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Research article

Changes in cell wall pectins and their relation to postharvest mesocarp softening of "Hass" avocados (*Persea americana* Mill.)

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ABSTRACT

The avocado is a climacteric fruit and begins a softening process after harvest. During ripening, the mesocarp changes in texture, and this affects fruit quality and cold storage capacity. Softening is commonly associated with cell wall disassembly in climacteric fruits. However, changes in the cell wall structure and composition during avocado softening are poorly understood. To understand this process, cell wall pectins in "Hass" avocado fruit were studied during ripening at 20 °C after harvest and after cold storage. Additionally, avocados were treated with 1-MCP to evaluate the delay in softening. Biochemical analysis showed a decrease in galacturonic acid (GalA) in alcohol-insoluble residues (AIR) and water-soluble pectin concomitant to softening, paralleled by an increase in polygalacturonase (PG) activity. In the same way, the β-galactosidase activity increased in soft avocado fruit, along with a reduction in galactose in cell wall material and the Na_2CO_3-soluble fraction. The arabinose content in the cell wall material did not change during softening. However, there was a change in arabinose ratios between the different fractions of pectin, mainly in the fractions soluble in water and in Na_2CO_3. The cold storage of avocado fruit did not induce softening of the fruit, but the content of GalA showed a substantial decrease, accompanied by an increase in PG activity. Thus, our work supports the hypothesis that the solubilization of neutral sugars such as arabinose and rhamnose, as well as the loss of galactose content mediated by the enzyme β-galactosidase, were the main factors that began the coordinated action of cell wall remodeling enzymes that resulted in the loss of firmness of avocado fruit.

1. Introduction

Avocado (*Persea americana* Mill.), a climacteric fruit, starts to ripen once harvested, triggered by surges in respiration rate and ethylene production (Kassim et al., 2013). During the avocado climacteric period, during which most of the ripening-related changes occur, the fruit presents changes in firmness, skin color and aroma (García-Rojas et al., 2016; Zamorano et al., 1994). Avocado undergoes an extensive softening of the mesocarp tissue during ripening (Jeong and Huber, 2004; O'Donoghue and Huber, 1992), influencing the marketing of avocado fruit in terms of not only fruit quality but also storability.

Fruit softening is mainly associated with cell wall disassembly as consequence of ripening-induced modifications to primary cell wall polysaccharides and their interactions (Brummell, 2006). The plant cell wall is composed of cellulose and hemicelluloses embedded in a matrix of pectin. Pectins are the most abundant polysaccharides in the cell wall matrix and middle lamella, playing an important role in the regulation of cell-to-cell adhesion (Billy et al., 2008). There are two main components of pectins, homogalacturonan (HGA) and rhamnogalacturonan-I (RG-I). Homogalacturonans are linear chains of galacturonic acid linked by α-bonds (1 → 4), and RG-I is a heteropolymer composed of repeats of the disaccharide (1 → 2) α-L-rhamnose- (1 → 4) α-D-galacturonic acid. Structures composed of RG-I contain arabinogalactan type I, which is mainly composed of arabinose and galactose (Buchanan et al., 2015). Consequently, changes in pectins, such as solubilization and depolymerization, have been proposed as the primary causes for fruit softening during ripening as a result of cell wall loosening and disintegration (Brummell, 2006; Jeong et al., 2002; O'Donoghue and

Abbreviations: 1-MCP, 1-Methylcyclopropene; AIR, Alcohol-insoluble residue; AV-GAL, Avocado Galactosidases; CDTA, Cyclohexane-trans-1, 2-diamine tetra-acetic acid; GalA, Galacturonic acid; HGA, Homogalacturonan; HPAEC-PAD, High performance anion exchange chromatography with pulsed amperometric detection; PG, Polygalacturonase; PME, Pectin methylesterase; PMSF, Phenylmethylsulfonyl fluoride; PNP, *p*-nitrophenol; RG-I, Rhamnogalacturonans type I; RH, Relative humidity; RT, Room temperature; TFA, Trifluoroacetic acid; β-Gal, β-Galactosidase

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Huber, 1992).

Modifications to cell wall polysaccharides are believed to be mediated by the coordinated action of several cell wall hydrolases, although other non-enzymatic mechanisms may be involved. Polygalacturonase (PG) and pectin methylesterase (PME) have been considered the main pectin-degrading enzymes in fruits. PME is responsible for catalyzing the reaction that removes methyl groups from HGA, while PG cleaves the galacturonic linkages of the HGA backbone (Brummell, 2006). The high levels of PG activity observed in avocado have been related to the extensive solubilization and depolymerization of pectins that take place during avocado ripening (Awad and Young, 1979; Jeong et al., 2002; O'Donoghue and Huber, 1992). On the other hand, several studies have suggested that HGA, which is present in a highly methylesterified form in the cell wall, is more susceptible to PG activity if this polysaccharide is partially de-esterified (Fischer and Bennett, 1991). The role of de-esterification of HGA is attributed to PME activity in several fruits including avocado (Wakabayashi et al., 2000).

There is significant evidence showing that PG and PME are not exclusively responsible for pectin degradation (Fischer and Bennett, 1991). β-Galactosidase (β-gal) activity, for instance, has been associated with a marked loss of galactose units, facilitating pectin solubilization during avocado ripening (Tateishi et al., 2007). Previous studies have identified three β-gal isoforms in avocado cultivars such as "Fuerte": AV-GAL I, II and III. Among them, AV-GAL III produced a substantial release of galactose units from avocado cell wall polysaccharides, and its enzyme activity was found to be positively associated with fruit softening (Tateishi et al., 2007). Although there are several studies that have addressed the changes in pectin composition during softening of "Hass" avocados, almost all of them have focused on ripening immediately after harvest, and many of the cell wall mechanisms associated with changes in avocado texture remain unclear (Redgwell et al., 1997; Sakurai and Nevins, 1997).

