

Chapter 9

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Avocado and Its By-products: Natural Sources of Nutrients, Phytochemical Compounds and Functional Properties

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ABSTRACT

Avocado (*Persea americana* Mill.) is a tropical and subtropical fruit that is native to Mexico and Central America. This fruit is gaining increasing worldwide acceptance and has received extensive marketing and a wide distribution due to its relevant nutritional benefits for human health. This work presents relevant information on the production, composition and application of avocado, with an emphasis on its by-products, focusing on the proper use of waste and the possibility of monetizing waste for nutritional and environmental purposes. The entire avocado is rich in bioactive compounds (pulp, peel and seed) and presents several health benefits, such as antimicrobial, antioxidant and anticancer activities, as well as dermatological uses and others. Therefore, several food grade ingredients can be obtained from avocado wastes, particularly premium-grade fats or extracts with a high functional power. Studies should continue to identify the profiles and phytochemicals available to the business sector, which can also be implemented to valorize the nutritional and functional potential of avocado seeds and peels.

Keywords: Avocado fruit; by-products; nutritional composition; bioactive compounds; antioxidant activity.

1. INTRODUCTION

Persea americana, commonly known as avocado fruit, has recently achieved significant popularity and is often marketed as a “superfood” because of its unique nutritional composition, phytochemical content, and health benefits. *P. americana* is native to Mexico and Central America, being a member of the flowering plant family Lauraceae [1]. Apart from its use as food, the avocado is traditionally used for several medicinal purposes including hypotensive, hypoglycemic, anti-viral and anti-diarrheal and cardiovascular diseases [2-8]. Thus, to these fruits are equally attributed analgesic, anti-inflammatory and antifungal properties [9] and their pulp is also used in several dermatological formulations namely, emulsions for the treatment of dry skin, protective agents against ultraviolet radiation, and anti-aging agents [10,11]. A large variety of phytochemicals commonly consumed in human diet, promote health benefits and may contribute to the prevention of many diseases. However, it is still difficult to make nutritional recommendations for these bioactive compounds. Current studies of phytochemicals are generally focused on specific compounds and their effects on a limited number of markers. New approaches are needed to take into account both the diversity of phytochemicals found in these fruits and the complexity of their biological effects. Firstly, it is necessary to mention that there are three major races with different growing conditions and characteristics: Mexican race (*P. americana* var. “drymifolia”), Guatemalan race (*P. nubigena* var. “guatemalensis”) and West Indian race (*P. americana* var. “Americana”). Thus, hybrid forms exist among all three types or races. Despite the pre-established morphological differences and botanical

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characterization, all varieties are known to contain, besides fat (monounsaturated (oleic acid), and polyunsaturated fatty acids (linoleic acid and linolenic acid), protein (highest among fruits), sugars (sucrose and D-mannoheptulose), natural pigments, polyphenols, phytoestrogens, and fiber [12]. Besides their association with biological factors, avocados have also been studied for their nutritional profile and phytochemical contents, revealing great variability among species and sometimes even within the same species. The reported differences are often modulated by soil and climatic conditions, besides being influenced by the avocado tree regeneration processes.

2. AVOCADO FRUIT AND AVOCADO BY-PRODUCTS

Different varieties of avocado are cultivated worldwide, and Hass and Fuerte are among the most consumed ones. Avocado industrial processing generates large quantities of agro-industrial by-products, mainly peel and seed, ranging from 18% to 23% of fruit dry weight [13]. Thus, it is interesting to reuse these by-products both to reduce their negative impact on the environment and to add value to them, in as much as they are sources of important phytochemical compounds.

2.1 Fruit Pulp

Avocado is a low sugar fruit with high contents of protein, fibre and vitamins (A, B, C and E). It is an excellent source of potassium and phosphorus, and contains mono-unsaturated fatty acids which effectively reduces the levels of low density lipoproteins, in the blood helping in the prevention of coronary heart diseases [14]. Thus, avocado pulp is recognized to present high phenolic compounds content of different chemical classes from simple phenolic acids such as gallic acid to larger flavonoids, anthocyanidins, and tocopherols with significant antioxidant, neuroprotective and cardioprotective activities [15-18]. Also, a natural compound (2R,4R)-1,2,4-trihydroxyheptadec-16-yne, extracted from the unripe pulp, may inhibit dengue virus replication [19].

