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DETERMINATION OF ORGANIC ACIDS, TOTAL PHENOLIC CONTENT, AND ANTIOXIDANT CAPACITY OF SOUR *Citrus aurantium* FRUITS

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Sour orange, known as bitter orange, is a well-known citrus rootstock, which is also used extensively in Turkey for citrus production [1]. Due to its sour and bitter taste, it has not been used as an edible fruit [2]. The juice of the fruit is used in salads for sour taste instead of lemon juice and the peel is used in jam production in some regions, of Turkey.

It is worthwhile referring to the recovery of hesperidin and naringin from orange peel [3], which is considered to be the most popular source: recovery of naringin from sour orange [4].

Much attention recently has been paid to the possible health benefits of dietary phenolics that have antioxidant activities stronger than that of vitamin C. However, information concerning the antioxidant capacities of sour orange peel and juice is not available. So, the purpose of this research is to determine the organic acids, total phenolic content, total flavonoid content, and antioxidant capacity of sour orange (peel and juice), which is grown in Turkey.

Some physicochemical properties of juice and peel are given in Table 1. Since the predominant acid was found as citric for juice, and oxalic for peel (HPLC assay), total titratable acidity (TTA) of samples were calculated on the basis of the predominant acid. Karadeniz [5] reported pH as 2.6, TTA as 4.9 g/100g, and Brix as 10.0 in sour orange juice, which is similar to our results.

4 organic acids in juice and 3 organic acids in peel were detected and quantified. The predominant organic acid was found as citric (97% of total acids) in juice and oxalic in peel (54 % of total acids), respectively. The other acids were oxalic, malic, and ascorbic in juice, and quinic and ascorbic in peel (Table 2). The recoveries of organic acids from the BondElut cartridges were within 87–105%. In the calculation of final results, recovery rates were not taken into consideration. The sum of all quantified acids by HPLC in juice and peel were 3.9 g/100 mL and 0.5 g/100 g, respectively. Citric and malic acids of sour orange juice from Antalya (Turkey) were reported by Karadeniz as 48.8 and 2.2 g/L, respectively [5]. Slight differences between the two studies are attributable to the fruit sources, ripeness, and analytical conditions used. The presence of ascorbic acid (only qualitative) in sour orange juice has been previously reported [6].

The total phenolic content, total flavonoid content, and EC_{50} values of the samples are given in Table 3. In the literature, there are no published results for sour orange, so the total phenolic content of sour orange peel and juice was compared with the total phenolic content of different citrus fruits analyzed with the same method.

Gorinstein et al. reported that peeled lemons, oranges, and grapefruit contain 164±10.3, 154±10.2, and 135±10.1 and their peels 190±10.6, 179±10.5, and 155±10.3 mg gallic acid equivalent/100 g of total polyphenols, respectively [7]. The total flavonoid content of grapefruit peels was found between the ranges 74.4–95.2 mg /100 g fresh weight [8]. In our study, both total phenolic content and total flavonoid content of sour orange peel were higher than literature values of different citrus fruit peel such as lemon, orange, and grapefruit.

It can be seen with increasing total phenolic content, the EC_{50} value decreases, so the necessary amount of sample needed to decrease the initial DPPH· concentration (EC_{50}) by 50% becomes lower [9, 10]. Another term for antioxidant capacity is antiradical efficiency (AE), which is widely used to compare the results. Higher AE means higher antioxidant activity. As can be seen in Table 3, the total flavonoid content of peel was approximately 50-fold higher than juice where the total phenolic content was 8-fold higher. Also the percentage of total flavonoid content to total phenolic content was higher in peel (~80 %) than juice (~14 %).

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TABLE 1. Physicochemical Properties of Sour Orange

Sour orange	pH	TTA[a] (g/100 g)	Brix	Total dry matter (g/100 g)
Juice	2.6	5.4	10.9	N.d.
Peel	5.2	0.7	N.d.	24.9

[a]TTA is given as citric acid equivalent for juice, oxalic acid equivalent for peel.
N.d.: not determined.

