Chemistry of Natural Compounds, Vol. 46, No. 1, 2010

CHANGES IN PHENOLIC COMPOSITION OF AVOCADO CULTIVARS DURING HARVESTING TIME

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UDC 547.972

Avocado, a tropical fruit native to Central America, is cultivated in tropical climates throughout the world. Avocado is also grown in Turkey, and plantations have rapidly expanded during the past decade. There are numerous cultivated varieties of avocados in the world [1].

Avocado has high oil content (8–32%) relative to other fruits and vegetables and, thus, has a relatively high-energy value [2]. Avocado is rich in vitamin E, ascorbic acid, vitamin B_6, β-carotene, and potassium [3]. The composition of avocado depends on ecotype, cultivar, degree of maturity, and growing conditions [1]. Fruit maturity and picking time are determined according to external markers (color and size), or by measuring dry matter and oil content in the flesh [4, 5]. The minimum oil content necessary for marketing avocado fruit is 8% [4, 6]. The phenolic content of fruits is also affected by the degree of maturity. Spanos and Wrolstad [7] determined that the phenolic content of pear depends primarily on variety and the level of maturity. For example, the phenolic content in apple and pear increases in the first three months and decreases later [8]. Avocado fruits have a long harvesting period depending on cultivars.

In previous study, although the phenolic compounds were characterized according to chemical structures, there was no information regarding the amount of the compounds in avocado. The phenolics were determined to be *p*-hydroxybenzoic, protocatechuic, β-resorcyclic, γ-resorcyclic, α-resorcyclic, gallic, isovanillic, vanillic, syringic, *o*-coumaric, *m*-coumaric, *p*-coumaric, caffeic, ferulic, and sinapic acids [9].

These compounds can contribute to the quality of the fruit in many ways, such as its color and aroma [10]. Thus, in fruits such as avocados, the phenolics contribute to the formation of a brown color, which manifests itself after these fruits are diced or stored for a long time [9, 11]. The most important quality parameter of avocado fruit and products (puree) is color [12]. The color of the product changes according to enzymatic activity and phenolic contents of the fruit. With enzymatic oxidation of the phenolic compounds catalyzed by polyphenoloxidase enzyme, they eventually turn into quinones, a polymerized structure responsible for the brown colour.

In the present study, the phenolic compound contents of four common avocado cultivars (Bacon, Zutano, Fuerte, Hass) grown in Turkey are analyzed to determine whether they change between cultivars depending on harvesting time.

The total amount of phenolic as well as individual phenolic compounds in three harvesting period of four different avocado cultivars (12 samples) were determined by UV-VIS spectroscopy plus analytical RP-HPLC, and the results are shown in Tables 1 and 2.

The avocado cultivars studied showed small differences in total phenolic content of the edible portion (wet weight). However, this differences also had statistical significances at $p<0.05$ level. On the basis of wet weight, Zutano and Fuerte cultivars had the highest phenolic content, followed by Bacon and Hass cultivars (in descending order) (Table 1). The total phenolic content was significantly affected by harvesting time in all cultivars. The lowest phenolic content was determined on the third harvesting time except for the Zutano cultivar. In addition, the total quantity of phenolic compounds determined by RP-HPLC showed similar trends as that determined by the spectrophotometric method. The decrease of total phenol amount is a well-known phenomenon during ripening. Torres et al. [9] determined the total phenolic content of the Hass and Fuerte cultivars as 1.8 mg/g and 1.1 mg/g respectively. Soong and Barlow [13] obtained 1.3 mg/g based on fresh weight (gallic acid equivalent). These values were similar to our result. Slightly differences might be due to differences in harvesting time (maturity degree), agricultural practices, and climatic factors.

