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Research Article
Bioactive Compounds and Antioxidant Activities of Avocado Peels and Seeds

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Abstract

Background and Objective: Bioactive plant constituents from food industrial wastes could be utilized in food industry, for the development of functional or enriched foods. The present study aimed to determine biochemical composition as well as the antioxidant activity of peels and seeds in summer and winter avocado varieties. **Materials and Methods:** Biochemical composition and antioxidant activity of peels and seeds were assessed in 2 varieties of avocado fruits (*Persea americana*). Total poly phenols, flavonoids, carotenoids and tannins were determined in Duke (summer) and Fuerte (winter) varieties. Polyphenols and flavonoids were fractionated and identified using HPLC. The radical scavenging activity was determined by DPPH assay. **Results:** Both varieties are different in all measured parameters. Solvent type strongly affected active constituents 'contents as well as their antioxidant activities. The highest phenolic recovery, by methanol 80% revealed greater efficiency as antioxidant potency for peels and seeds of summer variety with significant differences ($p \geq 0.05$). However, winter variety peels contained higher total carotenoids. Obvious relations were found among the extractable total phenolic components and DPPH scavenging potentials of avocado peel and seed extracts. Similar results were noticed for tannins content in the winter peels. Winter variety peels contained higher total carotenoids. The major polyphenols in avocado peels included catechin and 3-hydroxy tyrosol, while avocado seeds had catechin and pyrogallol. The major flavonoids; hesperidin, naringin and rutin in peels were significantly higher than those in seeds. **Conclusion:** Avocado seeds and peels could be explored as a valuable bioactive source and as a functional ingredient in food industries.

Key words: Avocado, antioxidant, phenols, flavonoids, tannins

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Avocado (*Persea americana*), Family; *Lauraceae*, is a tropical and subtropical fruit. Although, *P. americana* is a native plant in Central America (Mexico), it grows in places as far from America, Australia, South Africa and so on[1]. In 2014, the global production of avocado reached 4,717,102 t with 139% increase during the last two decades. Avocado production has considerably expanded in recent years due to rising consumption[2,3].

Natural antioxidants have been used in the food industry to prevent lipid and protein oxidation through different mechanisms. Most of these natural antioxidants included polyphenol components[4]. Using avocado as a functional food in diets has been recommended for its phytochemical content[5] and high nutritive value[6].

In the recent years, more attention has been paid to the antioxidants contained in fruits and its byproducts because the epidemiological studies have documented those antioxidants could be associated with the reduced incidence of cardiovascular diseases, diabetes and some types of cancer. Thus, developing strategies to enhance avocado fruit antioxidant composition has become a goal of avocado industries globally as a means to promote avocado consumption[7].

A large part of avocado (skin and seed or stone) is lost during industrial processing[2]. However, peels and seeds appeared to be normally discarded as useless when consuming or processing, causing environmental waste problems. Recovery of bioactive plant constituents from food industrial waste would be promising and a cost-effective and environmental friendly option[1]. Avocado seeds, representing an under-utilized waste stream, form a stable orange color when crushed in the presence of air as a potential source of new natural colorants for use in foods[8].

Processing avocado fruit might result in substantial waste, particularly from discarded seeds representing about 16% of fruit dry weight[4]. These by-products can cause environmental problems. They also generate financial losses due to the high cost of transport to disposal areas[5]. Efforts are ongoing to develop integrated use strategies for avocado fruit. Indeed, the seed's tannins and polyphenol compounds contents provide it a higher antioxidant activity than its edible portion[9].

Although not as thoroughly studied as the pulp, avocado peel and seed reportedly contain a great amount of phenolic compounds and display a higher antioxidant activity than the pulp. The phenolic content and antioxidant capacity of avocado peels and seeds are known for its high antioxidant capacity[10,11].

Worth mentioning, the peel or seed extracts showed radical scavenging capacity and antioxidant activity due to their polyphenol composition[12]. The peel and seed from overripe avocado fruit, the main by-products of avocado processing, have demonstrated to possess high concentrations of polyphenols. These characteristics make avocado by-products useful matrices to develop functional food, nutraceutical or cosmetics[10]. The response variables were total phenol content (TPC) and antioxidant capacity (DPPH). The recent research focused the light on transforming avocado by-products to useful commodities in food products for its health benefits[13].

