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RESEARCH ARTICLE

Chemical composition, bioactive compounds and antioxidant activity of six avocado cultivars *Persea americana* Mill. (Lauraceae) grown in Egypt

Mahmoud Abd Al-Galil Rozan[1], Esmail Galal Boriy[1], Hala Mahmoud Bayomy[1,2]

[1]Department of Food and Dairy Science and Technology, Faculty of Agriculture, Damanhour University, Damanhur, Egypt, [2]Department and Nutrition and Food Science, Tabuk University, Tabuk, Kingdom of Saudi Arabia

ABSTRACT

Amongst the most common tropical fruits is avocado (*Persea americana*), which is widely consumed in the world due to its high nutritional value and multiple applications. Hass avocado cultivar is the primary global cultivar of avocado. Recently, some other cultivars are included by some producers in Egypt namely Ettinger, Fuerte, Maluma, Pinkerton, and Reed. The point of the study is to evaluate the chemical composition, fatty acid profile, bioactive compounds, and antioxidant activity of various cultivars in the fruit flesh. The results indicated that the ripening season of avocados extends from early October to mid-March. Ettinger avocado is the earliest cultivar, while the Reed cultivar is the most delayed avocado in the ripening. Hass cultivar recorded a higher value of firmness (89.5 N). Dry matter was 32.12, 27.67% in Hass and Ettinger, respectively. Acidity and chemical composition differed significantly ($P < 0.05$) among all avocados under study. All cultivars can be considered as good sources of K, P, and Mg elements, and moderate sources of Ca and Na elements, while they contain relatively limited quantities of Fe, Zn, and Mn elements. Oleic acid was the main fatty acid present in the oil of all avocado cultivars, while myristic acid was the lowest in all of the cultivars. The acidic amino acids (glutamic and aspartic) were the greatest amino acids in all the studied avocados, while S-amino acids (methionine and cystine) and tryptophane were the lowest acids in the studied cultivars. Hass avocado significantly was the highest in its content of total carotenoids (6.91 µg/g, FW), α-carotene (0.58 µg/g, FW), β-carotene (1.43 µg/g, FW), lutein (3.84 µg/g, FW), total chlorophyll (29.5 µg/g, FW), Chlorophyll a (15.6 µg/g, FW), and Chlorophyll b (13.2 µg/g, FW), total phenols content (4.9 ± 0.38 mg/g FW), total flavonoids content (0.25 ± 0.01mg/g FW), Ascorbic acid content (12.8 ± 0.15 mg/100g FW), and DPPH value (1.3 ± 0.09 µmol TE/g). The individual phenolic compounds in the flesh of avocado fruits were significantly ($P < 0.05$) affected by cultivars. The major compound was epicatechin, which ranged between 130.12 µg/g DW (in Hass cultivar) and 184.15 µg/g DW (in Pinkerton cultivar).

Keywords: Avocado cultivars; Chemical composition; Fatty acid profile; Ascorbic acid; Carotenoids; Phenolics; Antioxidant activity

INTRODUCTION

Persea americana Mill. (Lauraceae) is an evergreen subtropical and tropical tree that is considered to have originated around 1000 years ago in Mexico, Guatemala, Colombia, Venezuela, Ecuador, and Peru and commonly known as the avocado (Ferreyra et al., 2016). Avocado is exceptionally high oil content, which can exceed 20% dry weight, so investigators have called it butter fruit (Takenaga et al., 2008).

Avocados have been known since ancient times and consumed for their high content of macronutrients, micronutrients, high phytochemicals content, flavor, and versatility of applications (Bartoli, 2008) and assorted as a functional food (ADA,1999) for their benefits to human health. Numerous scientific reports confirm the beneficial health effects of avocado oil and have likened the quantitative and qualitative nutritional properties of avocado oil to olive oil. Its consumption also results in a decrease of low-density lipoproteins content, and an increase in high-density lipoproteins in the blood, which contributes to reducing the blood content of triglycerides, which protects against the risk of vascular disease. (Ortiz et al., 2004; Mendez and Hernandez, 2007; Rodriguez-Sanchez et al., 2015; Gokkaya et al., 2021).

Many factors affect the chemical composition and bioactive compounds in avocado fruit, including the place of cultivation (Lu et al., 2009; Donetti and Terry, 2014; Nasri et al., 2021), the sort of cultivar (Takenaga et al.,

***Corresponding author:**
Hala Mahmoud Bayomy, Department of Food and Dairy Science and Technology, Faculty of Agriculture, Damanhour University, Damanhur, Egypt. E-mail: hala.biomy@agr.dmu.edu.eg

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2008; Ramos-Aguilar et al., 2021), conditions of maturity (Ozdemir and Topuz, 2004; Donetti and Terry, 2014), and even to the part of the fruit measured (Landahl et al., 2009).

Globally, Mexico is the world's leading avocado grower, producing 30% of this fruit, followed by the Dominican Republic, Peru, and Colombia (FAO, 2019). Locally, avocado is newly cultivated in some locations of Egypt, especially the El-Behira governorate, which produces 95% of Egypt's production of avocados (El-Beheira Directorate of Agriculture, 2019).

Hass avocado cultivar is the primary global cultivar of avocado (Dreher and Davenport, 2013). Despite the large number of studies conducted to evaluate Hass avocado fruits, there are no significant studies on other cultivars that have begun to expand in cultivation in Egypt, especially Ettinger, Fuerte, Maluma, Pinkerton, and Reed.

Naturally, increasing confirmation of the health benefits of avocado fruits supports increasing its production and consumption. That given the economic value of avocados as a promising crop in Egypt, it is required to undertake a comparative analysis of the chemical differences between avocado cultivars based on its nutritional value. So, the present work aims to evaluate the physicochemical, chemical composition, bioactive compounds, and antioxidant activity of six avocado fruit flesh cultivars grown in Egypt.

MATERIALS AND METHODS

Fruits collection and preparation for analysis

Ettinger, Fuerte, Hass, Maluma, Pinkerton, and Reed (Figure 1) avocado cultivars were grown in the same orchards of PICO Modern Agriculture Company (Latitude: 30.53°, Longitude: 30.794) in El-Beheira Governorate, Egypt. The altitude of the avocado orchard above sea level is 15 m on average, the soil is light clay with good drainage and ventilation, the local climate is moderate to hot and humid, the wind speed is light, and the temperature often ranges between 13 to 35 °C, the distance between plants on the line is 4 m, and the distance between the lines is 6 m, the trees are 12 years old, fifty fruits of each cultivar were randomly collected from 4 ha of the middle of the orchard. All fruits were harvested at the maturity stage after 190-200 days from full bloom, a non-damaged, absence of visible decay, and fresh avocado fruits were collected during the period from 15- October 2020 to 15- March 2021 (Table 1).

