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Changes in energy metabolism accompanying pitting in blueberries stored at low temperature

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ABSTRACT

Low-temperature storage and transport of blueberries is widely practiced in commercial blueberry production. In this research, the storage life of blueberries was extended at low temperature, but fruit stored for 30 d at 0 °C pitted after 2 d at room-temperature. Fruit cellular structure and physiological parameters accompanying pitting in blueberries were changed. The objective of this research was to characterise properties of energy metabolism accompanying pitting in blueberries during storage, including adenosine phosphates and mitochondrial enzymes involved in stress responses. Physiological and metabolic disorders, changes in cell ultrastructure, energy content and ATPase enzyme activity were observed in pitting blueberries. Energy shortages and increased activity of phenylalanine ammonia lyase (PAL) and lipoxygenase (LOX) were observed in fruit kept at shelf life. The results suggested that sufficient available energy status and a stable enzymatic system in blueberries collectively contribute to improve chilling tolerance, thereby alleviating pitting and maintaining quality of blueberry fruit in long-term cold storage.

1. Introduction

Blueberry (*Vaccinium* spp.) has recently been recognised as a functional food because of emerging evidence of its health-promoting properties, including nutrient richness and antioxidant potential. Blueberry showed the highest antioxidant activity among 25 commonly consumed fruits in the United States (Wolfe et al., 2008). *Vaccinium* spp. have been widely used as sources of natural antioxidants and in a variety of applications for preventing diseases of the nervous system (Youdim et al., 2000) and chronic disorders, such as coronary heart disease, stroke, and certain types of cancer (Stoner, Wang, & Casto, 2008).

Blueberries are highly appreciated for their quality but the postharvest life of this fruit is very short at ambient temperature because of susceptibility to microbial decay, mechanical damage, and moisture and nutritional loss (Hancock, Callow, Serce, Hanson, & Beaudry, 2008). Transfer of fruit from producer to consumer generally requires 5–10 d, which necessitates cold storage. In our previous studies (Zhou et al., 2014), low-temperature storage delays senescence and helps to preserve fruit quality, and is thus recommended for extending the postharvest life of blueberry; fruit placed directly in storage at 20 °C or at 0 °C after picking showed no apparent signs of pitting. Nonetheless, fruit quality can gradually decline during long term low-temperature storage and can rapidly deteriorate when fruit are subsequently moved to shelf life conditions at ambient temperature. Pitting, a physiological symptom of degeneration, often occurs during shelf life after cold storage and can be observed visually. Physiological manifestations of chilling injury (CI) usually precede or occur concomitantly with the appearance of visible symptoms (Purvis, 2002). Membrane integrity plays a key role in the pitting of blueberry fruit. Maintenance of membrane integrity requires energy and involvement of adenylate nucleotides in lipid metabolism (Pradet & Raymond, 1983).

Adenosine triphosphate (ATP) serves as the energy currency of the cell and is a major determinant of cell function and viability (Pradet & Raymond, 1983). Several important observations regarding energy metabolism in plants have led to a resurgence in research on intra- and extracellular energy metabolism (Jiang, Jiang, Qu, Duan, & Jiang, 2007). These observations include documentation of an ATP threshold for membrane lipid synthesis (Rawyler, Pavelic, Gianinazzi, Oberson, & Braendle, 1999); effective methods of preserving horticultural crops in association with elevated ATP levels (Duan et al., 2004; Saquet, Streif, & Bangerth, 2001); decreased fruit browning and decay (Saquet, Streif, &

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Bangerth, 2000; Yi et al., 2008); and extracellular nucleotides (eATP, eADP, and hydrolysable analogues) that function as regulatory agents in plant signal transduction (Roux, Song, & Jeter, 2006). In Conference pears stored under delayed controlled atmospheric conditions, inhibition of flesh browning was associated with maintenance of high levels of ATP and adenylate energy charge (*EC*) (Saquet, Streif, & Bangerth, 2003). Internal browning in pear fruit was suggested by Saquet et al. (2003), Veltman, Lentheric, Van der Plas, and Peppelenbos (2003) to be the result of limited energy availability and cellular decompartmentalisation. Lower levels of browning and ion leakage from the pericarp of lychee fruit treated with pure oxygen were correlated with higher ATP and adenosine diphosphate (ADP) levels, but were not related to content of adenosine monophosphate (AMP) (Duan et al., 2004).

Phenylalanine ammonia lyase (PAL), lipoxygenase (LOX), and peroxidase (POD) are key enzymes involved in plant stress responses. LOX catalyses peroxidation of plasma membrane lipids, increases lipid unsaturation, and thus changes membrane fluidity (Wang, 2001), with direct consequences for membrane integrity and permeability. PAL and LOX are thought to initiate lipolytic cascades in membrane deterioration during senescence and stress. Increases in membrane-associated PAL and LOX have been observed in response to chilling stress in maize (Pinhero, Paliyath, Yada, & Murr, 1998).

To date, there have been few reports on changes in energy metabolism accompanying pitting in blueberries during storage. Here, we explored the relationship between pitting and energy metabolism by exposing blueberries to damaging chilling temperatures. Our objective was to clarify the role of energy metabolism in blueberry pitting. To achieve this objective, the cell ultrastructure alterations induced by pitting and the physiological and metabolic changes have been studied along with a study of the energy metabolism.

