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Avocado-Derived Biomass: Chemical Composition and Antioxidant Potential †

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Abstract: Avocado has become fashionable due to its great organoleptic and nutritional properties. It is consumed as a fresh product and it is also processed to obtain salad oil and guacamole. In all cases, the only usable portion is the pulp. Therefore, to be a more sustainable and profitable agribusiness, it is important to recognize which compounds from the peel and the stone waste can be converted into valuable bio-products. Therefore, their chemical composition was determined according to the National Renewable Energy Laboratory, the total phenolic content by the Folin-Ciocalteu method and the antioxidant properties by the FRAP and TEAC assays. The main components of the peel and stone were acid-insoluble lignin (35.0% and 15.3%, respectively), polymeric sugars (23.6% and 43.9%, respectively), and the aqueous extractives (15.5% and 16.9%, respectively). Both biomasses contain lipids and protein; but a minor proportion (<6%). The valorization of lignin and sugars is of interest given the high content; particularly; stones are a rich source of glucose (93.2% of the polymeric fraction), which could be used to obtain biofuels or derivatives of interest. The extractive fraction of the peel contained the highest amount of phenolic compounds (4.7 g/100 g biomass), mainly; concentrated in the aqueous fraction (i.e., 87%) compared to the ethanol one; which was subsequently extracted. It correlated with a major antioxidant activity and thereby the peel can be applied to obtain antioxidants and water can be used as an environmentally friendly extraction solvent

Keywords: agroindustrial residue valorization; avocado; peel; stone; natural antioxidants; polymeric sugars

1. Introduction

Avocado is one of the most commercial fruits for its organoleptic properties. Based on average production between 1994 and 2018, the top 10 avocado producing countries were México, followed by the Dominican Republic, Indonesia, Colombia, United States, Peru, Brazil, Chile, Kenya and China. In 2018 the production was around 6.4 million tons worldwide, of which these countries produced 77.4% [1].

An avocado fruit consisted of pulp (65–72%), stone (20–21%) and peel (7–15%) [2,3]. The only usable portion is the pulp, which is consumed fresh or into products such as halves and frozen cubes [4]. Other processed products are also obtained from avocado such as guacamole, oil, jams, candies juice, ice cream, or as a sauce like dips, chutney, sandwich spreads, mayonnaise, etc. The

industrialization around avocado gives as a result a huge amount of waste, for every 1000 kg of avocado processed, only around 78 kg or 85 L of cold pressing avocado oil are produced [5], so that tropical fruit can be better exploited if the residual parts are used as alternative source of value-added compounds, with different industrial purposes. This includes natural antioxidants such as phenolic compounds because consumers prefer products that contain few or no synthetic compounds. Therefore, to enable a complete valorization of avocado peel and stone in multiple bioproducts, the chemical composition was determined as well as their phenolic content and antioxidant activity were studied.

2. Methods

2.1. Raw Material

Avocado waste was obtained from the ripe fruit (cultivar 'Hass') bought in a local supermarket (Jaén, Spain). The fruit contained 12.9% of peel and 13.8% of stone with moisture of 71% and 52%, respectively, determined in a fresh weight basis. The peel and stone were dried at room temperature and protected from sun light and then exposed to a first grinding (4.0 mm) in Retsch SM100 Mill (Hann, Germany) and to a second grinding (1.0 mm) in Retsch ZM200 Mill (Hann, Germany).

2.2. Extraction and Chemical Characterization

The extraction and characterization methods are summarized in Figure 1. The air-dried byproducts were characterized in terms of moisture (105 °C, 24 h) and ash (575 °C, 4 h) [6]. The elemental analysis (H, C, N, and S) was determined with a TruSpec Micro, Leco (St. Joseph, MI, USA). The O content was estimated by difference, considering the percentages of H, C, N, S and ash. The protein content was estimated from the N content and applying a conversion factor of 6.25.

Aqueous and ethanolic extracts were obtained using Soxhlet extraction according to the National Renewable Energy Laboratory (NREL) methodology [7], which is based in two consecutive extraction steps, firstly with water and then with ethanol, for 24 h each step. After the evaporation of the solvent, the yield of the extracts was estimated and referred to the mass of peel and stone loaded in the thimble. Also, the aqueous extract was subjected to acid hydrolysis to measure sugars by high-performance liquid chromatography (HPLC) (1200 series) connected with a refractive index detector from Agilent Technologies (Palo Alto, CA, USA). An ICSep ICE-COREGEL 87 H3 column (Transgenomic, Inc., Omaha, NE, USA) was used set at 65 °C and the mobile phase was 5 mM sulfuric acid at 0.6 mL/min.

