Journal of Agricultural Science; Vol. 5, No. 12; 2013
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Physicochemical Parameters, Phytochemical Composition and Antioxidant Activity of the Algarvian Avocado (*Persea americana* Mill.)

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Received: September 2, 2013 Accepted: September 22, 2013 Online Published: November 15, 2013

doi:10.5539/jas.v5n12p100 URL: http://dx.doi.org/10.5539/jas.v5n12p100

Abstract

The physical, chemical and nutritional properties of *Persea americana* fruits variety 'Hass' produced in the Algarve region were studied. Edible and non-edible parts of the fruits (pulp, seeds and peel) were compared considering their possible contribution to improve the sustainability of the food and pharmaceutical industries. The nutritional contents evaluated were moisture, ash, proteins, fat, total soluble solids and acidity. It were also evaluated the contents of bioactive compounds (phenolics, flavonoids, carotenoids, ascorbic acid and vitamin E) and their influence in the antioxidant activity exhibited by the fruit material. The results of the analysis demonstrated that the Algarvian avocado has physical and chemical characteristics comparable or superior to avocados from other growing regions around the world namely, Mexico and California. With regard to the contents of bioactive compounds, the pulp of the Algarvian avocado proved to be rich in carotenoids (0.815±0.201 mg/100g), phenolic compounds (410.2±69.0 mg/100g) and flavonoids (21.9±1.0 mg/100g). The skin was superior to the pulp in the contents of all these compounds with 2.585±0.117 mg/100g of carotenoids, 679.0±117.0 mg/100g of total phenolics and 44.3±3.1 mg/100g of flavonoids. The seed, in turn, was the part of the fruit with the highest total phenolic content (704.0±130.0 mg/100g) and flavonoids (47.97±2.69 mg/100g). Regarding the concentration of vitamins C and E, the highest values were found in the pulp (5.36±1.77 mg/100g of vitamin E) and skin (4.1±2.7 mg/100g of vitamin C). The extracts obtained from the seeds demonstrated higher *in vitro* DPPH$^{\bullet}$ assay antioxidant activity (43%) than those obtained from the skin (35%) and the fruit pulp (23%). The contents of carotenoids, phenolic compounds and flavonoids found in the non-edible parts of the Algarvian avocado demonstrated that these byproducts could be an interesting inexpensive raw material for the food and cosmetic industries.

Keywords: *Persea americana* Mill., Algarvian avocado, food byproducts, bioactive compounds, antioxidant activity, edaphoclimatic conditions

1. Introduction

The *Persea americana* Mill. tree belongs to the family Lauraceae, genus *Persea* and is a plant native of Central America. Apart from its use as food the avocado is traditionally utilized for various medicinal purposes including as hypotensive, hypoglycemic and anti-viral, and is applied for the treatment of ulcers and cardiovascular diseases (Anita et al., 2005; Nayak et al., 2008; Raharjo et al., 2008; Anaka et al., 2009; Kosińska et al., 2012). To the avocados are equally attributed analgesic and anti-inflammatory properties (Adeyemi et al., 2002) and the avocado pulp is also used in various dermatological formulations namely, emulsions for the treatment of dry skin, protective agents against ultraviolet radiation, and anti-aging agents (Korać & Khambholja, 2011). Given the variety of uses that are assigned to ethnobotanical species *Persea americana* several studies have been conducted in order to unveil their biological activity (Gomez-Flores et al., 2008; Yasir et al., 2010; Pahua-Ramos et al., 2012). For example the characterization of phenolic components and antioxidant activity of hydroethanolic extracts of the avocado skin and seed revealed a predominance of compounds belonging to the group of