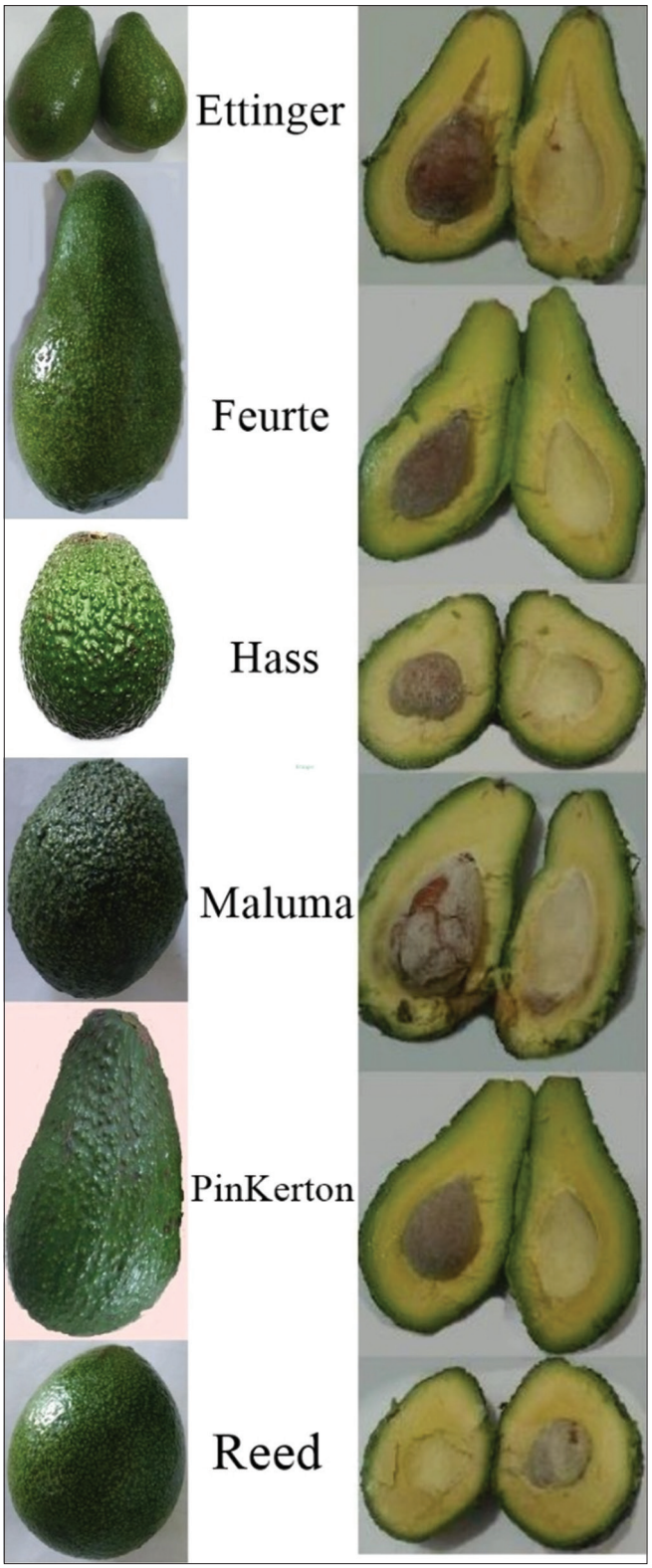

Fig 1. Appearance of whole and halves of avocado fruit cultivars.

On arrival at the lab., fruits were packed in carton boxes and held for 5 days at 20 °C in controlled temperature rooms with 90-95% relative humidity. Then, fruit weight, % total soluble solids and firmness were assessed, thereafter fruits were peeled, the seeds removed, and each part of the fruit was weighed. Then the flesh of the fruits was crushed and packed under vacuum in plastic bags and stored at 0 °C. All analyzes were done within two weeks.

Table 1: Ripening season and physicochemical parameters of six avocado cultivars

Cultivar		Ettinger	Fuerte	Hass	Maluma	Pinkerton	Reed
Ripening season		1 Oct. - 15 Nov.	15 Oct. – 1 Dec.	15 Nov. – 31 Jan.	15 Nov. – 31 Jan	15 Nov. – 1 Jan.	1 Feb. – 15 Mar.
Fruit average weight (g)*		255-470 (354) b	250-400 (323) c	100-300 (178) f	150-400 (192) e	255-375 (303) d	300-475 (375) a
% of whole fruit*	Peel	10.5±0.3a	8.8±0.4b	7.6±0.3c	8.0±0.2c	7.8±0.1c	8.1±0.3c
	Seed	22.4±0.5a	20.6±3b	14.6±0.3e	16.5±0.6c	15.7±0.4d	16.6±0.3c
	Flesh	67.1±0.4c	70.6±0.6b	77.8±0.4a	75.5±0.9a	76.5±0.4a	75.3±0.3a
Firmness (N)*		72±1.2c	74.3±2.6c	89.5±1.5a	87.5±1.3a	83.2±0.5b	72.8±2.3c

* Values (Mean±standard deviation, three replicates). Different letters in the same row means significantly differences among cultivars at $P<0.05$.

Standards and reagents

Sigma Chemicals (Sigma-Aldrich Company, USA) and El-Gomhoria Pharmaceutical Co., Cairo, Egypt, provided all reagents and chemicals utilized in this investigation. HPLC grade chemicals and solvents were employed for spectral and HPLC studies.

Weight and firmness of avocado fruits

Weight and firmness of avocado fruits were carried out on 15 fruits for each cultivar. Fresh avocado fruits were weighed using Sartorius Balances Model PT 600, Germany. The firmness of fresh avocados was determined. An Instron testing machine model 4301 with a 6 mm diameter convex probe was used to determine the maximum force (in Newton units) exerted up to tissue penetration. On either side of the middle zone, the fruit was measured twice.

Total soluble solids (TSS)

The TSS percent in avocado flesh juice was evaluated three times at 20 °C using an Atago RX-1000 digital Refractometer (Atago Co., Ltd., Tokyo, Japan).

pH and total acidity

Avocado fruits were homogenized in a grinder, made suspension by ten grams of fruit flesh stirring in 100 mL distilled water and filtered. The pH of the samples was assessed using a pH meter (type Hanne 9124) at 25 ºC. Total acidity was determined using the direct titration method with NaOH (0.01N) according to AOAC (2000). The titratable acidity of the avocado was measured in grams of tartaric acid per 100 g of avocado fruit flesh.

Chemical composition

Proximate chemical analysis of avocado fruits including moisture content, crude protein (N×6.25), in a Soxhlet units, crude fat is extracted with petroleum ether (b.p. 60-80 °C), crude fiber and ash were carried out according to the AOAC (2000) official method no. 962.09 and 981.10, respectively. Total carbohydrates content was determined by difference (100 - moisture content + crude protein + ash + ether extract + crude fiber).

