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Effect of air-drying temperature on extractable and non-extractable phenolics and antioxidant capacity of lime wastes

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ABSTRACT

The lime industry is generating more waste due to the increasing demand of juice. In this study, extractable and non-extractable phenolics in the Gallega lime waste, dried at different temperatures were determined and their antioxidant capacities were evaluated using DPPH (2,2-diphenyl-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6/sulphonic acid) and, ORAC (oxygen radical absorbance capacity) methods. The amount of non-extractable phenolics measured in lime waste dried at 60, 90, and 120 °C were 38.4%, 39.2%, and 33.5%, respectively higher, compared to the amount of extractable phenolics. Dried lime waste is rich in extractable and non-extractable hesperidin (213.87 and 181.32 mg/g, respectively), and eriocitrin (55.22 and 97.24 mg/g, respectively) and non-extractable gallic acid (42.45 μg/g,). The antioxidant capacities of extractable and non-extractable phenolics were from highest to lowest: ABTS > ORAC > DPPH and ABTS > DPPH > ORAC, respectively. The information reported here may encourage lime industry operators to re-evaluate their by-products, extending the application of lime fruits and reducing waste.

1. Introduction

Citrus juice is the most popular citrus product but only accounts for 50% of the total mass of the whole fruit (Lario et al., 2009); the rest of it is usually discarded after juice extraction. The process leaves an estimate of 15×10^6 t of waste per year, generating a serious environmental problem (Marin et al., 2007). Citrus waste consists of the peels (albedo and flavedo), seeds, and pulp (including carpellary membranes). Chemical characterization of juice and citrus waste has been mainly focused in orange and lemon; however, lime (*Citrus aurantiifolia*) has gotten attention in recent years due to the increasing demand of its juice; as a result, the production of lime waste or peel, has also increased. The main products obtained from lime peel are candy and liqueurs, while the oil is a minor product, used as flavoring in sweets and beverages (Loizzo et al., 2012). Despite lime peel health-related properties (Loizzo et al., 2012; Boshtam et al., 2013; Adina et al., 2014), there have been few efforts for their chemical characterization. For example, lime peel is rich in flavonoids (Loizzo et al., 2012), total soluble phenols and total flavonoids (Ubando-Rivera et al., 2005; Guimarães et al., 2010), phenolic acids (Larrauri et al., 1996), and has attractive antioxidant capacity (Ubando-Rivera et al., 2005; Guimarães et al., 2010). Moreover, very few studies have evaluated the effect of drying temperature on total phenolic, limonin, dietary fiber and antioxidant capacity of lime residues (Kuljarachanan et al., 2009; Peerajit et al., 2012).

Dietary fiber (DF) and antioxidants (AOX) are two recognized dietary factors in the prevention of various diseases (Yang et al., 2015; Del Rio et al., 2013). The literature in this field addresses DF and AOX separately as nonrelated compounds, probably because of substantial differences in their chemical and biological properties (Saura-Calixto, 2011; Pérez-Jiménez et al., 2013). The literature data on food polyphenols (PP), comes from the analysis of aqueous organic extracts, and consequently biological studies address only extractable PP (EPP) ignoring the PP that remains in the residues. However, it is known that a significant fraction of food polyphenols remains in the corresponding residues after the extraction; the so-called non-extractable polyphenols (NEPP). This fraction of dietary polyphenols has been fully neglected and studies of such compounds are still scarce (Pérez-Jiménez et al., 2013). In fact, Arranz

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et al. (2010) demonstrated that levels of NEPP in fruits, vegetables, cereals, nuts and legumes are higher than levels of EPP.

Heat treatment changes the nutritional value and microstructures of fruits and their products (Akter et al., 2010). The drying temperature could also affect PP content and antioxidant capacity of citrus peels (Xu et al., 2007; Ho and Lin, 2008; Chen et al., 2011). For instance, it was reported that heat treatment might free some low molecular weight phenolic compounds, increasing the antioxidant capacities of citrus peel (Seok-Moon et al., 2004). To our knowledge, no studies have been carried out to determine the effect of drying temperature on extractable and non-extractable phenolics of lime waste. This information will be of great value to industry when it turns its attention to such residue. The aim of the present study was to evaluate the effect of high-temperature treatments on the phenolic composition and antioxidant capacity of the whole lime waste including albedo, flavedo, carpellary membranes and pulp residues after juice extraction.

2. Materials and methods

2.1. Lime fruit and sample preparation

"Gallega" (*Citrus* × *aurantifolia* (Christm)) lime fruits from trees grown at El Marquez, Queretaro, Mexico were harvested in November 2013 when commercially mature; sanitized (aqueous HCl, 0.05%, 1 min) before processing, to avoid contamination. Fruits were selected for their homogeneous size, free from defects and randomly divided in four batches, 150 fruits each. Limes from each batch were cut in two pieces, seeds removed, and squeezed with a Hamilton Beach Electric juice squeezer (Hamilton, China) to produce juice and lime waste (albedo, flavedo, pulp residues and carpellary membranes), known hereafter as LW. Then, the LW was blanched in hot water at 95 °C for 15 min to inhibit enzymes responsible for browning reactions (Kuljarachanan et al., 2009). The samples were then immediately cooled in cold water (4 °C) and centrifuge at 1440 rpm for 5 min to remove excess water. Subsequently, the LW were chopped into small pieces by a chopper (Warning Commercial, Mexico City, Mexico). One batch of blanched LW was freeze-dried, hereafter known as fresh-LW; the other three batches were dried at 60, 90 and 120 °C (LW-60, LW-90, and LW-120) in a tray dryer (UOP 8 Tray Dryer, Armfield, Ringwood, England) equipped with controls for temperature and airflow velocity. The dryer air velocity was of 1.5 m/s. Weight loss and airflow velocity values were recorded during the drying process using a digital balance (Ohaus, Explorer, USA). Dehydration lasted until a moisture content of ~4.5% was achieved. The dried samples were grounded and passed through a 40 mesh sieve and stored at −20 °C until analyses were performed.

