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Characterization and quantification of fruit phenolic compounds of European and Tunisian pear cultivars

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ABSTRACT

The flesh and peel of 19 pear cultivars (8 Tunisian dessert cultivars, 8 European dessert cultivars and 3 French perry pear cultivars) were studied for their phenolic composition. Phenolic compounds were identified by HPLC/ESI-MS^2 and individually quantified by HPLC-DAD. Five classes of polyphenols were present: flavan-3-ols, phenolic acids, flavonols, anthocyanins and simple phenolics (hydroquinones). The total phenolic content ranged between 0.1 g/kg Fresh Weight (FW) ('Conference' cultivar) and 8.6 g/kg FW ('Plant De Blanc' cultivar) in the flesh and between 1.6 g/kg FW ('William vert' cultivar) and 40.4 g/kg FW ('Arbi Chiheb' cultivar) in the peel. Procyanidins, analyzed after thioacidolysis, were the main phenolic compounds in all pear cultivars either in the pulp or the peel, their constitutive units being essentially (−)-epicatechin. Tunisian dessert pears and French perry pears are richer in procyanidins with very high degree of polymerization (>100) for Tunisian pears. Peel procyanidins were less polymerized (from 4 to 20). Pear peel phenolic profile was more complex especially for Tunisian cultivars, with flavonols and in some cultivars anthocyanins.

1. Introduction

Pyrus communis L. is a typical fruit crop of temperate climates such as Europe, North America, North Africa and the temperate regions of the Southern hemisphere. It is the fifth most widely produced fruit in the world, being produced mainly in China, Europe, and the United States. *Pyrus communis* belongs to Rosaceae family. There are over 2000 pear cultivars, but only few are relevant in terms of volume of production and commercialization. Pears are typically eaten fresh and also used to produce juice, puree, and jam (Raffo, Ponce, Sozzi, Vicente, & Stortz, 2011; Silva, Souza, Barbieri, & Costa de Oliveira, 2014). The quality of pear fruit is known to be influenced by both external (size, shape, and color) and internal characteristics (nutritional and taste qualities) (Choi, Choi, Hong, Kim, & Lee, 2007). It has high nutritional value with reasonable amounts of sugars, amino acids and minerals like sodium, potassium, calcium, magnesium and iron (Yim & Nam, 2016). It has also higher dietary fiber level than most common fruits and vegetables, giving excellent results in the treatment of constipation and intestine inflammation (Silva et al., 2014). Pears also contain other nutritional and bioactive components as polyphenols.

Polyphenols are an important group of secondary metabolites widely distributed in the plant kingdom. They are linked to many health benefits and are strong natural antioxidants (El Gharras, 2009; Tsao, 2010). Phenolic compounds contribute also to the sensory quality of fruit (color, astringency, bitterness and flavor) (Fernandez de Simon, Perez-Ilzarbe, Hernandez, Gomez-Cordoves, & Estrella, 1992). Phenolic compounds are generally more concentrated in the peel than in the fruit flesh (Andreotti, Costa, & Treutter, 2006; Kolniak-Ostek, 2016a). Pear polyphenols have diverse structures and belong to different classes, namely as flavonoids (monomers and polymers of flavan-3-ols, flavonols and anthocyanins), phenolic acids (hydroxycinnamic acids derived from caffeic acid and *p*-coumaric acid) and simple phenolics (the *p*-hydroquinone-glucoside: arbutin) (Es-Safi, Guyot, & Ducrot, 2006; Kolniak-Ostek, 2016c; Öztürk, Demirsoy, Demirsoy, Asan, & Gül, 2015). Many studies on polyphenol composition conclude that hydroxycinnamic acids and arbutin are the main phenolic compounds in pear (Amiot, Aubert, & Nicolas, 1992; Cui, Nakamura, Ma, Li, & Kayahara, 2005; Galvis Sánchez, Gil-Izquierdo, & Gil, 2003; Lin & Harnly, 2008; Oleszek, Amiot, & Aubert, 1994; Yim & Nam, 2016). However, procyanidins are usually underestimated when alcoholic or hydro-alcoholic extractions are used; most of them are not extracted and remain in the insoluble part of the cortex (Pérez-Jiménez, Díaz-Rubio, & Saura-Calixto, 2013). Moreover, after extraction, their estimation by

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direct HPLC remains incomplete because polymeric forms do not give well-resolved peaks on chromatograms (Thompson, Jacques, Haslam, & Tanner, 1972). The thioacidolysis-HPLC analysis allows quantitative and qualitative information on the procyanidin contents of fruit by cleaving oligomeric and polymeric procyanidins into their constitutive units (Guyot, Marnet, & Drilleau, 2001; Guyot, Marnet, Laraba, Sanoner, & Drilleau, 1998). Ferreira et al. (2002), Renard (2005), Le Bourvellec et al. (2013) or Kolniak-Ostek (2016b), detected procyanidins in pear fruit and report that are the predominant class of phenolic compounds in some cultivars such as 'Bartolomeu', 'William' and 'Gieser Wildeman'.

Among *Pyrus communis* cultivars, perry pears are specific cultivars used to make perry, a slightly alcoholic fizzy drink; little data is available about their phenolic composition (Guyot, Marnet, Le Bourvellec, & Drilleau, 2002) and differences might exist between dessert and perry pear cultivars as observed between cider and dessert apple by Guyot, Marnet, Sanoner and Drilleau (2003). The aim of this study was to characterize and quantify the peel and flesh phenolic composition of dessert pears (Tunisian and European cultivars) and perry pears (French cultivars).

