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Phenolic Compound Profiles and Antioxidant Capacity of *Persea americana* Mill. Peels and Seeds of Two Varieties

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ABSTRACT: Avocado processing by the food and cosmetic industries yields a considerable amount of phenolic-rich byproduct such as peels and seeds. Utilization of these byproducts would be favorable from an economic point of view. Methanolic (80%) extracts obtained from lyophilized ground peels and seeds of avocado (*Persea americana* Mill.) of the Hass and Shepard varieties were characterized for their phenolic compound profiles using the HPLC-PAD technique. The structures of the identified compounds were subsequently unambiguously confirmed by ESI-MS. Compositional analysis revealed that the extracts contained four polyphenolic classes: flavanol monomers, proanthocyanidins, hydroxycinnamic acids, and flavonol glycosides. The presence of 3-*O*-caffeoylquinic acid, 3-*O*-*p*-coumaroylquinic acid, and procyanidin A trimers was identified in seeds of both varieties. Intervarietal differences were apparent in the phenolic compound profiles of peels. Peels of the Shepard variety were devoid of (+)-catechin and procyanidin dimers, which were present in the peels of the Hass variety. Peels of both varieties contained 5-*O*-caffeoylquinic acid and quercetin derivatives. The differences in the phenolic profiles between varietals were also apparent in the different antioxidant activity of the extracts. The peel extracts had a higher total phenolic compound content and antioxidant activity when compared to the seed extracts. The highest TEAC and ORAC values were apparent in peels of the Haas variety in which they amounted to 0.16 and 0.47 mmol Trolox/g DW, respectively. No significant ($p > 0.05$) differences were apparent between the TEAC values of seeds of the two varieties but the ORAC values differed significantly ($p < 0.05$). Overall these findings indicate that both the seeds and peel of avocado can be utilized as a functional food ingredient or as an antioxidant additive.

KEYWORDS: *avocado, seeds, peels, phenolics profile, antioxidant activity, byproduct, Persea americana Mill.*

INTRODUCTION

The avocado is an evergreen tree native to Central America and Mexico that can now be found in most of the tropical and subtropical regions of the world. A unique feature of this plant is that the avocado fruits mature on the trees and ripen after harvest. There are many varieties of avocado of which Hass is the most common worldwide. Hass avocado peel changes from a green to a dark purple color as it ripens. Hass avocados are more oval than other varieties. On average fruits of this variety weigh about 140−340 g, have a small seed, and contain a good amount of edible flesh. Shepard avocados are green-skinned and are available in Australia only. The average weight of a Shepard avocado is 200−320 g, and it has buttery yellow flesh.[1] The distinctive feature of fruit from the Shepard variety is that the flesh does not discolor readily when cut, and they thus exhibit excellent eating quality. Avocados are rich in unsaturated fatty acids, vitamins C, B, and E, and other nutrients.[2,3] They are also an abundant source of potassium and dietary fiber.[1] The edible part of the fruit contains high amounts of oleic, linoleic, and α-linolenic acids that are valuable constituents of the human diet.[4] Avocado is mainly consumed as a fresh fruit; however, efforts are being undertaken to expand the range of avocado value-added products available. Manufacturing of guacamole, avocado pulp, and avocado oil results in the production of a considerable amount of solid residue. Increasing environmental concerns have resulted in a drive to utilize the large amount of avocado residue generated. A current interest in the use of food industry byproduct as a potential source of natural antioxidants or functional food ingredients is apparent.[5−14] A review recently published by Ayala-Zavala et al.[15] pointed out the importance of utilizing the entire fruit for increasing profitability of fruit processing by lowering byproduct treatment costs and producing natural additives which can be sold. The natural additives from fruits have potential application in the food industry as antioxidants, antimicrobials, flavoring, colorants, and texturizing additives.[15] Avocado byproducts in the form of peels and seeds are currently of no commercial use; however, they are reported to contain great amounts of phenolic compounds and to display a

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higher antioxidant activity than the pulp of avocado fruit.[5,16,17] The phenolic content and antioxidant capacity of avocado seeds and peels are several-fold greater than that reported for raw blueberry which is known for its high antioxidant capacity.[16] Avocado seeds and peel contain high levels of B-type procyanidins with A-type procyanidins as minor components. Monomers, oligomers, and polymers with a degree of polymerization higher than 10 have been quantified in both peels and seeds of avocado of different varieties.[16] Another study of avocado seed and peel has reported the presence of phenolic compounds belonging to five different groups, i.e., catechins, hydroxybenzoic acids, hydroxycinnamic acids, flavonols, and procyanidins, with the latter group being the most abundant one.[17] However, the identification of phenolic compounds in avocado byproduct with confirmation by mass spectra analysis has not been published. Rodríguez-Carpena et al.[18] reported that the use of avocado peel and seed extracts in raw porcine patties inhibited lipid and protein oxidation and eventually led to improvement of the quality of these products.

