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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 its main by-products: the 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: an identification and quantification using HPLC, and an evaluation of the antioxidant capacity and ability to inhibit enzymatic overactivation. In the end, the 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

The avocado fruit, highly consumed throughout the last decade, is composed of pulp (around 65–73%), and its main by-products—the seed and peel—comprise more than one third of the total weight [1]. These parts are usually discarded after the industrialization of the avocado pulp, generating high amounts of waste each year. In addition, different studies have proven that these by-products are rich in bioactive compounds with interesting health-related properties, even presenting a 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 compound sources, an in vitro comprehensive evaluation was performed. Among the assays conducted were 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 oxidative stress. 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, the seed and peel, is performed for the first time on pre-industrial scale extracts, determining their future applications in different industries such as food, pharmacological or cosmetic.

2. Methods

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

Pre-industrial extracts from avocado seed and peel ('Hass' variety) were obtained. Then, three cycles of SLE were performed, using temperatures between 50 and 70 °C, with 200 L of a hydroalcoholic mixture (60/70%) for 20 kg of the 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 and protected from sunlight.

2.2. Determination of TPC and In Vitro Antioxidant Activity

The antioxidant properties from the 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 the Folin–Ciocalteu method. All the procedures were conducted as previously described in Rojas-García et al. (2022) [5]. The measurements were made in triplicate.

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

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

2.4. Evaluation of Enzymatic Inhibition Potential

The measurements were made in triplicate, and the IC_{50} was calculated using different avocado by-product extract concentrations. The procedures were carried out following the previous studies [5]. The tyrosinase and xanthine oxidase were evaluated by fully prepared kits.

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

Using a concentration of 5000 mg/L, the avocado seed and peel extracts were analyzed by HPLC (Waters) coupled to mass spectrometry (Waters Corp, Milford, MA, USA). The mobile phases were water with acetic acid and acetonitrile. The detection was conducted in the negative ionization mode from 50 to 1200 m/z. The MZmine 2.53 and Sirius 4.4.29 software were chosen to process and visualize the information. The identification was contrasted with the reviewed literature.

The quantification was performed using linear ($R^2 > 0.99$) calibration curves of reference compounds. A pattern mix was prepared with the standards diluted to concentrations from 0.5 to 500 mg/L. The selected standards were quinic acid, chlorogenic acid, procyanidin B1, catechin, quercetin, quercetin glucoside, myricetin-3-glucoside and verbascoside. For the quantification parameters, the 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 its 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.

The results obtained from the TPC and antioxidant activity evaluation are shown in Figure 1. Avocado peel extract seems to possess higher concentrations of phenolic compounds than that from the seed, which can be linked to differences in the environmental stress levels. While the seed is protected by the pulp, the 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 why the peel stands out as the best antioxidant extract by both the single electron transfer (SET) and hydrogen atom transfer (HAT) mechanisms.