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 0009-3130/10/4601-0112

TABLE 1. Changes in the Phenolic Compounds of Avocado Cultivars (mg/kg, mean±SE, n = 12)

Phenolic compound	Bacon	Zutano	Fuerte	Hass
Gallic acid	1.42^{c}±0.043	1.55^{b}±0.043	1.57^{b}±0.116	2.46^{a}±0.066
Protocatechuic acid	5.94^{b}±0.286	6.84^{a}±0.241	6.78^{a}±0.991	6.13^{ab}±0.354
α-Resorcyclic acid	5.45^{c}±0.323	5.75^{c}±0.143	7.59^{b}±0.430	8.32^{a}±0.294
(–)-Epicatechin	230.16^{b}±3.97	289.59^{a}±14.98	285.94^{a}±29.26	225.29^{b}±10.01
γ-Resorcyclic acid	0.91^{c}±0.068	1.05^{b}±0.184	2.41^{a}±0.118	2.47^{a}±0.142
Caffeic acid	14.60^{bc}±0.683	14.18^{c}±0.654	15.34^{b}±1.585	16.25^{a}±0.644
Ferulic acid	0.38^{a}±0.009	0.29^{c}±0.031	0.25^{d}±0.024	0.33^{b}±0.019
p-Coumaric acid	0.13^{d}±0.010	0.18^{c}±0.023	0.24^{b}±0.021	0.27^{a}±0.041
m-Coumaric acid	0.12^{c}±0.025	0.08^{d}±0.011	0.16^{b}±0.009	0.25^{a}±0.015
o-Coumaric acid	0.47^{a}±0.007	0.31^{b}±0.003	0.22^{c}±0.042	0.20^{c}±0.018
Rutin	10.82^{b}±0.34	13.78^{a}±2.74	4.17^{c}±0.75	1.41^{d}±0.14
Quercetin	0.50^{b}±0.015	0.58^{a}±0.026	0.59^{a}±0.030	0.51^{b}±0.052
Total phenolic[1]	1.21^{b}±0.059	1.25^{a}±0.029	1.25^{a}±0.037	1.20^{b}±0.021

The means followed by the different superscript letters in the same line are significantly different ($p<0.05$ by Duncan′s multiple range test). [1]Determined by spectrophotometric method.

Similar phenolic compound contents were determined in the edible portion of the Bacon, Zutano, Fuerte, and Hass avocado mesocarp part. The individual phenolic compound contents of avocado fruits were significantly affected by cultivars and harvesting time. Table 1 shows the phenolic compound content change between cultivars, and Table 2 shows the phenolic content change according to harvesting time of avocado.

The main phenolics determined in fresh edible portions of avocado fruits were (–)-epicatechin and rutin as flavonoids and caffeic acid and protocatechuic acid as phenolic acids. In all cultivars (–)-epicatechin was the most abundant phenolic compound present. The samples that have the highest amounts of (–)-epicatechin are Zutano (289.59 mg/kg), Fuerte (285.94 mg/kg), Bacon (230.15 mg/kg), and Hass (225.29 mg/kg) in descending order.

Relatively few researches on phenolic compounds of avocado fruit have been reported to date. According to Golan et al. [14], the phenolics in the mesocarp of Fuerte and Lerman varieties are caffeic, protocatechuic, ferulic, and *p*-coumaric acids. Torres et al. [9] identified 16 phenolic compounds (*p*-hydroxybenzoic, protocatechuic, β-resorcyclic, γ-resorcyclic, α-resorcyclic, gallic, isovanillic, vanillic, syringic, *o*-coumaric, *m*-coumaric, *p*-coumaric, caffeic, ferulic, and sinapic acids). However, there is no information available on the individual phenolic amounts in avocado fruit.

Quantitative differences in the phenolics content in avocado fruits from the studied cultivars have been observed. The highest gallic acid value (2.46 mg/kg) was determined in Hass cultivar. Fuerte (1.57 mg/kg), Zutano (1.55 mg/kg), and Bacon (1.42 mg/kg) cultivars, in descending order, follow this cultivar. In addition, the lowest amount of gallic acid was determined in the first harvesting time sample. The quantity of gallic acid increased 11% from the first harvest sample to the second harvest sample. In contrast, there was no significant difference ($p>0.05$) in gallic acid content between the second and third harvesting times in the samples. Samples that have the highest amounts of protocatechuic acid (6.84 mg/kg) and α-resorcyclic acid (8.32 mg/kg) are those obtained from Zutano, and (–)-epicatechin (289.59 mg/kg) from Hass cultivar (Table 1). The variation of protocatechuic and α-resorcyclic acid concentrations in avocado showed similar trends over the harvesting time. The protocatechuic and α-resorcyclic acids steadily increased from first harvesting to third harvesting in the avocado samples. However, the (–)-epicatechin concentration increased from first harvesting to second harvesting and decreased from second harvesting to third harvesting in the samples. The lowest (–)-epicatechin value (223.27 mg/kg) was determined in the first harvesting sample (Table 2). Examples exist showing the different phenolic contents of different cultivars in the same species (apple and pear) [15, 16].