Detailed information on avocado byproduct phenolic compound profiles and their antioxidant activity is still scarce. To our knowledge the Shepard variety, which is unique to Australia, has also not been investigated with respect to these characteristics. The objective of the study was therefore to characterize the phenolic compound profiles and antioxidant capacity of the peels and seeds of the Hass and Shepard avocado varieties.

MATERIALS AND METHODS

Materials. Avocado fruits (*Persea americana* Mill.) of two varieties, Hass and Shepard, were purchased from local suppliers in Queensland, Australia, between February and May and kept at room temperature until they reached ready-to-eat ripeness. Seeds and peels of individual avocados were manually separated from the flesh, freeze-dried (Christ Alpha 1−4 LSC freeze-dryer, Germany), and ground (SPEX sample prep, Metuchen, NJ). The ground components of three individual fruits of each variety were combined for analysis.

Chemicals. All solvents used were of analytical grade or higher. Folin-Ciocalteu reagent, acetic acid, methanol, sodium carbonate, iron(III) chloride hexahydrate, monosodium phosphate, disodium phosphate, potassium ferricyanide, and trichloroacetic acid were purchased from P.O.Ch. Company (Gliwice, Poland). (+)-Catechin, 5-*O*-caffeoylquinic acid, 2,2′-azobis(2-amidinopropane) (AAPH), fluorescein, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) diammonium salt, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), potassium persulfate, and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) were obtained from Sigma-Aldrich (Poznań, Poland). Acetonitrile of HPLC grade was acquired from Merck (Darmstadt, Germany). Quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-rutinoside, quercetin-3,4′-diglucoside, quercetin 3-*O*-arabinoside were purchased from Extrasynthese (Genay Cedex, France).

Extraction Procedure. The extraction of phenolic compounds was carried out according to Alasalvar et al.[11] Briefly, portions of approximately 10 g of freeze-dried and ground avocado peels and seeds of two varieties were extracted with 80% methanol at a solid to solvent ratio 1:8 in a thermostatic shaking water bath (357 Elpan, Lubawa, Poland), at 60 °C for 15 min. The supernatant was then filtered through filter paper, and the extraction step was repeated twice more. The supernatants were combined, solvent was evaporated using a Büchi rotavapor R-200 (Büchi Labortechnik, Flawil, Switzerland) at 40 °C, and the aqueous residue was lyophilized (Lyph Lock 6 freeze-dry system, Labconco, Kansas City, MO).

Total Phenolic Content (TPC). TPC was determined using a colorimetric assay with Folin-Ciocalteu phenol reagent according to Naczk and Shahidi.[19] Briefly, 0.25 mL of methanolic solution of extract (1 mg/mL) was mixed with 0.25 mL of Folin-Ciocalteu reagent (diluted 1:1 with distilled water), then 0.5 mL of sodium carbonate saturated solution and 4 mL water was added, and the mixture was vortexed thoroughly (Genie2, Scientific Industries, Bohemia, NY). After 30 min color development absorbance at 725 nm was measured with a Beckman DU-7500 spectrophotometer (Beckman Coulter, Fullerton, CA) with prior centrifugation of samples (5000 × *g* for 5 min, MPW-210, MPW Med. Instruments, Warsaw, Poland). (+)-Catechin was used as a standard for the calibration curve. TPC was expressed as mg (+)-catechin equiv (CE) per g of dry weight (DW). The measurements were performed in triplicate.

Trolox Equivalent Antioxidant Capacity (TEAC). TEAC was measured as the reducing activity of extracts against ABTS$^{\bullet+}$ according to Re et al.[20] Briefly, ABTS$^{\bullet+}$ solution was prepared by reacting 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt at a concentration of 7 mM with 2.45 mM potassium persulfate at room temperature for 16 h. The solution obtained was then diluted with methanol to an absorbance of 0.70 (±0.02) at 734 nm. For the assay 2 mL of prepared cation radical solution was mixed vigorously with 20 μL of the methanolic solutions of individual extracts at a concentration of 1 mg/mL and incubated at 30 °C for 6 min. The absorbance of the resultant mixture was measured at 734 nm. The percentage inhibition of absorbance was calculated in reference to blank absorbance (methanol instead of antioxidant). The results were expressed as mmol Trolox equiv per g of DW on the basis of calibration curve.