Atomic Absorption Spectrophotometer (Perkin-Elmer Model 2380 manufacture, USA) was using to determine the iron (Fe), magnesium (Mg), and zinc (Zn) in acidic ash solution according to AOAC, (2000) method no 995.11. Calcium (Ca), sodium (Na), and potassium (K) were determined using the Flame Photometer with reference to Pearson (1976).

Based on AOAC (2000) approved methodology No. 967.21, ascorbic acid was calculated using direct titration with 2,6 dichlorophenol indophenol reagent.

Determination of amino acids

Acidic hydrolysate of the avocados flesh by 6N HCl used to determine amino acids according to AOAC (2000) professional method 982.30 E [a, b, c], by using an amino acid analyzer (LC 3000 amino acid analyzer, High-performance system, a product of LC biochrom Eppdrop, Germany). A hydrolysate obtained by alkaline hydrolysis of samples were analyzed for tryptophan, as stated by Siddique et al., (2013) using dimethyl amino benzaldehyde (DMAB). 1 g of sample was reflexed with 10 mL of barium hydroxide for 20 hours to produce the alkaline hydrolysate. The hydrolysate was neutralized with 0.5 M Sulfuric acid to pH 7, then filtered to get rid of the precipitate. With a 0.2 % sodium nitrate solution, blue colour was produced. A standard curve of tryptophan prepared under the same conditions was used to calculate tryptophan content.

Chemical score

The chemical score of avocado pulp protein was calculated with reference to FAO/WHO (1985), this is calculated by comparing the quantity of the most limiting amino acid in the test protein to the quantity of that amino acid in reference egg protein.

Fatty acid analysis

The fatty acid fractions for oil avocados were identified as methyl ester by gas-liquid chromatography (Perkin Elmer Auto system XL) according to Radwan (1978) technique.

Total carotenoids determination

Total carotenoids content was determined according to Moore et al., (2005) using the spectrophotometric method at 470 nm and expressed as μg/g sample.

Total chlorophyll determination

Chlorophyll content was estimated by extracting the pigments from the pulp of avocado fruits with acetone and measuring the color density with a spectrophotometer (Bausch and Lamb Co. USA) as described by Lichtenthaler and Buschmann (2001).

Analysis of carotenoids and chlorophyll

Carotenoids and chlorophyll were extracted with reference to Ornelas-Paz et al., (2017). A 4 g aliquot of avocado fruit flesh was mixed with 0.2 g calcium carbonate and 25 mL methanol, filtering through Whatman filter paper No. 3 then add methanol until the particles became colorless. Following that, 50 mL of n-hexane/acetone (1:1, v/v) solution containing 0.1 % BHT was added to the methanolic extract and stirring flipping with addition of 40 mL sodium sulfate10%, then placed in a separating funnel to allow the two layers to be separated. The top layer was collected, rinsed three times with distilled water, and evaporated at 35°C. A C30 column (150 × 4.6 mm) (YMC Inc., MA, USA) used to separate the carotenoids and chlorophylls concurrently at 15 ◦C using the Agilent HPLC system. Water, methanol, and tert–butyl methyl ether (TBME) were used to make the mobile phase (0.75 mL/min) based on the following gradient: 4 % A/94.5% methanol/1.5 % C at 0 min, 4% water/68% methanol/28% TBME at 31 min, 4% water/30% methanol/66% TBME at 83 min, and 4% water/0% B/96% TBME at 85-90 min. High purity standards of (> 95%) all-E-lutein, all-E-β-carotene, all-E-α-carotene, chlorophyll a, chlorophyll b, used for naming and quantification aims. These bio active compounds were detected in avocado pulp by relating their UV–vis data and chromatographic behavior (spiking samples) with the standards' compounds.

Extraction and analysis of total phenolic content and total flavonoids

Total phenolic compounds and total flavonoids in avocado fruit flesh were extracted according to Di Stefano et al., (2017). Briefly, 10 g of fruit flesh were mixed with 40 mL of pure methanol. The mixtures were stirred for 30 min at ambient temperature, transferred in centrifuge tubes and finally centrifuged at 4500 rpm for 10 min; then supernatants were filtered, evaporated to dryness and kept at –18 °C. The total phenolic content was determined according to the Folin-Ciocalteu procedure (Zilic et al., 2012). 500 µl of extract was mixed with 250 µl of 5% NaNO2 for 6 min to determine the total flavonoid content. 2.5 ml of a 10% $AlCl_3$ solution was added, after 7 min, add 1.25 ml of 1 M NaOH and the mixture was centrifuged at 5000 g for 10 min. Absorbance of the supernatant was measured at 510 nm against the solvent blank. The total flavonoid content was expressed as mg of catechin equivalent per g of fresh sample (Zilic et al., 2012).

Determination of radical DPPH scavenging activity

The stable DPPH as developed by Hwang and Do-Thi (2014) was used to evaluate the avocado pulp's antioxidative ability. DPPH was used at a final concentration of 200 M, with a reaction volume of 3.0 mL. The solutions kept in the dark for 1 hour, then the absorbance was taken the absorbance against a pure methanol as a blank at 517 nm. Trolox was used to create the standard curve. The following equation was used to measure the DPPH free radical inhibition percentage:

$$\text{Inhibition (\%)} = 100 \times [(\text{A control-A sample})/\text{A control}]$$

Where:

- A control is the absorbance of the blank.
- A sample is the absorbance of the test samples.

DPPH free radical inhibition data were recoded as µM Trolox equivalents (TE)/100g sample.

Profile of phenolic compounds

Using an Agilent Technologies 1100 series liquid chromatograph with an autosampler and a diode-array sensor, the profile of phenolic chemicals in avocado samples were evaluated as stated by Kim et al., (2006). An Eclipse XDB-C18 particle size (150 X 4.6 m; 5 m) with a C18 guard column (Phenomenex, Torrance, CA) used as analytical column. The mobile phase was consisting of acetonitrile (solvent A) and aqueous solution of acetic acid 2% (solvent B). Total run time for 1 hour, the flow rate was held at 0.8 mL/min, and the gradient program was as follows: 100 % B to 85 % B in 30 min, 85 % B to 50 % B in 20 min, 50 % B to 0 % B in 5 min, and 0 % B to 100 % B in 5 min. The samples filtered with a 0.45 m Arcadis syringe filter (Gelman Laboratory, MI), then using 20 µl for injection. Peaks at 280, 320, and 360 nm were monitored at the same time and detected by comparing retention times and UV spectra to the standards.

Statistical analysis

Avocado samples were taken at random from each cultivar with no other constraints. The average value for N replicates (M ± SD) is used to express the results and analyzed by one-way ANOVA at significant level ($P < 0.05$) by SAS software (2017).

