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Research Article

Effect of Maturity State of Avocado (*Persea americana* Mill. cv. Hass) on Seed Characteristics

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Abstract: The ripe and overripe avocado seeds were evaluated in order to determine the maturity stage influence on the proximal composition. Lignocellulosic compounds, lipid profile, total phenols content, condensed tannins as well as antioxidant capacity by ABTS and DPPH were measured. The starch content of ripe and overripe avocado seeds was 63.70 and 58.7%; protein 3.1 and 2.9%, 14.72 and 16.36% for cellulose, respectively. The hemicellulose content was higher in ripe seeds (49.75%) than in overripe seeds (34.15%). The unsaturated lipids content, corresponding to linoleic acid and oleic acid in the seed oil was higher in the ripe fruit, while the overripe seeds showed higher linolenic acid concentration. The main fatty acid was oleic with 49% in the ripe seeds and concerning total unsaturated lipids. Total phenols were 43.04 and 41.02 mg GAE/g, while the condensed tannins were 146.45 and 148.47 mg catechin/g for the ripe and overripe fruit seeds, respectively. Therefore, the antioxidant capacity and essential acids concentration were higher in the ripe seeds than in the overripe seeds, while the condensed tannins content had no statistical difference.

Keywords: Agro-industrial residue, antioxidant capacity, phenolic compounds, proximal composition

INTRODUCTION

The avocado nutritional and sensorial quality has influenced the increase of its consumption in many countries. The above is reflected in the cultivated areas increase and therefore in world production. In 2014, the United Nations Food and Agriculture Organization (FAOSTAT, 2014) reported a global output of 5,028,756 avocado tons, an increase of 141% in the last 20 years, with cultivated Hass being of higher commercial importance (Lopez-Cobo *et al*., 2016).

In avocado industrialization, unlike the flesh, the skin and seeds are discarded as waste. However, the Hass avocado seed represents on average 16.5% of the total mass of the fruit (Márquez *et al*., 2014) resulting in the production of more than 829,000 tons of seed/year, which are discarded. As a result, this situation causes pollution and environmental damage (Barbosa-Martín *et al*., 2016). On the other hand, the avocado seed residues have potential use since they are rich in polyphenols with antioxidant characteristics, including catechin, epicatechin, proanthocyanidin and photocatalytic acid (Geissman and Dittmar, 1965; Soong and Barlow, 2004).

Other studies of the physicochemical characterization of avocado seed show their nutraceutical potential due to the presence of fatty acids, polyphenolic compounds (Soong and Barlow, 2004) and sterols (Lozano *et al*., 1993). Additionally, several beneficial properties of the compounds present in the seed and the avocado peel have been reported, which are related to the presence of high levels of phenolic compounds in the seeds (64%), in a higher proportion than in the shell (23%) and pulp (13%) (Pahua-Ramos *et al*., 2012). Seeds and avocado peels contribute 57 and 38% of the antioxidant capacity of all fruit respectively (Wang *et al*., 2010).

In this context, the avocado seed bioactive compounds extraction is an interesting alternative to take advantage of this byproduct that is abundantly available. Nonetheless, little is known about the effects of fruit maturity stages for some physiochemical, nutraceutical and physiological characteristics of avocado seed.

Due to the above, the investigation objective was to evaluate the effect of two avocado stages (*Persea americana* Mill cv. Hass) on proximal composition, lignocellulosic compounds, lipid profile, total phenol content, condensed tannins and antioxidant capacity of the avocado seed. The final purpose was determining the avocado seeds potential as a support for solid fermentation for the bioactive polyphenolic compounds extraction, aspects that have not yet been explored.

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MATERIALS AND METHODS

Vegetal material: The avocado fruits (*Persea americana* Mill cv. Hass) were obtained from a local crop, located in El Peñol municipality, Horizontes, Antioquia, Colombia. Fruit maturation was carried out in the laboratory at room temperature at 23°C±2 and 65%±5 relative humidity. On day 12 the avocado seeds were removed for the optimum maturation stage and at day 18 for the over-ripening stage (Márquez *et al*., 2014). The evaluated stages of maturity correspond to the stages in which the seeds are usually discarded by the consumption in table and industrialization of the fruit or by the loss of organoleptic quality as a result of the over-ripening of the fruit.