DPPH Radical Scavenging Activity. The scavenging activity against 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH$^{\bullet}$) was monitored according to the method described by Yen and Chen with slight modification.[21] A 0.1-mL methanolic solution containing 0.2−2.0 mg of individual extracts of avocado seeds or 0.1−1.0 mg of avocado peel extracts was mixed with 2 mL of methanol, to which a methanolic solution of DPPH$^{\bullet}$ (1 mM, 0.25 mL) was added. The mixture was vortexed vigorously for 15 s and then left to stand at room temperature for 20 min before absorbance was read at 517 nm. Mean values from 3 independent samples were calculated for each extract. The extract concentration providing 50% scavenging effect (EC_{50}) was calculated from the graph of scavenging effect percentage versus extract concentration in solution.

Oxygen Radical Absorbance Capacity (ORAC) Assay. The ORAC values of avocado seed and peel extracts were determined using fluorescein as a fluorescence probe.[13] Briefly, the reaction was carried out at 37 °C in 75 mM phosphate buffer (pH 7.4), and the final assay mixture (200 μL) contained fluorescein (70 nM), AAPH (12 mM), and antioxidant [Trolox (1−8 μM) or sample (at different concentrations)]. The plate was automatically shaken before the first reading, and the fluorescence was recorded every minute for 98 min. A Polarstar Galaxy plate reader (BMG Labtechnologies GmbH, Offenburg, Germany) with 485-P excitation and 520-P emission filters was used. The equipment was controlled by Fluostar Galaxy software version (4.11−0) for fluorescence measurement. Black 96-well untreated microplates (Nunc, Roskilde, Denmark) were used. AAPH and Trolox solutions were prepared daily, and fluorescein was diluted from a stock solution (1.17 mM) in 75 mM phosphate buffer (pH 7.4). All reaction mixtures were prepared in duplicate, and at least three independent runs were performed for each sample. Fluorescence measurements were normalized to the curve of the blank (no antioxidant). From the normalized curves, the area under the fluorescence decay curve (AUC) was calculated as

$$\mathrm{AUC} = 1 + \sum_{i=1}^{i=98} f_i/f_0$$

where f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at time *i*. The net AUC corresponding to a sample was calculated as follows:

$$\text{net AUC} = \mathrm{AUC}_{\text{antioxidant}} - \mathrm{AUC}_{\text{blank}}$$

The regression equation between net AUC and antioxidant concentration was calculated. The ORAC value was calculated by dividing the slope of the latter equation by the slope of the Trolox line

Table 1. TPC, Antioxidant Capacity (TEAC and ORAC), and DPPH Radical Scavenging Activity (EC_{50}) of Avocado Peel and Seeds from Two Varieties[a]

	variety	TPC (mg CE/g DW)	TEAC (mmol Trolox/g DW)	EC_{50} (mg DW)	ORAC (mmol Trolox/g DW)
peel	Hass	25.32 ± 0.242 a	0.161 ± 0.0024 a	0.358	0.47 ± 0.036 a
peel	Shepard	15.61 ± 0.241 b	0.112 ± 0.0034 b	0.927	0.29 ± 0.020 c
seeds	Hass	9.51 ± 0.161 d	0.094 ± 0.0007 c	0.920	0.21 ± 0.014 d
seeds	Shepard	13.04 ± 0.211 c	0.091 ± 0.0047 c	0.776	0.35 ± 0.021 b

[a]Means followed by different letters, within a column, are significantly different ($p < 0.05$).

obtained for the same assay. Final ORAC values were expressed as mmol of Trolox equiv per g of sample DW.

Ferric-Reducing Power Assay. The reducing power of phenolic compounds in the extracts was determined as described by Yen and Chen.[21] Briefly, a solution of each extract (0.2−1.0 mg) in 1 mL of distilled water was mixed with 2.5 mL of 0.2 M phosphate buffer (pH = 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide. The mixture was incubated in a water bath at 50 °C for 20 min. Following this, 2.5 mL of 10% (w/v) trichloroacetic acid was added, and the mixture was then centrifuged at 1750 × *g* for 10 min. A 2.5-mL of the supernatant was combined with 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) iron(III) chloride. Absorbance of the reaction mixture was read at 700 nm; an increased absorbance denotes greater reducing power. Mean values from 3 independent samples were calculated for each extract.

