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Changes in antioxidative metabolism accompanying pitting development in stored blueberry fruit

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ABSTRACT

Blueberries (*Vaccinium* spp. 'LanFeng') are harvested and consumed at maturation and have a short storage life at room temperature. Changes in blueberry quality and physiological parameters differ under room or low temperature storage conditions. The storage life of blueberries can be extended at low temperature, but pitting can develop associated with refrigeration, especially during subsequent shelf-life. The objective of this research was to understand the antioxidative metabolism accompanying pitting development of stored blueberry fruit, involving reactive oxygen species and antioxidant systems. Physiological and metabolic disorders, including low firmness, increased cell membrane electrolyte leakage and malondialdehyde (MDA) content, and changes in enzyme activity, were observed in pitting blueberries. Blueberries were stored at 20 °C and 0 °C for 10 days and 60 days, respectively. Hydrogen peroxide and superoxide radical production rate increased more rapidly during shelf-life after cold storage than at room temperature. On the other hand, blueberries during shelf-life after cold storage, when pitting occurred, had lower activity of antioxidant-related enzymes, including superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), than those stored at room temperature. The severity of pitting was paralleled by higher cell membrane electrolyte leakage and MDA content, and lower SOD, CAT, and APX activities.

1. Introduction

Blueberries (*Vaccinium* spp.) are highly appreciated for their quality but the postharvest life of this fruit is very short at ambient temperature because of microbial decay, mechanical damage, and moisture and nutritional loss (Hancock et al., 2008). Postharvest softening related to enzymatic reactions of polyphenols and oxidative stress from reactive oxygen species (ROS) is also a primary reason for the short shelf-life, and significantly reduces commercial value of blueberry fruit. Low-temperature storage delays senescence and helps to preserve quality, and is thus recommended for extending the postharvest life of blueberries. However, pitting can be observed when blueberries are stored at shelf-life conditions after cold storage. Fruit stored directly at 20 °C show no such problems.

Pitting, one of the physiological symptoms of blueberries, can be observed visually. Physiological manifestations of chilling injury (CI) usually precede or occur concomitantly with the appearance of visible symptoms (Purvis, 2002). In addition, oxidative damage is considered to be an early response of sensitive tissues to CI (Hariyadi and Parkin, 1991). For instance, free radical production in the thylakoid membranes of leaves in low temperature treated plants has been observed prior to the appearance of chilling injury (Hidey and Bjorn, 1996). When exposed to biologically stressful conditions, including low temperature (Sala and Lafuente, 2004), high temperature (Sairam et al., 2000), water deficit (Jin et al., 2006), and ozone pollution (Mehlhorn et al., 1990), production of ROS increases and causes oxidative damage (Shigeoka et al., 2002). Accumulation of ROS may cause oxidative damage to lipids, forming toxic products such as malondialdehyde (MDA), which is a secondary end product of polyunsaturated fatty acid oxidation. Thus, MDA is usually an indicator of the degree of plant oxidative stress (Hodges et al., 1999) and the structural integrity of membranes of plants that have been stored at low temperatures (Posmyk et al., 2005). It has been reported that the activities of catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD) also are related to CI. Plants have evolved an efficient antioxidant defense system that can prevent the accumulation of ROS and that can repair oxidative damage. This defense system involves lipid-soluble antioxidants (α-tocopherol and β-carotene), water-soluble reductants (ascorbate and glutathione), and enzymes such as CAT, APX, and SOD. The role of SOD is to scavenge H_2O_2 and O_2^- and protect cells from damage by superoxide radical reaction

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products that are potentially toxic compounds (Kochhar et al., 2003). In addition, the accumulation of H_2O_2 is a signal of oxidative stress in the compartments where it originates, and leads to an appropriate response in the cellular defense systems (Imahori et al., 2000).

To date, there have been no reports on the changes in antioxidative metabolism accompanying pitting of stored blueberry fruit. In this study, the relationship between pitting and ROS was explored by exposing blueberries to a damaging chilling temperature. Our aim was to investigate the role of active oxygen metabolism as a possible factor involved in pitting, and to achieve this objective, we determined the activities of enzymes related to oxidative metabolism.

2. Materials and methods

2.1. Fruit material

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. Defective fruit (crushed, cracked, or immature) were eliminated. Blueberries with uniform size and color were selected for further analysis.

2.2. Postharvest treatments

Blueberries were precooled in a refrigerator (0 °C) for approximately 8 h and then stored at 0 ± 0.5 °C and 20 ± 0.5 °C and relative humidity (RH) was maintained at 80%. Following 0, 15, 30, 45, or 60 d of cold storage, fruit were moved to a controlled-environment chamber and maintained at 20 ± 0.5 °C and 80% RH for a 10 d shelf-life before proceeding with the analysis. Samples of fruit flesh (approx. 200 g) were frozen in liquid nitrogen and stored at −80 °C for measurements.