The avocado seed was dried at 60°C for 24 h, then ground and stored in polypropylene bags at 22°C before use.

Avocado seed proximal composition: The starch content was determined according to the Ewers polarimetric method (ISO 10520, 1997). Calcium, sodium, copper, iron, magnesium, manganese, potassium and zinc contents were determined by using the atomic absorption spectrometry method according to the Colombian Technical Standard NTC 5151, 2003. The AOAC methods (AOAC, 2005) were applied to determine the total dietary fiber, protein, fat, ash and moisture.

The process used for the cellulose and hemicellulose determination was performed according to the ASTM 1695-77 (ASTM International, 2001) standard. The insoluble acid lignin content was evaluated according to the TAPPI method T222 om-02 (TAPPI Standard, 1988).

For the fatty acid lipid profile analysis, a derivatization process was performed to convert the fatty acids presentin the oil extracted from the avocado seed into non-polar low molecular weight derivatives, in order to improve the volatility and the sensitivity in the detention. To this end, 0.1 g of oil was weighed, 3 mL of cooled ethyl ether was added, 1 mL of trimethyl ammonium hydroxyl was stirred and added and then it was stirred again and the upper fraction was removed from the vessel.

The fatty acids in the oil and their concentration were evaluated according to the methodology proposed by Gómez-Coca *et al*. (2012). AGC-MS (Agilent Technologies® model 6890N, Santa Clara, USA), equipped with Split/Splitless Injector and the 5973N mass selective detector was used. A silica capillary column (5% diphenyl-dimethylpolysiloxane 95%) was used. The temperatures for the injector and the sensor were 300 and 325°C respectively, oil gas of the nitrogen carrier with a flow rate of 1 mL/min. The temperature ramp was 80°C, 1 min, then it rose to 15°C/min, at 140°C and finally, to 4.5°C/min at 335°C, 16 min.

Ethanolic extraction of polyphenolic compounds: An ethanolic extraction was performed and previously evaluated by other authors (Gómez *et al*., 2014), for which 4 g of material was dissolved in 15 mL of an ethanol/water solution (56:44, v/v). The mixture was placed in a 60°C bath for 20 min and then centrifuged for 10 min at 1000 g in a Hermle Z366K centrifuge (Gosheim, Germany). The extracts were purified on 0.2 µm cellulose filters and stored at -18°C until analyzed.

Total Phenol Content (TPC): The total phenol content was estimated by colorimetric analysis using Folin-Ciocalteau reagent (Merck, Germany). Four hundred and eighty µL of distilled water was taken and mixed with 20 µL of ethanolic extract, 1250 µL of sodium carbonate (20% w/v) was added, 250 µL of Folin reagent was poured and kept in the dark for 2 h. Absorbance was measured at 760 nm on a Thermo Scientific Genesys 10S spectrophotometer (Waltham, USA). The calibration curve was constructed using solutions of gallic acid (Panreac, Germany) at concentrations between 2 and 16 mg/L (R^2 = 0.993). The results were expressed as mg equivalents of gallic acid/g of material [mg GAE/g].

Content of condensed tannins: For the determination of condensed tannins, the HCl-Butanol technique described by Waterman and Mole (1994) was applied, which allowed quantifying the content of proanthocyanidins in catechin equivalents. Five hundred µL of ethanolic extract was taken into test tubes and mixed with 3 mL of HCl-Butanol and 100 µL of ferric reagent; then the tubes were heated in a metabolic bath at 100°C for 1 h and allowed to cool to room temperature. The absorbance was read at a wavelength of 460 nm on a Thermo Scientific Genesys 10S spectrophotometer (Waltham, USA). The calibration curve was constructed using catechin solutions (Sigma-Aldrich, USA) at concentrations between 0.25 and 1.0 mg/L (R^2 = 0.981). The results were expressed as mg of catechin per gram of material [mg catechin/g].

