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Comprehensive identification of bioactive compounds of avocado peel by liquid chromatography coupled to ultra-high-definition accurate-mass Q-TOF

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ABSTRACT

Industrially the avocado pulp is exploited principally as oil and paste, generating a huge quantity of peel and seed as by-products. Avocado peel is a promising inexpensive candidate for recovery phenolic compounds. The aim of this work was to identify the bioactive compounds present in an extract of avocado peel obtained by a green extraction technique. Accelerated solvent extraction was performed using water and ethanol as extraction solvents. Liquid chromatography coupled to ultra-high-definition accurate-mass spectrometry was used in order to identify the bioactive compounds. A total of sixty-one compounds belonging to eleven families were identified. Procyanidins, flavonols, hydroxybenzoic and hydroxycinnamic acids were the most common compounds. A sum of thirty-five compounds has been identified here for the first time in avocado peel. These results confirm the potential of avocado peel as a source of bioactive ingredients for its use in the food, cosmetic or pharmaceutical sector.

1. Introduction

Avocado, (*Persea americana* Mill., Lauraceae), is a very nutritious fruit endemic to the humid tropical areas of Mexico, although nowadays, it is widely grown worldwide on large scale in various subtropical countries (Rodríguez-Carpena, Morcuende, Andrade, Kylli, & Estévez, 2011). In 2014 the world production was 5 million tons, and Mexico was the largest producer with 1.5 million tons (FAO, 2017). The European Union is the major importer in the world (Rodríguez-Carpena et al., 2011), while the principal cultivars are Mexican, West Indian and Guatemalan. Current commercial varieties are hybrids of these cultivars, such as the 'Hass' variety, one of the most popular type grown and imported (Kosińska et al., 2012), which belongs to the Guatemalan-Mexican hybrid group (Wang, Bostic, & Gu, 2010).

Currently, the food and cosmetic industries are experiencing a constantly growing demand for new ingredients from natural sources as alternatives to synthetic substances. An example is the case of BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene), whose use in the food industry have been restricted because of their suspected carcinogenic effect and the growing consumer demand for natural products (Jayaprakasha, Ohnishi-Kameyama, Ono, Yoshida, & Jaganmohan Rao, 2006). In this sense, food industry by-products have a great potential to be used as a source of these demanded natural ingredients (Wang et al., 2010).

Industrially, only the avocado pulp is exploited as oil and paste generating a large quantity of seed and peel, which are discarded with no further applications. These by-products represent about 25% of the total fresh weight of the fruit, so its waste could cause environmental problems (Rodríguez-Carpena et al., 2011). Nevertheless, the avocado seed and peel had high amounts of extractable bioactive compounds, as polyphenols (Wang et al., 2010), which could be used in many applications due to their well-known bioactivity (Calderón-Oliver et al., 2016; Rodríguez-Carpena et al., 2011). Wang et al. (2010) reported that the avocado peel and seed showed much higher phenolic content than the pulp. Therefore, these by-products could be promising inexpensive candidates for recovery phenolic compounds, which could be used in the pharmaceutical, cosmetic and food industries as bioactive ingredients. Moreover, the utilization of these by-products add value to the avocado industry, minimize cost and environmental impact (Rodríguez-Carpena et al., 2011; Wang et al., 2010), whereas its

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extracts do not contain potentially toxic or harmful components (Rodríguez-Carpena et al., 2011).

Despite its great potential, the existing knowledge about phenolic profile in avocado by-products is scarce, especially in peel. Furthermore, the majority of existing literature use conventional extraction techniques based on maceration (Calderón-Oliver et al., 2016; Fidelis et al., 2015; Kosińska et al., 2012; Morais et al., 2015; Saavedra et al., 2017; Widsten, Cruz, Fletcher, Pajak, and McGhie, 2014; Wong et al., 2016) or this extraction procedure assisted by ultrasonic bath (López-Cobo et al., 2016; Wang et al., 2010). In this sense, only Rodríguez-Carpena et al. (2011) have used pressurized liquid extraction, but they do not presented an individual identification of the obtained extract. Generally, the extraction of phenolic compounds in these studies was based on the use of organic solvents, such as methanol and acetone. On the contrary, Accelerated Solvent Extraction (ASE) is a solid–liquid extraction process performed at high temperatures (50–200 °C) and high pressures (10–15 MPa), being its main advantages over traditional extraction methods the dramatic decreases in the amount of solvent used and the extraction time (H. Sun, Ge, Lv, & Wang, 2012). Moreover, ASE is a "green" technology that allows the use of ethanol, water or their mixtures, (generally recognized as safe or GRAS solvents) for the extraction of different classes of compounds, such as polyphenols. Furthermore, several studies have pointed out that ASE obtains similar or greater yields than conventional or ultrasonic assisted extraction (UAE) (Luthria, Biswas, & Natarajan, 2007; H. Sun et al., 2012). The aim of the present study was to determine the polar fraction of avocado peel using a powerful green extraction technique (ASE) with GRAS solvents in combination with a potent analytical platform (high resolution liquid chromatography coupled to ultra-high-definition accurate-mass spectrometry, HPLC-ESI-QTOF-MS) for the individual identification of bioactive compounds.