flavonoids, proanthocyanidins, and hydrocinnamic acids (Kosińska et al., 2012). Phenolics and flavonoids are bioactive compounds that have been related with a decrement of different deteriorative processes in the human body owing to their ability to reduce the formation and to scavange free radicals (Hidalgo et al., 2010). Rodríguez-Carpena and coworkers (2011) ascribed the high antioxidant activity exhibited by avocado extracts in various *in vitro* assays to these phenolic compounds. Chia and Dykes (2010) studied the essential oils of avocado and were able to demonstrate the antimicrobial activity of the skin and seeds of three different varieties of avocado ('Hass', 'Fuerte' and 'Shepard'). Other studies revealed that the avocado contains other classes of bioactive compounds with antioxidant properties and that are equally beneficial to Human metabolism, such as mineral constituents (phosphorus, magnesium and potassium), hydro and liposoluble vitamins (vitamin E, B, C and β-carotene, or provitamin A) (Honarbakhsh & Schachter, 2009; USDA, 2011). Given all the above, prominence has been given in certain countries, to public information about the avocado and its health promoting properties. An independent Australian organization, "The Heart Foundation" certified the fruit as healthy food for the heart and this certification with its appropriate logo is already used in advertising. The Californian Avocado Commission, has also driven efforts to publicize the fruit as health promoter, including conjoint publications with the American Dietetic Association, American Heart Association, and more recently, some press releases. For all the reasons above, the avocado is gaining worldwide recognition as healthy food and, consequently, a significant economic value. Hence, quite naturally, the avocado culture has attracted the interest of European farmers and, currently, it is already possible to find avocado orchards spread across Spain, Italy, Greece and Portugal. Regarding cultivars produced, 'Hass' and 'Fuerte' dominate the international market (Rodríguez-Carpena et al., 2011). In Portugal these fruits are being cultivated in the south (Algarve), where the soil and climatic conditions are more favorable. The cultivated area at present does not exceed 750 acres but it is rapidly expanding, as more and more farmers recognize the potential of this crop (Freire, 2012). The avocado tree is one of the most productive plants per unit of cultivated area. The Algarve region has a temperate Mediterranean climate, characterized by mild short winters and long, hot and dry summers. The soils of this region are mostly litholic not humic of sandstone, stoneware of Silves or similar. Given that the edaphoclimatic conditions play a fundamental role in plant metabolism and by this route in the chemical makeup of fruits, one of the objectives of this study was to evaluate the chemical and antioxidant composition of the Algarvian 'Hass' avocado and compare their content of phytochemicals with those of the same variety of fruit produced elsewhere. This is pioneering study, since, to the best of our knowledge, this is the first scientific characterization of the Portuguese avocado fruit. The non-edible parts of the fruit (skin and seed) were also studied in order to assess their potential use as cheap source of bioactive compounds for the food, pharmaceutical and dermocosmetic industries. The exploitation of non-edible parts of the fruits is an emerging trend which may prove to be very profitable in the near future. Firstly because it entails an important reduction in the production of waste, secondly, because the non-edible parts of some fruits, can concentrate high levels of valuable bioactive compounds, particularly natural antioxidants (Vinha et al., 2013).

2. Materials and Methods

2.1 Sample Collection and Preparation

All the avocado fruits, variety 'Hass' used in the present study came from an orchard located in the Faro area (Latitude: 37.019°, Longitude: -7.926°). The fruits, a total of 100 at the onset of ripening, were randomly collected and selected by their firmness, absence of mechanical damage and visible decay. Immediately after harvest the fruits were cleaned and prepared according to the requirements of the intended analysis. They were cut open to obtain their edible and non-edible portions (pulp, peel, and seeds, respectively) and stored at 4ºC. Six replicates of each sample were selected and analyzed. All analyzes were carried out over a period of time not exceeding two weeks after harvest.

2.2 Standards and Reagents

2,6-dichlorophenol-indophenol (Tillmans reagent), glacial acetic acid, meta-phosphoric acid, DL-α-tocopherol acetate sodium carbonate, β-carotene, petroleum ether, ascorbic acid, sodium phosphate, aluminium chloride and 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Methanol, the Folin-Ciocalteu reagent, sodium hydroxide, sulphuric acid, and gallic acid were purchased from Panreac Química S.L.U. (Barcelona, Spain). All aqueous solutions were prepared with Milli Q filtered water (resistivity >18 MΩ.cm) (Millipore, Bedford, MA).