2.2 Fruit By-products

Avocado seeds (an industrial by-product) can be used to prevent and treat gastric disorder due to its anti-inflammatory, antioxidant and antimicrobial properties [20]. *P. americana* leaves and seeds exhibited anti-cholinesterases and antioxidant activities [21].

Given the variety of uses that are assigned to ethnobotanical species of *P. americana* several studies have been conducted in order to unveil their biological activity [22,23]. In fact, avocado is currently considered "superfood" because of its unique nutritional and phytochemical composition compared to other fruits [12]. 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 [5]. Phenolic compounds, such as gallic acid, 3,4-dihydroxyphenylacetic acid, 4-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, ferulic acid and quercetin, existing in avocado pulp, are associated with antioxidant properties [18]. Ortega-Arellano et al. [17] concluded that avocado peels var. Colinred showed the highest total phenolic content, and specifically B-type procyanidins and epicatechin, as well antioxidant activity, when compared with seeds. These authors also showed that peel extract can protect transgenic parkin *Drosophila melanogaster* fly against paraquat-induced oxidative stress, movement impairment and lipid peroxidation, as model of Parkinson's disease. Segovia et al. [1] also demonstrated that antiradical activity is mainly due to polyphenols (+)-catechin, (-)-epicatechin, 3-*O*-caffeoylquinic acid (chlorogenic acid isomer), and three compounds of the flavonoid family. Flavonoids was one of the most representative group in seed and seed coat avocado, being quercetin, (\pm)-naringenin and sakuranetin identified by Figueroa et al. [24]. 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 scavenge free radicals [25]. Rodriguez-Carpena et al. [26] ascribed the high antioxidant activity exhibited by avocado extracts in various *in vitro* assays to these phenolic compounds. Chia and Dykes [27] 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'). Avocado ('Hass' and 'Fuerte') seeds, that contain diverse active compounds (phenolic acids and flavonoids), showed to act effectively against pathogenic bacteria, demonstrating high antimicrobial activity [28]. The peel of this fruit also showed a wide range of antimicrobial activities against Gram positive or Gram negative bacteria [29].

Other studies revealed that the avocado contains other constituents that are equally beneficial to Human metabolism, such as minerals (iron, phosphorus, magnesium, potassium, sodium, manganese, calcium, selenium, zinc and copper), hydro and liposoluble vitamins (vitamin E, B, C, K₁ and β -carotene, or provitamin A) [11,30,31]. The predominant carotenoid in this fruit is lutein, but α -carotene, β -carotene, zeaxanthin, neoxanthin and violaxanthin are also present in small quantities [11]. Cortés-Rojo et al. [8] found lutein and zeaxanthin and provitamin A (β -carotene and β -cryptoxanthin) carotenoids in avocado *Hass* from Costa Rica. Peel, seed and pulp of avocado, if consumed on a daily basis, have pharmacologically active constituents with lipid-lowering and antihyperlipidemic effects. This tropical fruit possesses effects on different components of metabolic syndrome, mainly on lipid profile, being LDL-cholesterol, HDL-cholesterol, triglyceride, total cholesterol and phospholipids the most affected biomarkers [32]. A study with diabetic rats demonstrated that avocado oil decreases ROS levels and lipidperoxidation, improving brain mitochondrial function [33]. Aqueous extracts of leaf and seed revealed to have inhibitory effect on angiotensin 1 converting enzyme activity, showing the seed a better antihypertensive activity than the leaf [34].

Given the above, public information about the avocado and its health promoting properties has been given in several countries. 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 [26]. In Portugal these fruits are being cultivated in the south (Algarve), where the soil and climatic conditions are more favorable [35]. The cultivated area has expanded, being currently about 1500 hectares spread over 180 farms [36]. 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 [37]. 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 [24,36]. 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 [37].

3. MATERIALS AND METHODS

3.1 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 4°C. 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.

3.2 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). Results were expressed in water percentage (%) according to the methodology described by the Association of Official Analytical Chemists [38]. Likewise, the following methods (AOAC, 2005) were used to determine protein (method 960.52), fat (method 920.39C) and ash content (method 923.03) in stored avocado pulp, peel and seed samples.

3.3 Bioactive Compounds Quantification

3.3.1 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/ 100 g (on a FW – fresh weight basis)) was quantified using an analytical validated method published in a previously work [39].

