Food Chemistry 242 (2018) 538–547

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Modifications of cell wall pectin in chilling-injured 'Friar' plum fruit subjected to intermediate storage temperatures

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ARTICLE INFO

Keywords:
Plum fruit
Chilling injury
Flesh translucency
Pectin
Neutral sugars
Nanostructure

Chemical compounds studied in this article:
Galacturonic acid (PubChem CID: 439215)
Galactose (PubChem CID: 6036)
Rhamnose (PubChem CID: 25310)
Arabinose (PubChem CID: 66308)

ABSTRACT

'Friar' plum (*Prunus salicina* Lindl.) fruit were stored at low (0 °C), intermediate (5 and 15 °C) and ambient temperature (25 °C). Flesh translucency was evidenced as the main chilling injury (CI) symptom and the CI developed rapidly at 5 and 15 °C but suppressed at 0 °C. Modifications of cell wall pectin in 'Friar' plums were investigated during storage. Sodium carbonate-soluble pectin (SSP) was found to be predominant in the fruit but it decreased more rapidly at 5 and 15 °C than 0 °C. Nevertheless, SSP possessed abundant galactose, arabinose and rhamnose at 5 and 15 °C. Nanostructural observations indicated that the detachment and degradation of linear backbone chains in SSP molecules were enhanced at 5 and 15 °C. Therefore, the development of CI of 'Friar' plums at intermediate temperatures was associated with the modifications of SSP in the cell wall pectin of the fruit subjected to chilling stress.

1. Introduction

Plums (*Prunus salicina* Lindl.) are highly perishable and deteriorate quickly during harvest, transportation, storage and the subsequent shelf life (El-Sharkawy, Sherif, Qubbaj, Sullivan, & Jayasankar, 2016; Pan et al., 2016). The fruit are commonly harvested at early mature stage before ripening and stored at 0–5 °C for prolonging postharvest life. However, plums are very sensitive to low temperature and the benefit of cold storage may be limited by the development of various physiological disorders, such as internal browning, flesh translucency, gel breakdown, reddening or bleeding, and loss of flavour after removal to ambient temperature (Candan, Graell, & Larrigaudière, 2011; Cantín, Crisosto, & Day, 2008; Crisosto, Garnera, Crisosto, & Bowerman, 2004; Manganaris, Vicente, Crisosto, & Labavitch, 2008; Minas, Crisosto, Holcroft, Vasilakakis, & Crisosto, 2013). These physiological disorders are also chilling injury (CI)-associated symptoms, which can extremely reduce the consumer acceptance of plums during marketing.

Generally, for most of the chilling-susceptible commodities, the severity of CI increases when the fruit are refrigerated for prolonged periods at close to 0 °C, but above fruit freezing point. In contrast, for stone fruits, such as plums, the CI symptoms develop more severely when stored at 5 °C than 0 °C (Crisosto, Mitchell, & Ju, 1999; Crisosto et al., 2004; Khan, Ahmed, & Singh, 2011; Manganaris et al., 2008), and even more severely at 8 °C (Velardo-Micharet et al., 2017), 10 °C (Minas et al., 2013), 15 °C (Wang, Pan, Wang, Hong, & Cao, 2016) or above (Fanning, Topp, Russell, Stanley, & Netzel, 2014). The wide range of temperature above 0 °C but below the ambient temperature is so called intermediate temperature. However, so far, few literatures are available on the mechanism that plums are more susceptible to CI at intermediate temperature than 0 °C.

Among CI symptoms, flesh translucency, which manifests itself as a translucent gelatinous breakdown in the mesocarp tissue, is the most predominant disorder occurred in plums that can lead to the collapse of fruit texture during cold storage and after removal (Minas et al., 2013; Wang et al., 2016). It has been demonstrated that the textural changes of plum fruit suffering CI are associated with the abnormal modifications of the cell wall pectin (Manganaris et al., 2008) and the occurrence of flesh translucency is related to the presence of water-soluble pectin in plums (Candan et al., 2011; Taylor, Rabe, Jacobs, & Dodd, 1995). Nevertheless, further understanding is needed on how the cell wall pectin is modified in the chilled plums (Sharma & Sharma, 2016; Wang et al., 2016).

