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Effect of maturity on the phenolic compositions of pear juice and cell wall effects on procyanidins transfer

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ABSTRACT

Perry pear polyphenols were characterized in fruit, juice and pomace for two cultivars and at two maturity stage. Cell walls were characterized only in fruits and pomaces. The phenolic contents and compositions of fruits did not change during overripening. However, their extraction to juice was modified. Juices of ripe fruits contained 38% (Plant de Blanc) and 28% (De Cloche) of initial polyphenols, whereas overripe pear juices contained only 26% and 15% respectively. Thus, procyanidins had more affinity for cell walls after overripening. Pear cell walls from De Cloche cultivar lost arabinose and galactose from pectic side chains during overripening promoting non-covalent interactions between procyanidins and cell walls.

1. Introduction

Perry pears are used in the west Midlands (UK) and in the regions of Bretagne and Normandie in France for the production of perry, a fermented fizzy beverage close to cider. They are specific cultivars characterized by their high content in polyphenols. Phenolic compounds have an important role in food industry. They contribute to flavor and color characteristics of fruit juices and wines (Spanos & Wrolstad, 1990). Polyphenols are divided into several classes, i.e. phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), flavonoids (flavonols, flavones, flavanols, flavanones, isoflavones, proanthocyanidins), stilbenes, and lignans (Collin & Crouzet, 2011). Procyanidins are the major polyphenols class in pear (dessert and perry pear cultivars) (Guyot, Marnet, Le Bourvellec, & Drilleau, 2002; Le Bourvellec et al., 2013; Renard, 2005) where they contribute to astringency of perry. In perry processing, the use of fruit at the overripe stage is the normal practice to decrease the astringency and to increase colloidal stability during storage. Further, preliminary studies have shown that the concentration of procyanidins in the juices varied with the fruit maturity at pressing (Alberti et al., 2016; Guyot, Marnet, Sanoner, & Drilleau, 2003; Spanos & Wrolstad, 1990). Low quantities of procyanidins are found in juices compared with the initial quantities measured in fruit (Guyot et al., 2002). Therefore, factors such as maturity that can induce change in the cell wall composition and structure can modulate the extractability of polyphenols and the organoleptic properties of perry.

The association between procyanidins and cell wall polysaccharides, especially pectins, can influence the transfer of procyanidins from fruit to juice (Guyot et al., 2003; Taira, Ono, & Matsumoto, 1997). The binding of condensed tannins to cell walls depends on one hand on the molecular characteristics of procyanidins, mainly their degree of polymerization but also the pyranic ring stereochemistry of the flavan-3-ols, and on the other hand on cell wall structure and composition. Associations differ depending on neutral sugar composition and the structure of pectic fractions. Arabinogalactan had the lowest affinity for procyanidins (Watrelot, Le Bourvellec, Imberty, & Renard, 2014). Watrelot, Le Bourvellec, Imberty, and Renard (2013) showed also that the strongest association was obtained with highly polymerized procyanidins and highly methylated pectins. The adsorption mechanism involves the establishment of non-covalent interactions, hydrogen bonds and hydrophobic interactions (Le Bourvellec, Bouchet, & Renard, 2005;

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Le Bourvellec, Guyot, & Renard, 2004; Lea & Arnold, 1978).

In general, cell wall modifications in ripening fruit involve hydrolysis of neutral sugars from side chains of pectin, depolymerization and increased solubilization of pectins (Brummell, 2006). Pear cell walls have a large heterogeneity compared to other fruits due to the presence of parenchyma and stone cells. Their cell walls have different polysaccharide compositions and evolve differently during ripening. In Spanish pears, changes were predominant in parenchyma cells and were accompanied by a decrease in pectic polysaccharides (arabinose, uronic acids) and an increase in their solubility (Martin-Cabrejas, Waldron, Selvendran, Parker, & Moates, 1994). Those changes can affect differently the affinity of cell walls for procyanidins thus the organoleptic properties of pear juice.

The aim of this work was to determine the polyphenol and cell wall compositions of two perry pear cultivars at two different ripeness stages and to investigate the impact of maturity on polyphenol transfers in pear juices.

2. Materials and methods

2.1. Chemicals and standards

Acetonitrile, dichloromethane and acetone were obtained from VWR (Leuven, Belgium). Methanol, acetic acid, hydrochloric acid, sodium tetraborate, sodium hydroxide and sodium hydrogen carbonate were from Merck (Darmstadt, Germany). Sodium borohydride, *N*-methyl imidazole, potassium hydroxide, 3-phenylphenol, lignin, acetic anhydride, acetyl bromide, perchloric acid, chlorogenic acid, (+)-catechin, (−)-epicatechin, quercetin, isorhamnetin, hydroxylamine hydrochloride and benzyl mercaptan were provided by Sigma Aldrich (Steinheim, Germany). Sugar standards were from Fluka-Biochemica (Sigma Aldrich, Steinheim, Germany).

