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Characterisation of phenolic compounds in processed fibres from the juice industry

Antoni Delpino-Rius [a], Jordi Eras [a,b,*], Francisca Vilaró [b], Miguel Ángel Cubero [c,d], Mercè Balcells [a,*], Ramon Canela-Garayoa [a]

[a] Chemistry Department, ETSEA, University of Lleida, 25198 Lleida, Spain
[b] Scientific Technical Service DATCEM, University of Lleida, 25198 Lleida, Spain
[c] Food Science and Technology Department, University of Lleida, 25198 Lleida, Spain
[d] Indulleida, S.A., N. 230, km 12, 25125 Alguaire, Lleida, Spain

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ABSTRACT

The content of phenolic compounds was determined in nine industrially processed fibres derived from the juice industry. Apple, peach, and pear as non-citrus fruit fibres were examined, as well as orange peel and flesh, tangerine peel and flesh, and lemon flesh as citrus fruit fibres, and carrot as vegetable fibre. The extractable phenolic profile of all fibres was obtained by UPLC-PDA-FLR-MS/MS. Forty phenolic compounds were identified and their concentrations determined. In addition, bound phenolic acids and proanthocyanidins were measured in solid residues in order to determine the phenolic compounds remaining. Also, to allow the comparison of the profiles and contents in the fresh fruit and fibres, we analysed extractable and bound phenolic compounds in lyophilized peel and pulp from fresh fruit. The profile and phenolic content of the fibres was similar to that of the fresh fruit, except for flavan-3-ols, which registered lower values.

1. Introduction

The recovery, recycling, and upgrading of waste material is particularly relevant in the food and food processing industry, in which wastes, effluents, residues, and by-products can be recovered and often upgraded to useful higher value products (Laufenberg, Kunz, & Nystroem, 2003). In this regard, a growing awareness of the relationship between diet and health has led to changes in dietary habits, these accompanied by an increasing demand for healthier foods. In particular, the health attributes of by-products obtained from fruit and vegetable processing are exploited in the food production industry. Ingredients of added value holding anti-oxidant compounds can be developed from by-products generated by the juice industry. One of the most relevant properties of fruit fibres is their bioactive compound content, such as anti-oxidants (Larrauri, 1999). In this context, some studies have reported the physical, chemical, and functional properties of fruit and vegetable by-products, highlighting their anti-oxidant potential (Figuerola, Hurtado, Estévez, Chiffelle, & Asenjo, 2005; Grigelmo-Miguel & Martín-Belloso, 1999).

Bioactive compounds, such as phenolic compounds, have beneficial effects in degenerative diseases and exhibit protective effects against cardiovascular diseases and cancer. Such compounds are present in the human diet, mainly through the ingestion of fruit and vegetables. Total phenolic content of several dried and fresh fruit and vegetable by-products has been described (Balasundram, Sundram, & Samman, 2006; O'Shea, Arendt, & Gallagher, 2012). Freeze-dried and dried apple by-products (Peschel et al., 2006) has already been described, also the profile of these anti-oxidants has also been studied in freeze-dried and air-dried apple pomace (Schieber, Keller, & Carle, 2001; Wijngaard, Rößle, & Brunton, 2009). However, little attention has been devoted to the potential of pear and peach by-products as sources of phenolic compounds. In this regard, only the total phenolic content in these matrices has been described (Deng et al., 2012; Peschel et al., 2006). In citrus fruits, total phenolic content in dried orange by-products has been described by de Moraes Crizel, Jablonski, de Oliveira Rios, Rech, and Flôres (2013) and in somewhat more detail by Fernández-López et al. (2009). Flavanones, hesperidin, and eriocitrin in waste from the industrial processing of lemons have been determined by Coll, Coll, Laencina, and

* Corresponding authors at: Chemistry Department, ETSEA, University of Lleida, 25198 Lleida, Spain. Tel.: +34 973 702589; fax: +34 973 238264 (J. Eras). Tel.: +34 973 702841; fax: +34 973 238264 (M. Balcells).
E-mail addresses: eras@quimica.udl.cat (J. Eras), balcells@quimica.udl.cat (M. Balcells).

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Tomás-Barberán (1998). Furthermore, the total phenolic content has been reported in carrot by-products (Chantaro, Devahastin, & Chiewchan, 2008).

The analysis of phenolic compounds usually involves a solvent extraction step using a combination of aqueous and organic solvents. Solvent extraction can be assisted by extraction techniques such as microwave or ultrasounds (Ignat, Volf, & Popa, 2011; Schieber et al., 2001). After extraction, in most articles the total phenolic content in by-products is commonly determined using the Folin–Ciocalteu reagent. However, this reagent interacts with other reducing non-phenolic substances (e.g., vitamin C), thus leading to an overestimation of the total phenolic content present (George, Brat, Alter, & Amiot, 2005). To analyse the individual phenolic compounds reversed-phase high-performance liquid chromatography (HPLC) is commonly used to analyse the different groups of phenols. In recent times, ultra performance liquid chromatography (UPLC) has been applied to improve the analysis of phenolic compounds in various matrices (Kalili & de Villiers, 2011). The solid residues from solvent extraction are generally considered exhausted material with regard to phenolic compounds. However, significant amounts of these anti-oxidants remain in the solid residue from fruit and vegetables (Arranz, Saura-Calixto, Shaha, & Kroon, 2009; Pérez-Jiménez & Torres, 2011). Phenolic compounds such as proanthocyanidins, hydrolysable tannins, and phenolic acids, have been classified as bound or non-extractable. Few studies have addressed bound phenolic compounds in fruit and vegetable by-products, with only one paper describing the non-extractable proanthocyanidins in freeze-dried apple waste (Tow, Premier, Jing, & Ajlouni, 2011).