RESULTS AND DISCUSSION

Ripening season and physicochemical parameters of avocado cultivars

Ripening season, fruit weight, Peel thickness, seed size, firmness, and the relative weights of the portions of the six avocado cultivars fruits are given in Table 1. Generally, the ripening season of avocados in Egypt extends from

early October to mid-March. Ettinger avocado is the earliest cultivar, followed by Fuerte, then Hass, Maluma and Pinkerton avocado, while the Reed cultivar is the most delayed avocado in the ripening season. In general, the length of the ripening season for each cultivar ranges from one and a half to two months.

Results revealed that the average weight of Reed cultivar fruit (375 g) is greater than that of the other cultivars. While, Hass cultivar is the lowest (178 g).

The percentage of flesh in the six avocado cultivars ranges from 67.1 (in Ettinger cultivar) to 77.8% (in Maluma cultivar). There are significant ($P < 0.05$) differences between the relative weights of the peel in the six cultivars, it ranged between 7.6% in the Hass cultivar and 10.5% in the Ettinger cultivar. Likewise, the relative weight of the fruit seed differed significantly, it ranged between 14.6% in Hass cultivar and 22.4% in Ettinger cultivar.

Amado et al., (2019) found that Hass cultivar grown in Brazil had a pulp with 57.63%, seed with 29.48%, and peel with 12.89% of whole fruit with an average weight of 197.65 g, while the Margarida cultivar had more pulp (81.0%) and less seed (10.5%), when comparing Hass, Quintal, and Fortuna avocados,

Avocado fruit firmness is one of the most consistent and commonly accepted indicators of avocado fruit ripeness. Firmness has a significant link with ripeness as evaluated by other factors including dry matter, oil content, and eating quality (White et al., 1999). Initially, firmness of avocado fruit declines gradually, then accelerates as the fruit matures (Magwaza and Tesfay, 2015). In our study, Hass cultivar recorded a higher value of firmness (89.5 N), followed by Maluma (87.5 N), Pinkerton (83.2 N), Fuerte (74.3 N), Reed (72.8 N), and Ettinger (72.1 N). The relatively low firmness of Ettinger fruit may be attributed to the thinness of its peel and its high moisture content. These results were high compared to results reported by Ahmed et al., (2010) for Fuerte cultivar fruits, who found that firmness decreased gradually after harvest till ripening at 20 °C from 77.24 N at the zero time to 10.75 N at 15 days after harvest.

Chemical composition

One of the most preferred indicators of maturity evaluated in fruits is moisture content, as it is a valid indication of their perishability (Kassim et al., 2013). Table 2 shows the chemical composition of the six avocado cultivars. Data revealed that moisture content ranged from 67.88% (in Hass cultivar) and 72.24% (in Ettinger cultivar). Dry matter revealed 32.12, 27.67% in Hass and Ettinger avocados, respectively.

The dry matter content is significant ($P < 0.05$) indicator of the ripeness degree of avocado fruits. Due to the increased oil content in the fruits during maturity, the total dry matter is increased. Avocado fruits with 20% of dry matter are minimally mature, while fruits with 40% of dry matter are considered very mature, therefore, the dry matter content parameter could potentially be used as an alternative to the oil content parameter to indicate the degree of ripeness of the avocado (Gamble et al., 2010). The dry matter concentration of avocado fruit pulp is wide contrast, varying from less than 20% for not good-tasting fruit that should be excluded for consumption to greater

Table 2: Chemical composition of flesh of six avocado cultivars on fresh weight

	Ettinger	Fuerte	Hass	Maluma	Pinkerton	Reed
Moisture (%)	72.24±0.47a	70.13±0.62b	67.88±0.2c	68.69±0.32c	70.38±0.5b	71.05±0.71ab
Dry matter (%)	27.76±0.13c	29.87±0.37b	32.12±0.67a	31.31±0.25a	29.62±0.27b	28.95±0.36b
Oil content (%, FW)	17.84±0.28d	20.42±0.19c	22.46±0.21a	21.49±0.23b	20.79±0.41bc	17.66±0.14d
Total proteins (%, FW)	2.04±0.25b	1.87±0.16c	2.21±0.19a	2.33±0.21a	2.16±0.14ab	1.98±0.17bc
Ash (%, FW)	1.16±0.11d	1.31±0.06bc	1.37±0.1c	1.49±0.08b	1.73±0.11a	1.38±0.06c
Crude fibers (%, FW)	3.36±0.23b	3.03±0.14b	2.22±0.1c	2.54±0.23c	1.72±0.12d	4.25±0.17a
Carbohydrate (%, FW)	3.36±0.2cd	3.24±0.19d	3.86±0.23a	3.46±0.18c	3.22±0.12d	3.68±0.27b
Ca (mg/100 g, FW)	13.1±0.04a	10.6±0.04a	13.4±0.07a	13.2±0.1a	11.8±0.05a	12.7±0.1a
Mg (mg/100 g, FW)	29.2±0.1a	28.5±0.08a	24.7±0.05b	21.6±0.1c	27.3±0.2a	27.0±0.1a
Fe (mg/100 g, FW)	0.41±0.01a	0.32±0.01c	0.36±0.02b	0.4±0.01a	0.24±0.04d	0.19±0.02e
P (mg/100 g, FW)	58.3±0.1ab	62.0±0.1a	60.5±0.14a	61.6±0.16a	54.7±0.14b	41.2±0.07c
K (mg/100 g, FW)	550.5±0.17a	405.0±2d	514.6±0.36b	492.7±0.3c	378.0±0.4e	385.5±1e
Na (mg/100 g, FW)	8.3±0.04c	8.4±0.05c	11.2±0.1b	11.0±0.1b	14.6±0.13a	11.4±0.1b
Zn (mg/100 g, FW)	0.62±0.02b	0.48±0.02c	0.52±0.04c	0.70±0.02a	0.58±0.03b	0.47±0.03c
Mn (mg/100 g, FW)	0.17±0.01c	0.45±0.02a	0.30±0.02b	0.38±0.01a	0.25±0.03b	0.29±0.02b
TSS (^{0}Brix)	6.26±0.13b	6.18±0.2bb	6.93±0.28a	6.84±0.25a	6.33±0.21b	6.14±0.17b
Acidity	0.84±0.07a	0.89±0.05a	0.85±0.11a	0.84±0.03a	0.88±0.12a	0.83±0.08a
pH	6.4±0.1a	6.4±0.1a	6.4±0.1a	6.4±0.1a	6.4±0.1a	6.4±0.1a

*Values (Mean±standard deviation, three replicates). Different letters in the same row means significantly differences among cultivars at $P<0.05$.

than 35% in fruit that is potentially appropriate for food processing treatments (Clark et al., 2007). Along with the literature, dry matter content in flesh of avocado fruits ranges from 13.25 to 31.4 %, depending on the harvest date (for example 28.8%, 31.4% for 'Hass' avocado harvested on 22 Jan, 9 Oct, respectively), region (for example 21%, 21.6-22.8% and 23% for Hass avocado grown in Australia, Brazil, and South America, respectively), and cultivar (for example 13.25, 13.75, 22.75 and 25.5% for Kallar Round, Pollock, Purple Hybrid and Fuerte cultivars in India, respectively) (Hofman et al., 2000; Carvalho et al. 2014; Magwaza and Tesfay, 2015; Krumreich et al., 2018; Nair and Chandran, 2018).

