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Fruit peels as sources of non-extractable polyphenols or macromolecular antioxidants: Analysis and nutritional implications

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ABSTRACT

Despite increasing interest in the relevance of non-extractable polyphenols (NEPP) or macromolecular antioxidants as food bioactive compounds, most studies on their presence in foods focus mainly on the edible part of specific fruits, but their potential presence in fruit peels is usually ignored. The aim of this study was to evaluate NEPP content in the peels from ten common fruits. The results showed that NEPP made up more than half of the total polyphenol contents in half of the studied samples. HPLC analysis showed that NEPP were constituted by phenolic acids, flavanols and flavonols. Also, it was found that peels accounted for > 40% of total NEPP in the fruit in four of the samples analysed. These results should encourage both the use of fruit peels in the fruit industry as ingredients and the consumption of whole fruits given the significant presence of NEPP in fruit peels.

1. Introduction

During the last two decades non-extractable polyphenols (NEPP) have emerged as important contributors to total polyphenol content in plant foods (Bravo, Abia, & Saura-Calixto, 1994; Pérez-Jiménez, Díaz-Rubio, & Saura-Calixto, 2013; Saura-Calixto & Pérez-Jiménez, 2018). Their name is derived from the fact that, during the aqueous-organic treatments commonly performed to evaluate polyphenol content in foods, they are not extracted, thus remaining in the discarded residues. This is due to the chemical nature of this fraction of dietary polyphenols, since NEPP include both high molecular weight polyphenols such as non-extractable proanthocyanidins (NEPA) and low molecular weight polyphenols associated with macromolecules (proteins, dietary fibre), in this case hydrolyzable polyphenols (HPP); in view of this, an equivalent term has become current, most recently macromolecular antioxidants (MACAN) (Pérez-Jiménez & Saura Calixto, 2015). Besides analytical reasons explaining why this fraction of food bioactive compounds has been ignored for decades, the point is that they make a major contribution to total polyphenol intake (Arranz, Silván, & Saura-Calixto, 2010; Saura-Calixto & Pérez-Jiménez, 2018) and there is promising evidence of their health-related properties (Pérez-Jiménez et al., 2013).

Nevertheless, data on NEPP content in foods are still limited. It is important to augment this information in order to develop databases focused on NEPP, to better estimate NEPP intake in different populations or to identify the richest dietary sources of NEPP so as to perform specific clinical trials. In this respect, fruits have been shown to be significant sources of NEPP, and several studies have reported NEPP content in the most widely-consumed fruits in Europe (Pérez-Jiménez & Saura Calixto, 2015) or in some tropical fruits (Rufino et al., 2010; Rufino et al., 2011). At the same time, several plant materials commonly discarded during food processing have been shown to be particularly rich sources of NEPP—for instance grape or pomegranate pomaces (Pérez-Jiménez, Arranz, & Saura-Calixto, 2009; Pérez-Ramírez, Reynoso-Camacho, Saura-Calixto, & Pérez-Jiménez, 2018). Their identification as sources of bioactive compounds may offer new functions for these materials, which are currently used only for low added value activities.

Common fruits generate large amounts of discarded peels, both when consumed in the home and when industrially transformed to produce juices, jams or other derived products. These materials are known to be of nutritional value due to their high dietary fibre content (Jiménez-Escrig, Rincón, Pulido, & Saura-Calixto, 2001); however, NEPP content has only been evaluated for some specific fruits, such as mango or pineapple (Larrauri, Rupérez, & Saura Calixto, 1997; Safdar, Kausar, & Nadeem, 2017).

Therefore, the starting hypothesis of this study was that peels from common fruits contain relevant amounts of NEPP. In order to test this, we performed a comparative evaluation of NEPP content in peels from a selection of common fruits, and to estimate their contribution to total

Abbreviations: EPP, extractable polyphenols; HBA, hydroxybenzoic acids; HCA, hydroxycinnamic acids; HPP, hydrolyzable polyphenols; MACAN, macromolecular antioxidants; NEPA, non-extractable proanthocyanidins; NEPP, non-extractable polyphenols

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polyphenol content.

2. Materials and methods

2.1. Samples

The following samples were acquired in local supermarkets: apple (var. Fuji from Italy, Golden from Italy and Granny Smith from France), banana (Del Monte, from Colombia), kiwi (var. Hayward, from Portugal), mandarin (var. Tang Gold from Spain), mango (var. Osteen from Spain), melon (var. Piel de sapo or Santa Claus from Spain), nectarine (var. Venus from Chile), orange (var. Newhall, from Spain), pear (var. Blanquilla from Spain) and watermelon (var. Imperial from Spain). Peels were removed taking as less as possible amount of pulp; then, peel and pulp were weighed in order to get the proportion of each fraction. Peels were later freeze-dried and milled in a centrifugal mill (Retsch ZM 200, Haan, Germany) to a particle size of 0.5 mm. Peels from three independent fruits were mixed to get a representative sample of each fruit item.

2.2. Reagents

Gallic acid and hydrochloric acid were from Sigma-Aldrich (St. Louis, MO, USA). The Folin–Ciocalteu reagent was from Panreac, Castellar del Vallés, Barcelona, Spain. All the reagents used were of analytical or HPLC grade, depending on the analysis.

Condensed tannin concentrate from Mediterranean carob pods (*Ceratonia siliqua* L) was supplied by Nestlé Ltd. (Vers-chez-les Blancs,Switzerland).