The γ-resorcyclic acid contents of Fuerte and Hass were similar but substantially different in that of Bacon and Zutano. The amounts of γ-resorcyclic acid in Hass (2.47 mg/kg) and Fuerte (2.41 mg/kg) were approximately two and a half times higher than Zutano (1.05 mg/kg) and Bacon (0.91 mg/kg). Caffeic acid was the most abundant phenolic acid, which was present in all cultivars. The highest caffeic acid value (16.25 mg/kg) was determined in Hass cultivar. Fuerte (15.34 mg/kg), Bacon (14.60 mg/kg), and Zutano (14.18 mg/kg) cultivars followed in succession. All the avocado cultivars showed similar ferulic and *p*-coumaric acid concentrations. The quantity of ferulic and *p*-coumaric acids was extremely low in the avocado samples (Table 1).

TABLE 2. Changes in the Phenolic Compounds of Avocado at Harvesting Time (mg/kg, mean±SE, n = 16)

Phenolic compound	1st Harvesting	2nd Harvesting	3rd Harvesting
Gallic acid	1.63^{b}±0.160	1.81^{a}±0.126	1.81^{a}±0.199
Protocatechuic acid	5.81^{b}±0.299	6.29^{b}±0.319	7.17^{a}±0.637
α-Resorcyclic acid	6.22^{b}±0.472	6.98^{a}±0.548	7.14^{a}±0.498
(–)-Epicatechin	223.27^{c}±11.59	282.60^{a}±16.89	267.36^{b}±18.40
γ-Resorcyclic acid	1.60^{b}±0.323	1.96^{a}±0.324	1.57^{b}±0.217
Caffeic acid	14.45^{b}±0.504	14.96^{b}±0.396	15.87^{a}±1.325
Ferulic acid	0.27^{c}±0.026	0.30^{b}±0.018	0.37^{a}±0.015
p-Coumaric acid	0.18^{b}±0.029	0.20^{ab}±0.018	0.22^{a}±0.039
m-Coumaric acid	0.13^{c}±0.023	0.15^{b}±0.022	0.18^{a}±0.032
o-Coumaric acid	0.30^{b}±0.047	0.34^{a}±0.035	0.22^{c}±0.048
Rutin	6.89^{b}±1.78	10.23^{a}±2.91	5.52^{c}±1.29
Quercetin	0.51^{b}±0.029	0.55^{ab}±0.032	0.58^{a}±0.031
Total phenolic[1]	1.22^{b}±0.033	1.29^{a}±0.013	1.18^{c}±0.037

The means followed by the different superscript letters in the same line are significantly different ($p<0.05$ by Duncan's multiple range test).

[1]Determined by spectrophotometric method.

The differences in the concentration of γ-resorcyclic and caffeic acids showed a similar trend during the harvesting period in samples. The highest γ-resorcyclic and caffeic acid contents were found in the second harvesting samples. The phenolic content of plants depends on genetic and environmental factors [10, 15]. These differences may be dependent on their genetic characteristics and climatic factors. The ferulic, *p*-coumaric, and *m*-coumaric acid contents of avocado steadily increased over the harvesting period (Table 2).

Contrary to the analysis of the quercetin content, which showed no differences in the cultivars, the contents of *m*-coumaric acid, *o*-coumaric acid, and rutin showed significantly differences in the cultivars, whereas the concentrations of *m*-coumaric acid, *o*-coumaric acid, and quercetin were extremely low in the samples except for rutin. The highest content of *m*-coumaric acid (0.25 mg/kg) was in the Hass cultivar, followed by the Fuerte (0.16 mg/kg), Bacon (0.12 mg/kg), and Zutano (0.08 mg/kg) cultivars. The highest *o*-coumaric acid was found in the Bacon cultivar, is followed by the Zutano, Fuerte, and Hass cultivars in that order. The rutin concentrations were 13.78 mg/kg in Zutano, 10.82 mg/kg in Bacon, 4.17 mg/kg in Fuerte, and 1.41 mg/kg in Hass. The quercetin concentrations of Bacon, Zutano, Fuerte, and Hass were similar in these cultivars, and the highest amount was in the Fuerte variety (Table 1).

