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Characterization of tissue specific differences in cell wall polysaccharides of ripe and overripe pear fruit

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ABSTRACT

Cell walls from flesh, parenchyma cells, stone cells and skin were isolated from ripe and overripe *Pyrus communis* L. cv "De Cloche" using the phenol-buffer method. Pear polysaccharides were solubilized from cell walls by sequential extractions with aqueous solutions of ammonium oxalate, Na_2CO_3, and increasing concentration of NaOH, to explore overripening impact. Cell walls were also differentiated using MIR spectral data. Stone cells contained high levels of xylose and lignin while parenchyma cells had high levels of glucose, uronic acids and arabinose. Sequential extractions revealed that pear pectins had highly branched rhamnogalacturonans and were extremely methylated. Xylans were the main hemicelluloseespecially for stone cells. Cellulose represented about half of all cell walls. This heterogeneous composition of pear affected differently cell wall evolutions and properties. Thus, overripening involved a decrease in arabinose and a loss of pectic side chains mostly from parenchyma cells. Changes in hemicellulose and cellulose were minor.

1. Introduction

Cell walls are important features of plant cells and are implicated in evolution of fruit firmness and texture during ripening. Cell walls have among others a major impact on fruit juice composition by their interactions with condensed tannins also known as proanthocyanidins (Le Bourvellec, Le Quere, & Renard, 2007). Pear juice but not pear fruit phenolic composition depends on fruit maturity at pressing. Previous studies showed that pear juice with very high procyanidin concentrations is obtained from ripe fruit whereas juice obtained from overripe fruit is almost devoid of procyanidins although they are strongly present in the fruit (Guyot, Marnet, Le Bourvellec, & Drilleau, 2002). The availability of procyanidins in the juice was limited by the formation of non-covalent adducts with cell walls (Guyot, Marnet, Sanoner, & Drilleau, 2003; Le Bourvellec et al., 2007; Smith, McRae, & Bindon, 2015), hence modifications of pear cell wall affinity for procyanidins might also explain this difference between ripe and overripe stage.

In most species of the Rosaceae family fruit flesh is very homogeneous and is predominantly composed of parenchyma cells. However, in pears, *Pyrus communis* L., the cortical tissue contains also stone cells. The later, also known as sclereids, give a gritty texture when pears are eaten or processed into juice, puree and jam. Stone cells are a type of sclerenchyma cell formed by the secondary thickening of cell walls, followed by the deposition of lignin on the primary walls of parenchyma cells. Occurrence of stone cells has also been reported in other fruits such as guava (Marcelin, Williams, & Brillouet, 1993). The formation of stone cells is influenced by several factors such as cultural practices, post-harvest handling but the most important factor is the genetic variability (Cai et al., 2010; Yan et al., 2014), from almost imperceptible in some cultivars to very gritty texture in perry pears. Their formation includes lignification, which usually occurs during plant growth, when cell growth is completed or cells undergo secondary growth. It plays crucial role in plant defense against biotic and abiotic stress (Vance, Kirk, & Sherwood, 1980). Sclereids contain abundant cellulose (Cai et al., 2010) and a high level of glucose and xylose (Martin-Cabrejas, Waldron, Selvendran, Parker, & Moates, 1994). Data which already exists on pear cell walls take into account only the whole flesh without separation between stone cells and parenchyma cells (Ahmed & Labavitch, 1980; Almeida, Gomes, & Pintado, 2009; Hiwasa et al., 2004; Jermyn & Isherwood, 1956; Murayama, Katsumata, Endou,

Abbreviations: FAD, Factorial Discriminant Analysis; MIR, Mid Infrared; SEM, Scanning Electron Microscopy; CWM, Cell Wall Materials; FL, cell walls isolated from the whole flesh; PC, cell walls isolated from parenchyma cells; ST, cell walls isolated from stone cells; SK, cell walls isolated from skin.

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Fukushima, & Sakurai, 2006; Raffo, Ponce, Sozzi, Vicente, & Stortz, 2011; Renard, 2005b; Yamaki, Machida, & Kakiuchi, 1979).

In general, cell wall modifications during fruit ripening involve hydrolysis of neutral sugars from pectin side chains, depolymerization and increased solubilization of pectins and hemicelluloses (Brummell, 2006). Minor changes can occur in cellulosic material (Houben, Jolie, Fraeye, Van Loey, & Hendrickx, 2011). During ripening of pears, the most commonly reported modifications taking into account only the whole flesh indicate a loss of arabinose (Ahmed & Labavitch, 1980; Jermyn & Isherwood, 1956), or arabinose and uronic acid (Martin-Cabrejas et al., 1994a,b; Yamaki et al., 1979). Hiwasa et al. (2004) suggested that cell wall degradation is correlated with a decrease in firmness during ripening and the modification of both pectins and hemicelluloses are essential for the development of melting texture in different species of pears. Although in lignified cell wall of stone cells the ultrastructure does not change during fruit maturation, in overripe fruit the parenchyma cell walls became extremely thin and fragile (Ben-Arie, Kislev, & Frenkel, 1979). Therefore, our hypothesis is that pear cell walls from parenchyma and sclereids have different compositions and evolve differently from ripe to overripe stage. These differences further may influence the affinity of cell wall for procyanidin and their extraction to juice. This cell wall-procyanidin interaction is background information and not tested here. However, little data was available on pear cell wall composition taking into account sclereid cells and parenchyma cells separately. The aim of this study was to characterize the composition and ultrastructure of pear cell walls differentiating skin and flesh tissue, and in the flesh differentiating parenchyma and stone cells. We report also the changes that occur in the various types of cell walls (whole flesh, parenchyma cells, stone cells and skin) to better understand the evolution of each tissue for ripe and overripe pears. This work provides the baseline data for further studies examining the impact of cell walls-procyanidins interactions in pear.