Then, lignin and carbohydrates were determined in the remaining solid fraction via two-step acid hydrolysis [11]. Briefly, H_2SO_4 (72% *w*/*w*) was added to the samples and the mixture placed in a water bath (30 °C) for 1 h. Later, water was added to dilute acid solution to 4% and to boil the samples for 1 h at 120 °C. This fractionates lignin into acid insoluble material and acid soluble material. The former is determined gravimetrically and the latter spectrophotometrically at 205 nm using a coefficient of extinction of 110 L/g cm. Moreover, during hydrolysis the polymeric carbohydrates are hydrolyzed into the monomeric forms, which are soluble in the hydrolysis liquid. They were then measured by HPLC using a 2695 liquid chromatographer from Waters (Milford, MA, USA) with refractive index detector and a CARBOSepCHO-782Pb (Transgenomic, Inc., Omaha, NE, USA) column. Ultra-pure water was used as eluent at a flow rate of 0.6 mL/min and a column temperature of 70 °C.

In addition, the lipid fraction was extracted using 190 mL of hexane recycling over 5 g of sample in a Soxhlet apparatus for 6 h [8]. Then, the solvent was removed at 60 °C and the yield was determined as percent of the mass of extracted lipid to the mass of peel and stone loaded in the thimble.

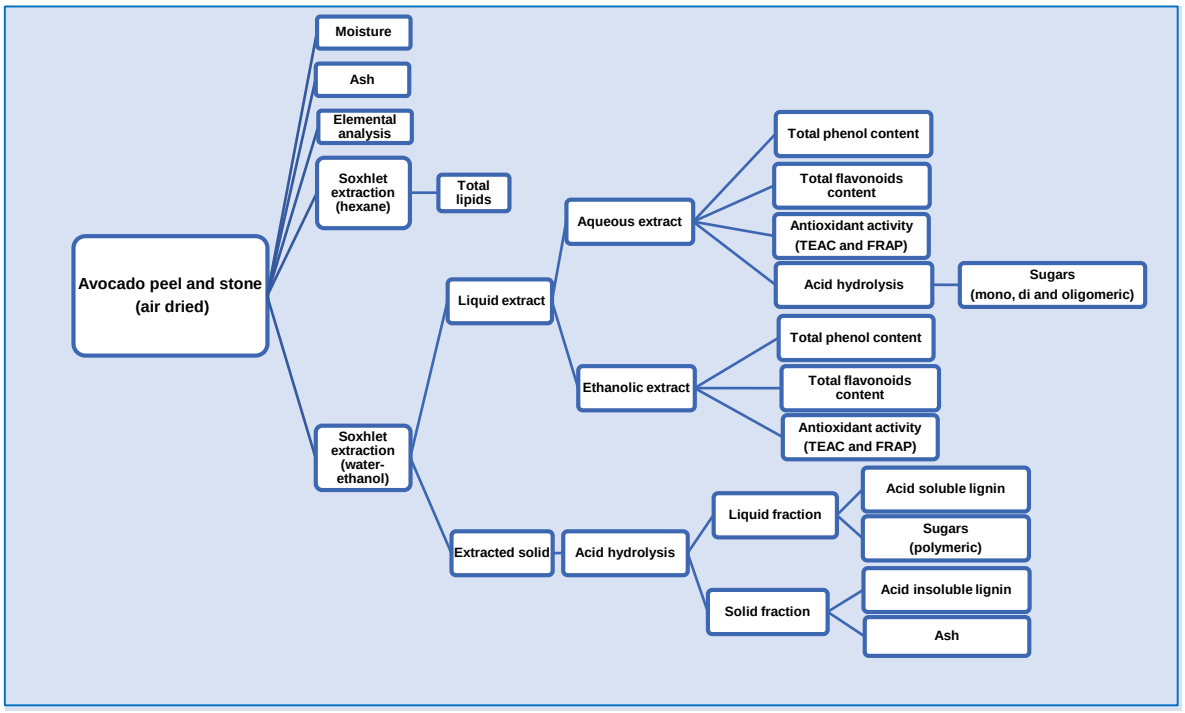

Figure 1. Avocado peel and stone characterization.

