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Proceeding Paper

Characterization and Biological Analysis of Avocado Seed and Peel Extracts for the Development of New Therapeutical Strategies †

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Abstract: The avocado is one of the most produced and consumed tropical fruits worldwide, so more than 1.5 million tons of waste are generated per year, especially due to their main by-products: seed and peel. In order to demonstrate whether these extracts are able to exert health benefits related to oxidative stress, a series of in vitro tests were conducted: identification and quantification using HPLC, evaluation of antioxidant capacity and ability to inhibit enzymatic overactivation. Finally, avocado peel stood out as the more antioxidant extract, due to its higher phenolic content. However, both extracts can be considered as great options for developing new high-added value products.

Keywords: avocado by-products; phenolic compounds; antioxidant properties; neuroprotection; revalorization

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1. Introduction

Avocado fruit, highly consumed through the last decade, is composed by pulp (among 65–73%) and the main by-products seed and peel, more than the third part of the total weight [1]. These parts are usually discarded after industrialization of avocado pulp, generating high amounts of wastes per year. Also, different studies have proven that these by-products are rich in bioactive compounds with interesting health-related properties, even presenting higher quantity of phenolic compounds than the pulp [2,3]. Some of the therapeutic effects already demonstrated are antiaging, anticarcinogenic, anti-inflammatory and antioxidant, among others [4].

In order to highlight the potential of avocado seed and peel as phenolic compounds sources, an in vitro comprehensive evaluation was performed. Among the assays conducted, the evaluation of the phenolic content of both matrixes, the assessment of the antioxidant activity, the evaluation of the free radical scavenging capacity, as well as the determination of the inhibitory concentration for the overactivation of different enzymes caused by stress oxidative. Finally, the identification of the main bioactive compounds of both matrixes was also performed using HPLC coupled to mass spectrometry.

In the present work, an in-depth study of avocado by-products seed and peel is performed for the first time on pre-industrial scale extracts, determining their future applications on different industries such as food, pharmacological or cosmetic ones.

2. Methods

2.1. Extraction of Avocado By-Products by Solid-Liquid Extraction (SLE)

Pre-industrial extract from avocado seed and peel 'Hass' variety were obtained. Then, 3 cycles of SLE were performed, using temperatures among 50 and 70 °C, with 200 L of hydroalcoholic mixture (60/70%) for 20 kg of raw material over 2 h. Next, after decantation and drying, both extracts were ground and sieved, obtaining 2 mm particles. The material was stored at room temperature protected from sunlight.

2.2. Determination of TPC and In Vitro Antioxidant Activity

Antioxidant properties from avocado seed and peel were evaluated by Ferric Reducing Antioxidant Power (FRAP), Trolox Equivalent Antioxidant Capacity (TEAC) and Oxygen Radical Absorbance Capacity (ORAC). Also, the Total Phenolic Content (TPC) was determined according to Folin-Ciocalteu method. All procedures were conducted as previously described in Rojas-García et al. (2022) [5]. Measurements were made in triplicate.

2.3. Evaluation of Reactive Oxygen Species (ROS) and Free Radical Scavenging Potential

Superoxide ($\cdot O_2^-$) was evaluated by a colorimetric method based on NBT reduction to diformazan. Nitric oxide ($\cdot NO$) and hypochlorous acid (HOCl) were assessed by fluorometric-based assay [5]. Results were expressed as the concentration needed to inhibit the ROS formation by half (IC_{50}). Measurements were performed in triplicate.

2.4. Evaluation of Enzymatic Inhibition Potential

Measures were made in triplicate, and the IC_{50} was calculated using different avocado by-products extracts concentrations. Procedures were carried out following previous studies [5]. Tyrosinase and xanthine oxidase were evaluated by full-prepared kits.