Antioxidant capacity: The antioxidant activity of the ethanolic extracts was evaluated using the ABTS and DPPH methods. The discoloration test of the cationic radical of ABTS was followed according to what was proposed by Re *et al*. (1999). A 7 mM solution of 2, 2'-azinobis (3-ethyl benzothiazole-6-sulfonic acid ABTS (Sigma-Aldrich, USA) was mixed with 2.45 mM potassium persulfate solution and it was allowed to react in the absence of light during 16 h for radical formation. Twenty µL Ethanolic extract solution was taken and mixed with 2000 µL from ABTS radical, vortexed and left in the dark for 7 min, then the absorbance was read at a wavelength of 734 nm. The calibration curve was constructed using solutions of Trolox (Sigma-Aldrich, USA) at concentrations

between 0 and 1616 µM diluted with ethanol (R^2 = 0.990). The results were expressed as mmol of Trolox equivalents per gram of material [mmol TE/g].

The procedure for evaluating the ability to stabilize the 2, 2-diphenyl-1-picrylhydrazyl DPPH free radicals reacting with H+ donor substances was performed according to the methodology reported by Berger *et al*. (2008). Twenty µL of ethanolic extract was mixed with 1980 µL of 0.05 µm DPPH (Sigma-Aldrich, USA) methanolic solution, it was allowed to react in the dark for 30 min and the absorbance was read at 517 nm. The calibration curve was constructed with solutions of Trolox (Sigma-Aldrich, USA) in concentrations between 0.16 and 0.46 mg/mL diluted with methanol (R^2 = 0.993). The results were expressed as mmol of Trolox equivalents per gram of material [mmol TE/g].

Statistic analysis: All experiments were performed in triplicate and the statistical analysis was performed in STATGRAPHICS Centurion XVI software, version 16.2.04. The Tukey test was used to compare the means of the compounds analysis with a level of significance of $p<0.05$.

RESULTS AND DISCUSSION

The composition results of the avocado seed confirm that this residue is an important source of starch, dietary fiber, protein and minerals.

The starch content of the ripe fruit seed (63.70%) was higher than the one found in the overripe fruit seed (58.7%) (Table 1). Bressani *et al*. (2009) reported a carbohydrate content in Hass avocado seed of 79.54%, which corresponds mostly to starch. Avocado seeds are a by-product of waste and have a high starch content. Nonetheless the product of the degradation processes during the over-ripening period some of these compounds can react product of the fermentation and other enzymatic actions, to generate new substances, they may even be consumed by seed respiration what may explain the decrease of starch in overripe fruit seeds relative to ripe fruit seeds (Chel-Guerrero *et al*., 2016).

A low-fat content was found in ripe fruit seeds and overripe fruits, 0.9 and 1.39% respectively (Table 1). Perea-Moreno *et al*. (2016) reported values between 1.47 and 1.97% in avocado seeds collected from guacamole producers in Andalusia, Spain, Saavedra *et al*. (2017) published values of 1.11%, which are similar to the overripe results.

The total dietary fiber content found was relatively high, 27.60% in the ripe fruit seed and 31% in the super-mature fruit seed (Table 1). Nevertheless, Pahua-Ramos *et al*. (2012) reported a higher content, corresponding to 34.8%, while Barbosa-Martín *et al*. (2016) found 47% of total dietary fiber.

The total dietary fiber content corresponds to non-digestible polysaccharide compounds (cellulose, hemicellulose, pectin) and non-polysaccharide compounds such as lignin (Barbosa-Martín *et al*., 2016). Dietary fiber consumption promotes beneficial effects on health as it reduces the risk of cardiovascular disease, cancer, diabetes and obesity (Elleuch *et al*., 2011; Pahua-Ramos *et al*., 2012; Ceballos and Montoya, 2013; Huang *et al*., 2015). In this context, avocado seed represents a viable alternative for use as a source of dietary fiber.