2. Materials and methods

2.1. Chemicals compounds

Acetonitrile of chromatographic grade quality was obtained from VWR (Leuven, Belgium). Methanol of chromatographic quality, acetic acid and hydrochloric acid were from Merck (Darmstadt, Germany). Formic acid, and benzyl mercaptan were provided by sigma Aldrich (Steinheim, Germany). Polyphenol standards (3′-caffeoylquinic acid, 5′-caffeoylquinic acid, (+)-catechin, (−)-epicatechin, quercetin-3-*O*-rutinoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, isorhamnetin, cyanidin-3-*O*-galactoside, peonidin-3-*O*-galactoside) were provided by Extrasynthèse (Lyon, France).

2.2. Plant material

Pears (*Pyrus communis* L.) from eight European dessert cultivars ('Abate', 'Comice', 'Conference', 'Passe-Crassane', 'Louise Bonne', 'Rochas', 'William vert', and 'William rouge') were purchased at commercial maturity in September 2015 in a local supermarket (Avignon, France). Perry pears ('Fausset', 'De Cloche' and 'Plant de Blanc') were supplied by the Institut Français des Productions Cidricoles (IFPC, Sées, France) in September 2015. Based on previous diversity studies (Brini, Mars, & Hormaza, 2008), eight Tunisian dessert cultivars ('Arbi Bouficha', 'Tourki', 'Arbi Sidi Bou Ali', 'Meski Arteb', 'Soukri', 'Arbi Chiheb', 'Jrani', 'Radsi') were harvested in July 2015 in Tunisian orchards (Table 1). For each cultivar, three batches of 10 pears were constituted. Each pear was peeled and cored manually and then the flesh was divided into 8 equal portions, of which two opposite quarters were used. For each cultivar and each batch, flesh and peel were freeze-dried and stored at −20 °C until analysis.

2.3. Extraction of phenolic compounds

Polyphenol extracts were prepared as described by Guyot et al. (2001). About 30 mg of freeze-dried flesh and peel were directly submitted to extraction ("crude" samples) or thioacidolysis. For thioacidolysis, 400 μL of dried methanol acidified by concentrated HCl (0.4 mol/L) and 800 μL of a toluene-α-thiol solution (50 mL/L in dried methanol) were added. The extraction was performed by incubation of mixture for 30 min at 40 °C with agitation on a vortex every 10 min. Samples were cooled in ice in order to stop thioacidolysis reaction. For crude extraction, samples were dissolved in 1200 μL of dried methanol acidified by acetic acid (10 mL/L). The reaction was carried out in an ultrasonic batch during 15 min. All samples ("thioacidolysis" and "crude" extracts) were filtered (PTFE, 0.45 μm) and injected (20 μL) into HPLC-DAD (see Section 2.5).

2.4. Identification of phenolic compounds by HPLC/ESI-MS^2

HPLC/ESI-MS^2 analysis was performed on an Acquity Ultra performance LC (UPLC) apparatus from Waters (Milford, MA, USA), equipped with a photodiode array detector (detection at 280, 320, 350 and 520 nm) coupled with a Bruker Daltonics (Bremen, Germany) HCT ultra ion trap mass spectrometer with an electrospray ionization source. Separations were achieved using a Licrospher RP-18 5 μm column (Merck, Darmstadt, Germany) protected by a guard column of the same material (Licrospher RP-18 5 μm column, Merck Darmstadt, Germany) operated at 30 °C. The mobile phase consisted of water/formic acid (99:1, mL/mL) (eluent A) and acetonitrile (eluent B). The flow rate was 1 mL/min. The elution program was as follows: 3–9% B (0–5 min); 9–16% B (5–15 min); 16–50% B (15-45 min); 50–90% B (45–48 min); 90–90% B (48–52 min). Samples (crud extracts) were injected at a level of 10 μL. For polyphenol characterization, a capillary voltage of 2 kV was used in the negative ion mode. Nitrogen was used as drying and nebulizing gas with a flow rate of 12 L/min. The desolvation temperature was set at 365 °C and the nebulization pressure at 0.4 MPa. The ion trap was operated in the Ultrascan mode from *m/z* 100 to

Table 1
Geographic origins, different uses, and astringency characteristic of studied pear cultivars.

Cultivars	Abbreviations	Origins	Main uses	Astringency perception
Abate	AB	Italy	Dessert pears	No astringent
Arbi Bouficha	AF	Bouficha, Sousse, Tunisia	Dessert pears	Perceivable astringency
Arbi Chiheb	AC	Monastir, Tunisia	Dessert pears	Perceivable astringency
Arbi Sidi Bou Ali	AS	Sidi Bou Ali, Sousse, Tunisia	Dessert pears	Perceivable astringency
Comice	CO	France	Dessert pears	No astringent
Conference	CF	England	Dessert pears	No astringent
De Cloche	DC	Sées, France	Perry pears	Very astringent
Fausset	FA	Sées, France	Perry pears	Very astringent
Jrani	JR	Monastir, Tunisia	Dessert pears	Perceivable astringency
Louise Bonne	LB	France	Dessert pears	No astringent
Meski Arteb	MA	Sousse, Tunisia	Dessert pears	Perceivable astringency
Radsi	RD	Sousse, Tunisia	Dessert pears	Perceivable astringency
Passe Crassane	CR	France	Dessert pears	No astringent
Plant De Blanc	PB	Sées, France	Perry pears	Perceivable astringency
Rochas	RC	Portugal	Dessert pears	No astringent
Soukri	SK	Sousse, Tunisia	Dessert pears	Perceivable astringency
Tourki	TR	Monastir, Tunisia	Dessert pears	Perceivable astringency
William rouge	WR	United Kingdom	Dessert pears	No astringent
William vert	WV	United Kingdom	Dessert pears	No astringent