2. Materials and methods

2.1. Standards and chemicals

Phenol, methanol and ammonium oxalate were from Merck (Darmstadt, Germany). Sodium carbonate, sodium hydroxide, sodium borohydride ($NaBH_4$), sodium acetate, N-methylimidazole, acetic anhydride, formic acid, lignin alkali, glucose, galacturonic acid, toluene-α-thiol, methanol-d3, (+) catechin, (−) epicatechin and inositol were from Sigma-Aldrich (Deisenhofen, Germany). Sugars (arabinose, fucose, galactose, xylose, mannose and rhamnose) were from Fluka (Buchs, Switzerland). Dextrans were from Pharmacia BioProcess Technology (Uppsala, Sweden). Acetonitrile of HPLC grade was obtained from VWR International (Radnor, USA). Ethanol and acetone were from Fisher Scientific (Strasbourg, France).

2.2. Plant material

Pyrus communis L. cv "De Cloche", a special cultivar used to make perry, was used. Perry manufacturers produce pear juice from fruits in advanced stage of maturity to decrease the astringency and to increase colloidal stability during storage of perry. Two maturity stages were chosen from this empirical assessment, " Ripe" corresponding to pears at harvest, i.e. December 03, 2013 in the orchard of Mr. Aubry (Clécy, France), and "Overripe" (described as "soft under the fingers") corresponding to fruits stored for 5 days at 10 °C and then for 3 days at room temperature. Ripe and overripe pears were peeled and cored manually. Formic acid solution (10 mL/L) was sprayed on the fresh material during these later operations to avoid phenolic oxidation. Skin and flesh were separately frozen (−20 °C) until used.

2.3. Firmness

Firmness was measured at harvest for ripe pears and after 8 days of storage corresponding to the 5 days at 10 °C and 3 days at room temperature for overripe pears using a multi purpose texturometer (Texture analyser TAplus: Ametek, Lloyd Instruments Ltd., Fareham, UK). Fruit without skin was placed on a stationary steel plate and penetrated to depth of 3 mm with a 2 mm diameter probe at a speed of 100 mm/min. A batch of five fruits was constituted for each maturity stage. Four determinations were conducted in the four sides for each fruit. The work to limit, defined as the energy of penetration into flesh up to 3 mm depth, was registered during the test and results were expressed in Joules (J) (Choi, Choi, Hong, Kim, & Lee, 2007; Sirisomboon, Tanaka, Fujita, & Kojima, 2000).

2.4. Cell wall isolation and extraction

Cell Wall Materials (CWM) were prepared by the phenol buffer method according to a method adapted from Renard (2005b) for each maturity and from fruit differentiating skin and flesh tissue, and in the whole flesh differentiating parenchyma and stone cells.

2.4.1. Phenol buffer method

Frozen pear flesh and skin (2 kg and 1 kg, respectively) were suspended in chilled buffer (2 L, 1.2 mM $CaCl_2$, 2.0 mM $MgCl_2$, 6.7 mM KCl, 60 mg/L ascorbic acid and 4 g/L malic acid, sodium disulfite 1 g/L, adjusted to pH 3.5 with 5 M NaOH; this solution will be called 'buffer' in the following paragraphs) with Triton ×100 (0.1 g/L) and mixed in a Kitchen blender until an homogenous mixture was obtained. The detergent was then washed off with chilled buffer until the washings were sugar-free (absence of sugars tested by the phenol-sulphuric method of Dubois, Gilles, Hamilton, Rebers, and Smith (1956). Cell walls were then suspended for 15 min in acetone: water (60:40, v:v) to eliminate polyphenols and transferred to a G3 sintered glass filter. Excess solvent was removed by aspiration under vacuum, the remaining paste was suspended in four times its weight of phenol for 1 h at room temperature. The saturated phenol solution was removed by extensive washing with buffer on G3 sintered glass filter (until the phenol smell disappeared). The sample was finally solvent-exchanged in 50% acetone (three times), 75% acetone (three times) and 100% acetone (three times) then overnight in an oven at 40 °C and ground. We obtained at this step CWM from the whole flesh (this sample was named FL) and CWM from skin (this sample was named SK).

To extract cell walls from stone cells and parenchyma cells separately, 2 kg of frozen pear flesh was used. The separation of stone cells from parenchymatous tissues was based on their higher density. After washing with acetone: water, the pulp was stored overnight at 4 °C in the same acetone: water solution. Stone cells sedimented and formed a distinctive yellow layer at the bottom of the container. The upper layer representing the parenchyma cells was carefully discarded and kept separately. Parenchyma cells (still containing some stone cells) were then centrifuged (20 min, 5000*g*) and collected carefully to further separate stone cells. The other extraction steps were applied to the two cell types as explained above and we obtained CWM from parenchyma cells (this sample was named PC) and CWM from stone cells (this sample was named ST).

2.4.2. Sequential polysaccharide extractions

The cell wall polysaccharides present in different CWM were extracted sequentially by ammonium oxalate, Na_2CO_3, NaOH 1 M,