2.3. Preparation of polyphenol fractions

Polyphenol fractions were obtained as previously described (Arranz, Saura-Calixto, Shaha, & Kroon, 2009):

2.3.1. Extractable polyphenols

Briefly, 0.5 g of sample was placed in a capped centrifuge tube, 20 mL of acidic methanol/water/HCl (50:50, v/v; pH 2) was added and the tube was thoroughly shaken at room temperature for 1 h. The tube was centrifuged at 2500*g* in a Thermo Heraeus Megafuge 11 (Thermo Fisher, Waltham, MA, USA) for 10 min and the supernatant was recovered. Twenty millilitres of acetone/water (70:30, v/v) was added to the residue, and the shaking and centrifugation were repeated. The methanolic and acetonic extracts were combined, corresponding to EPP solutions.

2.3.2. NEPP-hydrolyzable polyphenols (HPP)

Three residues from EPP extractions were subjected to hydrolysis with methanol and sulphuric acid for 20 h at 85 °C (Hartzfeld, Forkner, Hunter, & Hagerman, 2002) and the pH was later adjusted to 5.5. These hydrolysates were then subjected to SPE treatment with Oasis HLB cartridges (5400 mg, 3 cm^3, 30 μm) from Waters (Milford, MA, USA) in order to remove salts that may have damaged chromatographic columns: after activation with methanol (5 mL) and methanol 50% (5 mL), 1 mL of the sample was loaded and it was eluted successively with methanol (1 mL) and methanol 80% (1 mL). The eluates were combined and concentrated under nitrogen.

2.3.3. NEPP-non extractable proanthocyanidins (NEPA)

Three residues of EPP extraction were treated with butanol/HCl/FeCl3 at 100 °C for 1 h (Pérez-Jiménez et al., 2009; Porter, Hrstich, & Chan, 1985).

2.4. Analysis of polyphenols fractions

Total polyphenol content correspond to the sum of EPP, HPP and NEPA. While EPP and HPP may be determined either spectrophotometrically - providing the content of each polyphenol fraction- or by HPLC -providing polyphenol profile-, NEPA can only be estimated by spectrophotometric determination.

2.4.1. Polyphenol fractions

EPP and HPP content were determined in the solutions described above according to the Folin-Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventós, 1998). A test sample (0.5 mL) was mixed with 1 mL of Folin-Ciocalteu reagent and swirled. After 3 min, 10 mL of sodium carbonate solution (75 g/L) was added and mixed. Additional distilled water was mixed in thoroughly by inverting the tubes several times. After 1 h, the absorbance at 750 nm was recorded. The results were expressed as mg/100 g gallic acid equivalents (GAE).

NEPA content in the solution obtained by depolymerization by butanolysis was measured at 555 and 450 nm (Zurita, Díaz-Rubio, & Saura-Calixto, 2012) in order to detect anthocyanins and xanthylium compounds, respectively. The results were compared with a carob pod (*Ceratonia siliqua*) proanthocyanidin standard, which is rich in high molecular weight proanthocyanidins.

2.4.2. Polyphenol profile

Polyphenol classes were measured by HPLC-DAD in the EPP and HPP solutions described above, based on methodologies previously described (Pérez-Jiménez & Saura Calixto, 2015). Briefly, an Agilent 1200 series LC (Santa Clara, CA, USA) was used. A 20 μL sample was separated in a chromatography column with the following characteristics: Luna C18 (50 × 2.1 mm i.d.) 3.5-μm particle with Phenomenex Securityguard C18 (4 × 3 mm i.d.) column (Torrance, CA, USA). Gradient elution was performed with a binary system consisting of [A] 0.1% aqueous formic acid and [B] 0.1% formic acid in acetonitrile. The following increasing linear gradient (v/v) of [B] was used (t, %B): (0, 6), (10, 23), (15, 50), (20, 50), (23, 100), (25, 100), (27, 8) and (30, 8), followed by a re-equilibration step. The flow was set at 0.4 mL/min. Signals were collected at the maximum wavelengths reported for the different polyphenol classes: 214 nm (flavanols), 280 nm (hydroxybenzocic acids and flavanones), 320 nm (hydroxycinnamic acids), 365 nm (flavonols) and 520 nm (anthocyanins). The results were expressed as equivalents of the appropriate standards for each polyphenol class (calibration curves, 1–20 ppm): vanillic acid for hydroxybenzoic acids ($y = 19.003\times + 14.145$, $R^2 = 0.9953$); ferulic acid for hydroxycinnamic acids ($y = 28.192\times + 1.879$, $R^2 = 0.9957$); (−)-epicatechin for flavanols ($y = 32.346\times + 14.892$, $R^2 = 0.9971$); naringenin for flavanones ($y = 15.894\times + 6.081$, $R^2 = 0.9973$); and rutin for flavonols ($y = 2.985\times + 0.389$, $R^2 = 0.9987$). Anthocyanins were not detected in any of the samples, so calibration curves were not needed for this polyphenol class. When different classes shared a maximum wavelength, the complete UV-spectrum was evaluated to ascertain to which class the peak corresponded.

2.5. Statistical analysis

Data are shown as mean ± standard deviation. Since results did not exhibit normal distribution (assessed by Shapiro-Wilk test), the non-parametric Kruskal-Wallis *H* test, followed by Mann-Whitney *U* test were applied to determine the existence of significant differences ($p < 0.05$) between the samples. Data were analysed with the statistical SPSS IBM 24 package for Windows.

3. Results

3.1. Total EPP and NEPP contents in fruit peels

Table 1 shows total EPP and NEPP contents in peels from common fruits. EPP contents ranged between 305.3 mg/100 g dw in watermelon to 4224.7 mg/100 g dw in mango ($p < 0.05$). As for NEPP, HPP were