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Compositional changes in cell wall polyuronides and enzyme activities associated with melting/mealy textural property during ripening following long-term storage of 'Comice' and 'd'Anjou' pears

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ABSTRACT

Irregular ripening is a major deterrent for the purchase of European pears. Cell wall polyuronides and related-modifying enzymes were investigated in two European winter pears, 'Comice' and 'd'Anjou', during ripening at 20 °C following 5 and 8 months of storage at −1.1 °C. Both pear cultivars developed a melting (buttery-juicy) texture and higher water-soluble polyuronides (WSP) in flesh tissue after 5 months, but the pears' texture quality decreased and a mealy (coarse-dry) texture presented in 'Comice' pear after long-term storage. Activities of pectin methylesterase (PME) and α-arabinofuranosidase (α-ARF) in 'Comice' pears was positively correlated with texture development, whereas in 'd'Anjou' pears polygalacturonase (PG), PME, cellulase (CEL), and β-galactosidase (β-GAL) were more highly correlated with texture. 1-MCP reduced pear ripening by inhibiting ethylene production and the activities of cell wall-modifying enzymes, maintaining higher antioxidants, and alleviating membrane lipid peroxidation. These results indicated melting texture developed in European pears as a result of the synthesis of WSP and increase of PME activity during pear fruit softening.

1. Introduction

Two main produced 'Comice' and 'd'Anjou' are the most produced European winter pears (*Pyrus communis* L.) in the U.S. Pacific Northwest. The minimum ripening chilling requirement for these varieties is storage at −1.1 °C for 5 months (Sugar and Einhorn, 2011; Wang and Sugar, 2013). Such storage conditions contribute to development of fruit softening, sweetness, aroma and buttery-juicy (melting) texture. Whether pears develop a consumer-preferred buttery-juicy or a coarse-dry (mealy) texture mainly depends on fruit maturity at harvest, orchard elevation, storage conditions and postharvest management. An effective, practical approach to define and deliver pears with an assured buttery-juicy texture is crucial for fruit marketing. Therefore, the ability to accurately predict pears' textural status is ensures profitability after long-term shipping and storage.

Fruit textural changes are influenced by variations in cell wall architecture and compositions. The matrix of cell walls is comprised of cellulose, hemicellulose, pectin, and other essential components such as structural proteins, minerals, and phenolic esters (Sila et al., 2009). An attempt to understand the textural properties and softening processes prompted these investigation into changes and modifications of cell wall polyuronides and related modifying-enzyme activity. As the major component in cell wall architecture, pectic polyuronides are classed as homogalacturonan (HG), rahamnogalacturonan-I (RGI) and rhamnogalacturonan-II (RGII), depending on their galacturonic acid moieties (Payasi et al., 2009). Any alteration in petic polyuronides is associated with cell wall structural transformation, which could be determined and extracted by water, chelators, acid, and alkali aqueous solution analyses (Raffo et al., 2011). Furthermore, the depolymerization and solubilization of pectic polyuronides is complicated and occurs as the result of coordination and interaction among cell wall-modifying enzymes including polygalacturonase (PG), pectin methylesterase (PME), cellulase (CEL), pectate lyase (PL), β-galactosidase (β-GAL) and α-arabinofuranosidase (α-ARF) (Ruiz-May and Rose, 2013; Seymour et al., 2013). PG cleaves to the α-(1,4)-galacturonosyl linkages in unesterified pectin. PME removes the methanol groups from the methylesterification of HG. CEL cleaves the cellulose chain. PL cleaves to de-esterified pectin by β-elimination. β-GAL and α-ARF hydrolyze the β-(1,4)-linked galactosyl and α-(1,4)-linked arabinofuranosyl residues to release the galactose and arabinose (Marín-Rodríguez et al., 2002; Payasi et al., 2009; Sozzi et al., 2002). Analyzing changes in patterns of cell wall-modifying enzymes would be give a better understanding of the

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relationship between the textural properties and fruit softening during the ripening of pears.

To provide consumers with full buttery-juicy textured pears, we examined the changes of cell wall metabolism in two European winter pears, 'Comice' and 'd'Anjou', during ripening after 5 (regular) and 8 (long-term) months of storage at −1.1 °C, to evaluate the correlation of the development of a melting or mealy texture with fruit softening behaviors, quality, and physiological properties.