There has been an increasing consumer need for and scientific interest in new natural antioxidants. Thus, there is also a surge in avocado by-products, which needs assessment. The aim of this work is to compare the bioactive compounds and antioxidant activity of avocado peels and seeds. Hence, in the present study extracts from avocado peels (AP) and avocado seeds (AS) of 2 varieties were prepared with the aim of exploiting potential antioxidant properties of such extracts. To achieve this aim the bioactive compounds were determined, besides the fractionation and identification of the phenolic and flavonoids in the peels and seeds.

MATERIALS AND METHODS

The study was carried out at Food Science Department, Faculty of Agriculture, Cairo University, Giza, Egypt and Horticulture Crops Processing Research Department, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt from Jan, 2017-October, 2019.

Chemicals: All reagents, solvents and standards were of analytical grade and were purchased from Acmatic and Cornell lab Pharm. and Chem., Cairo, Egypt.

Folin-Ciocalteu reagent was purchased from Qualikems Laboratory Chemicals and Analytical Reagents, Cairo, Egypt.

Materials: Two varieties of avocado fruits (*Persea americana*) in mature stage, Duke (summer) and Fuerte (winter) were purchased from Qanater Farm, Horticultural Research Institute, Agricultural Research Center (ARC), Al Qanatir Al Khayriyah, Qalyubia Governorate, Egypt, during seasons of 2017 and 2018.

Methods

Sample preparation: Peels and seeds were removed from fruits and washed with distilled water. They were cut into small pieces, dried at 45°C and until final dehydration (5-7% moisture content) and ground to fine powder. Ground samples were stored at 4°C until utilized.

Extraction of bioactive compounds from peels and seeds: Ten grams of finely ground peels and seeds were separately extracted by shaking in an orbital shaker (IKA® KS260 control, Germany) with 100 mL of the 2 extraction solvents (80% methanol; 80:20 methanol:water v/v, 0.5 N acidified methanol; HCl: Methanol 4.5:95.5 v/v) for 24 h at room temperature, then the extracts were filtered and stored at 4°C to determine antioxidant potential, total phenols and total flavonoids according to Sultana *et al.*[14].

DPPH radical scavenging activity: The DPPH assay is based on electron-transfer that produces a violet solution with a maximum absorption at 517 nm. The DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay was performed according to the method described by Kelebek *et al.*[15]. Briefly, 0.1 mL of diluted extract mixed with 3.9 mL of DPPH solution (2.36 mg/100 mL methanol) and vigorously vortexed. The solution was kept in dark at room temperature for 30 min. The absorbance was monitored at 517 nm by a UV-Visible spectrophotometer. Trolox calibration curve was used to calculate antioxidant activity of each raisin extracts and to express the antioxidant capacity in µM Trolox equivalent/100 g of avocado. The mean and standard deviation (n = 3) were calculated for 3 replicates.

Inhibition percentage of DPPH (I%) was calculated by Yogesh *et al.*[16] as follows:

$$\text{Inhibition (\%)} = \frac{\text{Ac (0)} - \text{AA (t)}}{\text{Ac (0)}} \times 100$$

where, Ac (0) is the absorbance of the control at time = 0 min, AA (t) is the absorbance of the antioxidant at time = 0.5 h.

Ascorbic acid was used as a reference antioxidant at 200 ppm concentration.

Determination of total polyphenols content: Total polyphenols in extracts were determined according to Sahu and Saxena[17] using the Folin-Ciocalteu Spectrometric method at 760 nm. Quantification was carried out on the basis of a standard curve of gallic acid (10-100 mg L^{-1}). Results were expressed as mg gallic acid equivalents (GAE) g^{-1} of extract.

Determination of total flavonoids: Flavonoids content was determined using the method of Barros *et al.*[18], with some modifications. An aliquot (0.5 mL) of the extract solution was mixed with distilled water (2 mL) and subsequently with $NaNO_2$ solution (5%, 0.15 mL). After 6 min, $AlCl_3$ solution (10%, 0.15 mL) was added and allowed to stand further 6 min, thereafter, NaOH solution (4%, 2 mL) was added to the mixture. Immediately, distilled water was added to bring the final volume to 5 mL. Then the mixture was properly mixed and allowed to stand for 15 min. The intensity of pink color was measured at 510 nm. Quantification was carried out on the basis of a standard curve of quercetin and the results were expressed as mg g^{-1} of extract.