2.2. Plant material

Perry pear cultivars, “Plant de Blanc” and “De Cloche” were harvested in the experimental orchard of Institut Français des Productions Cidricoles (IFPC, Sées, France) on September 16 and October 5, 2015, respectively. They were stored at 1 °C until reaching the desired stage of maturity. Plant de Blanc pears were stored until September 22, De Cloche pears until October 8, 2015 to reach the ripe stage. The overripe stage was reached on October 21 for Plant de Blanc fruits, and on December 11, 2015 for De Cloche fruits.

2.3. Preparation of samples

Juice preparation from ripe and overripe pears was carried out by IFPC (Le Rheu, France).

The sugars/acids ratio serves as an industrial indicator because the balance between sugars and organic acids influences the taste of some beverages (Alberti et al., 2016; Pal & Sampath Kumar, 1995). It was used in our case as a ripening index. Sugars and acids were determined as described by Le Bourvellec et al. (2015) and their ratio was then calculated. Overripening was marked by an increase of sugars/acids ratio especially for Plant De Blanc (Data not shown). Pears were washed and then crushed under CO_2 atmosphere and with added sodium fluoride to prevent oxidation. Pears were pressed on a small laboratory high-pressure press (model HP5, 5 L, Hafico, Fischer and Co., Dusseldorf, Germany) for 15 min. The hydraulic pressure was set at 4×10^7 Pa, corresponding to an effective pressure of 234 N/m^2 on the plant material. For each cultivar and maturity 3 × 1 mL of juice were collected after pressing, freeze-dried and stored at −20 °C until analysis. Pomace samples, collected under CO_2 atmosphere, were divided in two lots for polyphenols and cell walls analysis. Fresh fruits were cored and freeze dried in our laboratory (SQPOV, INRA, Avignon) and stored at −20 °C.

2.4. Analysis of phenolic compounds

Polyphenols were determined in fruit, juice and pomace at two maturity stages by high performance liquid chromatography (HPLC/Diode Array Detection) with or without thioacidolysis as described by Guyot, Marnet, and Drilleau (2001) and Le Bourvellec et al. (2011).

2.5. Cell walls preparation and characterization

Alcohol Insoluble Solids (AIS) from fruit and pomace at the two maturity stages were prepared according to Renard (2005); neutral sugars, galacturonic acid and methanol were determined as described in Renard and Ginies (2009).

Lignin was analysed in AIS as described by Syros, Yupsanis, Zafiriadis, and Economou (2004) with some modifications. Samples (10–15 mg) were digested in 1 mL of buffer (250 mL/L acetyl bromide, 27 mL/L perchloric acid and 723 mL/L acetic acid). Then, samples were incubated for 30 min at 70 °C. 10 μL for each sample were added to 570 μL of a solution of [172.4 mL/L of NaOH 2 mol/L and 827.6 mL/L of acetic acid] and 20 μL of 7.5 mol/L hydroxylamine hydrochloride to stop the reaction. The volume was corrected to 2 mL with acetic acid and the absorbance was read at 280 nm using a spectrophotometer V-730 (Jasco, Tokyo, Japan).

2.5.1. Statistics

Results are presented as mean values, and the reproducibility of the obtained results was expressed by pooled standard deviation (Pooled SD) (Box, Hunter, & Hunter, 1978). One-way analysis of variance (ANOVA) was performed on perry pear fruit, juice and pomace polyphenol compositions concerning ripeness using the Excel Stat package of Microsoft Excel.

3. Results and discussion

3.1. Phenolic composition of perry pears, juice and pomace

The phenolic composition of fruits, juices and pomaces and their changes during overripening for Plant De Blanc and De Cloche cultivars are summarized in Table 1.

Procyanidins were the predominant phenolic class, with between 6 and 8 g/kg FW, higher than reported by Le Bourvellec et al. (2013) in William pears (dessert pears) and by Renard (2005) in Gieser Wildeman (cooking pears). Perry pear procyanidins had a high degree of polymerization (DPn = 20 for De Cloche and DPn = 33 for Plant de Blanc fruits), as reported by Guyot et al. (2002). The only flavan-3-ols monomer detected was (−)-epicatechin as observed by Le Bourvellec et al. (2013). Hydroxycinammic acids were represented by 5-caffeolyquinic acid, which has been reported as the main hydroxycinammic acid in pears (Galvis Sánchez, Gil-Izquierdo, & Gil, 2003; Le Bourvellec et al., 2013; Li et al., 2012; Yim & Nam, 2016). Flavonols, located in the peel, mainly quercetin and isorhamnetin glucosides, were present in low quantities <40 mg/kg FW for both cultivars. No significant changes in polyphenol concentrations, neither procyanidins nor flavonols, were detected during fruit overripening, irrespective of the cultivar.

Juice concentrations decreased almost by half for De Cloche and slightly less for Plant De Blanc after overripening. This decrease was mainly due to decrease in procyanidins, as observed by Guyot et al. (2002). Decrease of the degree of polymerization was observed for