2.3 Proximate Composition Analysis

Moisture, titratable acidity (TA), total soluble solids (TSS) were evaluated as quality fruit indices. The ash, total protein and total fat contents were also analyzed. A gravimetric assay was performed to evaluate the

physiological weight loss of the avocado fractions (pulp, peel, and seeds). It was calculated by the difference between initial and final weight. A porcelain capsule containing 5 g of each fresh avocado fraction was placed in a stove (WTC binder Klasse 2.0, Tuttlingen, Germany) at 105±1ºC, followed by regular weighing up to a constant weight. Results were expressed in water percentage (%). TA was determined by titrating 5 ml of avocado aqueous extract with 0.1 M NaOH, using phenolphthalein (1%) as indicator. Results were expressed as grams of tartaric acid per 100 g of sample, according to the methodology described by the Association of Official Analytical Chemists (2005). The TSS were quantified using a hand digital refractometer Leica Abbe Mark II (Leica, Buffalo, NY, USA) and expressed as °Brix.

As with all food analysis procedures it is crucial to carefully select a sample whose composition represents that of the food being analyzed and to ensure that its composition does not change significantly prior to analysis. The following methods (AOAC, 2005) were used to determine protein, fat and ash content in stored avocado pulp, peel and seed samples: micro Kjeldahl for protein (N x 5.7) (method 960.52) (Glass Model Pyrex-1); incineration at 550°C for ash (method 923.03) (PCSIR-Lhr); defatting in a Soxhlet apparatus (J.P.Selecta–Spain) with 2:1 (v/v) chloroform/methanol for lipids (method 920.39C). All experiments were repeated in sextuplicate and the values are presented as mean (±SD).

2.4 Bioactive Compounds Quantification

2.4.1 Extraction and Analysis of Ascorbic Acid

Avocado fruit fractions (5 g) were dissolved in a mixture of 200 ml of water, 5 ml of metaphosphoric acid (30%) and 20 ml glacial acetic acid. The mixture was titrated with Tillmans reagent. Ascorbic acid (expressed as mg/100g (on a FW – fresh weight basis)) was quantified using an analytical validated method published in a previously work (Vinha et al., 2012).

2.4.2 Colorimetric Determination of Tocopheryl Acetate (Vitamin E)

The determination of the vitamin E content in the different constituents of Algarvian avocado fruit followed the procedure described by Amin (2001). From a standard solution of α-tocopherol acetate in 100 ml of methanol, several dilute solutions were prepared by taking 10, 25, 50, 100, 200, 400 µl aliquots of the stock solution and placing them in 25 ml calibrated flasks.

α-tocopheryl acetate was converted into α-tocopherol by transesterification. Standards were prepared by taking 10, 25, 50, 100, 250 and 500 µl portions of stock solution in 25 ml calibrated flasks, adding a drop of sulphuric acid, to catalyze the reaction, and 20 ml of methanol to each, and heating at 90°C in a water-bath for 90 min; within this period, the flask contents were reduced almost to dryness. The end-product of transesterification was dissolved in 15 ml of methanol, and 5.0 ml of NaOH (0.2 M) were added. The absorbance at 526 nm was measured after 10 min of heating in a water-bath at 90±2°C. The experiments were performed in sextuplicate for each avocado fruit fraction (pulp, peel, and seed).

2.4.3 Total Carotenoids Assay

Total carotenoids were extracted according to Akin et al. (2008) with some minor modifications. Briefly, five grams of sample were homogenized using a high-speed homogenizer, at 5000 rpm for 30 minutes (Heidolph, Diax 900, Germany) and then transferred to a separating funnel for extraction with 100 ml of methanol/petroleum ether (1:9, v/v). The petroleum ether layer was then filtrated through sodium sulphate, transferred to a 100 ml volumetric flask and dissolved with petroleum ether. Finally, total carotenoid content was measured spectrophotometrically (Hitachi UV-2800 spectrophotometer) at 450 nm by using an extinction coefficient of 2592. Results were expressed as β-carotene equivalents (milligrams per 100 g of FW).

