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Changes in fruit firmness, cell wall composition and cell wall degrading enzymes in postharvest blueberries during storage

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ABSTRACT

Blueberries are now the second most economically important soft fruit. However, they are highly perishable and susceptible to rapid spoilage. One of the main factors limiting postharvest life of blueberries is softening. The changes of fruit firmness, cell wall degrading enzymes and cell wall composition of 'Brilliant' blueberry (*Vaccinium ashei* cv. Brilliant) were investigated in this study. The results showed fruit firmness declined concomitantly with the increase of the content of water soluble pectin (WSP) during storage paralleled by a decreasing amount of sodium carbonate soluble pectin (SSP), cellulose and hemicellulose. Blueberries stored at low temperature (5 °C) maintained higher fruit firmness than those stored at 10 °C, which was due to the lower WSP content and higher contents of SSP, cellulose and hemicellulose. Meanwhile, the lower activities of cell wall degrading enzymes such as polygalacturonase, cellulase, β-galactosidase and α-mannosidase in blueberries at 5 °C were associated with greater fruit firmness and lower WSP content as compared to those in fruit stored at 10 °C.

1. Introduction

Blueberries, one of the most widely consumed fruit in the world, contain high amounts of phenolic compounds, including anthocyanins, flavonols, chlorogenic acid and procyanidins (Koca and Karadeniz, 2009), and have been shown a wide diversity of bioactivities such as antioxidant, antidiabetic, antimicrobial, antiproliferative, apoptotic, liver protection, lifespan-prolonging, anti-inflammatory, cancer preventive and cardioprotective activities (Smith et al., 2000; Faria et al., 2005; Torri et al., 2007; Bingül et al., 2013; Bunea et al., 2013). Due to their various health benefits, unique taste, and nutritional value, worldwide production and consumption of blueberries have increased rapidly in recent years and they have become the second most important soft fruit species after strawberry (Giongo et al., 2013). However, blueberries are highly perishable and susceptible to rapid spoilage (Cantína et al., 2012). It is reported that fresh blueberries have a shelf life of 1–8 weeks depending on stage of fruit ripeness, method of harvest, presence of fruit disease, and storage conditions (Duan et al., 2011). One of the main factors limiting postharvest life of blueberries is softening (Angeletti et al., 2010), which may influence not only the quality of the fruit, but also its storage life, transportability and resistance of postharvest diseases (Deng et al., 2005).

Softening in any fruit is primarily due to the change in cell-wall carbohydrate metabolism, leading to a net decrease in certain structural components (Sethu et al., 1996). During fruit softening, the loss of firmness is associated with the decrease in total water soluble pectin and the disassembly of primary cell wall and middle lamella structures (Giongo et al., 2013). Hemicellulose depolymerisation and arabinose loss are the main cell wall modifications (Vicente et al., 2007a). It is well documented that the changes in cell wall composition and structure results from the coordinated action of hydrolytic enzymes in the fruit (Deng et al., 2005). Prominent enzyme, polygalacturonase (PG), as well as a variety of glycanases and glycosidases, plays important roles in cell wall degradation (Sethu et al., 1996).

Several preservation technologies, including cold storage (Connor et al., 2002), high oxygen atmospheres storage (Zheng

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et al., 2003), allyl isothiocyanate (Wang et al., 2010) and edible coating (Duan et al., 2011), have been used to maintain bioactive compounds, reduce deterioration, and prolong shelf life of fresh blueberries. However, the modification of cell wall components in postharvest blueberries during storage is still not clear, and its possible mechanism during softening is not understood. Maintaining textural quality during storage is of interest to the fruit growing and distribution industries of blueberries, therefore, in the present work, composition modifications in the cell wall and changes of the activities of cell wall degrading enzymes such as PG, cellulase, β-galactosidase and α-mannosidase of blueberries were measured to explore the softening mechanism in this kind of fruit during storage.

2. Materials and methods

2.1. Fruit material and treatment

Blueberry (*Vaccinium ashei* cv. Brilliant) fruit were hand-harvested from five-year-old blueberry plants in a commercial orchard located in Anji county (119°68′N, 30°63′E) of Zhejiang Province. All fruit were harvested at commercial maturity, as determined by complete blue skin colour, and transported within 2 h to the laboratory. Fruit with uniform size and colour were placed in plastic containers with snap-on lids and each contained two hundred fruit (about 300 g). The containers were divided into two groups randomly. One group was then stored at 5 °C and the other 10 °C. Samples were taken initially and at 7-day intervals during storage of 49 days.

2.2. Firmness determination

Fruit firmness measurement was conducted by a TA-XT plus texture analyzer (Stable Micro Systems Ltd., U.K.) with a 5 mm diameter stainless probe. Firmness was measured on the equatorial region of each fruit. Twenty fruit from each treatment were compressed 5 mm at a rate of 1.0 mm/s and firmness was expressed in kilogram per square centimeter (kg/cm^2).