HPLC-PAD and HPLC-ESI-MS Analysis. Avocado peel and seed extracts of two varieties were dissolved in methanol at a concentration of 2 mg/mL and filtered through a 0.45 μm cellulose acetate filter (Millipore, Billerica, MA) and then subjected to HPLC analysis. A chromatographic system (Waters, Milford, MA) composed of autoinjector, quaternary pump, a 2996 photodiode array detector, a Nov-Pak C_{18} column (4 μm; 3.9 mm × 300 mm; Waters), and Millenium software was used. The separation was performed according to Dueñas et al.[22] Briefly, two mobile phases were employed for elution: (A) water/acetic acid (98:2, v/v) and (B) water/acetonitrile/acetic acid (78:20:2, v/v/v). The gradient profile was from 0 to 80% of B 0−110 min. The flow rate was 1 mL/min from the beginning to 55 min and 1.2 mL/min from this point to the end. The column was re-equilibrated between sample injections with 10 mL of acetonitrile and then 25 mL of mobile phase A. A diode array detection was performed by scanning over a wavelength range from 210 to 400 nm at an acquisition speed of 1 s. A 100 μL portion of sample was injected.

After HPLC separation, mass spectra were obtained using a Hewlett-Packard 1100MS chromatograph (Palo Alto, CA) equipped with an electrospray ionization (ESI) interface. The solvent gradient and column employed were identical to those for HPLC-PAD analyses. The ESI conditions were as follows: negative-ion mode of analysis; N_2 as the nebulizing gas at 275 kPa, drying gas flow rate and temperature of 10 L/min and 340 °C, respectively; voltage at the capillary entrance was set at 4000 V; and variable fragmentation voltage at 100 V (m/z 200−1000) and 250 V (m/z 1000−2500). Mass spectra were recorded from an m/z of 100−2500.

Chromatographic peaks were identified by comparison of retention times and UV spectra with those obtained for standards and confirmed by analysis of the mass spectra recorded for each peak. Other compounds, for which no standards were available, were identified based on their UV and HPLC-MS spectra. The quantification was performed using an external standard method. The calibration curves were obtained by injection of varying volumes of the standard solution under the same conditions as for the samples analyzed. 5-*O*-Caffeoylquinic acid, (+)-catechin, quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-rutinoside, quercetin-3,4′-diglucoside, and quercetin 3-*O*-arabinoside contents were calculated in reference to their standards. 3-*O*-Caffeoylquinic acid and 3-*O*-*p*-coumaroylquinic acid were quantified on the basis of the standard curve of 5-*O*-caffeoylquinic acid, and procyanidin dimers and trimers were quantified on the basis of the standard curve of (+)-catechin. The quercetin derivatives were quantified with the calibration curve of quercetin 3-*O*-glucoside.

Statistical Analysis. Analyses were performed in triplicate. Results were expressed as mean ± standard deviation. TPC, TEAC, and ORAC data were analyzed by a one-way ANOVA statistical model with Tukey's post test using GraphPad Prism version 5.02 for Windows (GraphPad Software, San Diego, CA). The differences were considered significant at $p < 0.05$ level.