2.4.4 Total Polyphenolic Content Assay

Total phenolics were determined according to the improved Folin-Ciocalteu method (Zieliski & Kozowska, 2000). Briefly, 5 g of fresh avocado fruit fractions were homogenized by using a homogenizer (model F.60, Falc Instruments, Italy) in water (100 ml) kept at 40°C for one hour and then filtered. The avocado fruit extracts were then resuspended in water and the supernatant (0.5 ml) was mixed with 0.5 ml of Folin-Ciocalteu's solution. The solution was homogenized for 3 minutes and 1 ml of saturated Na_2CO_3 was added. The solution was then incubated for 1 hour in the dark to obtain color development, through the reduction of phosphomolybdic and phosphotungstic acids in alkaline medium. The absorbance readings were measured at 720 nm with an UV-VIS spectrophotometer (Shimadzu UV-2100), using gallic acid (GA) as standard. Total phenol content was expressed as milligrams of GA equivalent (GAE) per 100 grams of fresh fruit weight (mg GAE /100 g^{-1} FW).

2.4.5 Total Flavonoids Content

Flavonoid contents in the aqueous extracts of the pulp, peel, and seeds of avocado fruits were determined using a method described by Soares et al. (2013) with slight modifications. Aliquots of 1 ml of extract solution were mixed with 4 ml of water and 300 µL sodium nitrate 25%. After 5 min incubation at room temperature it was added 300 µl of $AlCl_3$ reagent (10%), and left to react for one minute before adding 2 ml of sodium hydroxide and 2.4 ml of water. The absorbance was recorded at 510 nm in a BioTek Synergy HT microplate reader (GENS5). The flavonoid contents were express in milligrams per 100 grams of FW.

2.5 DPPH• Radical-Scavenging Activity

Pulp, peel and seeds of avocado aqueous extracts (300 µl) were mixed with 2.7 ml of an ethanolic solution containing DPPH• (2,2-diphenyl-1-picrylhydrazyl radical) in a concentration of 6 $x10^{-5}$ M. The mixture was shaken vigorously and left to stand in the dark until stable absorbance readings at 517 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH• discoloration using the equation: % RSA = [(ADPPH• – AS)/ADPPH•] x 100, where AS represents the absorbance of the sample solution extract with DPPH•· and ADPPH• is the absorbance of the DPPH• solution.

2.6 Statistical Analysis

A completely randomized design was used, with six replications. Statistical analysis was performed using SPSS v. 21 (IBM Corp., Armonk, NY, USA). Data of all chemical analysis were expressed as mean ± standard deviation. The independent samples T-test or Analysis of Variance (ANOVA) were used to assess the statistical differences among means followed, in the case of ANOVA, by Tukey's HSD post-hoc test for multiple comparisons. Pearson correlation tests were used to ascertain the existence of linear relationships between the contents of bioactive compounds and antioxidant activity. The level of significance for all hypothesis tests (*p*) was 0.05.

3. Results and Discussion

As previously referred, the objectives of this study were to characterize the Algarvian avocado in terms of food and potential source of bioactive compounds for the food and cosmetics industries. The results obtained for the fruit physicochemical parameters are presented in Table 1.