2.3. Cell wall preparation and fractionation

Cell wall polysaccharides were obtained as ethanol insoluble residue using the methods described by Deng et al. (2005). Briefly, 10 g of flesh were ground, extracted by 80% (v/v) ethanol and maintained in boiling water to inactivate enzymes. Then the sample was centrifuged after cooling and the residue was re-extracted twice with 80% ethanol. The retained residue was incubated overnight with 90% (v/v) dimethysulphoxide at 4 °C to remove starch, and then washed twice with water, chloroform–ethanol (2:1), and acetone, respectively. The isolated cell wall materials (CWM) were dried in a vacuum oven at 40 °C and stored over silica gel in a vacuum desiccator.

The CWM was fractionated according to the methods of Deng et al. (2005) and Li et al. (2006). Briefly, water soluble pectin (WSP) was obtained by suspending CWM in 50 mM sodium acetate buffer (pH 6.5) for 6 h of shaking, and collecting supernatant by centrifuging at 4 °C. The water-insoluble residue was re-suspended in 50 mM sodium acetate buffer (pH 6.5) containing 50 mM EDTA, shaken for 6 h, and centrifuged. The supernatant was collected as chelator soluble pectin (CSP). Sodium carbonate soluble pectin (SSP) was pooled by re-suspending the residue in 50 mM Na_2CO_3 containing 2 mM EDTA, shaking, centrifuging and collecting the supernatant. The remaining residue was re-suspended in 4 mM NaOH containing 100 mM $NaBH_4$, shaken and centrifuged. The supernatant was collected as hemicellulosic fraction and the final residue was cellulosic fraction.

The pectin content in the fraction was measured by the *m*-hydroxydiphenyl method (Paul and Jerome, 1982) using galacturonic acid as standard. The cellulose and hemicelluloses contents were determined using the anthrone method (Vicente et al., 2005) using glucose as standard.

2.4. Enzyme extraction and assay

Two grams of fruit tissues were treated with liquid nitrogen, pulverized and extracted in 10 mL of 0.2 M sodium acetate buffer (pH 5.0) containing 1 mM EDTA-Na, 5% polyvinylpyrrolidone (w/v). The enzyme extract was obtained by centrifugation at 10,000×*g* for 20 min at 4 °C.

PG activity was assayed by the method described by Deng et al. (2005) and Pathak and Sanwal (1998) with slight modification. The reaction mixture contained 2.0 ml of 0.2 M sodium acetate buffer (pH 5.0), 1.0 ml of 1% (w/v) solution of citrus pectin, and 1.0 ml of crude enzyme. The amount of reducing sugar released was determined using the dinitrosalicylate method after reaction 30 min at 50 °C. One unit of enzyme was the amount which catalyses the formation of 1 μg of reducing sugar per hour per g of original fresh weight.

Cellulase activity was determined by measuring the reducing sugar released from carboxymethyl cellulose (Deng et al., 2005). The reaction mixture contained 2.0 ml of 1% (w/v) solution of carboxymethyl cellulose and 0.5 ml of crude enzyme. The amount of reducing sugar released was determined using the dinitrosalicylate method after reaction 30 min at 50 °C. One unit of enzyme was the amount which catalyses the formation of 1 mg of reducing sugar per hour per g of original fresh weight.

β-Galactosidase and α-mannosidase activity was determined by measuring p-nitrophenol released from p-nitrophenyl-β-galactopyranoside and p-nitrophenyl-α-mannopyranoside, respectively (Sethu et al., 1996; Deng et al., 2005). The reaction mixture contained 0.5 ml of sodium acetate buffer (0.2 M, pH 5.0), 0.18 ml of 16 mM *p*-nitrophenyl-β-galactopyranoside and 0.12 ml of crude enzyme. The amount of *p*-nitrophenol was measured after reaction 90 min at 37 °C. One unit of enzyme was the amount which catalyses the formation of 1 μmol of p-nitrophenol per hour per g of original fresh weight.

2.5. Statistical analysis

All the measurements were conducted in triplicate. Data presented were the means ± SD values. All statistical analyses were performed with using SAS statistical software 8.01 (SAS institute, Cary, NC).

3. Results and discussion

3.1. Changes in fruit firmness

Fruit firmness is an important quality attribute in blueberry, and excessive softening is one of the main factors reducing quality and limiting commercialization for fresh consumption (Angeletti et al., 2010). As shown in Fig. 1, irrespective of the storage temperatures, firmness of blueberries increased during the first 7 days of storage, and declined gradually afterwards. Fruit stored at 5 °C maintained greater firmness than those at 10 °C after 7 days of storage.

3.2. Changes in cell wall composition

Previous work analyzing cell wall changes in blueberry fruit during development showed that pectin solubilization increased during ripening (Vicente et al., 2007b). Less attention has been