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Effect of β-aminobutyric acid on cell wall modification and senescence in sweet cherry during storage at 20 °C

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ABSTRACT

The effects of postharvest β-aminobutyric acid (BABA) treatment on fruit firmness, pectin degrading enzymes, cell wall constituents and microstructural alterations of pericarp in sweet cherry fruit were investigated. BABA significantly delayed the decline of fruit firmness and inhibited the increase of membrane permeability and the accumulation of malondialdehyde in cherries. The BABA-treated fruit exhibited significantly higher contents of water-soluble pectin, CDTA-soluble pectin, Na_2CO_3-soluble pectin, total pectin, cellulose and hemicellulose than the control during storage. Activities of pectin degrading enzymes including polygalacturonase and pectinmethylesterase were markedly reduced by BABA treatment. Observations by scanning electron microscopy showed BABA maintained smooth cuticle and integrated structure of subepidermal cell in sweet cherry. These results suggest that the delay in fruit senescence by BABA may be due to depressed membrane permeability and malondialdehyde content, reduced activities of polygalacturonase and pectinmethylesterase, enhanced cell wall polysaccharides content, and integrated subepidermal cell structure in sweet cherry.

1. Introduction

Sweet cherry (*Prunus avium*) is popular for its attractive red color, good flavor and high value of nutrients. However, sweet cheery deteriorates rapidly during storage at ambient temperature. The main causes of sweet cherry deterioration are weight loss, softening and color changes (Serrano et al., 2009). These changes directly affect the quality of fruit, as well as their shelf-life, transportability and marketing. The effect of different storage methods or postharvest treatments on reducing decay, improving quality or inhibiting softening of sweet cherry has been investigated (Choi, Wiersma, Toivonen, & Kappel, 2002; Díaz-Mula, Serrano, & Valero, 2012).

The softening and textural changes during fruit ripening and storage are characteristic of particular fruit species (Brummell, Dal Cin, Crisosto, & Labavitch, 2004). Any rapid loss of desirable texture due to excessive softening will contribute to poor quality, postharvest loss, and negative consumer buying behavior. Softening of fruit during ripening and storage is associated to a series of molecular, biochemical, and physiological changes (Li, Xu, Korban, & Chen, 2010). Solubilization and depolimerization of cell wall constituents are a consequence of the coordinated action of cell wall-modifying enzymes, such as polygalacturonase (PG), pectinmethylesterase (PME) and cellulase (Cheng et al., 2009). It has been reported that postharvest treatments with calcium and naphthalene acetic acid could individually alter the degradation of cell wall during storage in strawberry (Figueroa et al., 2012). Amnuaysin, Jones, and Seraypheap (2012) found that hot water treatment reduced enzymes activities and gene expression associated with cell wall modification in banana. In addition, the decrease in cell turgor pressure is associated with fruit softening (Thomas, Shackel, & Matthews, 2008). The decrease in membrane permeability and malondialdehyde (MDA) content is involved in the maintenance of turgor pressure. Moreover, postharvest senescence in many fruits and vegetables can be investigated by measuring lipid peroxidation as estimated by MDA production (Hodges, Delong, Forney, & Prange, 1999). The integrated cell wall

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structure can delay the senescence process of fruit (Li et al., 2010). The softening process occurs in sweet cherry influences its shelf-life, facilitates the pathogen attacks and increases susceptibility to decay. In this sense, fruit treatments aiming to maintain preferable texture and extend shelf life of sweet cherry are urgently needed.

As a non-protein amino acid, BABA has been shown to induce resistance response to protect numerous plants against various pathogens (Ton & Mauch-Mani, 2004). In addition, BABA enhanced salt and drought stress tolerance in *Arabidopsis* (Jakab et al., 2005). Zhang, Wang, Zhang, Hou, and Wang (2011) reported that BABA provided an effective control of postharvest blue mould in apple fruit, which might be associated with its direct fungitoxic property and the induction of defense-related enzymes. However, no information is available about the effect of BABA on cell wall modification and fruit-softening enzymes in postharvest fruit. The objective of this study was to investigate the effects of BABA on delaying softening process in sweet cherry fruit stored at 20 °C, as well as to evaluate the microstructural alterations in sweet cherry pericarp.

2. Materials and methods

2.1. Fruit material and BABA treatment

Sweet cherry (*P. avium* L. cv. Hongdeng) fruit were hand-harvested at commercial maturity stage with healthy greenish stems from an orchard in Yantai, Shandong province, China. Fruit were transported to our laboratory in a refrigerated van at 2–5 °C within 6 h of harvest. The fruit were selected for uniformity of size, ripeness, and absence of physical injuries or infection. BABA was purchased from Sigma Chemical Co., Ltd.

The fruit were divided randomly into two groups with 300 fruit each. The first group was dipped into a solution of 30 mM BABA for 10 min at 20 °C based on our preliminary study. The second group was immersed into sterile deionized water for 10 min at 20 °C and served as control. All fruit were air-dried for approximately 1 h and stored at 20 °C with 95% relative humidity for 5 days. Fruit samples of 10 fruit were taken daily during storage for measurements of firmness, membrane permeability, and morphological and structural changes of sweet cherry pericarp. Another sample of 10 fruit was collected at every day intervals. Tissue samples were mixed and frozen immediately in liquid nitrogen and then stored at −80 °C for measurements of MDA content, cell wall polysaccharide composition, activities of PG (EC 3.2.1.15) and PME (EC 3.1.1.11). Each treatment was replicated three times and the experiment was conducted twice.