Table 1: The proximal composition of the avocado seed

Component	Ripe fruit seed	Overripe fruit seed
Native starch (%)	63.70	58.70
Fat (%)	0.90	1.39
Total dietary fiber (%)	27.60	31.00
Crude protein (%)	3.10	2.90
Humidity (%)	52.60	53.10
Potassium (%)	0.82	0.81
Ashes (%)	1.97	1.75
Phosphorus (mg/kg)	1000	873
Copper (mg/kg)	<5	<5
Calcium (mg/kg)	553	605
Iron (mg/kg)	7	<5
Magnesium (mg/kg)	544	423
Manganese (mg/kg)	5	<5
Sodium (mg/kg)	<500	<500
Zinc (mg/kg)	10	9

Table 2: The lignocellulosic content of the avocado seed

Component	Ripe fruit seed	Overripe fruit seed
Holocellulose (%)	64.46±6.46^a	67.42±1.13^a
Cellulose (%)	14.72±5.66^a	16.36±2.70^a
Hemicellulose (%)	49.75±0.80^a	34.15±3.88^b
Insoluble lignin (%)	9.82±1.36^b	15.25±0.54^a
Soluble lignin (%)	29.72±5.49^a	32.03±3.49^a

The results were expressed as means ± the standard deviation; The different letters a and b in the same row indicate significant differences

The protein values found in the seeds of ripe and over-ripe fruits were 3.1 and 2.9% respectively (Table 1). These values are in agreement with what found by Bressani *et al*. (2009) (3.44%) and Saavedra *et al*. (2017) (2.51%).

In the avocado seed, small amounts of minerals such as potassium, phosphorus, copper, calcium, iron, magnesium, manganese, sodium and zinc were found (Table 1).

For both seeds the lignocellulosic content was high. However, there were significant differences in the hemicellulose content, being higher for the ripe fruit seed, while the other lignocellulosic materials contents were higher for the overripe fruit seed, both for cellulose and lignin (Table 2). Barbosa-Martín *et al*. (2016) found lower values, 19.81% hemicellulose, 7.64% cellulose and 12.99% soluble acid lignin.

Lignocellulose is the main component of the cell wall plants and is composed of cellulose, hemicellulose and lignin, where the sugar polymers represent a large part of the biomass. It is an essential aspect since this type of waste is used for the substrate in fermentative processes (Behera and Ray, 2016).

Table 3 shows the lipid profile of oil extracted from avocado seeds.

Table 3: Avocado seed lipid profile

Analysis	Ripe fruit seed	Overripe fruit seed
Unsaturated fat (%)	0.53	0.057
Monounsaturated fat (%)	0.27	0.010
Polyunsaturated fat (%)	0.26	0.050
Saturated fat (%)	0.28	0.390
Linolenic acid omega 3 (%)	5.66	87.710
Linolenic acid omega 6 (%)	43.39	1.750
Oleic acid omega 9 (%)	49.05	10.520

Table 4: Total phenol content, condensed tannins and antioxidant capacity of avocado seed

Analysis	Ripe fruit seed	Overripe fruit seed
Total phenols (mg GAE/g)	43.04±4.04[a]	41.02±2.61[a]
Condensed tannins (mg catechin/g)	146.45[a]	148.47[a]
DPPH (mmol Trolox/g)	80.78±0.32[a]	77.01±2.27[b]
ABTS (mmol Trolox/g)	4.85±0.15[a]	4.60±0.04[b]

The results were expressed as means ± the standard deviation; The different letters a and b in the same row indicate significant differences

The unsaturated fat content, linoleic acid and oleic acid in the extracted oil were higher for the ripe fruit seeds, whereas the seed of the overripe fruit had higher linolenic acid content (Table 3). The primary fatty acid in the ripe avocado seed oil was oleic (Omega 9). The differences presented may be because the overripe seed presented oxidation of its fatty acids owing to the advanced stage of deterioration of the fruit. Davila *et al*. (2017) reported lower linolenic acid contents (1.66%) and linoleic acid (15.07%) but higher oleic acid content (50.96%). The different extraction methods used, as well as the harvesting time and the ripe fruit stage are conditions that can influence the oil composition (Pedreschi *et al*., 2016).

