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Cell wall modifications in chilling-injured plum fruit (*Prunus salicina*)

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Abstract

The aim of this study was to analyze the changes in cell wall pectins in normally ripening (juicy) and in chilling-injured plum fruit (*Prunus salicina* cv. Fortune) showing mealiness. Total cell wall neutral sugars and uronic acids, solubilization and depolymerization of pectins in water-, CDTA- and Na_2CO_3-soluble fractions of the cell wall (WSF, CSF and NSF, respectively), non-cellulosic neutral sugar compositions of these fractions, and the activities of the cell wall-degrading enzymes polygalacturonase (PG), pectin methylesterase (PME), 1,4-β-D-glucanase/glucosidase and β-galactosidase (β-gal) were determined. No differences in the total content of pectin and neutral sugars between normally ripening and chilling-injured fruit were detected. However, the mealy plums presented a higher level of tightly bound pectin (NSF) and a lower proportion of loosely bound pectin (WSF) than the juicy controls. Lower pectin depolymerization and reduced solubilization of neutral sugars in the WSF and CSF were also detected in the chilling-injured tissues, confirming an alteration in the normal ripening-associated pattern of polyuronide disassembly. While no differences were found in the activities of PG, PME and 1,4-β-D-glucanase/glucosidase between normally ripening and mealy fruit, the latter had reduced β-gal activity. This might have led to differential solubilization of polymers with galactan side chains, but further studies are required to determine if there is a causal relationship between these events. Overall, results indicated that the development of chilling injury symptoms in 'Fortune' plums is associated with abnormalities in cell wall metabolism, including a reduction in pectin solubilization and depolymerization and decreased ripening-associated modification of galactose-rich pectin polymers.

Keywords: Plum; Chilling injury; Internal breakdown; Mealiness; Gel breakdown; Cell wall

1. Introduction

Plums are highly perishable and low temperature storage is recommended to extend fruit postharvest life and maintain quality (Crisosto and Kader, 2000). However, extended cold storage leads to physiological disorders (Wang, 1990; Wang, 1993) and abnormal fruit ripening, reducing consumer acceptance (Crisosto and Kader, 2000). In many commodities the severity of chilling injury (CI) increases when the fruit is refrigerated for prolonged periods at close to 0 °C, but above fruit freezing point. In contrast, for plums, peaches and nectarines, CI symptoms develop more markedly when fruit are stored at temperatures in the range 2–8 °C (Crisosto et al., 1999; Nanos and Mitchell, 1991; Manganaris et al., 2006). These symptoms mainly develop during fruit ripening after cold storage, thus the problem is not noticed until the fruit reach customers (Crisosto et al., 1999).

The physiological basis of CI symptoms has been studied in detail in peach (reviewed in Lurie and Crisosto, 2005). Mealiness is the most prominent CI symptom and main factor that negatively affects peach fruit ripening after removal from cold storage. Mealiness is characterized by loss of juiciness and pectin gel formation. It is accepted that the textural changes occurring are associated with abnormal modifications in the activities of cell wall-degrading enzymes, generally leading to alterations in pectin metabolism (Brummell et al., 2004; reviewed in Lurie and Crisosto, 2005). It has been reported that when the fruit are stored at low temperature for extended periods, the normal increase in endo-PG activity does not occur during ripening and mealiness results (Ben Arie and Sonego, 1980; Zhou et al., 2000a,b). The degree of methyl-esterification of pectin also may be altered in mealy fruit (Ben Arie and Lavee, 1971; Lurie et al., 2003). A more recent study (Brummell et al., 2004) confirmed that the ripening-associated solubilization of high molecular weight pectins remains low, not showing the increase characteristic of juicy fruit. However, this report also showed that the nature of the chilling-injured fruit ripening pro-

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cess is more complex than simply an alteration in the balance between PG and PME, involving reduced disassembly of Ara and Gal-rich polysaccharides. These alterations in the normal metabolism of cell wall polysaccharides might affect the properties of the middle lamella leading to tissue breakage along enlarged air spaces, rather than across cells resulting in reduced availability of free juice upon tissue disruption.

As for peaches, one of the most common symptoms in chilling-injured plums is the development of mealy texture (Crisosto et al., 1999, 2004). There are some data regarding the influence of ripening stage, position on the tree and cultivar, as well as the cold storage temperature on the severity and extent of CI symptoms in plum (Taylor et al., 1993a,b, 1994, 1995; Crisosto et al., 1999). However, there are significant differences between the cell wall modifications reported for chilling-injured peaches and plums. For instance, while reduced solubilization of pectins is associated with mealiness in peach, for plums no major differences in polyuronide solubilization were observed between normally ripening and chilling-injured fruit that are characterized by gel breakdown (Taylor et al., 1995). In addition, the biochemical characterization of the disorder in plums is still partial. For example, while peach mealiness has been shown to be associated with a substantial alteration in pectin depolymerization, in plum no such studies have been done. The objective of the present work was to characterize the changes in pectin solubilization, depolymerization and composition as well as the modification in some cell wall-degrading enzymes associated with plum mealiness development.