Table 1. Physicochemical parameters of the different fractions of the Algarvian avocado variety 'Hass'. Moisture, proteins, ash and fat are expressed in percentage. The Total Soluble Solids in °Brix and the acidity in mg of tartaric acid equivalents /100g FW

	Fraction of the Algarvian avocado var. 'Hass'		
Parameter**	Pulp*	Skin*	Seeds*
Moisture (%)	70.83±3.53[a]	69.13±2.58[b]	54.45±2.33[c]
Ash (%)	1.77±0.16[a]	1.50±0.08[b]	1.29±0.03[c]
Proteins (%)	1.82±0.07[a]	1.91±0.08[a]	2.19±0.16[b]
Fat (%)	43.5±4.62[a]	2.20±1.65[b]	14.7±0.32[c]
Total Soluble Solids (°Brix)	6.68±1.02[a]	3.01±2.03[b]	3.54±1.97[b]
Acidity	1.07±0.02[a]	2.05±0.24[b]	2.67±0.17[c]

As shown in Table 1, *Values represented as mean±standard deviation obtained from six measurements; **A letter is used to express the result of the comparison between the different fractions. Different letters indicate significant statistical differences (95% significance).

The moisture content is one of the most important indices evaluated in foods, especially fruits. It is a good indicator of their economic value because it reflects solid contents and serves to assess its perishability. The results indicate that the Algarvian avocado pulp has a higher water content (70.83%) , followed the skin (69.13%) and seed (54.45%). The fat and ash quantified in pulp were significantly superior to those found in the skin. The seed was the part of the fruit that had higher amounts of total protein (2.19%) and lowest ash content (1.29%), nevertheless, relative to its fat content, showed higher percentages compared to those found on the exocarp. According to Hernández-Muñoz et al. (2006) the total acidity is a measure of the organic acid content. The predominant acid found in avocados is tartaric acid although, theoreticaly, every species capable of donating a proton, including fatty acids, also contribute to the total acidity of the fruit (Omar et al., 2012). Acidity and soluble solids content are the common quality attributes that are associated with the maturity index of

agricultural products, especially fruits. The total acidity tends to decrease during the ripening period as a result of the breathing process or conversion into sugars. In the period of maturation of the fruit there is an increase in metabolic activity and organic acids are, par excellence, a source of energy reserve of the fruit through the Krebs cycle. In the case of the mature Algarvian avocado, the seed has higher acidity than the skin or pulp. The acidity of the pulp was found to be superior to that exhibited by 'Hass' avocados of American origin (0.04±0.01% citric acid) (Arias et al., 2012). In any case Algarvian 'Hass' avocados may be considered a non acidic fruit.

Among the various components of fruit, the total soluble solids (i.e., the percentage of solids that are dissolved in the matrix of the food) in °Brix, have a primary role in their quality due to the influence on thermophysical, chemical and biological properties. It is also a parameter which tends to increase with the progress of ripening due to the biosynthesis of the plant and degradation of polysaccharides. As expected, given that this physico-chemical parameter represents one of the best ways to evaluate the degree of sweetness of the fruit, and the fruit pulp is the only edible part of the avocado, the total soluble solids are higher in the pulp. This parameter follows a trend that is opposite to acidity. Nevertheless the content of soluble solids, although superior to those reported for 'Hass' avocados of American origin (5.1±0.1°Brix) (Arias et al., 2012), can be considered low, favoring the consumption of the Algarvian avocado *in natura*. Superior values of TSS have been reported for 'Hass' avocados from New Zeland (~9° Brix) (Burdon et al., 2007).

The characteristics of a fruit depend on the cultivar, the edaphoclimatic conditions of the region of provenance, ripeness and storage conditions (Ahmed et al., 2010). Tango et al. (2004) studied 24 varieties of avocado, and found levels for moisture and fat in the pulp of 'Hass' variety fruits of 57.3% and 31.1%, respectively. These values are significantly lower than those found in the Algarvian avocado studied here. Regarding the avocado seeds, Olaeta et al. (2007) observed higher protein concentrations and ash, compared with those recorded in this study (3.18% and 1.51%, respectively). Lu et al. (2009) on the other hand, reported a value of 25% fat for the pulp of 'Hass' avocados cultivated in California. The Algarvian avocado develops mainly during the winter because during the rest of the year the orchards in the Algarve are subjected to water stress. This is an important factor to justify the results presented in Table 1.