Table 4 presents the total phenols content, condensed tannins and antioxidant capacity of ripe and overripe avocado seeds.

The total phenols content and the antioxidant capacity by DPPH and ABTS in the ethanolic extracts were higher in the ripe seed, while the condensed tannins content had no statistical difference regarding the overripe seed stage, like the total phenols.

Ripe and overripe seed phenols found were similar to those reported by other researchers, Wang *et al*. (2010) (51.60 mg GAE/g), Gómez *et al*. (2014) (45.01 mg GAE/g) and Saavedra *et al*. (2017) (40.93 mg GAE/g). In studies carried out by other researchers, it has been found that in the avocado seeds the majority of polyphenolic compounds correspond mainly to catechin, epicatechin and procyanidins (Wang *et al*., 2010; Dabas *et al*., 2013).

The condensed tannins were relatively lower in the ripe fruit seed than in the overripe fruit. However, no reports have been found on the condensed tannins content in avocado seeds, although these compounds have been extensively studied in the grape seed. Bosso *et al*. (2016) found 8.3 mg/g using 50% acetone as a solvent and 4.3% mg/g with 50% ethanol.

The condensed tannins, also known as proanthocyanidins, are polymers formed by flavan-3-ol structures, have high antioxidant power due to the complex structural diversity and their related physicochemical properties (Dixon *et al*., 2005).

The ripe fruit seeds antioxidant capacity for DPPH and ABTS (80.78 and 4.85 mmol Trolox/g) had significant differences compared to the overripe fruit (77.01 and 4.60 mmol Trolox/g). These results may be because the overripe fruit showed an advanced deterioration, therefore affecting the antioxidant see compounds, which leads to a decrease in its antioxidant capacity. Other studies have found that the phenols concentration in several fruits decreases during maturation, probably due to the reduction of the primary metabolism in the excessively ripe fruit, resulting in the lack of substrates necessary for the biosynthesis of phenolic compounds. Which is seen also reflected in a loss of antioxidant capacity (Gruz *et al*., 2011; Ornelas-Paz *et al*., 2013; Siriamornpun and Kaewseejan, 2017).

In the DPPH assay found values were much higher than those ABTS assay found. Although the antioxidant capacity DPPH and ABTS assays are based on Electron Transfer (ET), the ABTS discoloration assay can be applied to hydrophilic and lipophilic antioxidants, whereas DPPH can only be dissolved in organic media (Arnao, 2000; Antololovich *et al*., 2002), suggesting that the antioxidant avocado seed compounds are mainly of Lipophilic nature.

Other researchers have also found high avocado seed antioxidant capacities using different techniques, e.g., Saavedra *et al*. (2017) reported an antioxidant capacity by DPPH of 165.97 mmol Trolox/100 g, Soong and Barlow, 2004 found by ABTS, 1160 μmol of ascorbic acid equivalents/g, even 55 times higher than the value found in the pulp, Wang *et al*. (2010) found 428.2 μmol Trolox/g by applying the ORAC oxygen radical absorbance test. The differences between the reported results likely due to the different analytical techniques used, as well as the solvents used in the extraction process (Durling *et al*., 2007; Maisuthisakul and Gordon, 2009).

CONCLUSION

Avocado seeds have high starch content, it being significantly higher in ripe fruit seeds than in overripe ones. The highest Omega 6 and 9 unsaturated fatty acids concentration were found in oil extracted from seeds of ripe avocados, whereas Omega 3 fatty acid was found mostly in oil extracted from seeds of overripe avocados. The overripe avocados seeds had a higher lignocellulosic material concentration, except the hemicellulose that was higher in the ripe avocados seeds. The highest antioxidant capacity and phenolic substances concentration were found in ripe fruits seeds.

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CONFLICT OF INTEREST

The authors declare that there is no interest conflict.

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