrus reticulata*)	64.14 ± 0.27 c	18.95 ± 0.42 b	38.80 ± 0.63 b	8.50 ± 0.30 e
10-18 (Clementine × Kara)	67.59 ± 0.64 ac	24.73 ± 0.61 ab	66.80 ± 1.21 a	8.50 ± 0.27 e
39-9 (Clementine × Cocktail)	67.38 ± 1.15 ac	25.65 ± 0.16 ab	69.22 ± 1.17 a	10.50 ± 0.38 d
38-13 (Clementine × Cocktail)	74.45 ± 1.13 ab	17.69 ± 0.18 b	75.96 ± 1.09 a	16.40 ± 0.19 a
20-2 (Clementine × Valencia)	68.37 ± 1.26 ac	33.46 ± 0.40 a	74.62 ± 1.57 a	11.90 ± 0.67 bc
7-19 (Clementine × Kara)	70.05 ± 0.93 ac	24.77 ± 0.20 ab	72.26 ± 1.98 a	10.20 ± 0.32 d

*There are significant ($P < 0.01$) differences between values with different letters in the same lines.

of Satsuma mandarin were given as 13.08% and 1.06%, respectively (Xu et al., 2008). Goulas and Manganaris (2012) determined the *L**, *a**, *b**, SSC, and total acidity of citrus fruits (*Citrus reticulata* × *Citrus sinensis*, cultivar Mandora) as 50.86, 26.34, 41.34, 10.5 °Brix, and 1.47 g/100 g, respectively. In the study by Stuetz et al. (2010), ascorbic acid content in mandarin juice (organic) was determined as 145.2 mg/L. Research has demonstrated that morphological and biochemical characteristics of fruits are affected by genetic factors, climatic factors, climate, and soil structure. While some findings of this study are in agreement with those of other researchers, some findings are in discord. This is attributed to the variability of citrus species and mandarin hybrids used in the studies as well as other environmental factors.

In this study, chemical compounds of the citrus species and mandarin hybrids were examined and nutritional values and importance of these cultivars and hybrids for human health were determined. The region of study is one of Turkey's most important regions in terms of production of citrus species. The demand for fruit species containing antioxidants and carotenoids has been increasing due to the identification of flavonoids having anticarcinogenic effects in studies in recent years. *Citrus reticulata* is also included among these fruit species. This increases the importance of *Citrus* fruits for human health. In the literature there are a limited number of studies on biochemical content in citrus fruits. The present study included citrus species and mandarin hybrids, and hence this study is considered to be a valuable reference for forthcoming studies on morphological and biochemical characteristics of mandarin hybrids to find the most favorable one to introduce into commercial production. The results suggest a high potential of health benefits. However, more detailed biological and pharmacological studies are needed for the demonstration and clarification of health benefits of *Citrus* species and mandarin hybrids fruits.

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