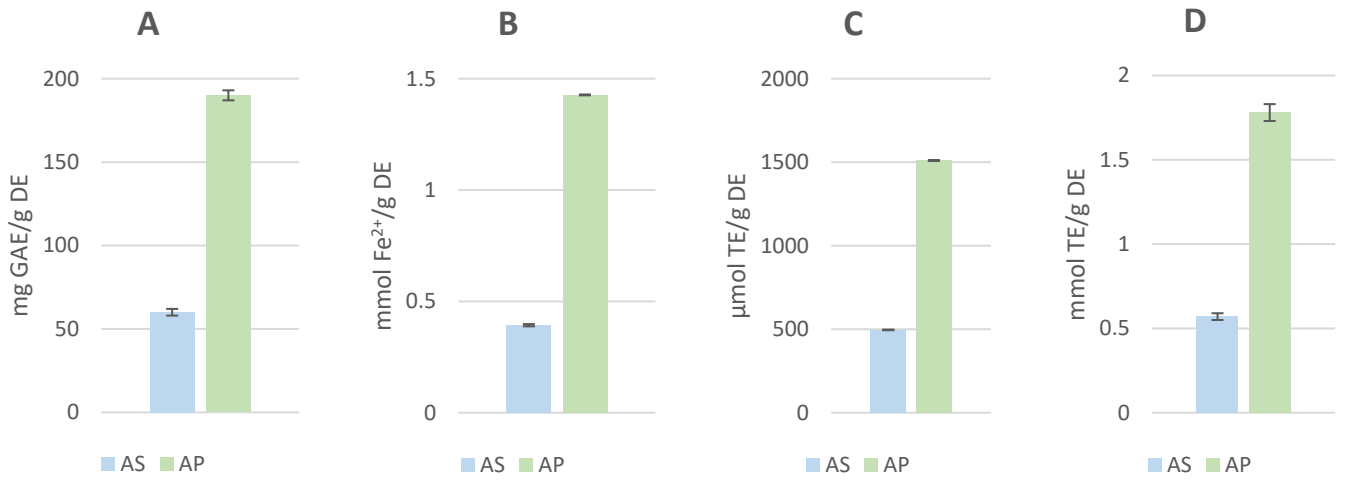

Figure 1. Quantification and antioxidant evaluation of avocado seed (AS) and peel (AP). (**A**) TPC, (**B**) FRAP, (**C**) TEAC and (**D**) ORAC.

3.2. Assessment of ROS and Free-Radical Scavenging Capacity

Endogenous ROS and free radicals are normal 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: O_2^{-}, NO and HOCl. The results are shown in Figure 2, with gallic acid (GA) and epicatechin (EPI) as the positive controls.

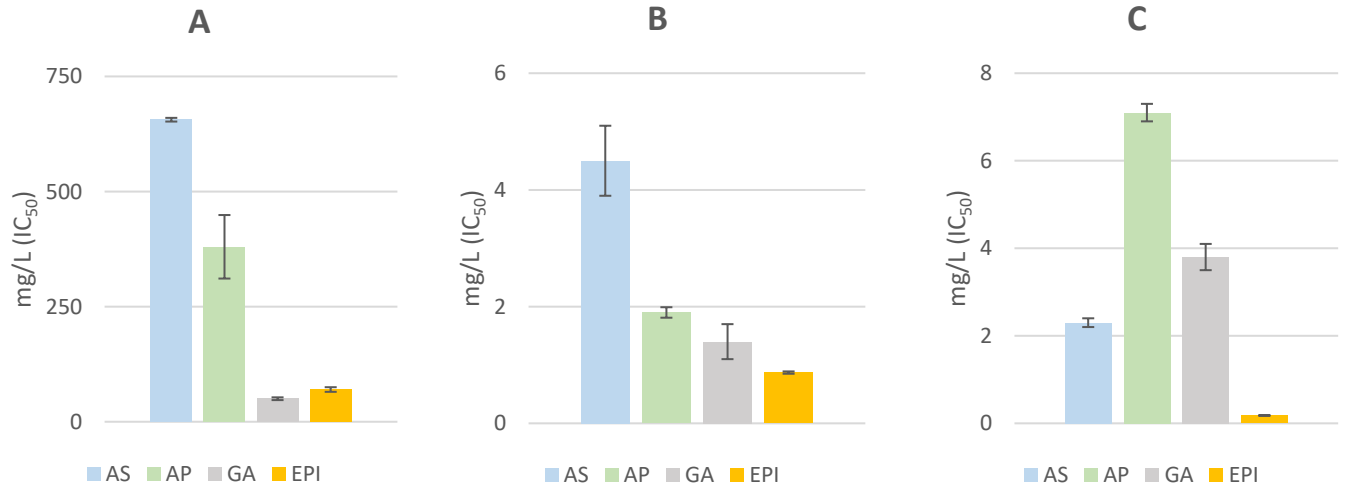

Figure 2. Radical scavenging assessment of (**A**)·O_2, (**B**)·NO and (**C**) HOCl by avocado seed (AS) and peel (AP). Results are expressed as the concentration in mg/L needed to inhibit the response by 50% (IC_{50}). Data are means ± standard deviation (n = 3) Positive controls used were GA, gallic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

The avocado peel extract showed better results scavenging ROS and free radical species than the seed, except for HOCl scavenging. Excluding O_2^{-}, the peel extract exerted similar scavenging results to those shown by the GA and EPI, so its performance as an antiradical extract is highly remarkable.

3.3. Analysis of Enzymatic Inhibition Capacity

The excessive generation of ROS and radical species can lead to deleterious effects on the 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].

The results from the enzyme inhibition evaluation are shown in Figure 3. According to its IC_{50} or IC_{30} values, and comparing to the positive controls tested and shown, the peel extract was shown to inhibit the hyaluronidase, XO and collagenase enzymes, while the seed especially worked to inhibit the 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.