The *o*-coumaric acid and rutin contents were increased from the first to the second harvesting time and decreased from the second to the third harvesting time in the samples. In addition, the lowest *o*-coumaric acid (0.22 mg/kg) and rutin values (5.52 mg/kg) were found in the third harvesting sample. In contrast, the quercetin content of all of the avocado cultivars steadily increased over the harvesting period (Table 2).

As a result, the total phenolic content was the highest in Zutano, followed by the Bacon, Fuerte, and Hass cultivars. The total phenolic content increased at the beginning of the harvesting period up to the second harvesting time, and it decreased at the end of the harvesting time (third harvesting time). The phenolic composition of avocado fruits showed significantly differences in terms of the cultivars and their harvesting time, and the main phenolic compounds of avocado fruit were found to be protocatechuic acid, caffeic acid, (–)-epicatechin, and rutin.

Materials. Four avocado varieties, Bacon, Zutano, Fuerte, and Hass (*Persea americana* Mill.), were selected for this experiment. Avocado cultivars Bacon, Zutano, Fuerte, and Hass were harvested from the orchard of Bati Akdeniz Agricultural Research Institute Antalya, Turkey, during 2004–2005 seasons. Mature Bacon and Zutano fruits were picked in the first week of November (early season), December (mid-season), and January (last season). Fuerte and Hass cultivars were harvested in the first week of January (first harvest), February (second harvest), and March 2005 (third harvest). Sufficient fruits were handpicked at one time, from the outer layer avoiding the tops and bottoms of the trees, which had been treated by the same agricultural practices. Fruits were graded for appearance (i.e., free from damage and sunburn) and randomized for analyses.

Methods. The total amount of polyphenols in the samples were measured by the Folin-Ciocalteu method [7]. The total amount of phenolic compounds was calculated and expressed as gallic acid equivalents (mg/g). Approximately 10 g of

the edible portion of the avocado cultivars was accurately weighed and extracted at room temperature in the absence of light with methanol containing 1% butylated hydroxyanisole (BHA) as an antioxidant in an ultrasonic water bath. The extraction was carried out according to the method of Escarpa and Gonzales [17]. Samples were extracted twice with 10 mL of solvent for 1 hour, 10 mL for 30 min, and then with 5 mL for 30 min. The extracts were combined to a final volume of 25 mL. The extraction mixtures were centrifuged for 10 min in a tabletop centrifuge at 4°C. The extracts for HPLC analysis were filtered through membrane filters (0.45 μm pore size) and stored at –18°C before chromatographic analysis. The extracts were filtered through 0.45 μm membranes before analysis.

Standard phenolic compounds were dissolved in methanol. The phenolics in the HPLC chromatogram were identified by comparison with the retention times of authentic samples, and the quantity of phenolics in the chromatogram was quantified by comparison with standard solutions of known concentration detected at 300 nm. This wavelength was chosen for monitoring because all phenolics examined in this study are absorbed at this wavelength.

HPLC Conditions. Instrument: Varian 1100 liquid chromatograph with UV detector; column: Nucleosil 5 C_{18} reverse-phase analytical column (250 × 4.6 mm, 5 μm particle size, Merck Licrosorb); guard column: Nucleosil 5 C_{18} (10 × 4.1 mm, Alltech); detector: UV-visible detector, 300 nm; column temperature: 40°C; injection volume: 20 μL; flow rate: 1.0 mL/min, mobile phase: A = methanol, B = trifluoracetic acid–H_2O (2:998); gradient: 5% B (0 min), 95% B (28 min), 5% B (35 min).

ACKNOWLEDGMENT

The Scientific Researches Support Department of Akdeniz University supported this study through project number 2003.03.0121.005.

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