RESULTS AND DISCUSSION

Total Phenolic Content. The results obtained revealed significant variation ($p < 0.05$) in the TPC of avocado byproduct both with respect to the fruit component (peels or seeds) and the variety (Table 1). Peels of the Hass variety contained more phenolic compounds at 25.32 mg CE/g than peels of Shepard variety at 15.61 mg CE/g, on the basis of dry weight. The TPC of peels which accounted for 9.51 and 13.04 mg CE/g for Hass and Shepard varieties, respectively, was higher when compared to that of seeds. A higher content of phenolic compounds in avocado seeds amounting to 88.2 mg of gallic acid equiv/g of DW was reported by Soong and Barlow.[5] Another study on avocado fruits of different varieties revealed that the seeds and pulp of the Hass variety contained the highest level of phenolic compounds on fresh weight basis among the eight tested.[16] The TPC content reported for the seeds of the different varieties in the same study ranged from 19.2 to 51.6 mg gallic acid equiv/g of fresh weight (FW). In contrast to the results obtained in our study, other studies reported a higher content of phenolics in avocado seeds than in peels, which in the case of peels ranged from 4.3 to 13.9 mg gallic acid equiv/g FW. Comparison of our results to those reported for the mesocarp in other studies confirmed the observation that fruit pulp in general contains much less phenolic compounds than its byproduct.[5,9,16,23] The TPCs in peel and seeds of various exotic fruit have been reported in the literature. Kinnow, litchi, and grape seeds contained 3.68, 17.9, and 37.4 mg gallic acid equiv/g DW, respectively. On the other hand, the peel accounted for 17.5, 24.6, and 3.8 mg gallic acid equiv, for kinnow, litchi, and banana, respectively.[10] Mango, tamarind, and longan seeds had TPC values which ranged from 62.6 to 117 mg gallic acid equiv/g DW.[5] In general TPC readings are highly affected by the distinctive features of fruits studied and, specifically, variety,[8,16,24] ripening stage,[4,7] agronomic conditions, and postharvest handling.[25]

Antioxidant Capacity. The results of the determination of the antioxidant capacity of avocado residues of two varieties as indicated by TEAC, EC_{50}, and ORAC values are presented in Table 1. The TEAC values ranged from 0.091 to 0.161 mmol Trolox equivalents per g DW (Table 1), with extracts of peel showing higher ABTS radical scavenging activity in comparison to extracts of seeds. As for the TPC content reported above, Hass peel exhibited significantly higher ($p < 0.05$) antioxidant activity than that of the Shepard variety. In addition, there was no significant difference between the TEAC values of the seeds of the two varieties. The TEAC levels obtained in our study

were higher than those reported for kinnow seeds, litchi pericarp, and banana peel, but were lower by comparison to litchi seeds.[10] Figure 1 depicts the scavenging effect of

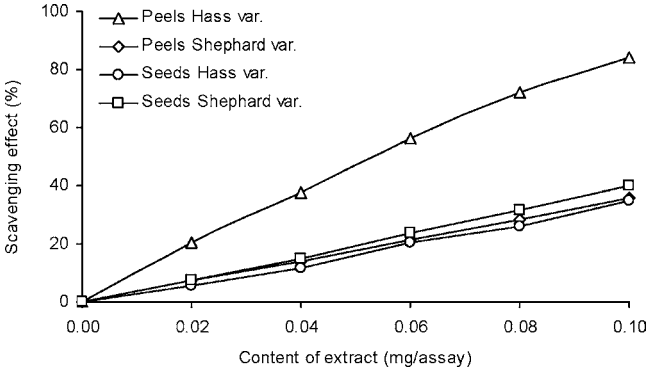

Figure 1. DPPH radical scavenging activity of methanolic extracts from avocado peel and seeds.

methanolic extracts of avocado peel and seeds against DPPH radicals. DPPH$^{\bullet}$ is a stable free radical which readily accepts electrons and becomes a stable diamagnetic molecule. The purple or violet color of the DPPH$^{\bullet}$ solution changes to yellow as the diamagnetic molecule forms and the absorbance at the 517 nm wavelength maximum decreases. The Hass peel methanolic extracts showed a considerably higher scavenging effect when compared to the other extracts investigated. The EC_{50} value (Table 1) indicates that Hass avocado peels exhibited the highest radical scavenging activity (EC_{50} = 0.358 mg), followed by Shepard seeds, Hass seeds, and Shepard peel. In contrast to the results obtained for the TPC and TEAC assays the seeds of the Shepard variety showed higher scavenging activity than the peels of the same variety. Wang et al.[16] reported the results of the DPPH assay for avocado byproduct of eight various varieties. In the case of the Hass variety the values reported were 189.8 and 164.6 μmol Trolox equiv/g of FW, for peels and seeds, respectively. The ORAC values obtained for avocado residues in our study ranged from 0.21 to 0.47 mmol Trolox/g DW (Table 1). The ORAC assay is based on the hydrogen atom transfer mechanism. This assay involves using the peroxyl radical as an oxidant and provides information on the radical chain breaking capacity of samples tested.[26] Similarly for the antioxidant assays of avocado peel described above, the Hass variety had the highest antioxidant capacity as measured by the ORAC method. The ORAC results presented previously for avocado byproduct are difficult to compare to our study since they were presented on a fresh weight basis.[16] It is worth noting, however, that the authors reported wide range of ORAC values for the different varieties studied which ranged from 58.2 to 631.4 μmol Trolox equiv/g FW in the case of peels, and from 229.0 to 464.4 μmol Trolox equiv/g in the case of seeds. Figure 2 depicts the reducing power of methanolic extracts from avocado peels and seeds. In the assay used, the presence of reductants (i.e., antioxidants) in the fraction causes a reduction in the Fe^{3+}/ferricyanide complex to the Fe^{2+} state. Activity can therefore be monitored by measuring the formation of Perl's Prussian blue color, which has a maximum absorbance at 700 nm. The methanolic extract of Hass avocado peel exhibited the strongest reducing power followed by Shepard peel, Shepard seeds, and Hass seeds. The same order was observed in the case of the TPC and TEAC assays. This can be explained by the similarity in the chemistry