To date, the characterisation of phenolic compounds in by-products has been performed on unprocessed materials or materials with processes optimised at laboratory scale. However, after the processing undergone during the industrial production of juice or related products, the fruit and vegetable by-products are subjected to several steps to obtain commercial fibres. In addition, in order to minimise damage to bioactive substances, all these processes have to be optimised, in particular the drying temperature (Peschel et al., 2006).

Here we report on the content of extractable and bound phenolic compounds in nine industrially processed fibres. The fibres were obtained from various parts of several fruits and also from carrot. All fibres were obtained from processed industrial raw materials obtained from an actual line of juice processing. Additionally, we analysed the phenolic content of lyophilized peel and pulp from fresh fruit. The content in fresh fruit and industrial fibres was compared. Finally, we examined the heterogeneity of phenolic compound content in various production batches of five industrially produced fibres. These values might be conditioned by factors such as the cultivar or variety, climate, stage of maturity, storage, and processing (Peschel et al., 2006).

2. Material and methods

2.1. Reagents, solvents and phenolic standards

Methanol and acetone (HPLC grade purity) were supplied by J.T. Baker (Deventer, The Netherlands). The chromatographic solvents were acetonitrile (LC–MS grade), also from J.T. Baker, and water was purified in a Milli-Q system from Millipore (Bedford, MA, USA). The chromatographic eluent additive was acetic acid (HAcO) (LC–MS grade), provided by Fluka (Sigma–Aldrich, Madrid, Spain), formic acid (HFor) and hydrochloric acid 37% (analytical grade), supplied by Merck. Sodium chloride was obtained from Fluka, sodium hydroxide from Panreac (Barcelona, Spain) and ascorbic acid from Acros (Pittsburgh, PA, USA).

Standards of phenolic compounds were supplied as follows: (+)-catechin, (−)-epicatechin, procyanidin B1, procyanidin B2, arbutin, eriodictyol-7-*O*-rutinoside, naringenin-7-*O*-rutinoside, naringenin, hesperetin-7-*O*-rutinoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-rhamnoside, quercetin-3-*O*-rutinoside, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside by Extrasynthèse (Genay, France); and arbutin, gallic acid, phloretin-2′-*O*-β-glucoside, 5′-caffeoylquinic acid, caffeic acid, *p*-coumaric acid, ferulic acid and sinapic acid by Sigma–Aldrich Chemie (Steinheim, Germany). All stock standard solutions of phenolic compounds were prepared in MeOH and stored at −80 °C. Working solutions were prepared from stock solutions by sampling an aliquot and diluting it with the injection solvent H_2O (0.1% HAcO).

2.2. Samples

A local juice company (Indulleida S.A., Alguaire (Lleida), Spain) provided the fibre samples from the following: apple (6), pear (5), peach (5), carrot (1), orange flesh (6), orange peel (1), tangerine flesh (1), tangerine peel (1), and lemon flesh (5). A picture of fibres and scheme of the production process is shown in Supplementary data.

We purchased approximately 1 kg of randomly chosen "Golden" apples, "Blanquilla pear, "Amarillo de septiembre" peaches, carrots, "Navelina" oranges, tangerines, and lemons, from a local market. After distilled water washing, the peel of all the fruits was immediately separated from the pulp. The pulp from each kind of fruit was homogenised in a blender (Grindomix GM 200; Retsch, Haan, Germany) at 5000 rpm for 2 min and ascorbic acid (ca. 10 g/kg) was added to prevent oxidation. The peel was dipped in liquid nitrogen and ground into a fine powder using a pre-chilled mortar and pestle. Next, the peel and pulp were immediately frozen at −80 °C and lyophilized at −50 °C, 1.1 Pa for 24 h in a Cryodos-50 lyophilizer (Telstar, Terrassa, Spain). Finally, the lyophilized samples were powdered and stored at −20 °C until analysis.

2.3. Extraction methods

2.3.1. Extractable phenolic compounds

Samples (0.25 g) were first extracted with 5 mL of methanol/water (50:50) (acidified at 0.1%, v/v with acetic acid) and then twice with 5 mL of acetone:water (70:30) in a ultrasonic bath for 30 min. Extracted solutions were pooled, diluted 1:4 with Milli-Q water (acidified at 0.1%, v/v with acetic acid), and filtered through 0.20 μm PTFE filters. Solutions were kept at 6 °C until UPLC analysis. All solvents contained ascorbic acid (0.2% w/v).

2.3.2. Bound phenolic compounds

Basic hydrolysis of bound phenolic acids: The lyophilized solid material obtained from the previous extractions was hydrolysed directly following a method previously described but with some modifications (Nardini et al., 2002). Part of the remaining material (50 mg) was mixed with 1.5 mL of 2 M NaOH containing 10 mM EDTA and 1% ascorbic acid and then shaken (Eppendorf© Thermomixer Comfort, Hamburg, Germany) for 30 min at 45 °C. The reaction mixture was acidified (*c.a.* pH 3) by adding 0.285 mL of 7.2 M HCl and centrifuged at 1400×*g* for 5 min (Hettich Eppendorf Centrifuge MIKRO 22 R; Germany).

Acidic hydrolysis of bound proanthocyanidins: For the determination of bound flavan-3-ols, approximately 10 mg of the lyophilized solid residue was incubated with 5 mL of *n*-butanol/HCl (95:5) and 200 μL of Ferric reagent (2% ferric ammonium sulphate in 2 M HCl) for 60 min (Porter, 1986). After cooling the solution in ice water and centrifugation, absorbance was measured at λ_{max} = 550 nm. Total flavan-3-ol concentration was determined using cyanidin chloride as reference compound.