Changes in Cell Wall Composition during Development of Persimmon Fruit

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Abstract

Cell walls were isolated from persimmon (Diospyrus kaki L.) fruit pericarp, at four developmental stages. Isolated walls were analyzed for changes in the composition and solubility of constituent polysaccharides during development. Pectic polysaccharides decreased from approximately 20% of total wall polysaccharides during development stages. Decreases in uronic acid, arabinose and galactose were accompanied during development. The loss of polyuronides was also accompanied by a depolymerisation of the polysaccharides. Overall, the results indicate that major changes in cell wall polysaccharide composition occurred during softening of ripening.

INTRODUCTION

Plant cell wall consists of cellulose microfibrils and matrix substance (pectins, hemicelluloses, proteins and phenolics). The cellulose microfibrils are thought to support the strength of plant cell and tissues and are generally bound to xyloglucans (Hayash, 1989). Other hemicelluloses polysaccharides seemed to form a continuum with pectic polysaccharides in the matrix phase of the cell wall (Carpita and Giberaut, 1993; Cutillas-Iturralde et al., 1993; Giberaut and Carpita, 1994; Karakurt and Huber, 2003).

Chemical changes in pectins and middle lamellae are closely related to the softening and changes in persimmon texture (Kwang-Soo-Kim et al., 1995; Tsuchida et al., 2003). Increases in the water-soluble pectin are usually caused by the action of hydrolase enzyme.

During the development, variation in the composition of the different compounds occurs. This experiment describes the changes in the polyuronides, neutral carbohydrates and hemicellulose released from the cell walls during the development of persimmon Fuyu.

MATERIALS AND METHODS

Plant Material

Persimmon fruits (Diospyrus kaki L.) were harvested weekly from orchards in Pilar do Sul, São Paulo State, Brazil. After harvest, fruits were selected based on their growth kinetics in four categories: I, II, III and IV growth stages (Fig. 1). Immediately transported on the day of harvest to the Postharvest Laboratory (~450 km), where they were washed with chlorinated water containing 150 μL L⁻¹ free chlorine, graded. After that fruits were stored at -20°C until used.

Preparation and Analysis of the Persimmon Fruit Cell Wall

A modified method of cell wall preparation by Cutillas-Iturralde et al. (1993) was used. Fruits at the four selected stages were homogenized in 300 ml 5 mM sodium metabisulphite for 3 min with an Polytron homogenizer (Brinkmann, PT 10-35, Lenz

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Kruenz, Switzerland). The homogenate were filtered through a miracloth (Calbiochem). The residue was slightly squeezed to remove remaining liquid and washed twice by homogenization in 100 ml of cold 96% ethanol. The alcohol insoluble residue was suspended in 96% ethanol, boiled for 30 min, to inactivate enzymes and then filtered through GF/C filter papers (Whatman Inc. Clifton, NJ, USA) in an aspiration flask and washed twice with 95% ethanol and stored overnight at 4°C. The suspension was centrifuged (8,500 g, 5 min) and the pellet washed twice with acetone followed by methanol:chloroform (1:1, v/v) and air dried to a constant dry weight. Iodine test for the presence of starch in cell wall preparations was negative.

The neutral sugar content of each sample was analyzed as described by Albersheim et al. (1967). After derivatization (alditol acetate derivatives) total non-cellulosic cell wall neutral sugars were dissolved in 200 μl of acetone. A portion of the sugars was introduced into a gas-liquid chromatography (Gas Chromatograph Varian model 3800) equipped with a capillary column (SP-2340, Supelco, 0,25 mm i.d × 30 m, Sigma-Aldrich Japan Co., Japan). The oven temperature was raised from 220 to 240 °C at the rate of 2°C min⁻¹. Rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose were determined and myo-inositol was used as an internal standard.

**Extraction and Chromatography of Hemicellulose from Four Growth Stages**

Extraction of polyuronides was performed by incubating cell wall material (20 mg) in 7 ml of deionized water for 4 h at 23°C with stirring. The suspension was filtered through GF/C filter paper and the filtrate was retained for gel chromatography. The suspension was filtered through GF/C filters and the filtrate was retained for chromatography. Uronic acids in the filtrates were determined using the procedure of Blumenkrantz and Asboe-Hansen (1973).

