LWT - Food Science and Technology 43 (2010) 26–32

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Changes in firmness, pectin content and nanostructure of two crisp peach cultivars after storage

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ARTICLE INFO

Article history:
Received 20 January 2009
Received in revised form
29 May 2009
Accepted 16 June 2009

Keywords:
Firmness
Nanostructure
Atomic force microscopy (AFM)
Peach
Pectin

ABSTRACT

To investigate the fundamental of firmness changes of crisp peaches, firmness and pectin contents of two peach (*Prunus persica* L. Batsch) cultivars ('Cangfangzaosheng' and 'Songsenzaosheng') stored at 2 °C, 8 °C and 15 °C were investigated. Sodium carbonate-soluble pectin (SSP) extracted showed the highest correlation (positive) with firmness among the three kinds of pectins (water-soluble pectin, chelate-soluble pectin and SSP). The qualitative and quantitative information about SSP nanostructures were determined by atomic force microscopy (AFM). The widths of the peach SSPs were very consistent. The SSP chain widths of both peach cultivars were similar and were composed of several basic units. Schematic models of the changes of the chain widths were proposed. The results indicate that the firmness of peach was closely related with the contents and nanostructural characteristics of SSP, which might be hydrolyzed by enzymes in fruit flesh.

1. Introduction

Fruit texture is one of the most important quality properties that influence acceptability by consumers. It has been well established that texture changes are largely determined by the fruit cell wall and middle lamella polysaccharides (Manrique & Laiolo, 2004; Roeck, Sila, Duvetter, Loey, & Hendrickx, 2008). Cell wall polysaccharides mainly consist of pectin, hemicellulose and cellulose, while the middle lamella consists predominantly of pectin polysaccharides cross-linked with Ca^{2+}. Compared with hemicellulose and cellulose catabolism, fruit softening was more related to pectin solubilization and depolymerization (Rosli, Civello, & Martínez, 2004). Generally, softening of most fruit flesh is accompanied by changes in pectin structure (Ketsa, Chidtragool, Klein, & Lurie, 1999). Biochemical and/or chemical changes of fruit pectin were believed to result in textural changes of fruit flesh (Roeck et al., 2008). Pectin is a complex heteropolysaccharide, the chain of which contains many different monosaccharides that are composed of several components including homogalacturonan, rhamnogalacturonan I, rhamnogalacturonan II and xylogalacturonan (Pelloux, Rustérucci, & Mellerowicz, 2007). Investigating the structural changes of pectin chains will benefit to illustrating the fundamental of texture changes during cold storage.

Atomic force microscopy (AFM), as one of the nanotechnology tools, has been successfully applied in characterizing fruit and vegetable polysaccharides (Chen et al., 2009; Kirby, MacDougall, & Morris, 2008; Sriamornsak et al., 2008; Zhang et al., 2008), describing pectin degradation during storage (Yang, An, Feng, Li, & Lai, 2005; Yang, Feng, An, & Li, 2006; Yang, Lai, An, & Li, 2006) and pectin molecular manipulation (Yang, An, & Li, 2006). Except for providing the information of individual molecular chains and polymers (An, Yang, Liu, & Zhang, 2008), AFM also provides quantitative results at nanoscale without complex preparation of samples (Yang et al., 2007).

Peach is one of the favorite fruits due to its nutrition and quality value. However, peach easily develops chilling injury and corresponding physiochemical and textural changes during cold storage if under some inappropriate conditions, which limits its quality and storage life (Manganaris, Vasilakakis, Diamantidis, & Mignani, 2006). Many factors are involved in fruit softening during storage, and many measures including delayed storage, intermittent warming and calcium application have been used to reduce chilling injury and prolong cold storage life (Girardi et al., 2005; Manganaris, Vasilakakis, Diamantidis, & Mignani, 2007; Zhou et al., 2000). However, to our best knowledge, the fundamental of degradation of pectin under cold storage at nanostructure level has not been elucidated. Our previous research shows that firmness has

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0023-6438/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.lwt.2009.06.015

close relationship with sodium carbonate-soluble pectin (SSP) (Zhang et al., 2008). Our work only focused on the changes of pectin chains in this paper.

The aim of this work was to investigate the fundamental of firmness changes including chilling injury of peaches under storage. The relationship among pectin contents, firmness, and SSP nanostructures were illustrated. Two crisp peach cultivars at commercial maturity were compared.

2. Materials and methods

2.1. Fruit material

Two crisp peach (*Prunus persica* L. Batsch) cultivars ('Cangfangzaosheng' and 'Songsenzaosheng' peaches) were harvested at commercial maturity according to skin background color of fruits. The fruits were harvested by hand at a farm in Zhengzhou, Henan province, China and transported to our laboratory within 2 h after harvest. Fruits with uniform size, weight, color, disease free and no other defects were selected, then each cultivar peaches were divided into three lots and stored at 2 °C, 8 °C, and 15 °C, respectively. Each group had about 60 fruits.

2.2. Firmness determination

Fruit firmness was measured using a TA-XT2i Texture Analyzer (Stable Micro Systems Ltd., Godalming, Surrey, UK). Two cylindrical slides (diameter 10 mm, height 5 mm) cut from peeled fruits were used for fruit firmness determination. Five fruits were measured for each condition. A cylindrical probe with a diameter of 35 mm was used. The operating parameters were: pre-test speed: 5.00 mm/s, test speed: 0.50 mm/s, post-test speed: 0.50 mm/s, period between cycles: 10 s, sample strain: 30%, trigger force: 3.0 g (Shao, Tu, Zhao, Chen, & Zhao, 2006).