2. Materials and methods

2.1. Fruit materials and experimental design

'Comice' and 'd'Anjou' pears were hand-harvested at commercial maturity (flesh firmness ≈ 51.9 and 60.3 N, respectively) on mature trees from the orchard of the Mid-Columbia Agriculture Research and Extension Center in Hood River, OR, USA (45.71°N, 121.51°W, elevation 150 m and average annual rainfall ~800 mm). Fruit of each cultivar fruit were selected to be free of any visual damage or fungal infection, and were selected for similar size and color. These were placed in twelve wooden boxes (~80 fruit per box), and placed in cold storage at 0 °C and > 90% relative humidity on the day of harvest. After 24 h of storage, six boxes of each cultivar were exposed to 0.15 μL L^{-1} 1-methylcyclopropene (1-MCP, SmartFresh®, AgroFresh, Spring House, PA, USA) in an airtight room (39.8 m^3) with a circulation fan at 0 °C for 24 h as recommended by the manufacturer. After treatment, all fruit were stored at −1.1 °C for 5 or 8 months. There replicates of ten fruit each were established at one point time during ripening. 'Comice' were held at 20 °C for 1, 3 or 5 d. 'd'Anjou were held at 20 °C for 1, 3 or 7 d. Pulp samples were quick-frozen and ground in liquid nitrogen, then stored at −80 °C for subsequent analysis.

2.2. Flesh firmness and sensory quality

Fruit flesh firmness (FF) was measured on opposite sides of the equator of each fruit after removing 2-mm thick peel discs using a texture analyzer (model GS-14, Güss Manufacturing Ltd., Strand, South Africa) equipped with a 8-mm probe, at 9 mm of penetration and at a speed of 9 mm s^{-1}. The maximum force was recorded and expressed as newton (N). Sensory quality (buttery-juicy texture score) of ripened pear fruit was evaluated organoleptically on a 5-point hedonic scale (Chen et al., 1993) at Hood River by two experienced three-member panels. Fruit with highly, moderately, or slightly buttery-juicy texture were rated as 5, 4, or 3, respectively, and those rated as moderately or very firm (i.e., underripe pears) or moderately or very mealy flesh texture (i.e., overripe pears) were rated as 2, or 1, respectively. Scale anchor points and definitions were determined in an orientation session before the first evaluation. An average score of 3 or higher was defined as commercially acceptable. Each assessor tasted one small fruit slice from each of the five fruits.

2.3. Extraction and fractionation of cell wall polyuronides

According to the method described by Murayama et al. (2002), pear samples (15 g) were homogenized in 20 mL of 80% ethanol using a hand-held homogenizer (D1000, Benchmark Scientific Inc., Sayreville, NJ, USA) with 14-mm generator probe, then boiled for 30 min. After centrifuging at 8000 *g* for 15 min at 20 °C, the supernatant was decanted and the residue was suspended with ethanol and centrifuged. This procedure was repeated twice. Next, the residue was suspended with acetone twice, then dried at room temperature for 48 h. The samples were the alcohol insoluble residue (AIR) fraction.

AIR (50 mg) were fractionated with 5 mL of distilled water and continuously shaken with a variable rotator (R4139-5A; Tek-Pro Tek-Tator V, Miami, FL, USA) for 1 h at 20 °C, then centrifuged at 8,000*g* for 10 min at 20 °C. This procedure was repeated and two supernatants were collected as water-soluble polyuronides (WSP). The residue was then suspended with 5 mL of 50 mM sodium acetate (pH 6.5) containing 50 mM cyclohexanetrans-1, 2-diaminetetra (CDTA) and subjected to continuous shaking for 1 h at 20 °C. The supernatant was centrifuged and repeated. The two supernatants were collected as CDTA-soluble polyuronides (CSP). The residue was suspended with 5 mL of 50 mM sodium carbonate containing 10 mM sodium borohydride, subjected to continuous shaking for 1 h at 20 °C, centrifuged, and repeated. The two supernatants were collected as sodium carbonate-soluble polyuronides (SSP).