2.3. Total Phenolic Content (TPC)

The TPC was measured using the Folin-Ciocalteu colorimetric method, according to Lama-Muñoz [9], with some modifications. In brief, 0.02 mL of samples and 0.08 mL of Na_2CO_3 (0.7 M), with 0.01 mL of Folin-Ciocalteu reagent (0.2 M) was added. The solution was left at room temperature and its absorbance was measured with a microplate reader (Bio-Rad iMark™, Hercules, CA, USA) at 655 nm. The TPC was reported as g gallic acid equivalents (GAE)/100 g by using gallic acid calibration curve.

2.4. Total Flavonoids Content (TFC)

The TFC was determined using the aluminum chloride colorimetric method [9]. Brief, 1 mL of extract (adequately diluted) was mixed with 0.03 mL of 5% $NaNO_2$, after 5 min 0.03 mL of 10% $AlCl_3$ was added and mixed, 6 min later the extracts were neutralized with 2 mL of 1 N NaOH, and incubated for 5 min at room temperature in the dark. Absorbance was measured with the aforementioned microplate reader at 510 nm. The total flavonoid content was calculated from a calibration curve build with rutin, and the results were expressed as g rutin equivalents (RE) per 100 g.

2.5. Antioxidant Activity

The antioxidant activity was appraised by measuring the ability of the antioxidants to scavenge or reduce the cation $ABTS^{\bullet+}$ (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) and Fe^{3+} using the Trolox equivalent antioxidant capacity (TEAC) and ferric ion reducing antioxidant power (FRAP) assays, respectively, according to Medfai et al. [10]. The absorbance was measured with Bio-Rad iMark™ at 734 nm for TEAC and 593 nm for FRAP. Absorbance readings of the antioxidant extracts were compared to standard calibration curves of the hydrophilic vitamine E analog, Trolox (6–330 μM), and the results were expressed as mmol Trolox equivalents (TE) per 100 g.

3. Results and Discussion

3.1. Chemical Characterization of Avocado Waste

The avocado peel and stone presented similar elemental composition but the peel contained slightly higher percentages of nitrogen and oxygen (Table 1). The results of the stone agreed with the study by Perea-Moreno [11]. It showed a similar composition to that of olive stone and almond shell, two byproducts commonly used as biofuel for heating. As these authors highlighted, the avocado stone has a potential for this use but from an industrial point of view some drawbacks are the ash content and the humidity, which are higher than those reported in the reference materials. Sulfur was not detected and so the formation of SO_x can be diminished. Similar attention can be given to the avocado peel (Figure 1, Table 1). Therefore, obtaining valuable compounds before burning could be profitable to provide another value to this waste.

Table 1. Elemental composition of avocado peel and stone.

Element [1] (%)	Peel	Stone	Element [1] (%)	Peel	Stone
N	0.97 ± 0.07	0.66 ± 0.01	H	5.71 ± 0.02	5.58 ± 0.02
C	49.83 ± 0.42	42.05 ± 0.05	O	42.2 ± 2.62	50.79 ± 1.56
Ash	3.81 ± 0.05	2.76 ± 0.28	Humidity	70.9± 0.2	52.0 ± 0.4

[1] S was not detected.

The chemical composition of avocado waste is shown in Figure 2. In general, both biomasses showed higher differences in the polymeric glucose content and acid insoluble lignin; the former was the highest component in the avocado stone and the latter in the avocado peel.