TABLE 2. Organic Acid Content of Sour Orange Samples

Sour orange	Organic acids[a]				
	Oxalic	Malic	Ascorbic	Citric	Quinic
Juice (mg/L)	89.5±2.4	384.6±12.9	312.2±19.7	39153.3±328.8	N.d.
Peel (mg/100 g)	257.5±25.3	N.d.	117.6±7.3	N.d.	98.5±10.4

[a]Results are expressed as mean and standard deviation of three determinations.
N.d.: not detected.

TABLE 3. Total Phenolic Content, Total Flavonoids Content, and EC_{50} Values of Sour Orange Samples

Analysis	Sour orange		Analysis	Sour orange	
	Juice	Peel		Juice	Peel
TPC[a]	56.9±2.4	487.1±5.1	Ae[d]	0.003	0.019
TFC[b]	7.7±0.8	387.4±6.9	Slope[e]	-0.116	-0.300
EC_{50}[c]	385.4±2.1	53.4±2.1	Correlation coefficient[f]	0.999	0.993

[a]Total phenolic content, mg GAE/100 mL for juice and mg GAE/100 g for peel.
[b]Total flavonoid content, mg CE/100 mL for juice and mg CE/100 g for peel.
[c]Efficient concentration (EC_{50}: mg sample/mg DPPH).
[d]Antiradical efficiency (AE: $1/EC_{50}$).
[e]Exponential regression, ln (DPPH %rem) = x (mg sample/mg DPPH)+y.
[f]Correlation coefficients of exponential regression.

It is known that sour orange peel is used as a pharmaceutical supplement because of its health benefits. Due to the high amount of bioactive components in sour orange peel, it can be a very cheap raw material for the production of functional foods as an additive by application of applicable processes or preperation methods. Due to the high citric acid content, sour orange juice can be industrially exploited as an alternative source for citric acid production. It is important to increase its acceptability in food products despite its sour and bitter taste. Further research should be carried out to produce new food products containing sour orange peel or juice and to study the changes of the biological active compounds during food processing applications.

Samples and Reagents. Sour orange (*Citrus aurantium* L.) fruit samples were grown in Aydýn city located in the western part of Turkey in 2005 during the December season and stored at 4°C until analysis (maximum 2 days). The fruit samples were cut into two halves and the juice was collected by a domestic squeezer. The peels of the squeezed fruit samples were separated manually. The analysis of the peel and juice were performed using freshly prepared samples.

1,1-Diphenyl-2-picrylhydrazyl (DPPH·), Folin & Ciocalteau's phenol reagent, catechin hydrate, gallic, oxalic, tartaric, malic, malonic, fumaric, ascorbic, citric, and quinic acid standards were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Analytical grade methanol were obtained from Riedel-de Haen (Seelze, Germany). All other reagents and solvents commercially obtained were of analytical grade.

Determination of pH, Total Titratable Acidity, Brix, and Total Dry Matter. pH, total titratable acidity, and Brix parameters were determined according to the conventional methods [11]. Total Dry Matter content of samples was analyzed by using a vacuum oven at 70°C [12].

Analysis of Organic Acids. Extraction of organic acids was carried out with meta-phosphoric acid (3%) at room temperature for 30 min using a shaker [13]. Extracts were analyzed according to the standard HPLC method [12]. HPLC analysis was conducted with a Hewlett-Packard 1050 series pumping system and a Waters 486 UV-detector. Reversed phase separations were carried out using a 250 × 4.6 mm i.d., 5 μm Hichrom C18 column.

Determination of Total Phenolic Content, Total Flavonoid Content, and Antioxidant Capacity. Total phenolic content [14], total flavonoid content [15], and antioxidant capacity [16] of samples were determined spectrophotometrically using a Cary 50 UV-vis. spectrophotometer.

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