Determination of tannins: Tannins were determined in the samples according to the method described by Herald *et al.*[19] with some modifications. Two grams of samples were shaken on an orbital shaker (IKA® KS260 Control, Germany) with 50 mL of 1% HCl in methanol for 24 h at ambient temperature. Then, 1 mL was reacted with 5 mL of vanillin reagent (50:50 mixtures of 4% vanillin/ 8% HCl in methanol) for 20 min and absorbance was read at 500 nm. A catechin standard curve was used in tannin levels.

Determination of total carotenoids: Total carotenoids were determined in the samples according to the method described by Marković *et al.*[20]. The samples were taken for the analysis, weighed to 5 g were shaken on an orbital shaker (IKA® KS260 control, Germany) with 30 mL of acetone (85%) for 24 h at ambient temperature, the filter was washed with acetone (85%) so that the rest of the filter was completely white. The resulting filtrate was an extract of pigments, which was transferred and diluted up to 100 mL with acetone (85%). As the concentrations of pigments in most cases were high, the obtained extracts were diluted to enable spectrophotometric readings. Absorptions for the prepared extracts were read on the spectrophotometer at wavelengths of 662, 644 and 440 nm.

Identification of phenolic and flavonoid compounds: A high performance liquid chromatography system equipped with a variable wave length detector (Agilent technologies, Germany) 1200 series. Also the HPLC was equipped with auto sampler, Quaternary pump degasser and column compartment set at 35°C. Analysis were performed on a C_{18} reverse phase (BDS 5 µm, Labio, Czech Republic) packed stainless-steel column (4×250 mm, i.d.).

To determine phenolic acids and flavonoids, samples were prepared according to the method described by Jakopic *et al.*[21]. Samples were extracted with 10 mL methanol in ultrasonic bath for 45 min. Then the samples were centrifuged for 7 min at 4200 rpm. The supernatant was filtered through polyamide filter Chromafil AO-45/25, transferred into vial prior analyses.

The HPLC method started with linear gradient at a flow rate of 1.0 mL min^{-1} with mobile phase of water/acetic acid (98:2 v/v, solvent A) and methanol/acetonitrile (50: 50, v/v, solvent B), starting with 5% B and increasing B to levels of 30% at 25 min, 40% at 35 min, 52% at 40 min,

70% at 50 min, 100% at 55 min. The initial conditions were re-established by 5 min wash in both solvents. All chromatograms were plotted at 280 nm to estimated phenolic acids and at 330 nm for flavonoids.

Statistical analysis: Data were by statistically analyzed using one-way analysis of variance ANOVA Silva and de Azevedo[22]. Each experiment was repeated at least 3 times, means and standard deviations were calculated.

RESULTS

Effect of extraction method on bioactive compounds content: Results in Table 1 represent the effect of extraction methods on the bioactive compounds of avocado peels and seeds. It was found that the extraction with methanol 80%, gave the highest content of antioxidant activity, total phenols and total flavonoids with significant difference ($p \leq 0.05$) in avocado seeds summer, compared to ascorbic acid (200 ppm) and the other variety (winter).

Tannins content in avocado peels and seeds: It was observed that tannins content in peels and seeds winter variety were significantly higher than those in peels and seeds of summer variety (Fig. 1). Meanwhile, tannins content in peels were significantly higher than those in seeds of the 2 varieties. However, peels of winter variety contained higher amount of tannins (19.75%).

Carotenoids content in avocado peels: The obtained results shown in Fig. 2 ascertained that the total carotenoids of avocado peels of winter variety were significantly higher than those in summer variety (176.35 and 168.75 mg/100 g DW, respectively).

Fractionation and identification of phenolic and flavonoid compounds: Phenolic compounds were fractionated and identified using HPLC. The results are presented in Table 2. It could be noticed that 22 compounds were identified, the major polyphenols presented in avocado peels are catechin, 3-hydroxyl tyrosol, pyrogallol and protocatechoic acid. However, catechin, pyrogallol, 3-hydroxyl tyrosol and catechol were the main phenols in avocado seeds. Moreover, catechol, ellagic acid, gallic acid, salicylic acid, pyrogallol, catechin, protocatechoic acid and 3-hydroxy tyrosol were significantly higher in peels than those in seeds. The concentration of catechin ranged at (25.402-113.826 mg/100 g DW), while pyrogallol varied from 15.412-22.479 mg/100 g DW.