3.3.2 Colorimetric determination of vitamin E

The determination of the vitamin E content in the different constituents of Algarvian avocado fruit followed the procedure described by Amin [40]. The experiments were performed in sextuplicate for each avocado fruit fraction (pulp, peel, and seed).

3.3.3 Total carotenoids assay

Total carotenoids were extracted according to Akin et al. [41] with some minor modifications. Results were expressed as β -carotene equivalents (milligrams per 100 g of FW).

3.3.4 Total polyphenolic content assays

Total phenolics were determined according to the improved Folin-Ciocalteu method [39]. Total phenol content was expressed as milligrams of GA equivalent (GAE) per 100 grams of fresh fruit weight (mg GAE /100 g-1 FW) [42]. Flavonoid contents were determined using a method described by Soares et al. [43] with slight modifications. The flavonoid contents were express in milligrams per 100 grams of FW.

3.4 DPPH[•] Radical-scavenging Activity

Pulp, peel and seeds of avocado aqueous extracts were determined in accordance with the Shimada [43] method, which is based on the principle of scavenging the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical DPPH[•]. 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.

3.5 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 \pm 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 ($P=.05$).

4. RESULTS AND DISCUSSION

To determine the patterns of biologically active compounds accumulation in pulp fruit and agro-industrial by-products (peel and seed), it is important to identify their composition and content in separate parts. 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 (TSS) in °Brix and the acidity in mg of tartaric acid equivalents /100 g FW

Parameter**	Fraction of the Algarvian avocado var. "Hass"		
	Pulp*	Peel*	Seeds*
Moisture (%)	70.83 \pm 3.53 ^a	69.13 \pm 2.58 ^b	54.45 \pm 2.33 ^c
Ash (%)	1.77 \pm 0.16 ^a	1.50 \pm 0.08 ^b	1.29 \pm 0.03 ^c
Protein (%)	1.82 \pm 0.07 ^a	1.91 \pm 0.08 ^a	2.19 \pm 0.16 ^b
Fat (%)	43.5 \pm 4.62 ^a	2.20 \pm 1.65 ^b	14.7 \pm 0.32 ^c
TSS (°Brix)	6.68 \pm 1.02 ^a	3.01 \pm 2.03 ^b	3.54 \pm 1.97 ^b
Acidity	1.07 \pm 0.02 ^a	2.05 \pm 0.24 ^b	2.67 \pm 0.17 ^c

*Values represented as mean \pm standard deviation (n=6). **A letter is used to express the results of the comparison between the different fractions. Different letters indicate significant statistical differences ($P=.05$)

Avocados have been recognized for their high nutritional value and therapeutic importance for centuries. 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 higher water content (70.83%), in agreement with values of United States Department of Agriculture [31] (72.3%), followed the peel (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, pulp showed higher percentages. Morais et al. [44] also reported superior total lipids values for pulp 28.6 \pm 7.8 g/100 g in dried basis than for avocado by-products, in agreement with Alkhalaf et al. [45] (seed 0.85%, fruit 30.86%), being oleic acid the most predominant unsaturated fatty acid in both samples. Ejirofor et al. [46] referred higher values for protein (15.55 \pm 0.36 g/100 g), lipid (17.90 \pm 0.4 g/100 g) and ash (2.26 \pm 0.23 g/100 g) for Nigerian avocado seeds. According to Hernández-Muñoz et al. [47] the total acidity is a measure of the organic acid content. The predominant acid found in avocados is tartaric acid although, theoretically, every species capable of donating a proton, including fatty acids, also contribute to the total acidity of the fruit [48]. 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 peel or pulp. The acidity of the pulp was found to be superior to that exhibited by 'Hass' avocados of American origin (0.04 \pm 0.01% citric acid) [49]. Astudillo-Ordóñez and Rodríguez [50] reported values of acidity (percentage of tartaric acid) for Colombian avocado pulp between 19.47 to 9.24%, decreasing acidity with storage time. 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 ($5.1 \pm 0.1^\circ$ Brix) [49], can be considered low, favoring the consumption of the Algarvian avocado in natura. Astudillo-Ordóñez and Rodríguez [50] referred values of $^\circ$ Brix degrees between 5.07 and 7.26, depending on maturity stage of Colombian avocado. Superior values of TSS have been reported for 'Hass' avocados from New Zeland ($\sim 9^\circ$ Brix) [51].