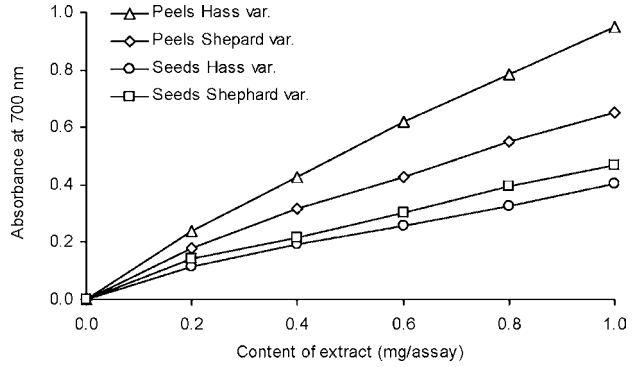

Figure 2. Reducing power of methanolic extracts avocado peel and seeds.

between all three assays as they are based on an electron transfer reaction. The TPC assay with Folin-Ciocalteu reagent measures a sample's reducing capacity.[26]

Identification and Quantification of Phenolic Compounds. Retention time (t_R), maximum absorption wavelength (λ_{max}), deprotonated molecular ions, and fragment ion masses as well as quantification of identified compounds were compiled in Table 2 for avocado peel and in Table 3 for avocado seeds. The presence of 5-*O*-caffeoylquinic acid, (+)-catechin, quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-rutinoside, quercetin-3,4′-diglucoside, and quercetin 3-*O*-arabinoside were identified by comparing their retention times and UV spectra with those of corresponding commercial standards, and their structures were confirmed by HPLC-ESI-MS. Compounds eluting at retention times of 17.3, 18.6, and 23.8 min possessed similar UV spectra with a λ_{max} at 278−279 nm, which is characteristic for flavanol monomers and procyanidins. HPLC-ESI-MS analysis revealed the presence of negative molecular ions M − H^- at m/z of 577 or 575 and a negative fragment ion at a m/z of 289, corresponding to (+)-catechin or (−)-epicatechin. These compounds were identified as procyanidin dimers of A type (M − H^- at 575 m/z) and B type (M − H^- at 577 m/z). In addition, to the five previously mentioned quercetin glycosides, four other compounds exhibited UV spectra characteristic for quercetin with a λ_{max} at 353−356 nm and with fragment ions at m/z of 301 originating from the quercetin moiety (Table 2). The peak eluting at a retention time of 26.4 min was identified as quercetin-3,4′-diglucoside since it displayed a negative molecular ion at m/z of 625, which corresponds to quercetin bound to a disaccharide composed of two hexoses. The peak eluting at 29.6 min displayed a negative molecular ion at m/z of 595, which corresponds to quercetin bound to a disaccharide composed of pentose and hexose, and a fragment ion at m/z of 463, which corresponds to a quercetin 3-*O*-arabinosyl-glucoside, compound identified by Slimestad et al.[27] in black chockeberries. Three late eluting peaks (t_R > 40 min) were designated as quercetin derivatives based on the presence of a fragment ion at 301 m/z in their ESI-MS spectra; however, further identification was not possible. The phenolic composition of peels from two different avocado varieties differed considerably. Only two compounds were identified in both samples, namely 5-*O*-caffeoylquinic acid and quercetin 3-*O*-galactoside. Procyanidin dimers and catechin were present in peel from the Hass variety only. Peel from both varieties contained a number of quercetin glycosides; however, their