2. Material and methods

2.1. Chemicals and reagents

For identification purposes, the standards compounds benzoic acid, (+)-catechin, chlorogenic acid, citric acid, (-)-epicatechin, gentisic acid, protocatechuic acid, quercetin, quercetin-3-β-glucoside, rutin and (±)-naringenin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Moreover, 4-hydroxybenzoic acid was sourced from Fluka Chemika (Buchs, Switzerland), quinic acid was supplied from Acros Organics (Geel, Belgium), while dihydrocaffeic acid, procyanidin dimer A2 and B2 standards were purchased from Extrasynthese (Genay Cedex, France). For mobile phase preparation, formic acid was purchased from Fluka, Sigma-Aldrich (Steinheim, 126 Germany), HPLC-MS-grade acetonitrile were purchased from Fisher Scientific (Leicestershire, UK), and ultrapure water was obtained with a Milli-Q system (Millipore, Bedford, MA, USA). Ethanol and Ottawa sand for extraction were purchased from Fisher Scientic (Leicestershire, UK).

2.2. Samples

'Hass' avocados from the subtropical coast of Granada were provided by the commercial group La Caña, Miguel García Sánchez e Hijos, S.A. (Motril, Spain). All fruits were kept at room temperature until full maturity. Fully ripened fruits were manually separated into pulp, peel and seed. Peel was cleaned under continuous flow of tap water. Then, the obtained by-product was chopped and oven-dried at 85 °C while turned periodically to ensure uniform dryness until a percentage moisture content of less than 10%. After that, the dried peel was milled in an ultra-centrifugal mill ZM 200 (Retsch GmbH, Haan, Germany). The resulting avocado peel powder had an average particle size of 0.5 mm. The material was stored at room temperature and protected from light until its extraction and analysis.

2.3. Accelerated solvent extraction (ASE)

For bioactive compounds extraction, an accelerated solvent extractor model ASE 350 (Dionex Corp., Sunnyvale, CA) equipped with a solvent controller was used. A portion of dried peel (1.5 g) was mixed homogeneously with 5 g of sand and transferred into 34 mL stainless-steel extraction cells. Disposable cellulose filters were placed into the cell's inlet and outlet in order to prevent clogging in the metal frits of the extraction cell. The extractions were performed at 200 °C with a mixture of ethanol/water (1:1, v/v) as solvent. Prior to use, solvents were sonicated for 15 min to remove the dissolved oxygen in order to avoid any possible oxidation. The extraction of avocado peel bioactives was performed with a static extraction cycle of 20 min at 11 MPa. An extraction cell heat-up step of 9 min was carried out prior to the static cycle. After that, the cell was flushed with solvent (60% of the cell volume), purged with nitrogen (100 s) and immediately cooled in ice to attain a temperature of 20–25 °C. The obtained extracts were centrifuged at 12,000 rpm for 15 min at 4 °C in a Sorvall ST 16 R centrifuge (Thermo Scientific, Leicestershire, UK) and the supernatants were evaporated at 35 °C to dryness in a Savan SC250EXP Speed-Vac (Thermo Scientific, Leicestershire, UK). The extract was stored at −20 °C until further use. All experiments were performed in duplicate.

2.4. Determination of phenolic and other polar compounds by HPLC-DAD-ESI-QTOF-MS

The extracts were reconstituted with the same solvent used in the extraction at a concentration of 10,000 mg/L. Then, the extracts were filtered with regenerated cellulose syringe-filters of 0.2 µm pore size (Millipore, Bedford, MA, USA). The purified extracts were analysed using an Agilent 1260 series Rapid Resolution LC coupled to a diode-array detector (DAD) and an Agilent 6540 Ultra High Definition (UHD) Accurate Mass Q-TOF with a Jet Stream dual ESI interface (Agilent Technologies, Palo Alto, CA, USA). The instrument was equipped with a vacuum degasser, a binary pump, and a thermostated autosampler and column compartment. Compounds were separated using a Zorbax Eclipse Plus C18 column (4.6 × 150 mm, 1.8 µm; Agilent Technologies, Palo Alto, CA, USA). Acidified water (0.1% formic acid, v/v) and acetonitrile were used as mobile phases A and B, respectively. The elution gradient was conducted at a constant flow rate of 0.5 mL/min as follows: 0 min, 95% A; 25 min, 50% A; 33 min, 0% A; 36 min, initial conditions until 40 min as a re-equilibration step. The sample volume injected was 10 µL. The autosampler and column temperatures were maintained at 4 and 25 °C, respectively. The UV spectra were recorded from 190 to 600 nm, whereas the compounds were monitored at 240, 280 and 340 nm.

MS analyses were carried out using the following operating conditions: drying nitrogen temperature at 325 °C with a flow of 10 L/min; nebulizer pressure, 20 psi; sheath gas temperature at 400 °C with a flow of 12 L/min; capillary, nozzle, fragmentor, skimmer and octopole radiofrequency voltages of 4000, 500, 130, 45 and 750 V, respectively. The spectra were acquired in negative ionization mode over a mass-to-charge (m/z) range 100–1700, and the detection window was set to 100 ppm. Data acquisition (2.5 Hz) in the centroid mode was performed by MassHunter Workstation software (Agilent Technologies, Palo Alto, CA, USA). To maintain mass accuracy during the runtime, a continuous infusion of Agilent TOF mixture containing two references masses was performed: trifluoroacetic acid ammonium salt (m/z 112.9856) and hexakis (1H, 1H, 3H–tetrafluoropropoxy) phosphazine (m/z 1033.9881) was used. Data analysis was realized on MassHunter Qualitative Analysis B.06.00 (Agilent Technologies, Palo Alto, CA, USA). Literature search for published spectral information was carried out by using SciFinder®.