Research Article

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

1,2 Yepes Diana, 1 Marquez Carlos and 1 Cadena Edith

1 Universidad Nacional de Colombia, Calle 59A No 63-20,

2 Servicio Nacional de Aprendizaje, Calle 104 No 69-120, Medellin, Colombia

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 (Marquez 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-Martin 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 physicochemical, 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.

Corresponding Author: Yepes, Diana, Universidad Nacional de Colombia, Calle 59A No 63-20 Medellin, Colombia

This work is licensed under a Creative Commons Attribution 4.0 International License (URL: http://creativecommons.org/licenses/by/4.0/).
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 present in 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 detection. 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). A GC-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 (R² = 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 (R² = 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'-azino bis (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 (R² = 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 (R² = 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-Martin 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.

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-Martin 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 1: The proximal composition of the avocado seed

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

### Table 2: The lignocellulosic content of the avocado seed

<table>
<thead>
<tr>
<th>Component</th>
<th>Ripe fruit seed</th>
<th>Overripe fruit seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holocellulose (%)</td>
<td>64.46±0.46</td>
<td>67.42±1.13</td>
</tr>
<tr>
<td>Cellulose (%)</td>
<td>14.72±0.66</td>
<td>16.36±0.70</td>
</tr>
<tr>
<td>Hemicellulose (%)</td>
<td>49.75±0.80</td>
<td>34.15±0.88</td>
</tr>
<tr>
<td>Insoluble lignin (%)</td>
<td>9.82±1.36</td>
<td>15.25±0.54</td>
</tr>
<tr>
<td>Soluble lignin (%)</td>
<td>29.72±5.49</td>
<td>32.03±3.49</td>
</tr>
</tbody>
</table>

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 avocado seed.

The ripeness of the fruit led 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 Kaewsueejan, 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. 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 content in avocado seeds, although these compounds, which leads to a decrease in its antioxidant capacity.

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.

ACKNOWLEDGMENT

The authors thank the Universidad Nacional de Colombia and the Servicio Nacional de Aprendizaje, SENA for funding the study. To the biologist Jaison Martínez Saldarriaga, for his services and assistance in the experimental phase of the work.

CONFLICT OF INTEREST

The authors declare that there is no interest conflict.

REFERENCES