Table 2. Contents of Individual Phenolic Compounds of Avocado Peel from Two Varieties

compound name	t_R (min)	λ_{max} (nm)	$[M - H]^-$ (m/z)	fragment ions (m/z)	content (μg/g DW)	
					Hass	Shepard
5-*O*-caffeoylquinic acid	15.4	324	353	191, 179	81.8 ± 5.95	77.4 ± 6.07
procyanidin dimer B (I)	17.3	278	577	289	135.4 ± 7.44	ND
procyanidin dimer A	18.6	279	575	289	26.8 ± 4.46	ND
catechin	20.2	278	289	-	148.8 ± 5.95	ND
procyanidin dimer B (II)	23.8	279	577	289	55.1 ± 4.46	ND
quercetin-3,4′-diglucoside	26.4	356	625	301	46.1 ± 2.98	ND
quercetin 3-*O*-rutinoside	27.2	354	609	301	23.8 ± 2.98	ND
quercetin-3-*O*-arabinosyl-glucoside	29.6	355	595	301	80.4 ± 5.95	ND
quercetin-3-*O*-arabinoside	29.7	354	433	301	ND	94.1 ± 6.07
quercetin 3-*O*-galactoside	33.9	354	463	301	31.2 ± 4.46	144.1 ± 12.14
quercetin-3-*O*-glucoside	35.5	356	463	301	ND	54.6 ± 7.59
quercetin derivative (I)	40.3	353	479	301	ND	63.7 ± 9.10
quercetin derivative (II)	40.9	353	609	301	62.5 ± 29.76	ND
quercetin derivative (III)	44.4	355	565	301	ND	81.9 ± 9.10

Table 3. Contents of Individual Phenolic Compounds of Avocado Seeds from Two Varieties

compound name	t_R (min)	λ_{max} (nm)	$[M - H]^-$ (m/z)	fragment ions (m/z)	content (μg/g DW)	
					Hass	Shepard
3-*O*-caffeoylquinic acid	10.0	326	353	191, 179	57.5 ± 6.49	53.5 ± 4.54
3-*O*-*p*-coumaroylquinic acid	13.5	314	337	191, 163	13.6 ± 3.24	8.1 ± 3.03
procyanidin trimer A (I)	19.1	280	863	289	81.7 ± 6.49	98.9 ± 7.57
procyanidin trimer A (II)	21.7	280	863	289	89.3 ± 9.73	73.0 ± 4.54
catechin/epicatechin gallate	33.8	266, 299sh	441	283, 269	152.8 ± 14.60	105.4 ± 6.05

compositions varied in respect to the saccharide moiety present in the molecule.

In avocado seeds from both varieties five compounds were identified based on their UV and MS spectra (Table 3). The compound eluting at a t_R of 10.0 min displayed an absorption maximum λ_{max} at 326 nm, which is characteristic of isomers of caffeoylquinic acid. The ESI-MS spectrum of this compound displayed a negative molecular ion at a m/z of 353 and fragment ions at m/z of 191 and 179. Taking into consideration the intensity of fragment ions, 191 was the main peak corresponding to a quinic acid moiety. A comparatively intense signal from caffeic acid (m/z 179) meant the compound was assigned as 3-*O*-caffeoylquinic acid.[28] The compound with a retention time of 13.5 min and maximum adsorption at 314 nm displayed a deprotonated ion at a m/z of 337 and fragment ions at a m/z of 191 (predominant signal) and 163, and was identified as 3-*O*-*p*-coumaroylquinic acid.[28] On the basis of the λ_{max} at 280 nm two compounds eluting at a t_R of approximately 20 min were tentatively identified as flavanol derivatives. Due to the presence of a deprotonated molecular ion at a m/z of 863 and a fragment ion at a m/z of 289 in their ESI-MS spectra they were assigned as procyanidin trimers of the A type. The UV spectrum of the latter compound present in the chromatograms of avocado seed methanolic extracts was characterized by an absorption maximum at 266 nm and a shoulder at 299 nm. The compound possessed a negative molecular ion at a m/z of 441 and a fragment ion at a m/z of 283 and was tentatively identified as catechin or epicatechin gallate. All phenolic compounds identified in avocado seeds were present in extracts of both varieties.