2.5. Characterization and Quantification of Phenolic Compounds by HPLC-ESI

Using a concentration of 5000 mg/L, avocado seed and peel extracts were analysed by HPLC (Waters) coupled to mass spectrometry (Waters Corp). Mobile phases were water with acetic acid and acetonitrile. Detection was conducted in the negative ionization mode from 50 to 1200 *m/z*. MZmine 2.53 and Sirius 4.4.29 softwares were chosen to process and visualise information. Identification was contrasted with the reviewed literature.

Quantification was performed using linear ($R^2 > 0.99$) calibration curves of reference compounds. A pattern mix was prepared with standards diluted to concentrations from 0.5 to 500 mg/L. Selected standards were quinic acid, chlorogenic acid, procyanidin B1, catechin, quercetin, quercetin glucoside, myricetin-3-glucoside and verbascoside. For quantification parameters, data are collected in Rojas-García et al. (2022) [5].

3. Results and Discussion

3.1. Evaluation of TPC and Antioxidant Capacity

Antioxidant activity determines the level of protection of the biological system against oxidative stress and all their related harmful effects. Therefore, an approximate way to know the beneficial effect of certain extracts is to in vitro evaluate their antioxidant potential, as well as their phenolic content.

Results obtained from TPC and antioxidant activity evaluation are shown in Figure 1. Avocado peel extract seems to possess higher concentrations of phenolic compounds than seed, what can be linked to differences in environmental stress levels. While seed is protected by the pulp, peel protects the rest of the fruit from exogenous agents such as sunlight, which triggers a massive generation of phenolic compounds in order to avoid oxidative damage [6]. This higher phenolic richness can explain also that peel stands out as the best antioxidant extract by both single electron transfer (SET) and hydrogen atom transfer (HAT) mechanisms.

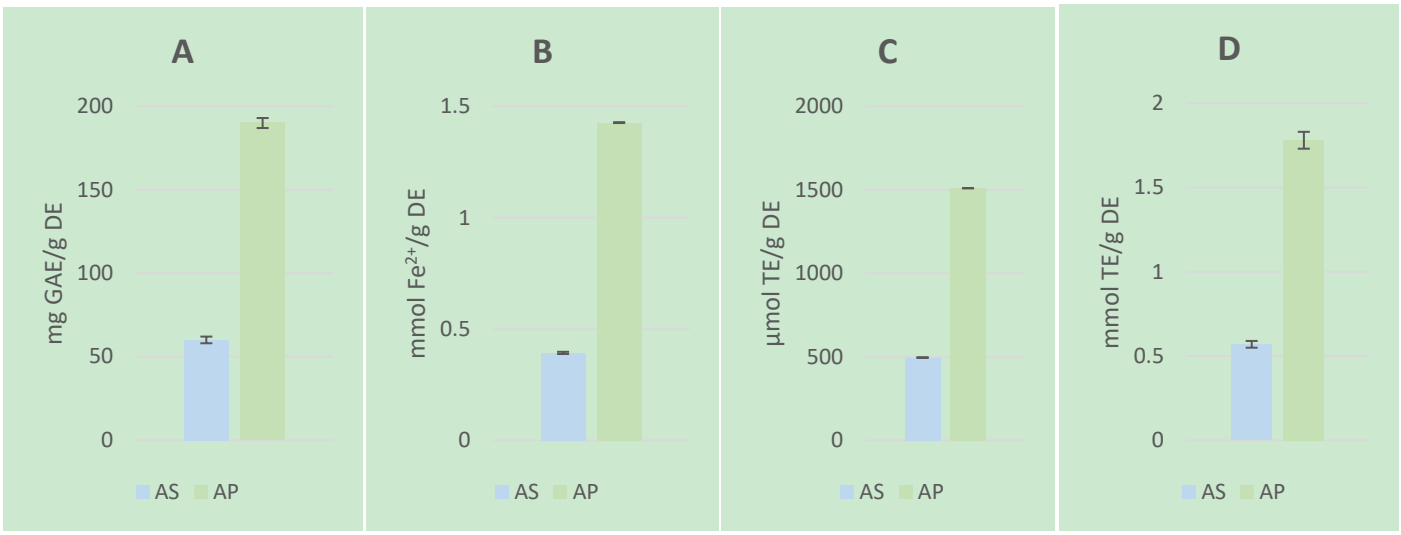

Figure 1. Quantification and antioxidant evaluation of avocado seed (AS) and peel (AP). (**a**) TPC, (**b**) FRAP, (**c**) TEAC and (**d**) ORAC.