2.3. Measurement of fruit pitting incidence

The incidence of pitting was assessed after 0, 15, 30, 45, and 60 d of 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.

2.4. Measurement of fruit weight loss

Weight loss was determined using 5 replicates (n = 100 fruit per replicate) and was recorded initially and finally during storage. Weight loss was calculated as follows: weight loss = $(W_i - W_f)/W_i \times 100$, where W_i is the initial sample weight and W_f the final sample weight (g). Results were reported as percentage weight loss. A digital balance (BSA224S Beijing Sartorius, China) with 0.001 g precision was used for these weight measurements.

2.5. Measurement of fruit decay incidence

Fruit showing symptoms of rot, visible fungal growth, or bacterial lesions on the fruit surface were considered as a loss irrespective of the severity of the symptoms, and the percentage of decay incidence was recorded after 0, 15, 30, 45, and 60 d of storage at 0 °C followed by 8 d of shelf-life at 20 °C. Three independent replicates (n = 100 fruit per replicate) were conducted for each treatment.

2.6. Measurement of fruit firmness

Fruit firmness was measured on two paired sides of 10 different fruit from each replicate with a CT3 texture analyzer (Brookfield Engineering Laboratories, Inc., USA) and a 5 mm diameter probe at a speed of 0.5 mm s^{-1}. Results were expressed in grams (g).

2.7. Measurement of cell membrane electrolyte leakage

Electrolyte leakage was determined using 30 disks (2 mm diameter, 1 mm thick) from 10 fruits. Disks were washed with double-distilled water and then immersed in 40 mL double-distilled water for 3 h. Ion leakage was measured as the conductivity of the solution (DDS-307, Leici, China). Disks were boiled for 30 min at 95 °C, cooled at room temperature, and then the total conductivity was measured. Electrolyte leakage was expressed as relative conductivity (%).

2.8. Measurement of lipid peroxidation

Malondialdehyde (MDA) production was used as the analytical parameter to determine the index of lipid peroxidation, and was measured using the thiobarbituric acid reactive substrates (TBARS) assay. Extraction and determination of MDA content were performed according to Martinez-Solano et al. (2005) with slight modifications: 2 g of lyophilized blueberry tissue was homogenized in 5 mL of 0.1% trichloroacetic acid (TCA) solution. After centrifugation at 10,000 × g for 20 min, a 2-mL aliquot of the supernatant was mixed with 2 mL 10% (w/v) TCA containing 0.67% (w/v) thiobarbituric acid (TBA). The mixture was heated to 100 °C for 20 min, quickly cooled, and centrifuged at 10,000 × g for 10 min. The supernatant was collected and absorbances at 450, 532, and 600 nm were then measured using a spectrophotometer (TU-1810, PuXi, China). The MDA concentration was calculated according to the formula: $6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$. Three independent replicates were conducted for each treatment.

2.9. Determination of superoxide radical (O_2^-) production rate

Superoxide radical production rate was measured according to the method of Yan et al. (1996) with some modifications. Blueberry tissue (5 g) was homogenized with 20 mL of 50 mM Na phosphate buffer (pH 7.8). The homogenate was centrifuged at 12,000 × g for 15 min and the supernatant was used to measure the superoxide production rate. A 1 mL aliquot of the supernatant was mixed with 1 mL of 50 mM Na phosphate buffer (pH 7.8) and 1 mL of 10 mM hydroxylammonium chloride. After incubation for 20 min at 25 °C, 1 mL of the above reaction mixture was added to 1 mL of 17 mM 4-aminobenzene sulfonic acid and 1 mL of 7 mM α-naphthylamine, mixed, and then separated into two layers using ether to remove the interference caused by pigments. The absorbance of the lower pink water-phase was measured at 530 nm.

2.10. Measurement of hydrogen peroxide (H_2O_2) content

The content of H_2O_2 in blueberry tissue was measured by monitoring the absorbance of the titanium-peroxide complex at 412 nm, following the method of Patterson et al. (1984) with some modifications. One gram of the fruit was homogenized with 6 mL of chilled 100% acetone and then centrifuged at 12,000 × g for 10 min at 4 °C. The extracted solution (1 mL) was mixed with 0.1 mL of 5% $Ti(SO_4)_2$ and 0.2 mL of concentrated NH_4OH solution. The titanium-peroxide complex precipitated and this sediment was dissolved in 4 mL of 2 M H_2SO_4 after centrifugation at 3000 × g for 10 min.