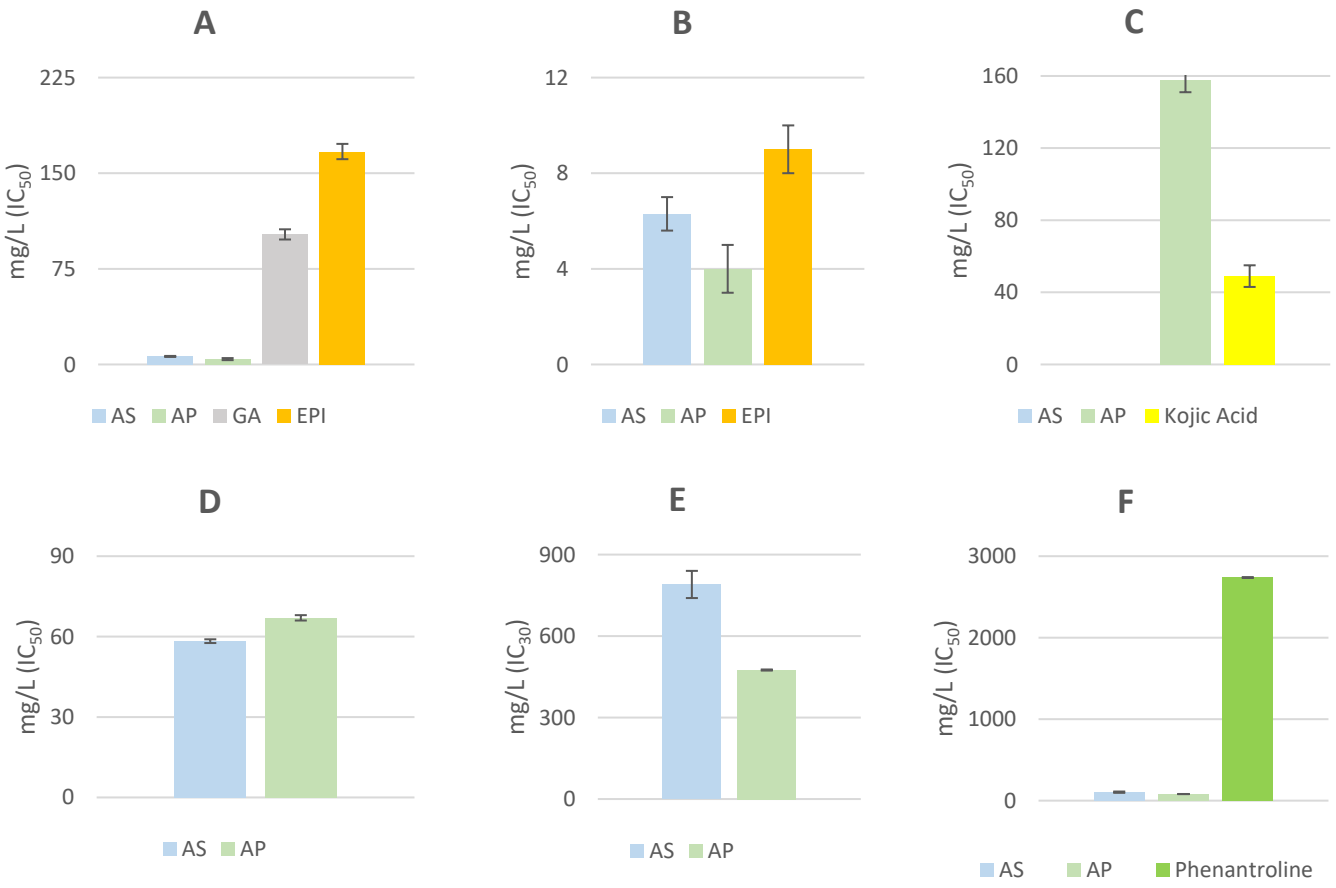

Figure 3. Inhibitory capacity analysis of (**A**) hyaluronidase, (**B**) xanthine oxidase, (**C**) tyrosinase, (**D**) acetylcholinesterase, (**E**) elastase and (**F**) collagenase, by avocado seed (AS) and peel (AP). Data are means ± standard deviation (n = 3). Results are expressed as IC_{50} (mg/L).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

3.4. Characterization 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 mainly performed using structurally

related substances. The results are expressed as the mean ± standard deviation in mg of analyte per gram of dry extract (DE). Further information about the characterization and quantification of the main phenolic compounds is 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 the avocado peel extract, which would explain its better results in antioxidant activity. In the 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 the quantification, the avocado seed showed a concentration of 14 ± 1 mg/g DE, while the avocado peel showed 144 ± 2 mg/g DE. The presence of these bioactive compounds explains the larger 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 the 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 the avocado by-product properties, we can conclude that both extracts are great sources of antioxidants that could be employed for therapeutical benefits. However, the avocado peel did show higher bioactivity than the 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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