There is already evidence that the ingestion of fruits confers protection against human chronic diseases, neurological disorders and some types of cancer (Middleton et al., 2000; Pandey & Rizvi, 2009; Hamid et al., 2010). These properties are assigned to the presence of significant levels of bioactive antioxidant compounds in fruits. For this reason, those molecules are attracting a growing interest from the scientific community. During the last decades, ample evidence of the benefits of avocado on health has been gathered (Yasir et al., 2010; Al-Dosari, 2011). This promoted their consumption, stimulating also the research about their pharmacological potential. The maturation of any fruit promotes an increase of bioactive compounds (Arancibia-Avila et al., 2008). Among the different secondary metabolites with antioxidant properties, phenolics, flavonoids and carotenoids are the most cited. The levels of these compounds, as well as those of the vitamins C and E, found in the Algarvian avocado are presented in Table 2.

Table 2. Concentration of bioactive compounds present in different Algarvian avocado 'Hass' var. fruit fractions

	Avocado fraction var. 'Hass'		
Bioactive compound **	Pulp*	Skin*	Seed*
Total Phenolics	410.2±69.0[b]	679.0±117.0[a]	704.0±130.0[a]
Flavonoids	21.9±1.0[b]	44.3±3.1[a]	47.9±2.7[a]
Carotenoids	0.815±0.201[b]	2.585±0.117[a]	0.966±0.164[b]
Vitamin C	1.2±0.7[c]	4.1±2.7[a]	2.6±1.1[a, c]
Vitamin E	5.36±1.77[a]	2.13±1.03[b]	4.82±1.42[a]

As shown in Table 2, *Values represented as mean±standard deviation mg/100g FW obtained from six measurements; **A letter is used to express the result of the comparison between the different fractions. Different letters indicate significant statistical differences (95% significance).

The results reveal that is in the avocado seed that the highest levels of total phenolics and flavonoids are found. This agrees with the results reported for avocados cultivated in Mexico (Wang et al., 2010). The skin of the fruit had the highest carotenoid content, as expected, since this tissue is usually the fraction were these phytochemicals

are concentrated. Recently a study proved that the composition of carotenoids and vitamin E in fruits is affected by several factors, including the degree of maturation and edaphoclimatic (Arancibia-Avila et al., 2008). Significant differences were found in the levels of carotenoids and vitamin E in 'Hass' avocados cultivated in four different Californian counties. It was concluded in the same study that the levels of carotenoids in the fruit pulp increased with the fat present in it and that the xanthophylls, in particular lutein and cryptoxanthin, were the predominant phytochemicals of this group, contributing approximately to 90% of the total carotenoids present in the 'Hass' avocado (Lu et al., 2005). When one compares the contents of bioactive compounds of the Algarvian fruit with those of other fruits produced in different parts of the globe, it may be noted that it has levels of phenolics in the pulp comparable to those found in Mexican 'Hass' avocados (4.9±0.7 mg GAE/g FW), inferior levels in the skin (12.6±0.3 mg GAE/g FW) and seeds (51.6±1.6 mg GAE/g FW) (Wang et al., 2010) while possessing comparable levels of flavonoids (26.36 QE/100 g FW) (Rodríguez-Carpena et al., 2011). The phenolic levels are also superior to those reported for the same fruit of Turkish provenance (1.20±0.02 g/kg FW) (Golukcu & Ozdemir, 2010). The content of carotenoids is inferior to that found in Californian avocados (42.2 μg/g) (Lu et al., 2009) and higher than that found in the corresponding fractions of Mexican 'Hass' avocados ((7.1±0.6 μg/g (pulp), 15.2±2.7 μg/g (skin), 6.3±0.9 μg/g (seed)) (Wang et al., 2010). Furthermore the Algarvian avocado has superior levels of carotenoids in the pulp than the 'Hass' avocados cultivated in New Zeland (~5.2 μg/g), but inferior levels in the skin (~50 μg/g) (Ashton et al., 2006). The mesocarp of the Algarvian avocado presented higher levels of vitamin E, with a value that is statistically similar to that found in the seeds and above that found in the skin. The amount of this vitamin found in the pulp is comparable to that found in avocados grown in Brazil (6.4 mg/ 100g) (Salgado et al., 2008) but superior to that of avocados from california (27 μg/g) (Lu et al., 2009). The concentration of ascorbic acid is inferior to that reported for Californian avocados (17.3 mg/100g) (USDA, 2011).