The total soluble solids (TSS) significantly differed in six cultivars, they were 6.93% in the Hass cultivar and 6.14% in the Reed cultivar. The suggestion to utilize total soluble solids as an indication of fruit ripeness, quality, and sweetness sprang from a desire for an accessible and quick analytical approach for measuring sugar concentration. Özdemir et al., (2009) found that fruit flesh firmness decreases with the increase of the number of days after full bloom, it was 196.13 N on the 230th days after full bloom in Fuerte cultivar. Then, it decreased to 156.02 N on the 245th days after full bloom and 67.66 N on the 275th days after full bloom. The same observation is recorded in the Bacon and Zutano cultivars.

Acidity is also a typical quality feature linked to fruit ripeness. Pedreschi et al., (2019) reported that the tartaric acid is the main common acid found in avocados.

The total acidity for the pulp of six studied avocados was not significantly ($P < 0.05$) different, it ranged between 0.83 and 0.89% for Reed and Fuerte cultivars, respectively (as tartaric acid).

This result is low as compared to that found by Vinha et al., (2013), who found that the pulp of the mature Algarvian avocado has 1.07% (as grams of tartaric acid per 100 g of sample), but it is relatively high as compared to that reported by Krumreich et al., (2018) for Breda cultivar (0.53%).

Avocado is sorted as a non-acidic fruit since it contains only trace levels of citric and malic acids, with tartaric acid acting as the predominant organic acid. Avocados' organic acids do not greatly contribute to their quality and flavor resulting in high oil content, which significantly improves the quality and flavor of the fruit, unlike other fruits that contain organic acids such as malate, citrate, tartaric, and others, (Ahmed et al., 2010; Pedreschi et al., 2019).

The pH value in the flesh of avocado fruits was 6.4 for all cultivars. This result makes avocado pulp a quite suitable fruit for use in sweet or salty preparations due to its low acid content (Krumreich et al., 2018).

Avocado fruits are rich in oil content relative to other fruits and vegetables (Donetti and Terry 2014). The oil content of the flesh of the six cultivars of avocado is shown in Table 2. There are significant differences ($P < 0.05$) between the cultivars in their oil content, which was higher in the Haas cultivar (22.46% on the wet basis) and less in the Reed cultivar (17.66% on the wet basis).

In the literature, the lipid content of different cultivars of avocado varied markedly. Based on the cultivar, environmental and agricultural factors, oil content in avocados pulp varies from a slightest of 8 to 30%. (Magwaza and Tesfay, 2015), for example, it was 18.9%, 19.4%, 19.52%, 20.2% in Fuerte, Purple Hybrid, Kallar Round, and Pollock avocados cultivated in India, respectively (Nair and Chandran, 2018), and it was 15.8% in Breda cultivar cultivated in Brazil (Krumreich et al., 2018).

Based on dry weight, the oil content of avocado flesh can be 70%, considering differences in the amount and composition of the oil due to variations in regions of growth, cultivar, ripening season, agricultural and climatic factors, time of picking and portion of fruit (Landahl et al., 2009; Ferreyra et al., 2016).

Considering oil amount and composition differences related to cultivation of areas, cultivar, maturing season, agriculture and climate conditions, harvest times, and portion of fruit (Landahl et al., 2009; Ferreyra et al., 2016).

Oil content is a significant ($P < 0.05$) indicator of fruit maturity in many avocado-producing countries, therefore, usually defines the optimum harvest date (Woolf et al., 2004).

The results show significant ($P < 0.05$) differences in protein content between the six cultivars. The highest protein content value ever recorded in the Maluma cultivar (2.33% on the wet basis), while the lowest value ever recorded in the Fuerte cultivar (1.87% on the wet basis), our findings not accepted by Oliveira et al., (2013), who scored a protein content that ranged from 0.74% to 1.9% for eleven varieties of avocado. Carbohydrate content in the pulp among all avocado cultivars differed significantly ($P < 0.05$), it ranged between 3.22% (in Pinkerton cultivar) and 3.86% in the Hass cultivar. Crude fiber content was also significant difference among all avocado cultivars ($P < 0.05$), Reed cultivar was the highest with 4.25% of crude fiber, while Pinkerton cultivar was the lowest one with 1.72% of crude fiber (on the wet basis). These results are high as compared to the presented by Daiuto et al., (2010) and Krumreich

et al., (2018) who found 1.62, 1.6% of fibers in Hass and Breda cultivars, respectively.

Ash content ranged from 1.16% in the Ettinger cultivar to 1.73% in the Pinkerton cultivar on a wet basis.

For the estimated minerals, all cultivars can be considered as good sources of K, P, and Mg elements, and moderate sources of Ca and Na elements, while they contain relatively limited quantities of Fe, Zn, and Mn elements (Table 2).

Fatty acid profile

The fatty acid profile is mostly dependent on environmental and growth variables (Blakey, 2011; Carvalho et al., 2014; Pedreschi, et al., 2016). Consequently, it is suggested the fatty acid profile as a potential biomarker to detect avocado fruit cultivating zones (Donetti and Terry, 2014).

Fatty acid contents are shown in Table 3. Under our work conditions, GC analysis of avocado oil gave 7 fatty acids: myristic, palmitic, stearic, palmitoleic, oleic, linoleic, and linolenic acids. The results showed that oleic acid was the most common acid present in the acylglycerols in the oil of all avocado cultivars. It ranged between 62.98% (in Pinkerton cultivar) and 71.88% (in Fuerte cultivar), while myristic acid was the lowest in all of the cultivars, it was ranged from 0.04% (Hass) to 0.19% (in Ettinger). Palmitic acid was the second fatty acid present in Pinkerton (17.02%), Ettinger (16.42%), and Reed (13.34%), while linolenic acid was the second fatty acid present in Hass (13.29%), Maluma (12.94%), and Fuerte (11.64%). Generally, total saturated fatty acids content ranged from 10.91% (in Fuerte) to 18.63% (in Pinkerton), total monounsaturated fatty acids content ranged from 69.35% (in Pinkerton) to 75.67% (in Fuerte), and polyunsaturated fatty acids content ranged from 9.5% (in Ettinger) to 14.89% (in Reed).