3.2. Assessment of ROS and Free Radical Scavenging Capacity

Endogenous ROS and free radicals are usuals in average biochemical processes. However, an abnormal generation could lead to the promotion of oxidative stress, a pathology related to different diseases such as cancer, neurodegeneration, diabetes, cardiovascular illness, etc. [7,8]. So, the radical scavenging capacity of avocado extracts was tested against three common ROS: $\cdot O_2^-$, ·NO and HOCl. Results are shown in Figure 2 with positive controls tested gallic acid (GA) and epicatechin (EPI).

Avocado peel extract showed better results scavenging ROS and free radical species than seed, except for HOCl scavenging. Excluding $\cdot O_2^-$, peel extract exerted similar scavenging results than those showed by GA and EPI, so its performance as antiradical extract is highly remarkable.

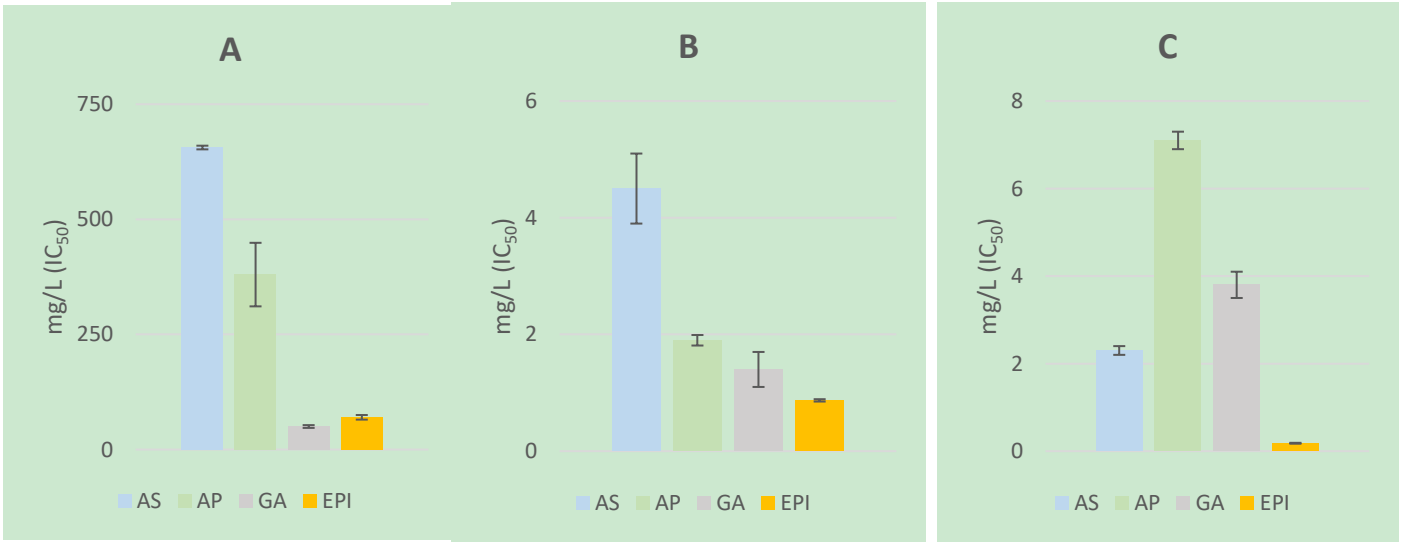

Figure 2. Radical scavenging assessment of (**a**) $\cdot O_2$, (**b**) ·NO and (**c**) HOCl by avocado seed (AS) and peel (AP). Results are expressed as the concentration in mg/L needed to inhibit the response by 50% (IC_{50}). Data are means ± standard deviation (n = 3) Positive controls used were GA, Gallic acid, and EPI, Epicatechin.