Overall these results also demonstrate the potential of the non-edible parts of the avocado as a source of bioactive compounds. The skin of the Algarvian 'Hass' avocado contains 59% of the carotenoids and the seeds 39% of total phenolic compounds and 42% of the flavonoids present in the fruit. Instead of being wasted as trash, fruit skin could constitute an inexpensive source of carotenoids in the dermocosmetic and food industries. Indeed the avocado is the fruit with the highest content of carotenoids in the exocarp. The carotenoid compounds are known to exert a protective action against cell damage caused by UV rays and pollution, which make them an essential ingredient of several dermatological formulations. Additionally the carotenoids, phenolics and flavonoids are known to prevent the risk of developing certain diseases related to age, such as premature aging, cancer and heart disease (Hidalgo et al., 2010). Both the skin and the seeds can also be harnessed as a source of these compounds to use as food additives (Ayala-Zavala et al., 2011). Remarkably the skin and seeds of avocado have higher levels of these compounds than those that exist in many other fruits and vegetables such as apple (*Malus domestica*), banana (*Musa cavendish*), tomatoes (*Lycopersicum esculentum*) or red cabbage (*Brassica oleracae var. botrytis*) just to name a few (Marinova et al., 2005; Lin & Tang, 2007; Sulaiman et al., 2011; Vinha et al., 2013).

Consistent with the fact that they contain higher levels of bioactive antioxidant compounds, it was found that the avocado seeds also exhibit higher, and statistically different, values of *in-vitro* antioxidant activity (measured in this work through the ability to scavenge the 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$)), Figure 1.

These results differ slightly from those reported in the literature since both Wang et al. (2010) and Rodríguez-Carpena et al. (2011) showed that the skin had superior antioxidant activity. In fact it turns out that both the skin and the seeds of avocado fruit are very rich in antioxidant compounds however the seed has greater content of flavonoids and phenolic compounds while the skin is richer in carotenoids. In general, the contribution of vitamin C to the total antioxidant capacity of extracts varies with the type of fruit. In fact, vitamin C due to its hydrophilic character is unique among the vitamins present in the avocado matrix, the majority of which, namely vitamins A, D and E, are all liposoluble. It is well known fact that the bioactive compounds do not all have the same antioxidant activity, thus, an increase in the level of a compound does not mean a proportional increase of antioxidant activity of the matrix (Sanjust et al., 2008). Furthermore for a complex extract, as the one in question, it is also necessary to take into account the synergistic or antagonistic effects among the various compounds present, which makes not only the antioxidant activity dependent of the concentration of each compound but also of the interaction between different compounds, antioxidants or not. Perhaps this is why when the concentration of the extracts doubles, the antioxidant activity exhibited by the pulp increases but remains unaltered in case to the skin and seeds.