These findings typically correspond with reports from earlier research that avocados have proven their uniqueness with their high amount of unsaturated oils that may be over 80% of fatty acids and 13.5% of these are polyunsaturated (Takenaga et al., 2008; Ariza et al., 2011; Donetti and Terry, 2014). Avocado oil is an excellent source of omega fatty acids that benefit humans, in particular cardiovascular and other chronic diseases. (Ortiz et al., 2004).

Donetti and Terry (2014) found that avocado oil mainly consists of oleic (50 - 60 % of the fatty acid content), palmitic (15 - 20%), linoleic (11-15%), palmitoleic (6 -10%), and linolenic acid (approximately 1%). Yanty et al., (2011) revealed that the most frequent fatty acid of the Australian and Malaysian 'Hass" cultivars was oleic acid. Similar results were reported by Carvalho et al., (2014), who studied the effect of orchard height and fruit ripening stage on fatty acid formation in Hass avocado and found that oleic acid is the predominant fatty acid and that its percentage decreases significantly ($P < 0.05$), while amount of palmitolic and linoleic acids in avocado fruits of these orchards increases. They also demonstrate a high linear link to the stage of harvest stage, in particular palmitoleic acid, which significantly increased ($P < 0.05$) with the stage of ripeness of avocados at all regions.

Amino acid composition

The nutritional value and quality of dietary protein are among the key determinants in amino acid composition. Amino acids are the basic building blocks of proteins and are traditionally classified: (1) essential amino acids, which cannot be synthesized in the human body, therefore, it required to be taken in by diet, (2) non-essential amino acids, which can be synthesized in the human body. Amino acids have remarkably different biochemical properties and functions. The deficiency of essential amino acids leads to impaired growth, impaired immunity, slow wound repair, muscle weakness and dull looking skin and hair (Wu, 2009). Moreover, amino acids and their derivatives have been reported to exert influence on taste and quality in fruits (Ardo, 2006). Generally, high protein sources

Table 3: Fatty acid profile of oils from six different avocado cultivars

Fatty acid*	Ettinger	Fuerte	Hass	Maluma	Pinkerton	Reed
14:0	0.19±0.05a	0.11±0.04b	0.04±0.01c	0.05±0.01c	0.13±0.02b	0.13±0.03b
16:0	16.42±0.25a	10.43±0.31b	11.86±0.12b	12.71±0.15b	17.02±0.29a	13.34±0.24b
18:0	1.30±0.02a	0.37±0.02c	0.31±0.03c	0.36±0.02c	1.48±0.15a	0.72±0.1b
16:1	5.33±0.23b	3.79±0.26c	4.36±0.21c	6.75±0.34a	6.27±0.32a	6.14±0.3a
18:1	67.26±1.75b	71.88±2.61a	68.56±2.54b	65.54±3.12bc	62.98±3.19c	64.78±2.97c
18:2	7.87±0.92c	11.64±1.24b	13.29±1.53a	12.94±1.14a	11.17±1.37b	13.05±2.07a
18:3	1.63±0.09a	1.78±0.13a	1.58±0.17a	1.65±0.07 a	0.95±0.06b	1.84±0.13a
SFA	17.91	10.91	12.21	13.12	18.63	14.19
MUFA	72.59	75.67	72.92	72.29	69.25	70.92
PUFA	9.50	13.42	14.87	14.59	12.12	14.89

*% of the total fatty acid methyl ester content. Values (Mean±standard deviation, three replicates). Different letters in the same row means significantly differences among cultivars at $P<0.05$.

are also high in contents of amino acids including the nine essential amino acids: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Plant proteins are often characterized by their relatively low content of essential amino acids. High quality proteins usually derive from animal-based sources of nutrition, except for soy (Lopez and Mohiuddin, 2021).

Amino acid composition in avocado fruits is shown in Table 4. Results show that the acidic amino acids (glutamic and aspartic) were the greatest amino acids for all studied avocados, while S-amino acids (methionine and cystine) and tryptophane were the lowest acids for the six studied cultivars.

Chemical score

The chemical score procedure is the first attempt to evaluate the protein sources based on their ability to meet essential amino acids. requirements. This procedure aims to compare the percentage of an amino acid in the tested protein to the percentage of the same acid in a standard protein, which is usually the egg protein. The essential amino acid that greatest deficit in the evaluated protein diet is expressed as the first limiting amino acid (Whitaker and Tannenbaum, 1977).

Table 5 shows the chemical score of avocado protein. The results show that the amount of all the essential amino acids except for methionine was greater in avocado than its counterpart in the reference protein. Data indicate that avocado flesh protein is a very rich source of valine, tryptophan, and isoleucine with a chemical score of 142.29- 168, 119- 159, and 139.64- 150.7, respectively. It also contains a considerable score for other amino acids, except for methionine, which was are the first limiting amino acid followed by lysine as a second one.

Pigments content in avocados flesh

Carotenoids are responsible for the color of many vegetables and fruits. They are a family of over 700 various compounds in nature, but the most common carotenoids include α-carotene, β-carotene, lutein, lycopene, β-cryptoxanthin, and zeaxanthin. In general, the three major compounds that have provitamin A activity are α-carotene, β-carotene, and β-cryptoxanthin (Rozan, 2017). Table 6 shows pigments content in avocado flesh of the six cultivars cultivated in Egypt. Generally, total carotenoids, α-carotene, β-carotene, and lutein contents ranged from 5.66 to 6.91 μg/g, FW, 0.43 to 0.58 μg/g, FW, 1.08 to 1.43 μg/g, FW, and 2.94 to 3.84 μg/g, FW, respectively. There are significant ($P < 0.05$) differences in total carotenoids content between the cultivars. Hass avocado was the highest in its content of total carotenoids, α-carotene, β-carotene, and lutein, while Reed avocado was the lowest in total carotenoids content, Fuerte avocado was the lowest in α-carotene content, and Pinkerton avocado was the lowest in β-carotene and Lutein contents. Vinha et al., (2013) found that β-carotene content in the pulp of Hass avocados grown in the Portugal region is (0.810 mg/100 g sample). Also, Hass avocado was the highest cultivar in its content of total chlorophyll (29.5 μg/g, FW), Chlorophyll a (15.6 μg/g, FW), and Chlorophyll b (13.2 μg/g, FW), while Ettinger avocado was the lowest in total chlorophyll (7.4 μg/g, FW), Chlorophyll a (2.1 μg/g, FW), and Chlorophyll b (4.5 μg/g, FW).