The variety, grade of ripening, climate, the composition of the soil, and fertilizers are the major factors that largely influence the nutritional profiles of avocados [52]. Tango et al. [53] 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. [54] observed higher protein concentrations and ash, compared with those recorded in this study (3.18% and 1.51%, respectively). On the other hand, Lu et al. [55] reported a value of 25% fat for the pulp of 'Hass' avocados cultivated in California. Dias et al. [56] obtained for peel powder higher values for ash (2.94%) and lipids (35.22%), and lower values for moisture (37.79%) and proteins (0.25%). Comparing with the values of United States Department of Agriculture [31], Algarvian avocado pulp had similar values for protein (1.96%) and ash (1.66%). 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 [57,58]. These properties are assigned to the presence of significant levels of bioactive antioxidant compounds in fruits. During the last decades, ample evidence of the benefits of avocado on health has been gathered [58,59]. This promoted their consumption, stimulating also the research about their pharmacological potential. The maturation of any fruit promotes an increase of bioactive compounds [60]. 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. Bioactive compounds quantification in different Algarvian avocado var. "Hass" fruit fractions (mg/ 100 g FW)

Bioactive compound**	Avocado fraction var. "Hass"		
	Pulp*	Peel*	Seeds*
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

**Values represented as mean±standard deviation (n=6). **A letter is used to express the results of the comparison between the different fractions. Different letters indicate significant statistical differences (P=.05)*

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 [13]. The skin of the fruit had the highest carotenoid content, as expected, since this tissue is usually the fraction where 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 [60]. 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 [15]. 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 peel (12.6 ± 0.3 mg GAE/g FW) and seeds (51.6 ± 1.6 mg GAE/g FW) [13] while possessing comparable levels of flavonoids (26.36 QE/100 g FW) [26]. The phenolic levels are also superior to those reported for the same fruit of Turkish provenance (1.20 g/kg FW) [61]. Amado et al. [62] obtained total phenolic content (TPC) of 16.15 (peel), 2.04 (pulp) and 8.07 (seed) mg GAE /g dry weight (DW) for Brazilian avocado. The same authors presented 0.26 mg QE/g DW for flavonoid content of seed. Kosińska et al. [5] obtained values of TPC of 25.32 mg CE/g DW for peel and 9.51 mg CE/g DW for seed of "Hass" cultivar. These authors compared TPC values with those of "Shepard" variety and concluded that peels from "Hass" are richer in phenolic compounds, but seeds present less TPC. Tremocoldi et al. [63] demonstrated that "Hass" and "Fuerte" varieties peels presented superior phenolic contents (63.5 and 120.3 mg GAE/g, respectively) than seed (57.3 and 59.2 mg GAE/g, respectively). These studies, were in disagreement with our results. Thus, it seems that seeds of the "Hass" Algarve cultivar contained more phenolic compounds than avocado seeds of other parts of world, but all studies are in line with the fact that the pulp is the part of the fruit that has less TPC values. Gómez et al. [64] reported values of polyphenolic content between 35.10 and 46.95 mg GAE/g FW, depending on extraction conditions (etanol concentration and time).

The content of carotenoids is inferior to that found in Californian avocados (42.2 μ g/g) [55], and higher than that found in the corresponding fractions of Mexican 'Hass' avocados ((7.1 μ g/g (pulp), 15.2 μ g/g (peel), 6.3 μ g/g (seed)) [13]. Furthermore, the Algarvian avocado has superior levels of carotenoids in the pulp than the 'Hass' avocados cultivated in New Zealand (~5.2 μ g/g), but inferior levels in the peel (~50 μ g/g) (Ashton et al., 2006). Cortés-Rojo et al. [8] separated and quantified carotenoids in Costa Rican Hass avocados, obtaining astaxanthin, lutein, zeaxanthin, β -cryptoxanthin and β -carotene (0.64, 15.13, 0.02, 3.37, 2.28 mg/100g, respectively).

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/ 100 g) [65], (Salgado et al., 2008) but superior to that of avocados from California (27 μ g/g) [55]. The concentration of ascorbic acid was inferior to that reported for Californian avocados (17.3 mg/100 g) [31]. More recently, Talabi et al. [66] described in Nigerian avocado seeds contents of 14.63 and 0.65 mg/100 g for vitamin C and E, respectively.