2. Materials and methods

2.1. Plant material

Plum fruit (*Prunus salicina* Lindell cv. Fortune) were harvested at commercial maturity stage according to fruit size and skin background color. Forty fruit were allowed to ripen at 20 °C for 4 d (juicy) while another 40 fruit were stored for 4 weeks at 5 °C (90% RH) and subsequently transferred to 20 °C for 4 d. Fruit stored at 5 °C developed CI symptoms, evident as mealiness, based on the perceived sense of dry texture when tasted and the lack of juice when squeezed. In order to analyze fruit with similar firmness a Fruit Texture Analyzer equipped with a 7.9-mm-diameter, flat-tipped probe was used to perform compression tests at a speed of 0.17 mm s^{-1}. The maximum force during the test was determined and fruit within the most common range of tissue firmness (4.1 ± 0.4 N) were selected for further analysis. Longitudinally cut wedge-shaped slices from each fruit were cut, frozen in liquid nitrogen and stored at −40 °C until use.

2.2. Preparation of cell walls

Fifty grams of fruit pulp tissue were homogenized with an Ultraturrax (IKA Werke, Janke & Kunkel GmbH & Co. KG, Staufen, Germany) in 200 mL of 95% ethanol and boiled for 30 min to extract low molecular weight solutes and to prevent autolytic activity. The insoluble material was filtered through glass fiber filters (Whatman GF/C) and sequentially washed with ethanol, chloroform:methanol (1:1, v/v) and acetone and allowed to dry at 37 °C, yielding the alcohol insoluble residue (AIR).

2.3. Neutral sugars (NS) and uronic acids (UA)

Ten milligrams of AIR were solubilized in H_2SO_4 as described by Ahmed and Labavitch (1977) and aliquots of the AIR solution were subsequently assayed for uronic acid (Blumenkrantz and Asboe-Hansen, 1973) and total sugars (Yemm and Willis, 1954). Results were calculated by using a standard curve of galacturonic acid (UA) or glucose (NS). Three independent samples were analyzed for each treatment, and results were expressed as grams of galacturonic acid or glucose equivalents per kg of AIR for UA and NS, respectively.

2.4. Fractionation of cell wall pectins

Fractions of different cell wall components were obtained by sequential chemical extraction of the cell wall material (AIR). Approximately 200 mg of AIR residue from each sample were suspended in 15 mL of water and stirred at room temperature for 12 h. The samples were then centrifuged at 6000 × *g* and 4 °C for 10 min, the supernatant was filtered through glass fiber filters (Whatman GF/C), and the pellet was washed with water. The filtrate and water washings were combined and designated the water-soluble fraction (WSF). The residue was then extracted for 12 h at room temperature with 15 mL of 50 mmol L^{-1} CDTA, pH 6.5 with stirring. The slurry was centrifuged and passed through glass fiber filters, as above, and the pellet was washed with CDTA solution. The combined filtrates were collected, extensively dialyzed against water and designated the CDTA-soluble fraction (CSF). The CDTA-insoluble pellet was then extracted with 15 mL of 50 mmol L^{-1} Na_2CO_3 containing 20 mmol L^{-1} $NaBH_4$ at 4 °C for 12 h. After filtration (as above) the filtrate obtained was neutralized with glacial acetic acid, extensively dialyzed against water and the sample was designated Na_2CO_3-soluble fraction (NSF).

2.5. Neutral sugar composition

Tubes containing aliquots from the WSF, CSF and NSF were blown dry with air in a water bath at 40 °C. After that the samples were hydrolyzed in 2 mol L^{-1} trifluoroacetic acid (Albersheim et al., 1967), and converted to alditol acetates (Blakeney et al., 1983) for gas chromatographic analysis of neutral sugar composition. The derivatized samples were dissolved in acetone and 1 μL-aliquots were injected into a gas chromatograph fitted with a 30 m × 0.25 mm DB-23 capillary column (J&W Scientific, Folsom, CA, USA) and a flame ionization detector. Temperature in the injector was 250 °C and a linear oven temperature gradient (initial temperature 160 °C, 0 min; the oven increased at 4 °C/min to 250 °C) was used to improve separation. The different alditol acetates were identified by comparison with standards containing myo-inositol (internal standard), rhamnose (Rha), fucose (Fuc), arabinose (Ara), xylose (Xyl), mannose