Gel chromatography of polyuronides was performed as described by Chun and Huber (2000) on a bed (1,5 cm width, 27 cm length) of Sepharose CL-2B-300 and Sepharose 6B-100 to hemicelluloses packaged and operated in 200 mM ammonium acetate, pH 5.0. Polyuronides (approximately 0,5 mg galacturonic acid equivalents in a volume of 2.5 ml buffer) were applied to the column and eluted with the ammonium acetate buffer. Fractions of 2 ml were collected, and 0,5 ml aliquots of these were used for the determination of uronic acids. Uronic acids from each growth stage were chromatographed in triplicate.

**RESULTS AND DISCUSSION**

Molecular size features showed that polyuronides were a little degraded during development (Fig. 2). Changes not were evident in polyuronides isolated from four developmental stages. Slight decreases of hemicellulose during growth were observed (Fig. 3). Cutillas-Iturralde et al. (1993) have pointed out that the molecular size characteristics of persimmon fruit polyuronides may be explained, in part, by fact that D-galacturonases (polygalacturonases) activity was not detected in this fruit. In a number of fruit types, increased levels of soluble polyuronides are closely correlated with the appearance of pectohydrolytic enzymes (El-Zogbhi, 1994; Huber, 1984).

During persimmon fruit growth and ripening a large decrease in firmness was observed and that particularly evident during the latter periods of development (Data not shown). We also observed a large decrease in galactose and arabinose from the cell wall during development, and the main components of the non-cellulosic neutral sugars were galactose, arabinose, xylose and glucose (Table 1).

**CONCLUSION**

The effects of development on persimmon fruits (cv. Fuyu) fruit deterioration and cell wall composition were investigated. Arabinose (Ara) and galactose (Gal) in the pectic fraction of mesocarp cell walls decreased significantly at late stage of fruit softening as did the hemicellulose fraction. Fruit firmness was significantly correlated to contents of Ara and Gal of (WS), and hemicellulose, which suggest that these components influence
the integrity of mesocarp cell walls of persimmon fruit.

**Literature Cited**


**Tables**

Table 1. Cell wall composition (neutral sugars) of pericarp tissue during development (growth stage) of persimmon fruit.

<table>
<thead>
<tr>
<th>Growth stage (sugars μg mg⁻¹ DW)</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ramnose</td>
<td>17,6</td>
<td>19,6</td>
<td>31,5</td>
<td>9,8</td>
</tr>
<tr>
<td>Fucose</td>
<td>8,0</td>
<td>3,3</td>
<td>6,3</td>
<td>7,4</td>
</tr>
<tr>
<td>Arabinose</td>
<td>118,5</td>
<td>72,7</td>
<td>57,0</td>
<td>33,0</td>
</tr>
<tr>
<td>Xylose</td>
<td>35,6</td>
<td>33,6</td>
<td>39,8</td>
<td>53,1</td>
</tr>
<tr>
<td>Mannose</td>
<td>8,6</td>
<td>18,9</td>
<td>9,6</td>
<td>6,3</td>
</tr>
<tr>
<td>Galactose</td>
<td>140,5</td>
<td>73,8</td>
<td>58,5</td>
<td>32,8</td>
</tr>
<tr>
<td>Glucose</td>
<td>16,2</td>
<td>10,4</td>
<td>21,0</td>
<td>23,2</td>
</tr>
</tbody>
</table>
Fig. 1. Four growth stage of persimmon fruit.
Fig. 2. Sepharose – CL-2B-300 chromatography of soluble persimon polyuronides. Profiles represent polyuronides from (A) first, (B) second, (C) third and (D) fourth growth stage fruit.
Fig. 3. Sepharose – CL-6B-100 profiles of hemicelluloses from persimmon cv. Fuyu, (A) represent large green, (B) Brake, (C) pink and (D) red fruit.