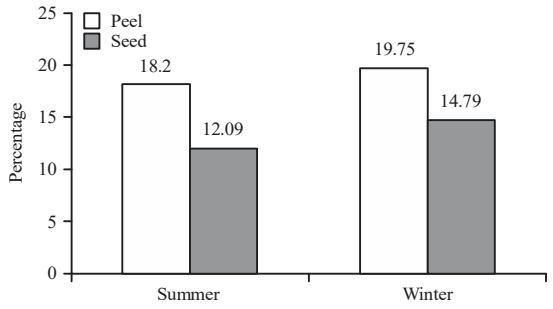

Fig. 1: Tannins percentage of avocado peels and seeds for 2 varieties (on dry weight basis)

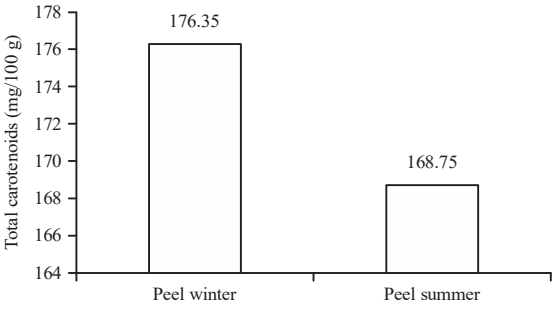

Fig. 2: Total carotenoids (mg/100 g) of avocado peels for 2 varieties (summer and winter) on dry weight basis

Table 1: Effect of extraction methods on bioactive compounds of 2 varieties avocado peels and seeds (on dry weight basis)

	Avocado peels			Avocado seeds		
Extraction method	Antioxidant activity (%)	Total flavonoids (as quercetin, mg g^{-1})	Total phenol (GAE mg g^{-1})	Antioxidant activity (%)	Total flavonoids (as quercetin, mg g^{-1})	Total phenol (GAE mg g^{-1})
Extraction with methanol 80%						
Summer	94.81±0.51^{a}	26.41±1.09^{a}	26.82±1.24^{a}	97.84±0.23^{a}	27.14±1.05^{a}	27.29±1.08^{a}
Winter	93.50±0.07^{a}	23.53±1.14^{b}	24.37±1.35^{b}	96.46±0.26^{b}	21.46±0.47^{b}	21.86±0.43^{b}
Ascorbic acid (200 ppm)	89.32±0.30^{b}			89.32±0.30^{c}		
Extraction with 0.5 N acidified methanol						
Summer	84.01±2.93^{c}	21.97±0.96^{b}	24.70±0.11^{b}	84.97±0.42^{d}	19.16±0.46^{c}	23.20±1.05^{b}
Winter	82.66±0.36^{c}	22.20±0.50^{b}	27.14±0.44^{a}	85.17±1.56^{d}	18.49±0.35^{c}	26.28±1.13^{a}
Ascorbic acid (200 ppm)	90.09±0.06^{b}			90.09±0.06^{c}		

Each value represents Means±SD of the 3 replicates, means with the same letter in the same column are not significantly different at $p<0.05$

Table 2: Phenolic compounds in avocado peels and seeds by HPLC (mg/100 g, on dry weight basis)

	Peels		Seeds	
Phenolic compounds (mg/100 g)	Summer	Winter	Summer	Winter
Gallic acid	1.767	1.653	1.728	1.212
Chlorogenic acid	5.609	13.174	2.605	1.175
Catechin	94.870	113.826	25.842	25.402
Ellagic acid	6.426	7.534	4.721	4.305
Ferulic	0.928	0.252	0.622	0.718
P-hydroxy benzoic acid	1.356	1.190	3.750	4.834
Protocatechuic acid	12.731	5.714	5.390	8.410
Caffeic acid	0.560	0.276	0.465	0.338
P-coumaric acid	0.977	0.732	0.639	0.620
Pyrogallol	22.479	20.440	21.980	15.412
4-amino benzoic acid	3.948	2.395	1.359	1.755
Catechol	8.703	10.459	8.142	9.406
Caffeine	6.350	3.951	5.080	4.708
3-hydroxy tyrosol	28.153	15.549	12.270	11.391
Vanillic acid	0.973	1.793	0.339	0.523
Iso-ferulic	1.292	0.963	0.831	0.825
Oleuropein	2.964	2.557	2.821	2.090
Benzoic acid	3.444	6.486	5.309	5.963
Salicylic acid	6.790	3.962	5.504	2.571
3,4,5 methoxycinnamic	0.283	0.321	0.970	1.634
Coumarin	0.941	0.557	0.165	0.292
Cinnamic acid	0.147	0.130	0.087	0.070