2.2. Determinations of fruit firmness

Fruit firmness was measured by the method described by Serrano, Guillén, Martínez-Romero, Castillo, and Valero (2005) with some modifications. For each fruit, 1 cm^2 of the skin was removed, and flesh firmness was individually recorded using a TA-XT2i texture analyzer (Stable Micro System Ltd., UK) on twenty fruit from each replicate with a 5 mm diameter probe at a speed of 1 mm S^{-1}, and the results were expressed in N.

2.3. Membrane permeability of sweet cherry pericarp and MDA content

The membrane permeability of cherry pericarp was expressed in terms of relative electrical conductivity with a conductivity meter (DDS-11A, China). Pericarp samples (2 mm thickness) of sweet cherry were excised from the equatorial zone of 10 fruit with a double edged blade. One gram sample were incubated in 40 mL double-distilled deionized water for 20 min at 20 °C and the initial electrolyte leakage (C1) was assessed. The solution was then boiled for 20 min and re-adjust to a volume of 40 mL before the final electrolyte leakage (C2) was measured. Relative electrical conductivity (%) = (C1/C2) × 100.

MDA concentration was measured according to the method of Hodges et al. (1999). Tissue samples were homogenized in 10 mL 10% trichloroacetic acid and centrifuged at 10,000*g* for 15 min. The supernatant was used to measure the absorbance at 532, 600 and 450 nm. The MDA concentration = $6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$. MDA content was expressed as nmol g^{-1} fresh weight (FW). Each experiment was repeated three times.

2.4. Measurement of cell wall polysaccharides

Cell wall polysaccharides of sweet cherry flesh were extracted according to the method of Zhou, Li, Yan, and Xie (2011) with some modifications. Five grams of frozen tissue was homogenized in 100 mL of 80% (v/v) boiling ethanol for 20 min. The homogenate was cooled and centrifuged at 3000*g* for 10 min, and the residue was thoroughly washed three times with 15 mL of 80% (v/v) ethanol. The residue containing cell-wall material was dissolved in 15 mL of 90% (v/v) dimethylsulfoxide at 4 °C for 15 h to remove starch. The crude cell wall extract were dried at 37 °C for 12 h. The dry sample was stirred for 6 h at 20 °C with 10 mL of 50 mM sodium acetate buffer (pH 6.5), and centrifuged at 10,000*g* for 10 min, the precipitate was re-extracted twice with sodium acetate buffer (pH 6.5). The three supernatants were combined and designated as water-soluble pectin (WSP). The residue was then extracted in 10 mL of 50 mM sodium acetate buffer (pH 6.5) containing 50 mM CDTA for 6 h at 20 °C with stirring and centrifuged at 10,000*g* for 10 min. This procedure was repeated twice. The three supernatants were pooled and designated as CDTA-soluble pectin (CSP). Afterwards, the remaining residue was dispersed in 10 mL of 50 mM Na_2CO_3 containing 2 mM CDTA with constant stirring for 6 h, and centrifuged at 10,000*g* for 10 min. The sediment was re-extracted twice by the abovementioned procedure. The three supernatants were combined as Na_2CO_3-soluble pectin (NSP). The remaining residue was extracted with 10 mL of 4 M NaOH containing 100 mM $NaBH_4$, mechanically shaken for 6 h, and centrifuged at 10,000*g* for 10 min. This step was repeated twice and the three supernatants were combined as hemicellulose-containing fractions. The remaining residue was washed with double-distilled deionized water until neutralisation, and was designated as cellulose-containing fractions. The pectin contents in the fraction were determined by the m-hydroxydiphenyl method using galacturonic acid (GA) as standard, and expressed as mg g^{-1} FW. The total pectin (TP) content was obtained by adding WSP, CSP, and NSP values. The contents of hemicellulose and cellulose fractions were estimated as glucose by using the anthrone method. Results were expressed as mg g^{-1} FW.

2.5. Enzymes assays

PG and PME were extracted by homogenizing 1 g of frozen tissue sample at 4 °C with 5 mL of 40 mM sodium acetate buffer (pH 5.2) containing 100 mM NaCl, 2% (v/v) mercaptoethanol, and 5% (w/v) polyvinyl polypyrrolidone. The extracts were then centrifuged at 25,000*g* for 20 min at 4 °C. Desalting by dialysis was unnecessary because of the low salt concentration in extraction buffer. The supernatants were used to determine enzyme activity. Duplicate activity assays were performed immediately.

PG activity was determined by the method of Andrews and Li (1995). The reaction mixture contained 40 mM sodium-acetate buffer (pH 5.2), 0.1% (w/v) polygalacturonic acid, 100 μL enzyme extract. The reaction was terminated with 2 mL of 10 mM $Na_2B_4O_7$