Table 4: Amino acid content (% on 100 protein) of oils from six different avocado cultivars

Amino acids	Ettinger	Fuerte	Hass	Maluma	Pinkerton	Reed
Lysine	5.11±0.36c	5.61±0.56a	5.59±0.43a	5.63±0.71a	5.22±0.58c	5.41±0.31b
Isoleucine	3.91±0.22b	4.02±0.5ab	4.20±0.35a	4.22±0.29a	3.99±0.21b	4.04±0.28ab
Leucine	7.95±0.19a	7.06±0.5b	7.27±0.47b	7.19±0.61b	8.02±0.64a	7.89±0.32a
Phenylalanine	5.11±0.45b	4.71±0.38b	4.84±0.62b	4.86±0.37b	5.93±0.27a	5.97±0.32a
Tyrosine	2.31±0.24b	2.1±0.14c	2.42±0.21a	2.47±0.19a	2.23±0.17b	2.26±0.18b
Histidine	2.29±0.12b	2.2±0.12b	2.47±0.21a	2.52±0.22a	2.25±0.2b	2.31±25b
Valine	4.98±0.36c	5.88±0.32a	5.38±0.27b	5.36±0.1b	5.74±0.19a	5.29±0.36b
Tryptophan	1.63±0.07a	1.57±0.12a	1.25±0.14b	1.28±0.17b	1.19±0.11b	1.59±0.21a
Threonine	4.05±0.23c	3.85±0.52c	3.61±0.46d	3.69±0.27d	4.81±0.2a	4.48±0.25b
Methionine	1.66±0.1bc	1.59±0.19c	1.78±0.11b	1.92±0.23a	1.61±0.14c	1.74±0.28b
Aspartic	8.94±0.94d	10.74±0.57b	11.83±0.69a	11.86±0.48a	9.85±0.64c	9.12±0.85d
Glutamic	11.78±0.88c	13.45±0.6b	14.45±0.56a	14.39±0.47a	14.68±0.95a	14.86±0.71a
Proline	5.61±0.2a	4.76±0.38c	4.93±0.27c	4.92±0.43c	5.26±0.18b	5.91±0.3a
Serine	6.42±0.63a	5.81±0.41b	5.74±0.52b	5.77±0.49b	5.84±0.47b	5.67±0.56b
Glycine	5.92±0.45c	5.87±0.4c	6.20±0.37b	6.25±0.51b	6.34±0.27b	6.97±0.63a
Alanine	6.79±0.73a	6.06±0.34b	5.42±0.47c	5.49±0.33c	5.82±0.62bc	5.66±0.74bc
Arginine	4.68±0.36a	4.16±0.41c	4.47±0.19b	4.44±0.68b	4.79±0.67a	4.72±0.36a
Cystine	1.52±0.14a	1.49±0.07a	1.41±0.1a	1.39±0.17a	1.64±0.34a	1.54±0.12a

* Values (Mean±standard deviation, three replicates). Different letters in the same row means significantly differences among cultivars at $P<0.05$.

Table 5: The chemical score of avocado protein

Amino acids	FAO/WHO/UNU	Low value	High value	% amino acid chemical score
Lysine	5.80	5.11 Ettinger	5.63 Maluma	88.1-97.07**
Isoleucine	2.80	3.91±0.22 Ettinger	4.22±0.29 Maluma	139.64-150.71
Leucine	6.60	7.06±0.49 Fuerte	8.02±0.64 Pinkrton	106.97-121.52
Phenylalanine+Tyrosine	6.30	6.83 Fuerte	8.23 Reed	108.41-130.63
Histidine	1.90	2.18 Fuerte	2.52 Maluma	114.74-132.63
Valine	3.50	4.98±0.36 Ettinger	5.88±0.32 Fuerte	142.29-168
Tryptophan	1.00	1.19 Pinkrton	1.59 Reed	119-159
Threonine	3.40	3.61 Hass	4.81 Pinkerton	106.17-141.47
Methionine	2.20	1.59 Fuerte	1.92 Maluma	72.27-87.27*

Chemical score was calculated as a percentage of the FAO/WHO/UNU, 1985. *First limiting amino acid. **Second limiting amino acids.

Table 6: Pigments content in avocado flesh of six cultivars

Components	Ettinger	Fuerte	Hass	Maluma	Pinkerton	Reed
α-carotene (μg/g, FW)	0.47±0.04bc	0.43±0.03c	0.58±0.03a	0.51±0.05b	0.44±0.03c	0.48±0.05bc
β-carotene (μg/g, FW)	1.22±0.1c	1.16±0.13c	1.43±0.08a	1.30±0.1b	1.08±0.05d	1.17±0.11c
Lutein (μg/g, FW)	3.39±0.12b	3.05±0.2cd	3.84±0.21a	3.18±0.16c	2.94±0.1d	2.98±0.1cd
Total carotenoids (μg/g, FW)	5.94±0.15b	5.79±0.16b	6.91±0.1a	6.04±0.21b	5.84±0.19b	5.66±0.15b
Chlorophyll a (μg/g, FW)	2.1±0.09d	6.3±0.1c	15.6±0.12a	12.5±0.18b	7.3±0.2c	8.1±0.23c
Chlorophyll b (μg/g, FW)	4.5±0.07c	5.8±0.09c	13.2±0.17a	9.4±0.1b	7.5±0.15bc	7.2±0.14bc
Total chlorophyll (μg/g, FW)	7.4±0.12c	12.9±0.2bc	29.5±0.34a	23.9±0.31a	16.1±0.23b	16.9±0.21b

Values (Mean±standard deviation, three replicates). Different letters in the same row means significantly differences among cultivars at P<0.05.

The carotenoids are usually concentrated in the skin of the fruit, so the flesh of the fruits after peeling loses a large amount of these vital compounds. Vinha et al., (2013) reported that Carotenoids content in pulp, seed and skin of Hass avocado were 0.815, 0.966 and 2.585 mg/100 g FW, respectively.

Ahmed et al., (2010) recorded a significant decrease in the concentration of chlorophyll a and b, which led to color changes during the ripening process in Fuerte cultivar fruits.

Bioactive compounds

Bioactive compounds are mainly primary or secondary metabolism compounds that form within plant parts and have a beneficial human health impact when the parts containing them are eaten in the diet. The many nutrients, phytochemicals, and bioactive compounds in avocado fruits may promote a number of health advantages due to their role as antioxidants (Yasir et al., 2010 and Bayomy 2017). Among the most important compounds with antioxidant properties, phenols, flavonoids, and ascorbic acid, along with carotenoids, are the most well-known and present parts of many vegetables and fruits (Giuffrè et al., 2018). Avocados were known as the functional fruit due to its bioactive compounds which has beneficial effects on human health, including ascorbic acid, tocopherols, carotenoids and phenolic compounds (Mfonobong et al., 2013). Table 7 shows the concentration and significant differences (P <0.05) of total phenols, total flavonoids, ascorbic acid, and antioxidant activity in fruit flesh of six avocado cultivars grown in Egypt.