3.3. Analysis of Enzymatic Inhibition Capacity

The excessive generation of ROS and radical species can lead to the deleterious state of human body, with the overaction of different enzymes among them. Those tested in this assay were acetylcholinesterase (AChE) for neurodegeneration; xanthine oxidase (XO) for oxidative stress; and tyrosinase, elastase, hyaluronidase and collagenase for skin aging [9].

Results from enzyme inhibition evaluation are shown in Figure 3. According to their IC_{50} or IC_{30} values, and comparing to positive controls tested and shown, peel extract highlighted inhibiting hyaluronidase, XO and collagenase enzymes, while seed especially worked inhibiting AChE and hyaluronidase enzymes. Both extracts exerted notable therapeutic potential, in addition to the fact that their activity seemed complementary to each other, thus reaching broader targets. Moreover, in some cases these extracts showed higher inhibitory activity than the positive controls, thus enhancing their therapeutic interest. Further research must be performed in order to determine how synergistic both extracts are. In the case of AChE and elastase, the positive controls physostigmine and elastatinal exerted an inhibitory activity several orders higher than the extracts, so they were omitted.

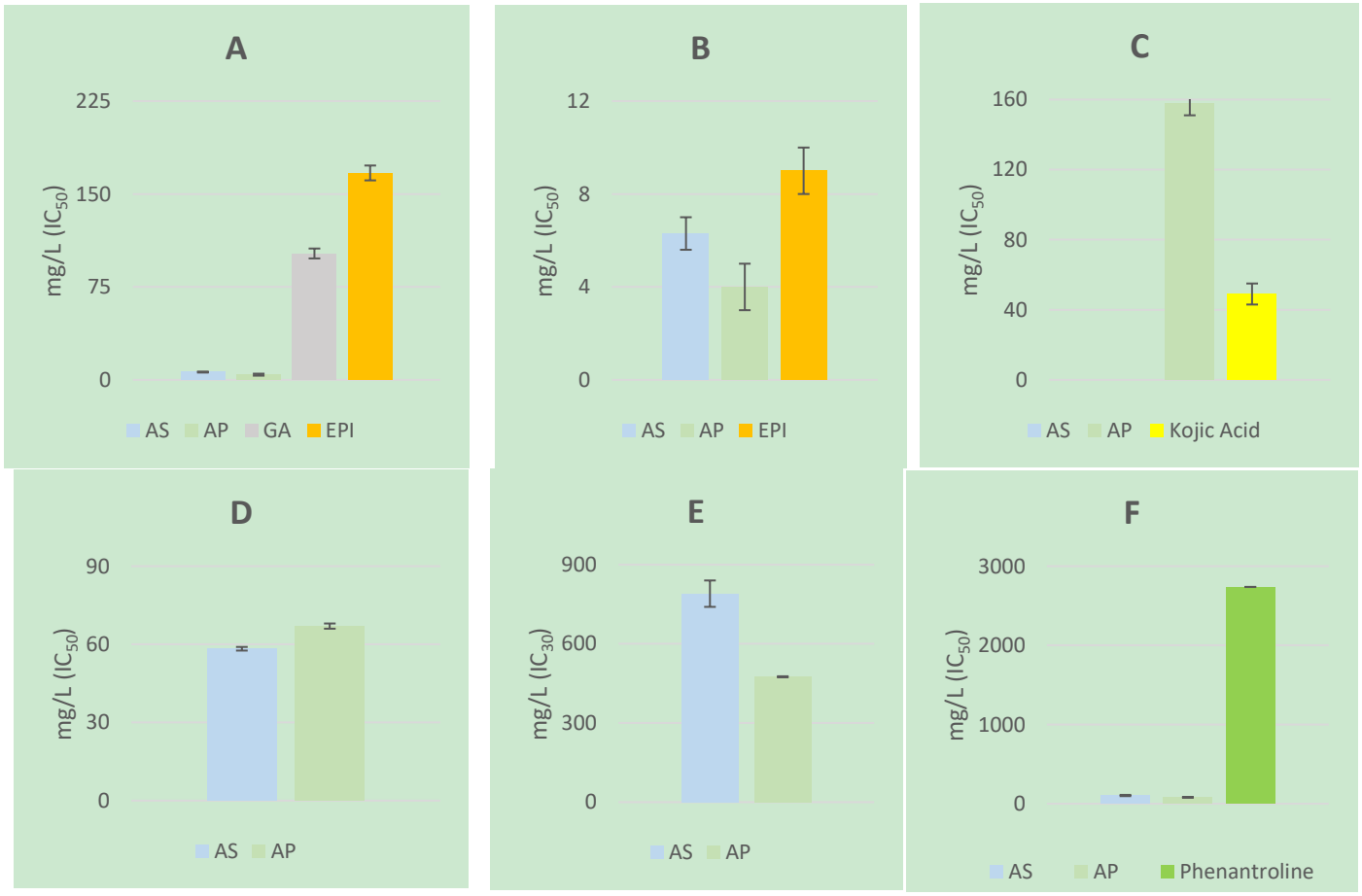

Figure 3. Inhibitory capacity analysis of (**a**) hyaluronidase, (**b**) xanthine oxidase, (**c**) tyrosinase, (**d**) acetylcholinesterase, (**e**) elastase and (**f**) collagenase, by avocado seed (AS) and peel (AP). Data are means ± standard deviation (n = 3). Results are expressed as IC_{50} (mg/L). EPI (epicatechin), kojic acid and phenantroline were used as positive controls, in addition to physostigmine and elastatinal for acetylcholinesterase and elastase, respectively.

3.4. Characterisation Using HPLC-ESI of Avocado Seed and Peel Extracts

From the characterization, 69 compounds were found in both matrixes, some of them for the first time [10,11]. The quantification was majorly performed using structurally