Abbreviations: AFM, atomic force microscopy; AIR, alcohol insoluble residues; Ara, arabinose; CI, chilling injury; CSP, chelator-soluble pectin; Gal, galactose; GalA, galacturonic acid; Glu, glucose; HG, homogalacturonan; Man, mannose; MDA, malondialdehyde; MW, molecular weight; RG-I, rhamnogalacturonan I; RG-II, rhamnogalacturonan II; Rha, rhamnose; SSP, sodium carbonate-soluble pectin; WIP, water-insoluble pectin; WSP, water-soluble pectin; Xyl, xylose

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http://dx.doi.org/10.1016/j.foodchem.2017.09.090
Received 2 May 2017; Received in revised form 9 August 2017; Accepted 18 September 2017
Available online 19 September 2017
0308-8146/

Pectin participates in cell-to-cell adhesion, by forming a gel matrix embedding cellulosic microfibrils to make up the cell wall architecture. Pectic substances constitute a highly complex and heterogeneous group of polysaccharides that possess backbones consisting of a linear chain of (1→4)-α-D-GalA (galacturonic acid) in both the primary cell wall and the middle lamella (Paniagua et al., 2014, 2017; Round, Rigby, Macdougall, & Morris, 2010). Pectin exists usually with several structural polymers, including homogalacturonan (HG), rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II) and other branched HG. HG is a linear homopolymer of GalA in backbone chains, which is often partially methylesterified at the *O*-6 carboxyl. RG-I contains a backbone of the repeating disaccharide unit of (1→2)-α-L-Rha-(1→4)-α-D-GalA that is predominantly substituted at the *O*-4 of rhamnose (Rha) residues by side chains rich in neutral sugars, mainly arabinose (Ara) and galactose (Gal). RG-II also contains side chains containing abundant neutral sugars. Regarding the way in which these structural domains are inter-connected, the most accepted model for pectin assembly that have been proposed shows the pectin composite as a linear backbone composed by HG (smooth regions), that can be interspersed with rhamnosyl residues, alternated at regular intervals with RG-I, arabinan and arabinogalactan, etc. (hairy regions) (Maxwell, Belshaw, Waldron, & Morris, 2012; Paniagua et al., 2017). Loss of neutral sugars, leading to the degradation of side chains, results in the depolymerisation of these structural complexes of pectin. The solubilisation and depolymerisation of pectic polysaccharides causes the disassembly of cell wall architecture, ultimately leading to textural changes of fruit tissue (Paniagua et al., 2014; Redgwell, Fischer, Kendal, & MacRae, 1997). Still, it is not very clear on how the modifications of cell wall pectin occurred in plums exposed to chilling stress.

'Friar' plum is one of the most famous cultivars worldwide, but it commonly suffers severer CI during storage at 2–15 °C than 0 °C (Crisosto et al., 1999; Wang et al., 2016). Flesh translucency is identified as one of the main CI symptoms of 'Friar' plums, which can develop to gel breakdown and lead to the destructive damage on the fruit texture (Abu-Kpawoh, Xi, Zhang, & Jin, 2002; Candan et al., 2011; Cantín et al., 2008; Wang et al., 2016). It is unavoidable that plums are often exposed to a higher temperature than 0 °C, for the temperature regime used for harvesting, postharvest treatments, storage, sorting, packaging, shipping and marketing alters with the objective of improving the flavour and achieving uniform ripening (Minas et al., 2013; Velardo-Micharet et al., 2017). Therefore, a better understanding of textural changes and the involved modifications of cell wall pectin in plums subjected to intermediate temperature stress is necessary.

Accordingly, the aim of this work was to investigate the CI occurrence, textural changes and the modifications of cell wall pectin of 'Friar' plum fruit during storage at different temperatures, by comprehensively characterising the biochemical and compositional alterations of cell wall pectic polysaccharides. In particular, the nanostructural characterisation of sodium carbonate-soluble pectin (SPP) fraction was to be explored by atomic force microscopy (AFM) and the modification of SSP was to be identified in plums subjected to chilling stress. The data would provide a reference point at a molecular level for understanding the textural changes of 'Friar' plum fruit as responded to intermediate temperatures.