Cold storage is widely used by the avocado industry to delay ripening-associated softening and extend the postharvest life of avocados. Despite the importance of this technology in modulating avocado softening, there is very limited information regarding the changes in cell wall pectins contributing to the process of firmness loss in cold stored avocados. This research aimed to study the changes of cell wall pectins during the softening process of "Hass" avocados ripened immediately after harvest and following cold storage. For this purpose, pectin composition and the activity of key pectin-degrading enzymes were characterized during ripening. Additionally, the role of ethylene in avocado cell wall disassembly after treatment with 1-MCP was investigated to further elucidate the pectin modifications.

2. Materials and methods

2.1. Fruit material

Avocado fruits cv. "Hass" were collected at commercial harvest maturity (32% average dry matter) from a commercial orchard located in Melipilla Metropolitan Region, Chile. Fruits were selected based on shape and size uniformity and transported to the postharvest laboratory at INIA. Within 5 h of harvest, fruits were randomly divided into four groups and subjected to the following postharvest treatments: Control fruits (without 1-MCP treatment) were ripened immediately after harvest at 20 °C and 45% relative humidity (RH) until reaching the ready-to-eat stage (firmness less than or equal to 1 N); fruits in the 1-MCP group were treated with 300 nL L^{-1} 1-MCP (SmartFresh, Agrofresh) for 24 h at 20 °C/45% RH and then ripened until reaching the ready-to-eat stage. For cold storage fruit, control fruits (without 1-MCP treatment) were stored at 5 °C and 92% RH for 25 days with subsequent ripening at 20 °C and 45% RH until reaching the ready-to-eat stage; fruits in the 1-MCP group were treated with 300 nL L^{-1} 1-MCP for 24 h at 5 °C/92% RH and then stored at 5 °C and 92% RH for 25 days with subsequent ripening at 20 °C and 45% RH until reaching the ready-to-eat stage. Four fruits from each postharvest treatment were selected for biochemical analyses during ripening at 20 °C. The fruits that were chosen for analysis showed firmness values reflecting a gradual softening of ripe avocados after harvesting or after cold storage (García-Rojas et al., 2016). Each fruit sample was evaluated for ethylene production rate following measurement of fruit firmness. Mesocarp tissue obtained from the equatorial region, where fruit firmness was determined, was then immediately frozen in liquid nitrogen, homogenized and stored at −80 °C until use for cell wall analyses.

2.2. Ethylene production rate

Each avocado sample was placed in a 1.6 L plastic container and sealed for ethylene accumulation at 20 °C during a given period of time. This time depended on the ripening stage of the fruit and avoiding excessive accumulation of carbon dioxide. Then, 1 mL of gas was taken from the headspace and injected into a gas chromatograph (Shimadzu GC 8A, Tokyo, Japan) equipped with a flame ionization detector (FID) and an alumina column (Supelco 80/100 Porapak column, 75 cm × 5 mm × 3 mm). The oven and injector temperatures were 40 °C and 150 °C, respectively. The results were expressed as μL C_2H_4 kg^{-1} h^{-1}.

2.3. Fruit firmness measurement

Fruit firmness was determined by puncture test using a TA-XT plus Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK) equipped with a 2-mm diameter cylindrical probe. A tissue slice approximately 15 mm thick, including peel, mesocarp and core, was excised from the equatorial plane of each avocado fruit. The slice was placed horizontally on a platform. The middle part of the mesocarp, between the core and the peel, was penetrated with the probe to a depth of 10 mm in four points that were equidistant across the slice. Complete force-time curves were obtained for each point, with a test speed of 1 mm s^{-1}. The maximum force (N) was calculated with Exponent Lite software (Exponent Lite, version 5.0.9.0; Stable Micro Systems Ltd., Surrey, UK).

2.4. Cell wall characterization

2.4.1. Preparation of alcohol-insoluble residue (AIR)

Cell wall material was extracted from each avocado fruit tissue as alcohol-insoluble residue (AIR) using the method described by Fry. (1988). A total of 5 g of frozen tissue was ground with a mortar and pestle with 80% ethanol. After grinding, 80% ethanol was added to a final volume of 80 mL. The mixture was boiled for 20 min and then cooled to room temperature (RT). The mixture was filtered over Miracloth (CalBiochem, San Diego, CA, USA), and the residue was again homogenized in 25 mL of 80% ethanol by stirring for 30 min. The filtration/homogenization process was repeated three times. The solid residue was homogenized in 25 mL of 95% ethanol and mixed via vortex for 5 min. The solution was then filtered through Miracloth and resuspended in 25 mL of 100% acetone. The solution was homogenized via vortex for 5 min after filtration. The final residue (AIR) was dried overnight and stored at RT.

2.4.2. Extraction of cell wall fractions

AIR was sequentially extracted to fractionate the cell wall material into: loosely bound pectins (water-soluble pectin), ionically bound pectins (cyclohexane-trans-1,2-diamine tetra-acetic acid (CDTA) -soluble pectin) and covalently bound pectins (Na_2CO_3-soluble pectin). Cell wall fractions were obtained based on 10 mg of AIR by sequential extraction with 800 μL of the following solutions: a) Milli-Q water; b) 50 mM CDTA in 50 mM acetate buffer (pH 6); and (c) 0.1 M Na_2CO_3, 1 mg/mL $NaBH_4$. Extractions were performed by stirring the suspension for 2 h, followed by centrifugation at 8000*g* for 8 min. The supernatants from each step were collected and assigned to the different fractions.