The identifications of avocado fruit (pulp) phenolic compounds of three varieties, including Hass, have been recently published;[23,29] however, as previously stated the phenolic profile of avocado byproduct has not been extensively investigated. The characterization of procyanidin in avocado tissue has been reported by Wang et al.[16] Hurtado-Fernández et al.[29] developed a HPLC-DAD-ESI-TOF method for the identification of phenolic compounds in methanolic extracts of avocado fruit. These authors reported the presence of protocatechuic, *p*-coumaric, ferulic, sinapic, benzoic, *trans*-cinnamic acids, and (−)-epicatechin in avocado fruit from the Hass variety, none of which were detected in our study of avocado peel or seeds, whereas (+)-catechin was detected in both the peel in our study and the fruit in previous studies. The same authors[29] found gentisic, 4-hydroxybenzoic, chlorogenic, caffeic acids, laricitrin, naringenin, chrysin, and kaempferide in the avocado fruit of the Rugoro variety. It is also worth noting that, in a similar way to our study, a large number of quercetin glycosides were identified in the peel but not in seeds of mangos of the Ubá cultivar.[8]

To summarize, the compositional analysis in our study revealed that seed extracts contained polyphenols belonging to only three classes of compounds, namely flavanol monomers, proanthocyanidins, and hydroxycinnamic acids, whereas the peel extracts contained all of these compounds and flavonol glycosides. The study presented by Rodríguez-Carpena et al.[17] reported the presence of catechins, procyanidins, flavonols, and hydroxybenzoic and hydroxycinnamic acids in both peels and seeds of avocado.

The phenolic compounds identified in our study are reported as μg/g DW of avocado residue. The predominant compounds from the Hass variety peel were (+)-catechin (148.8 μg/g DW) and its dimers (sum of 217.3 μg/g DW). Wang et al.[16] identified and quantified monomers, oligomers, and polymers of procyanidins in different parts of the avocado fruit of eight varieties. The amounts of these compounds reported are higher than observed in our study, but this can be explained by differences in extraction method with the other authors aiming

to extract procyanidins (70% acetone). The 80% methanol used in our study enables the extraction of a wider range of low molecular weight phenolic compounds which we aimed to identify. It is worth noting that avocado peel of the Hass variety analyzed in our study contained A-type procyanidins, the health-benefits of which have been emphasized in recent reports to include the prevention of urinary tract infections.[30] Peel from the Shepard variety was devoid of flavan-3-ols or their polymers, and they contained a large amount of quercetin glycosides including quercetin 3-*O*-galactoside (144.1 μg/g DW), quercetin 3-*O*-arabinoside (94.1 μg/g DW), and quercetin 3-*O*-glucoside (54.6 μg/g DW). The presence of substantial amounts of two other quercetin derivatives with unidentified structures was also noted. The quercetin derivatives containing disaccharide moieties in the structure (quercetin 3-arabinosyl-glucoside, quercetin-3,4′-diglucoside and quercetin rutinoside) were predominant in the peel from the Hass variety.

It is of interest that the phenolic composition of seeds from both varieties did not vary considerably in contrast to the phenolic composition of peel from the same two avocado varieties. The predominant compound in seeds from both varieties was catechin gallate with a content amounting to 152.8 and 105.4 μg/g DW for Hass and Shepard varieties, respectively. The content of 3-*O*-caffeoylquinic acid was also similar in both varieties at approximately 55 μg/g DW, whereas the content of 3-*O*-*p*-coumaroylquinic acid was approximately 10 μg/g DW. The presence of substantial amounts of A type procyanidin trimers, which rarely occur in plant material, is important to note owing to their health-promoting properties.[30] Both chlorogenic acids and procyanidin trimers are of vital importance in regard to the antioxidant activity of phenolic compounds.[31,32]

Pronounced intervarietal differences have been observed in terms of the qualitative and quantitative phenolic composition of avocado peel, which was reflected in their different antioxidant activity. Higher antioxidant activity of the Hass peel was probably associated with the presence of procyanidin dimers and catechins, which were not found in the Shepard variety.[32,33] Overall findings indicate that both seeds and peel from avocado can be utilized as the basis for ingredients of functional foods or as antioxidant additives. A recently published paper confirms this potential as the authors describe the utilization of avocado byproduct as oxidation inhibitors during chilled storage of raw pork patties.[17,18]

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Notes

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ABBREVIATIONS USED

AAPH, 2,2′-azobis(2-amidinopropane); ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; CE, (+)-catechin equivalents; DPPH$^{\bullet}$, 2,2-diphenyl-1-picrylhydrazyl radical; DW, dry weight; ORAC, oxygen radical absorbance capacity; TEAC, Trolox equivalent antioxidant capacity; TPC, total phenolic content; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

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