2.2. Chemicals

Gallic acid (PubChem CID: 370); 3,4-hydroxybenzoic acid (PubChem CID: 4989777); vanillic acid (PubChem CID: 8468); syringic acid (PubChem CID: 10742); (−)-epigallocatechin gallate (EGCg) (PubChem CID: 65064); naringerin (PubChem CID: 932); naringin (PubChem CID: 442428); eriocitin (PubChem CID: 83489); hesperidin (PubChem CID: 10621); 2,2-diphenyl-1-picrylhydrazine (PubChem CID: 74358). All were purchased from Sigma–Aldrich (Ciudad de Mexico, DF, Mexico).

2.3. Soluble and insoluble fiber

Soluble and insoluble fiber was assessed according to Manthey et al. (1999). In short, sample was weighed into a screw-capped centrifuge container and incubated at 100 °C with Termayl (A0164, SIGMA) and 70 mL of 0.1 M sodium acetate, pH 5.0, and containing 70 ppm of Ca from $CaCO_3$ for 45 min. Sample was mixed thoroughly at 0, 15, and 30 min during the incubation. Samples were cooled to 50 °C and amyloglucosidase (A9913, SIGMA) was added to the sample. The sample was cooled to room temperature, centrifuged at 2500 × *g* for 15 min, and vacuum-filtered (Whatman No. 54). The pellet was washed with 70 mL of distilled water, sonicated 5 min, centrifuged, and vacuum-filtered. The original filtrate and wash filtrated were pooled and dialyzed against distilled water (4 L) for 48 h, changing the water every 12 h. The dialyzed was freeze-dried and weighed (soluble fiber). The pellet was washed twice in 50 mL of 100% ethanol and once in acetate. Each wash was followed by 10 min centrifugation at 2500 × *g*, and vacuum filtration. The supernatant was discarded. The remaining pellet was dried at 50 °C overnight, cooled to room temperature, and weighed (insoluble fiber).

2.4. Extractable phenolic compounds

The samples were extracted three times with aqueous methanol (80%) in an ultrasonic bath at room temperature for 60 min. After that, the samples were centrifuged at 5000 rpm for 10 min (Ye et al., 2011). The lime waste residues (LWRs) were reserved for extraction of NEPP. Supernatants (extracts) were combined and eventually analyzed for total phenolics (TP) (Wolfe et al., 2003) and total flavonoids (TF) (Ying and Wan, 2012). Simple phenolics (phenolic acids, flavonoids, and flavanones) of supernatants were also quantified through a HP 1100 series HPLC equipped with a diode array detector (HPLC-DAD) (Tsao et al., 2003). The identification and quantification of the peaks were carried out from (1) the retention times, (2) the spectra derived from DAD in comparison with those from authentic standards, and (3) by spiking with standards of the suspected compounds. Gallic, 3,4-hydroxybenzoic, 4-hydroxybenzoic, vanillic, syringic acids, rutin and quercetin were detected at 260 nm; (+)-catechin, epicatechin (EC), (−)-epigallocatechin gallate (EGCg) and benzoic and ellagic acid as well as naringerin, naringin, eriocitin and hesperidin at 280 nm; chlorogenic, caffeic, *p*-coumaric, ferulic, salicylic and sinapic acids, as well as vanillin and resveratrol were detected at 320 nm and miricetin and kaempherol at 360 nm.

2.5. Non-extractable phenolic compounds

The LWRs were hydrolyzed with 10 mL of methanol/H_2SO_4, (90:10, v/v) for 20 h at 85 °C (Arranz and Saura-Calixto, 2010). Samples were centrifuged (5000 rpm, 10 min) and non-extractable TP, TF, phenolic acids, flavonoids, and flavanones in supernatants (extracts) were determined using the same methods used to assess EPP.

2.6. Antioxidant capacities

ABTS (7 mM) radical cation ($ABTS^{\bullet+}$) solution was produced by reacting ABTS with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The $ABTS^{\bullet+}$ radical was diluted with potassium phosphate-buffered saline (PBS) to give an absorbance of about 0.700 ± 0.020 at 734 nm. For measuring antioxidant capacity 10 μL of sample was mixed with 990 μL of radical solution. Absorbance was monitored at 734 nm for 6 min. The decrease in absorption at 734 nm, 6 min after addition of the sample, was used for calculating the TEAC value (Re et al., 1996). Results were expressed in terms of μmol Trolox equivalent per kg of sample, FW (lmol TE/kg, FW).

The ORAC method used, with fluorescein (FL) as the "fluorescent probe," was described by Ou et al. (2001). The ORAC assay was carried out on a fluorimeter counter (PerkinElmer, USA) with fluorescence filters for an excitation wavelength of 485 nm and an