Overall these results also demonstrate the potential of the non-edible parts of the avocado as a source of bioactive compounds. The peel 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 peel 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 [25,67,68]. Both peel and seeds can also be harnessed as a source of these compounds to use as food additives or functional food ingredients [69-71]. Remarkably the peel 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 (*Lycopersicon esculentum*) or red cabbage (*Brassica oleraceae* var. botrytis) [37,72,73]. According to Amado et al. [62] avocado by-products could also prevent food contamination with the advantage of not showing toxicity, and consequently peel and seed appear to be a promising alternative for food applications.

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•)) (Fig. 1).

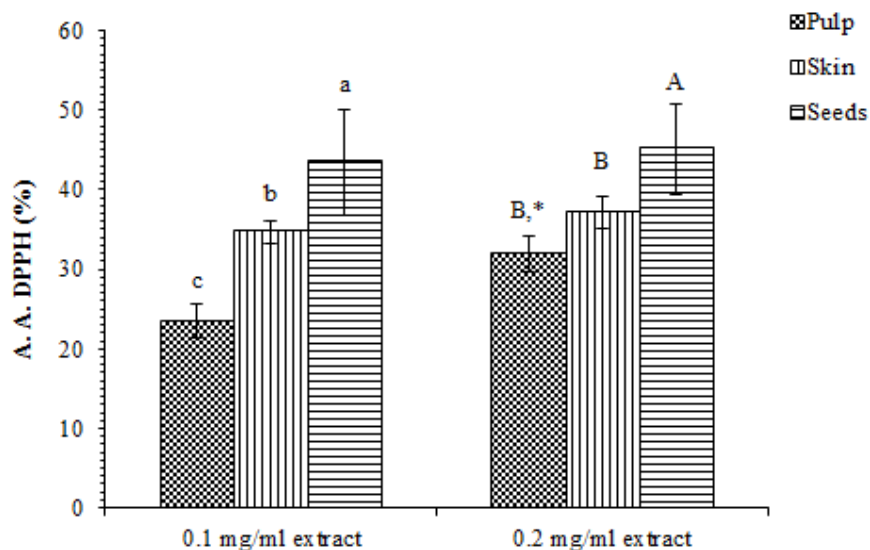


Fig. 1. Antioxidant activity (A.A.) of aqueous extracts obtained from the various avocado fractions on 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•). The symbol “*” indicates the existence of significant statistical differences ($P=0.05$) among the antioxidant activity exhibited by the two aqueous extracts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

These results differ slightly from those reported in the literature since both Wang et al. [13], Rodríguez-Carpena et al. [26] and Amado et al. [62], showed that the peel presented superior antioxidant activity, but in agreement with Tremocoldi et al. [63]. However, according with the referred studies, and in agreement with this work, the antioxidant activity was higher for avocado by-products. Fruit seeds and peel are very rich in antioxidant compounds, however the seed showed higher contents of flavonoids and phenolic compounds while fruit peel 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 [74]. 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 peel and seeds.

Most studies have demonstrated a linear correlation between total phenolic content and the antioxidant activity evaluated by different methodologies in fruits and vegetables [75-77]. 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 content of bioactive compounds and antioxidant activity

Extracts	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
Peel	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 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 peel extracts.

5. CONCLUSIONS

Several studies carried out in the last decades report and highlight the exceptional nutritional and phytochemical composition of avocado, as well as their biological potential in the treatment and prevention of different diseases. Some studies have underlined its importance as the source of lead molecules for pharmaceutical industry due to the abundance of novel chemical compounds. The cumulative effects of avocado components described in the pulp fruits as well as in their by-products (peel and seed) in the prevention and treatment of oxidative stress and age-related degenerative diseases are also described. However, more comprehensive *in vitro*, *in vivo*, and clinical investigations are fundamental to significantly expand the understanding of the molecular mechanisms of action of its phytochemicals for developing subsequent therapeutic and nutritional interventions against cancer, diabetes, inflammatory, microbial, and cardiovascular diseases. Interestingly, despite its popularity as a “superfood”, clinical studies evaluating the therapeutic potential of avocado for the prevention and management of different diseases are few in the literature. More investigations to understand the bioavailability and pharmacokinetics of avocado phytochemicals and antioxidants are also crucial to determine their clinical efficacy and potential toxicity.

Therefore, consuming avocados as a part of the diet can be beneficial to human health. The fact that the non-edible parts of the fruit (peel 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 by-products 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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