2. Materials and methods

2.1. Fruit material

'Friar' plums (*Prunus salicina* Lindl.) were harvested at a firm mature stage from a commercial orchard located in Yanqing County, Beijing, China. The fruit were transported to the laboratory immediately after harvest and sorted according to uniformity of shape, colour and size. Those plums with physical injuries, visual blemishes or infections were discarded.

Plums were placed into plastic baskets and each basket was packed with a 30 μm-thick perforated low density polyethylene (LDPE) bag. Some packed fruit were directly stored at 15 or 25 °C, 80–90% relative humidity (RH). Others were pre-cooled in air to 5 °C overnight and then stored for 8 weeks at 0 °C or for 4 weeks 5 °C, 85–95% RH. Examinations were conducted in certain intervals during storage. Flesh tissue were sampled and dipped immediately into liquid nitrogen and then frozen at −40 °C for biochemical measurements.

2.2. Evaluation of chilling injury

Plum fruit were longitudinally cut into halves for the evaluation of the occurrence of chilling injury (CI) according to the severity of flesh translucency (Khan et al., 2011). CI was estimated visually as the percentage of the affected area compared with the total surface area of each section on a scale where: 0 = no change; 1 = less than 10%; 2 = 10–25%; 3 = 25–50%; 4 = 50–75%; and 5 = more than 75%. The CI index was calculated using the following formula: CI index = Σ(number of the fruit within the scale × each scale)/(total number of fruit × the highest scale). Thirty plums were cut for CI evaluation in a replicate, with three replicates in each treatment.

2.3. Determination of malondialdehyde content and electrolyte leakage

The malondialdehyde (MDA) content was determined according to the method described by Sharma and Sharma (2016). Flesh tissue (2.0 g) was ground in liquid nitrogen and extracted in 10% (w/v) trichloroacetic acid (TCA). After centrifugation at 10,000 × *g* for 20 min, 2 ml of the supernatant was mixed with 2 ml 10% (w/v) TCA containing 0.6% (w/v) thiobarbituric acid (TBA) and then subjected to heating up to 100 °C for 20 min, quick cooling and centrifugation. The resultant supernatant was collected and absorbance was recorded spectrophotometrically at 532, 600 and 450 nm. The MDA concentration was calculated according to the formula: $6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$. The MDA content was expressed as nmol g^{-1} fresh weight (FW). Each treatment was repeated three times.

The electrolyte leakage was measured according to the method of Carvajal, Palma, Jamilena, and Garrido (2015). Briefly, fruit discs were excised from the plum flesh tissue with an 11-mm diameter stainless-steel cork borer. The discs were rinsed with 50 ml of deionized water three times for 5 min. After being incubated for 30 min and shaken at 100 rpm in 50 ml of deionized water, the solution was measured for conductivity at room temperature using a conductivity meter (DDS-11A, China). Total conductivity was determined after boiling the flasks for 10 min and cooling at room temperature. The electrolyte leakage was expressed as percentage of total conductivity. Three replicates from each treatment were measured.

2.4. Analysis of textural properties

Plums were longitudinally cut into 5-mm thick cylinders. Texture profile analysis was performed on the flesh cylinders using a texture analyzer (CT3, Brookfield Engineering Labs. Inc., USA), equipped with an aluminium cylinder probe (25.4 mm diameter). Hardness (N), restorable deformation (mm) and chewiness (mJ) were determined according to the method described by Singh, Guizani, Al-Alawi, Claereboudt, and Rahman (2013). Fifteen plums were measured individually as one repetition and the operation was performed in triplicate.

2.5. Preparation, fractionation and analysis of cell wall materials

2.5.1. Preparation of alcohol insoluble residues (AIR)

Fifty grams of plum flesh tissue was homogenised in 200 ml of 80% (v/v) ethanol, boiled for 30 min and then vacuum-filtered after cooling. The residue was boiled in fresh 80% ethanol three times. The insoluble