2. Materials and methods

2.1. Fruit material and treatments

Blueberries (*Vaccinium* spp. LanFeng) were picked at commercial maturity in Shenyang, Liaoning Province, China, and transported to the food analysis laboratory at Shenyang Agricultural University on the day of collection. In the laboratory, the fruit were screened for uniform size, maturity, and absence of mechanical damage. Blueberries with uniform size and colour were selected for further analysis. Blueberries were precooled in a refrigerator at 0 °C for approximately 8 h and then stored at 0 ± 0.5 °C or 20 ± 0.5 °C and 80% relative humidity (RH). The blueberries were stored for 0, 2, 4, 6 and 8 d at 20 °C respectively. Following 0, 15, 30, 45, or 60 d cold storage, fruit were moved to a controlled-environment chamber and maintained at 20 ± 0.5 °C and 80% RH for 2, 4, 6 and 8 d of shelf life before proceeding with the analysis. Pitting incidence of blueberries was measured on the samples consisting of 100 fruits per replicate. Then samples of fruit flesh (approximately 200 g) were frozen in liquid nitrogen and stored at −80 °C for the measurement of ATP, ADP and AMP content and enzymatic activities. Three independent replicates were conducted.

2.2. Measurement of fruit pitting incidence

Pitting incidence was assessed after 0, 15, 30, 45, and 60 d storage at 0 °C followed by 8 d of shelf life at 20 °C. Three independent replicates ($n = 100$ fruit each) were conducted. Pitting incidence was calculated as follows: pitting incidence = $A_n/A_m \times 100\%$, where A_n is the number of blueberries with pitting and A_m is the total number of blueberries.

2.3. Transmission electron microscopy

Blueberry sections were embedded as described by Fernandez-Garcia, Lopez-Perez, Hernandez, and Olmos (2009). Sections (1 × 3 mm) were fixed with 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.2) for 2.5 h. After three 15-min washes with buffer, the samples were post-fixed for 2 h in 1% osmium tetroxide in the same buffer, followed by washing three times in phosphate buffer. All fixed tissues were dehydrated in a graded series of ethanol (35%, 50%, 70%, 96%, and 100%), and then infiltrated with propylene oxide followed by propylene oxide and Spurr's resin. Samples were immersed in Spurr's resin overnight at 4 °C and then were embedded in Spurr's resin. Blocks were sectioned on an LKB-VI ultramicrotome (LKB Instruments, Inc., Mt Waverley, Australia), collected on copper grids, and stained with uranyl acetate followed by lead citrate. Sections were examined using an H-7650 (Hitachi Instruments Inc., Tokyo, Japan) transmission electron microscope (TEM).

2.4. Measurement of ATP, ADP, and AMP contents and energy charge

ATP, ADP, and AMP contents were determined according to Liu, Jiang, Luo, and Jiang (2006) with minor modification. Tissue samples (5.0 g) from 10 blueberries were frozen in liquid nitrogen and then homogenised to powder. Adenosine phosphates were extracted with 10 mL 0.6 M perchloric acid for 1 min in an ice bath. The extraction mixture was centrifuged at 4 °C for 15 min at 12,000*g* (CT14RD centrifuge, Tianmei Instruments, Shanghai, China). A 6-mL aliquot of the supernatant was quickly neutralised (pH 6.5–6.8) with 1 M KOH, then diluted to 10 mL, passed through a 0.45-μm filter, and used for ATP, ADP, and AMP measurements. Separation and identification of ATP, ADP, and AMP was conducted by high-performance liquid chromatography (HPLC) (1525 Binary HPLC Pump; Waters Instruments, Milford, MA). The HPLC was equipped with a Thermo ODS-2 250 mm × 4.60 mm column and a UV detector. The elution programme used mobile phases A and B (percentages, defined below) as follows (1.0 mL/min flow rate): 0 min, 100% A; 7 min, 80:20 (A:B); 9 min, 75:25 (A:B); and 10 min, 100% A. The programme took a further 2 min to return to the initial conditions and stabilise. Mobile phase A consisted of 0.06 M K_2HPO_4 and 0.04 M KH_2PO_4 dissolved in deionized water and adjusted to pH 7.0 with 0.1 M KOH. Mobile phase B was 100% methyl alcohol. Sample aliquots (20 μL) were injected into the HPLC, and peaks were detected at 254 nm. ATP, ADP, and AMP peaks and contents were determined by comparison with external standards and expressed on a fresh weight (FW) basis. The adenylate energy charge (*EC*) was calculated as: ([ATP] + 0.5 × [ADP])/([ATP] + [ADP] + [AMP]).

2.5. Extraction of blueberry mitochondria

Mitochondria were extracted according to Walter and Bonner (1967). Each frozen sample (50 g) was homogenised in 100 mL extraction buffer. The crude homogenate was filtered through double nylon gauze and the extraction tubes were rinsed with an additional 50 mL solution. The homogenate was centrifuged at 4000*g* for 10 min at 4 °C, and the supernatant was removed to a fresh tube and centrifuged at 12,000*g* for 10 min at 4 °C for sedimentation of mitochondria. The mitochondrial pellet was resuspended in 20 mL of the extraction buffer and again centrifuged at 4000*g* for 10 min at 4 °C. The pellet was then resuspended in washing buffer. The extraction buffer consisted of 50 mM Tris, 0.3 M mannitol, 1.0 mM EDTA, 0.1% (w/v) cysteine, 0.1% (w/v) bovine serum albumin (BSA), and 0.5% polyvinylpyrrolidone (pH 7.5). The washing buffer consisted of 10 mM Tris, 0.3 M mannitol, 1.0 mM EDTA, and 0.1% (w/v) BSA (pH 7.2).