2.4. Measurement of uronic acids content

Uronic acids were measured according to the method described by Blumenkrantz and Asboe-Hansen (1973). Extracted polyuronides solution (200 μL) was added to 1 mL of sulfuric acid containing 75 mM sodium borate. After boiling for 10 min, 20 μL of 5 g L^{-1} NaOH containing 1.5 g L^{-1} *m*-phenylphenol was added to the mixture and loaded into a 96-well plate. Absorbance was measured at 550 nm using a plate reader (ELx800, Bio-Tek Instruments Co., Winooski, VT, USA). A standard calibration curve was constructed using galacturonic acid and the data were expressed on an AIR weight as mg kg^{-1}.

2.5. Activities of cell wall-modifying enzymes

Polygalacturonase (PG) was measured according to the method described by Gross (1982). Pear samples (1.5 g) were homogenized in 7.5 mL of 0.3 M NaCl and centrifuged at 10,000*g* for 15 min at 4 °C. The supernatant (75 μL) was added to 0.6 mL of 1 g L^{-1} polygalacturonic acid (from oranges, Sigma-Aldrich Co.), 0.725 mL of 50 mM sodium acetate (pH 4.5) and 0.6 mL of distilled water, then incubated for 2 h at 37 °C. After adding 4 mL of 0.1 M borate buffer (pH 9.0) and 0.6 mL of 10 g L^{-1} 2-cyanoacetamide, the mixture was boiled for 10 min. One unit (U) of PG activity was defined as 1 mg of galacturonic acid released per min at 276 nm absorbance using a UV/visible spectrophotometer (Ultrospec 3100 pro, Biochrom Ltd, Cambridge, England).

Pectin methylesterase (PME) was measured according to the method described by Basak and Ramaswamy (1996). Pear samples (4 g) were homogenized in 5 mL of 0.3 M NaCl and centrifuged at 10,000*g* for 15 min at 4 °C. The supernatant was collected and adjusted to pH 8.1 with 0.05 M NaOH, then 4 mL of the supernatant was added to 36 mL of 5 g L^{-1} pectin (from citrus, Sigma-Aldrich Co.) adjusted to (pH 8.1) and incubated for 1 h at 37 °C. After the mixture titrated to pH 8.1 using a titration system (DL15, Mettler-Toledo Inc., Columbus, OH, USA), the consumption volume of NaOH solution was recorded. One unit of PME activity was defined as 1 mM of ester hydrolyzed released per min.

Cellulase (CEL) was measured according to the method described by Jurick et al. (2012). Pear samples (1.5 g) were homogenized in 7.5 mL of 0.3 M NaCl and centrifuged at 10,000*g* for 15 min at 4 °C. The supernatant (75 μL) was added to 1 mL of 10 g L^{-1} carboxymethyl-cellulose and 0.925 mL of 50 mM sodium acetate (pH 4.5) and incubated for 2 h at 37 °C. After adding 4 mL of 0.1 M borate buffer (pH 9.0) and 0.6 mL of 10 g L^{-1} 2-cyanoacetamide, the mixture was boiled for 10 min. One unit of CEL activity was defined as 1 mg of galacturonic acid released per min at 276 nm.

Pectate lyase (PL) was measured according to the method described by Chourasia et al. (2006). Pear samples (1.5 g) were homogenized in 7.5 mL of 50 mM Tris-HCl (pH 8.5) containing 0.6 mM $CaCl_2$, 5 mM EDTA and 0.5 mL L^{-1} Triton X-100 and centrifuged at 10,000*g* for 15 min at 4 °C. The supernatant (0.5 mL) was added to 5 mL of 50 mM Tris-HCl (pH 8.5) containing 0.6 mM $CaCl_2$ and 2.4 g L^{-1} polygalacturonic acid (from oranges, Sigma-Aldrich Co.), incubated for 30 min at 37 °C and boiled for 10 min. One unit of PL activity was defined as 1 mM of 4,5-unsaturated product per min at 232 nm.

β-Galactosidase (β-GAL) and α-arabinofuranosidase (α-ARF) were measured according to the methods described by Brummell et al. (2004) and Sozzi et al. (2002). Pear samples (1.5 g) were homogenized