The results reveal that the Hass avocado cultivar is the highest one for total phenols content (4.9±0.38 mg/g FW), total flavonoids content (0.25±0.01mg/g FW), Ascorbic acid content (12.8±0.15 mg/100g FW), and DPPH value (1.3±0.09 μmol TE/g), while Reed cultivar shown the lowest total phenols content with 3.56±0.42 mg/g FW, and the lowest DPPH value with 0.5±0.04 μmol TE/g FW, and the lowest total flavonoids content on par with Ettinger and Fuerte cultivars with 0.19 mg/g FW, while Fuerte cultivar shown the lowest ascorbic acid content with 7.6±0.1 mg/100g FW. DPPH results are similar to that found by Wang et al., (2010), for the Hass variety (1.3 μmol TE/g) and Mardigan et al., (2018) for Breda variety (1.11 μmol TE/g). Vinha et al., (2013) found that total phenolics, flavonoids and ascorbic acid contents in pulp of Hass avocado were 410.2, 21.9 and 1.2 mg/100g FW, respectively.

Table 7: Concentration of bioactive compounds in the flesh of the six avocado cultivars

compounds	Ettinger	Fuerte	Hass	Maluma	Pinkerton	Reed
Total phenols (mg/g, FW)	3.92±0.36c	3.95±0.29c	4.9±0.38a	4.1±0.31b	3.89±0.25c	3.56±0.42d
Total Flavonoids (mg/g, FW)	0.19±0.03b	0.19±0.03b	0.25±0.01a	0.24±0.017a	0.2±0.04b	0.19±0.04b
Ascorbic acid (mg/100g, FW)	8.5±0.1b	7.6±0.13b	12.8±0.15a	11.3±0.1a	7.8±0.16b	8.6±0.2b
DPPH μmol TE/g)	0.7±0.03d	0.8±0.08c	1.3±0.09a	0.9±0.07b	0.8±0.07c	0.5±0.04e

* Values (Mean±standard deviation, three replicates). Different letters in the same row means significantly differences among cultivars at $P<0.05$.

Table 8: Phenolics profile of flesh of six avocado cultivars (mean±SD, n=3)

Compounds	Ettinger	Fuerte	Hass	Maluma	Pinkerton	Reed
Gallic	1.68±0.07b	1.52±0.03b	3.06±0.09a	2.94±0.11a	1.76±0.13b	1.45±0.09b
Chlorogenic	9.11±0.92a	9.47±0.75a	11.15±1.05a	10.95±0.87a	6.48±0.47b	6.80±0.69b
p-Hydroxybenzoic	0.34±0.03a	0.56±0.11a	0.46±0.09a	0.45±0.04a	0.62±0.1a	0.56±0.08a
Epicatechin	132.17±4.5e	153±3.6c	130.12±3.5e	140.51±4.1d	184.15±5.9a	165.27±4.3b
Vanillic acid	14.05±0.71c	17.23±1.5a	15.17±1.23b	14.78±0.97c	15.19±1.03b	16.19±0.98a
Ferulic	0.36±0.04c	0.47±0.07a	0.42±0.06b	0.33±0.03c	0.41±0.09b	0.47±0.07a
Sinapic	0.29±0.02d	0.39±0.04a	0.35±0.06c	0.35±0.05c	0.34±0.05c	0.38±0.06a
p-Coumaric	0.13±0.017b	0.26±0.014a	0.08±0.013c	0.09±0.015c	0.17±0.012b	0.11±0.01c
m-Coumaric	0.16±0.012c	0.31±0.01a	0.25±0.012b	0.12±0.013c	0.17±0.014c	0.18±0.001c
o-Coumaric	0.22±0.015c	0.35±0.01b	0.41±0.014a	0.37±0.016b	0.21±0.017c	0.24±0.004c
Protocatechuic	7.84±0.61b	4.52±0.31c	11.32±0.74a	12.37±1.02a	4.67±0.24c	6.74±0.84b
Rosmarinic	0.45±0.08c	0.29±0.01c	1.03±0.09a	1.11±0.12a	0.78±0.09b	0.83±0.1b
Cinnamic	0.74±0.11a	0.32±0.09c	0.34±0.011c	0.37±0.01c	0.68±0.06a	0.49±0.03b
Quercetin	1.32±0.96b	0.47±0.02e	2.09±0.14a	1.87±0.11a	0.67±0.09d	0.94±0.15c
Apigenin	0.91±0.12b	0.36±0.01d	1.20±0.09a	1.14±0.1a	0.86±0.09c	0.75±0.08c
Kaempferol	0.62±0.04a	0.28±0.01d	0.67±0.02a	0.48±0.02b	0.29±0.01d	0.34±0.01c

Values (μg/ g, DW) Mean ± standard deviation, three replicates). Different letters in the same row means significantly differences among cultivars at $P < 0.05$.

Phenolics profile

Phenolic compounds accumulate in plant parts as a defensive response against cellular stress, cold injury and/or ethylene exposure. Moreover, they may have a role in plant resistance to microbial diseases (Howard et al., 1994). Moreover, phenolic compounds can contribute to the quality of the fruit, especially it's color and aroma (Tomás-Barberán and Espin, 2001).

Under the present work conditions, 16 phenolic compounds were identified in the flesh of the fruits of the six avocado cultivars (Table 8). The individual phenolic compounds quantities in the flesh of avocado fruits were significantly ($P <0.05$) affected by cultivars. The major compound was epicatechin, which ranged between 130.12 μg/g DW (in Hass cultivar) and 184.15 μg/g DW (in Pinkerton cultivar), vanillic acid which ranged from 14.05 μg/g DW (in Ettinger cultivar) to 17.23 μg/g DW (in Fuerte cultivar), protocatechuic acid which ranged from 4.52 μg/g DW (in Fuerte cultivar) to 12.37 μg/g DW (in Maluma cultivar), and chlorogenic acid which ranged from 6.48 μg/g DW (in Pinkerton cultivar) to 11.15 μg/g DW (in Hass cultivar).

CONCLUSION

The length of the ripening season for each cultivar ranges from one and a half to two months. Hass cultivar recorded a higher value of firmness (89.5 N). All cultivars can be considered as good sources of K, P, and Mg elements, and moderate sources of Ca and Na elements, while they contain relatively limited quantities of Fe, Zn, and Mn elements. Total monounsaturated fatty acids content ranged from 69.35% (in Pinkerton) to 75.67% (in Fuerte), and polyunsaturated fatty acids content ranged from 9.5% (in Ettinger) to 14.89% (in Reed). Avocado oil is a good source of omega fatty acids that are beneficial for human health. The amount of all the essential amino acids except for methionine was greater in avocado than its counterpart in the reference protein. Methionine is the first limiting amino acid followed by lysine as a second one. The individual phenolic compounds quantities in the flesh of avocado fruits were significantly ($P <0.05$) affected by cultivars. The major compound was Epicatechin followed by vanillic acid and chlorogenic.

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