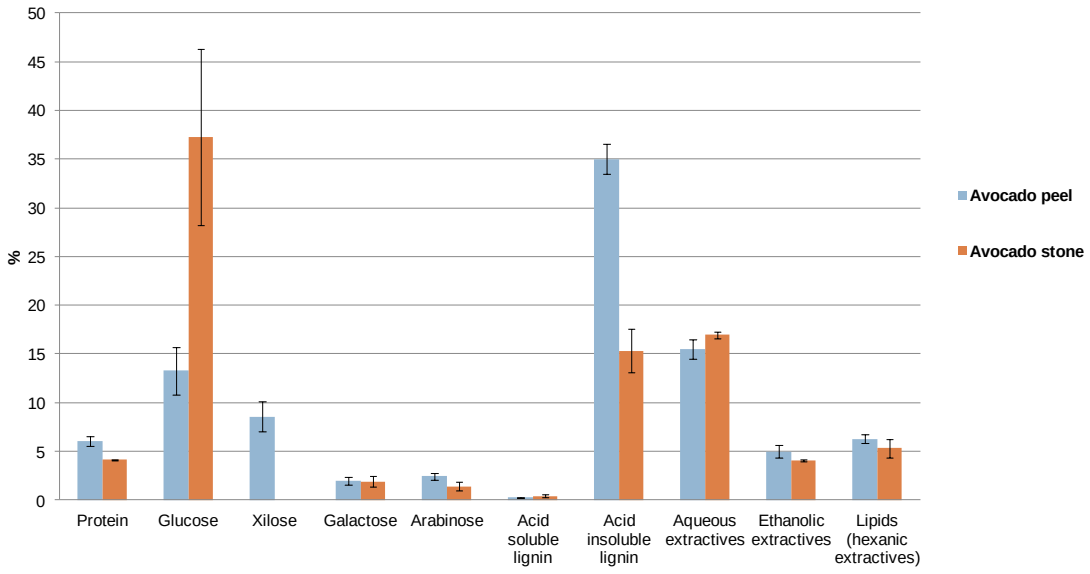

Figure 2. Chemical composition of the avocado peel and stone.

Therefore, the valorization of lignin and sugars is of interest given the high content, which could be used to obtain biofuels or derivatives of interest, such as aromatic compounds, furans and organic acids. Moreover, in terms of content, another interesting fraction was the extractive fraction, which contains non-structural components. The aqueous fraction was 15.5% and 16.9% of the peel and stone, respectively. It contained monomeric sugars, which was 15.0% and 72.5% of the aforementioned amount, respectively.

3.2. TPC, TFC and Antioxidant Activity

The extractive fraction also presents other non-structural components such as phenolic compounds. In avocado waste the content of this fraction was high, as commented before (Figure 2). Therefore, as a preliminary way to know the potential of avocado waste to obtain antioxidants, the TPC, TFC and the antioxidant activity were determined in the aqueous and ethanolic extracts obtained by the Soxhlet extraction. As Table 2 shows, the extractive fraction of the peel contained the highest amount of phenolic compounds (4.7 g/100 g biomass), mainly, concentrated in the aqueous fraction (i.e., 87%) compared to the ethanol one, which was subsequently extracted. It correlated with a major antioxidant activity and thereby the peel can be applied to obtain antioxidants and water can be used as an environmentally friendly extraction solvent.

The TPC found for avocado peel in the present study is within the range reported in the consulted literature, which goes between 2.0 and 10.7 g GAE/100 g of avocado waste [12]. Alternatively, the aqueous extracts obtained here from the peel were richer in phenolic compounds compared to those found in literature (values up to 9.0 g GAE/100 g) [13]; also, the antioxidant activity was higher. The difference in the values is attributed to the different pre-treatments, type of extractions and solvents used, in addition, place of origin and degree of maturity of the avocado, among others.

Table 2. Total phenolic content (TPC), total flavonoids content (TFC) and antioxidant activity determined by TEAC and FRAP assays.

Part	TPC		TFC		TEAC		FRAP	
	AE	**EE**	**AE**	**EE**	**AE**	**EE**	**AE**	**EE**
In terms of biomass weight (g GAE or g rutin or mmol TE/100 g, d.w.)								
AP	4.13 ± 0.56	0.60 ± 0.12	5.35 ± 1.36	0.75 ± 0.09	17.48 ± 3.12	0.47 ± 0.05	15.20 ± 2.02	1.49 ± 0.34
AS	0.31 ± 0.06	0.18 ± 0.03	0.45 ± 0.13	0.67 ± 0.02	1.66 ± 0.31	0.32 ± 0.08	1.29 ± 0.32	0.66 ± 0.05
In terms of extract weight (g GAE or g rutin or mmol TE/100 g, d.w.)								
AP	26.56 ± 2.77	12.60 ± 3.17	34.23 ± 6.90	15.63 ± 1.25	112.15 ± 13.35	9.67 ± 2.11	97.78 ± 7.83	37.77 ± 1.68
AS	1.81 ± 0.34	4.39 ± 0.88	2.66 ± 0.82	16.49 ± 0.80	9.85 ± 2.03	7.84 ± 2.04	7.71 ± 1.93	16.31 ± 1.62

AE, aqueous extract; AP, avocado peel; AS, avocado stone; EE, ethanolic extract; GAE, gallic acid equivalents; TE, trolox equivalents.

4. Conclusions

The avocado peel and stone have a high potential to obtain various valuable compounds from their chemical composition in a biorefinery context. Particularly, the stone is rich in glucose, mainly, from the polymeric fraction and the peel in lignin. In addition, the peel is a rich source of antioxidants and water can be used as an environmentally friendly extraction solvent.

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Conflicts of Interest: The authors declare no conflict of interest.

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