2.3. Cell wall preparation and pectin extraction and determination

Cell wall material of peach flesh was extracted by methods described by Deng, Wu, and Li (2005), and Zhang et al. (2008) with slight modification. Ten gram peeled peach flesh from five peaches (same as the five that were used for firmness determination) was ground rapidly in an ice-cold mortar, then transferred to 200 ml 80% (v/v) boiling ethanol for 20 min. The sample was cooled to room temperature, and then filtrated with vacuum pump. The residue was re-extracted with 200 ml 80% ethanol two times as described above. After that, the residue was incubated overnight at 4 °C with 50 mL dimethysulphoxide (DMSO, Tianjin Resent Chemical Co., Ltd., China): water (9:1, v/v) to remove starch. Then it was water-washed and transferred to 200 ml chloroform: ethanol (2:1, v/v) for 10 min. The sample was filtrated and washed with 200 ml acetone until total whitening, the residue was cell wall material.

The cell wall material was suspended in 10 ml distilled water, agitated at 25 °C for 4 h. After centrifugation at 10,000*g* for 10 min at 4 °C, the residue was subject to two additional distilled water extractions according to the same experimental procedure. The three supernatants were collected as water-soluble pectin (WSP). For further extraction of the residue, 10 ml 50 mM trans-1,2-diaminocyclohexane-*N, N, N′, N′*-tetraacetic acid (CDTA) (Tianjin Zinco Fine Chemical Institute, China) was used, the solution was shaken for 4 h at 25 °C and centrifuged as above. The remaining pellet was further extracted twice with 10 ml 50 mM CDTA and spun. The three supernatants were collected as chelate-soluble pectin (CSP). The final extraction was performed with 10 ml 50 mM Na_2CO_3 containing 2 mM CDTA, shaken and spun as above. The procedure was repeated twice and the three supernatants were combined as SSP.

The content of peach pectin was assayed by the Carbazole colorimetry method through determining the concentration of pectin solution, using galacturonic acid (Sigma-Aldrich Co., Ltd., St. Louis, MO, USA) as standard (Zhang et al., 2008). Pectin solution (2 ml) was mixed with 12 ml sulfuric acid (98%,w/w) in a test-tube and cooled immediately with ice water, then boiled for 10 min and cooled using running tap water. Then carbazole ethanol solution (0.5 mL) was added to the mixture and the mixed solution was incubated at room temperature for 30 min. The absorbance at 530 nm (A_{530} nm) was then determined with a UV-2000 spectrophotometer (Unico(Shanghai) Instrument Co., Ltd.) at room temperature. The concentration of pectin solution can be modified to a reasonable range for determination of pectin content. All the experiments were performed in triplicate.

2.4. Determination of molecular weight

The molecular weight of peach pectin was determined from the viscosity of the pectin solution on the basis of the Mark–Houwink equation $\eta_i = K \cdot M^{\alpha}$, where η_i is the intrinsic viscosity, K and α are constants of the pectin solutions.

A series of concentrations of pectin solutions were prepared, the solutions were heated to 20 °C, 15 ml heated pectin solutions were pipetted into the Ubbelohde viscometer for viscosity measurement. The determination of the intrinsic viscosity is to extrapolate the reduced viscosity (C, η_{sp}/C) to its value at zero solute concentration ($C \rightarrow 0$).

$$\eta_i = \lim_{C \rightarrow 0} \frac{\eta_{sp}}{C}$$

where C is the concentration of pectin solution (kg/m^3), η_{sp} is the specific viscosity, η_i is the reduced viscosity (m^3/kg) (Kar & Arslan, 1999; Lai & Yang, 2007).

2.5. AFM determination

AFM determination was conducted according to the previous methods (Yang et al., 2005; Yang, Feng et al., 2006; Yang, Lai et al., 2006). SSP solutions were diluted to a series of concentrations (about 0.5–30 μg/mL) and the diluted solutions were agitated with a vortex mixer (Fisher Scientific, Pittsburgh, PA, USA). Then about 20 μL of the diluted solution was deposited onto a piece of freshly cleaved mica sheets (Muscovite Mica; Electron Microscopy Sciences, Hatfield, PA, USA), modified molecular combing technique was applied with a glass slide for straightening the pectin (Yang, An et al., 2006), then the solution was air-dried at room temperature. The mica with the sample was attached to a 15-mm diameter AFM specimen disc (TED Pella Inc., Redding, CA, USA) using double-sided adhesive tabs. The imaging was conducted in air using a Nano-R2™ AFM (Pacific Nanotechnology Inc., Santa Clara, CA, USA) in "noncontact" mode. The NSC 11/no A1 tip (MikroMasch, Wilsonville, OR, USA) was used with scan rate of 0.5–2.0 Hz. The resonance frequency and force constant of the tip were 330 KHz and 48 N/m, respectively. Three samples of each group were observed using AFM, and for each group five to six fields were investigated.

The integrity of the AFM imaging could be verified through scanning standard references with certain roughness (Yang, Feng et al., 2006) or with regular surface shape that is provided by the AFM company (Pacific Nanotechnology Inc., USA).

2.6. AFM image analysis

The AFM images were analyzed offline using NanoRule+™ AFM software provided by the AFM company. Images of error signal