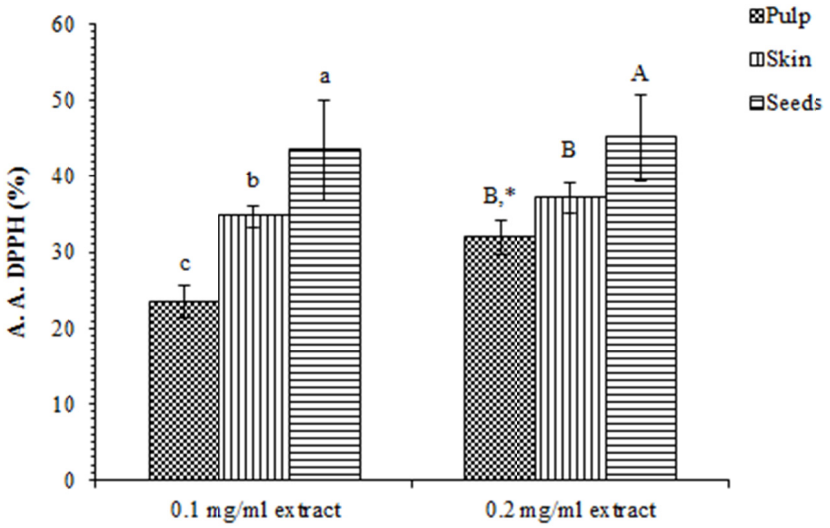

Figure 1. Antioxidant activity (A.A.) of aqueous extracts obtained from the various avocado fractions on 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•). The symbol "*" indicates the existence of significant statistical differences ($p<0.05$) among the antioxidant activity exhibited by the two aqueous extracts of the same fraction. Identical letters signalize extracts that exhibit the same antioxidant activity

Most studies have demonstrated a linear correlation between total phenolic content and the antioxidant activity evaluated by different methodologies in fruits and vegetables (Mahattanatawee et al., 2006; Corral-Aguayo et al., 2008). Regression analyses were performed to correlate the antioxidant activity of avocado samples with the antioxidants quantified in the avocado tissues (Table 3).

Table 3. Correlation among the contents of bioactive compounds and DPPH• antioxidant activity

Extract matrix	Flavonoids x DPPH•	Phenolics x DPPH•	Carotenoids x DPPH•	Vitamin C x DPPH•	Vitamin E x DPPH•
Pulp	-0.436	-0.094	-0.314	0.238	0.123
Skin	0.678	-0.430	-0.132	0.220	-0.880
Seeds	-0.506	0.715	0.703	0.011	0.641

Considering all the different antioxidant compounds, a good correlation was found between total phenolic content determined by the Folin-Ciocalteu and flavonoids contents and DPPH radical scavenging capacity ($r = 0.783$) and ($r = 0.820$), respectively. However, analyzing the fruit fractions separately, good positive correlations were only found for the contents of carotenoids, total phenolics and vitamin E and antioxidant activity exhibited by the seeds extracts and the contents of flavonoids in the case of the skin extracts.

4. Conclusions

Despite not being native of the region, the Algarvian avocado variety 'Hass' is a fruit with excellent physical and chemical characteristics, with moisture, protein, fat and ash comparable or superior to 'Hass' avocados from Mexico and California. Its levels of bioactive compounds are also comparable in the different constituent fractions of the fruit. Thus, for every 100 g of Algarvian avocado var. 'Hass', its edible portion (pulp) has, on average, 410.2±69.0 mg of total phenolics, 21.9±1.0 mg of flavonoids, 0.815±0.201 mg of carotenoids, 1.2±0.7 mg de vitamin C and 5.36±1.77 mg of vitamin E. The non-edible parts, i. e., the skin and seeds were found to have an average of 679.0±117.0 mg total phenolics, 44.3±3.1 mg of flavonoids, 2.585±0.117 mg of carotenoids, 4.1±2.7 mg of vitamin C and 2.13±1.03 mg of vitamin E (skin) and 704.0±130.0 mg of total phenolics, 47.97±2.69 mg of flavonoids, 0.966±0.164 mg of carotenoids, 2.6±1.1 mg of vitamin C and 4.82±1.42 mg of vitamin E (seeds). In accordance with the higher levels of bioactive compounds the extracts obtained from the seeds and skin of avocado

presented higher antioxidant activity against the DPPH• (43% and 35%, respectively) compared to that exhibited by the pulp (only 23%). The fact that the non-edible parts of the fruit (skin and seeds) contains such high levels of carotenoids, flavonoids and phenolics makes the idea of their exploitation, as a cheap source of these compounds in the food industry and dermo-cosmetics, very appealing. The mass of byproducts obtained as a result of processing tropical exotic crops, such as that of avocado, may approach or even exceed that of the corresponding edible part affecting the economics of growing of these crops.

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