Table 3: Flavonoid compounds in avocado peels and seeds by HPLC (mg/100 g on dry weight basis)

	Peels		Seeds	
Flavonoid compounds (mg/100 g)	Summer	Winter	Summer	Winter
Rutin	2.950	1.565	0.427	0.290
Quercetin	0.152	0.117	0.083	0.073
Kaempferol	0.152	0.122	0.082	0.039
Qurectrin	2.483	1.941	0.229	0.119
Naringin	6.751	5.807	1.291	0.876
Hesperidin	61.939	25.305	2.618	1.322
Acacetin neo. rutinoside	0.993	0.768	0.332	0.145
Naringenin	0.043	0.040	0.018	0.011
Hesperetin	0.205	0.189	0.167	0.038
Apigenin	0.063	0.018	0.059	0.021

Regarding flavonoids components, results ascertained that the major flavonoids included hesperidin, naringin and rutin in peels that were significantly higher than those in seeds (Table 3). The concentration of hesperidin ranged from 1.322 to 61.939 mg/100 g DW, while naringin fluctuated between 0.876 and 6.751 mg/100 g DW.

DISCUSSION

By-product that contains a large amount of extractable polyphenols, have attracted the attention of food and cosmetic industries due to their high antioxidant capacity. Results recorded for avocado seeds, where extraction with methanol 80%, gave the highest antioxidant potency, especially for summer variety, with significant difference ($p \leq 0.05$), compared to ascorbic acid (200 ppm) and the other variety (winter). Segovia *et al.*[11] found that fact makes noticed a linear relationship between total poly phenolic content and antioxidant capacity.

On the other hand, peels of winter variety recorded a significant higher content of carotenoids avocado, compared with summer variety. Hurtado-Fernández *et al.*[23] found that differences in the carotenoid content accounted for the different color characteristic between the 2 varieties. Moreover, avocado has considerable amounts of pigments [carotenoids (a-carotene, b-carotene, cryptoxanthin, lutein, isolutein, zeaxanthin, etc.)].

The major polyphenols and flavonoids included were significantly higher in avocado peels than those in seeds. Calderon-Oliver *et al.*[12] found that peel extract presented more antioxidant and radical scavenging capacity than seed extract due to its polyphenols content, such as epicatechin and other compounds. Further, flavonoids have been considered potent antioxidants with positive effects against diverse diseases such as cancer, neurodegenerative or cardiovascular disease. Monika and Geetha[24] found that hydro-alcoholic fruit extract of avocado (HFEA) wastes contain flavonoids: rutin, quercetin, phenolic compounds: Gallic acid, ellagic acid and vanillic acid. The HFEA is also found to contain vanillic acid and benzoic acid derivatives, which is a flavoring agent. It is antioxidized form of vanillin produced on converting vanillin to ferulic acid. This study would be suggested for utilizing avocado by-products as inexpensive clinical and pharmacological agents.

CONCLUSION

The obtained results ascertained that both avocado peels and seeds are considered good sources of bioactive compounds (total phenols, total flavonoids, carotenoids, tannins and antioxidant capacities). Avocado by-products that would otherwise be discharged into the environment could be utilized in different food products as well as their easy extraction technique. Further study would be suggested for utilizing avocado peels and/or seeds as inexpensive clinical and pharmacological agents.

SIGNIFICANCE STATEMENT

This study highlights the qualitative and quantitative analysis of bioactive compounds of peel and seed extracts of two avocado varieties (Duke and Fuerte). Besides, the antioxidant activity of such extracts which could be utilized as promising sources of phenolic, flavonoid and tannin compounds in functional foods for diverse diseases such as cancer, neurodegenerative or cardiovascular diseases.

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