related substances. Results are expressed as mean ± standard deviation in mg of analyte per gram of dry extract (DE). Further information about characterization and quantification of main phenolic compounds are shown in Rojas-García et al. (2022) [5].

The tentative characterization and quantification of both avocado extracts reflected a higher quantity and variety of phenolic compounds in avocado peel extract, which would explain its better results at antioxidant activity. In avocado seed, the main composition is formed by trimer procyanidins and derivatives of chlorogenic acid, while avocado peel harbors a substantial amount of glycosylated quercetins (arabinosyl, diglucoside, rhamnoside, xylosyl rhamnoside, rutinoside, etc.) and procyanidins with several degrees of polymerization (dimers, trimers, tetramers, etc.). Other compounds were (epi)catechin, luteolin, kaempferol, and different acids such as quinic acid or shikimic acid. Regarding quantification, avocado seed showed a concentration of 14 ± 1 mg/g DE, while avocado peel showed 144 ± 2 mg/g DE. The presence of these bioactive compounds explains bigger TPC value, and is responsible for the higher antioxidant activity of avocado peel; i. e. flavonoids such as quercetin or kaempferol show an arrangement of hydroxyl groups attached to aromatic rings which promotes avocado peel bioactivity profile, or the presence of different procyanidins, which have been formerly related to higher antioxidant activity and chelating properties for scavenging ROS [12].

4. Conclusions

After a deep evaluation of avocado by-products properties, both extracts can be highlighted as great sources of antioxidants that could be employed for therapeutical benefits. However, avocado peel did show higher bioactivity than seed, exerting great antioxidant activity, ROS scavenging capacity and the ability to inhibit XO and elastase enzymes. Also, its identification resulted in a matrix especially rich in phenolics such as flavonoids, procyanidins and acids, which are responsible for its remarkable therapeutic potential.

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