Postharvest Biology and Technology 67 (2012) 84–91

Contents lists available at SciVerse ScienceDirect

Postharvest Biology and Technology

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

Sulfur dioxide fumigation alone or in combination with CO_2-enriched atmosphere extends the market life of highbush blueberry fruit

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ARTICLE INFO

Article history:
Received 1 July 2011
Accepted 12 December 2011

Keywords:
Antioxidant activity
Controlled atmosphere
Decay
Phytochemicals
Polyphenols
Sensory evaluation

ABSTRACT

The combined effect of sulfur dioxide (SO_2) fumigation and different carbon dioxide (CO_2)-enriched atmospheres (3% O_2 + 3, 6, 12, or 24% CO_2) on quality attributes, postharvest decay, phytochemical content, and antioxidant capacity of eight fresh blueberry cultivars (*Vaccinium corymbosum* L.) was determined. The SO_2 treatments did not harm berry quality, but did significantly reduce decay incidence, especially when it was followed by storage in elevated CO_2 atmospheres (>6%). However, very high CO_2 atmospheres (24%) induced fruit softening and 'off-flavors'. *Botrytis* and *Alternaria* spp. were the dominant fungal pathogens causing decay of blueberries during storage, but differences in the species of decay microorganisms were found among cultivars. Postharvest strategies that included SO_2 fumigation and/or enriched CO_2 atmospheres did not negatively affect phytochemical content or antioxidant activity of the fruit; however, the polyphenolic content and total antioxidant activity varied greatly among cultivars. Overall, SO_2 fumigation followed by controlled atmosphere storage (3% O_2 + 6 or 12% CO_2) is a promising postharvest strategy for fresh blueberries to reduce decay, extend market life, and maintain high nutritional value.

1. Introduction

Highbush blueberry has been included recently in a special category of functional foods because of its favorable combination of nutrient richness, antioxidant potential, and emerging evidence of its health-promoting properties (USHBC, 2011). Blueberries are an excellent source of natural antioxidants, which have been shown to have health benefits in the prevention of several chronic diseases, coronary heart disease, stroke, and certain types of cancer (Olsson et al., 2004; Neto, 2007; Stoner et al., 2008). Indeed, blueberry had the highest antioxidant activity among 25 selected fruits commonly consumed in the United States (Wolfe et al., 2008). However, blueberries are highly perishable, susceptible to rapid spoilage, and have a short market life, which is highly dependent on fruit ripening stage, method of harvest, disease incidence, and storage conditions (Hancock et al., 2008).

The major causes of losses are fungal decay and rapid maturation that accelerates senescence (Duarte et al., 2009). The most important postharvest fruit rots of highbush blueberry include Botrytis rot or gray mold (*Botrytis cinerea*), alternaria rot (*Alternaria* spp.), and anthracnose fruit rot or ripe rot (*Colletotrichum* spp.) (Wang et al., 2010).

Postharvest technologies such as low-temperature storage, controlled atmosphere (CA), calcium dips, UV irradiation, ozonation, hot water, plant-originated antimicrobial agents, and edible coatings have been applied to reduce decay and extend the market life of fresh blueberries (Schotsmans et al., 2007; Fan et al., 2008; Perkins-Veazie et al., 2008; Wang et al., 2008; Duan et al., 2011). In addition, several packaging technologies such as modified atmosphere packaging (MAP), equilibrium modified atmosphere packaging (EMAP), and active packaging (AP), in combination with an adequate temperature control, can extend fresh produce market life by maintaining the nutritional and sensory quality and the microbiological safety of the product during storage and distribution to market (Almenar et al., 2006; Schotsmans et al., 2007; Hancock et al., 2008). Postharvest treatments that alter the natural conditions of the fruit may also affect its phytochemical content (Duarte et al., 2009; Gonzalez-Aguilar et al., 2010). Sulfur dioxide (SO_2) is widely used on table grapes to prevent decay during storage, by either initial fumigation of fruit from the field followed by weekly fumigation of storage rooms or the presence of in-package pads containing sodium metabisulfite (Palou et al., 2010). The SO_2 technology has also been tested for control of postharvest decay on other fruit species such as litchi (Sivakumar et al., 2010), fig (Cantín

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doi:10.1016/j.postharvbio.2011.12.006

et al., 2011), banana (Williams et al., 2003), lemon (Smilanick et al., 1995), or apple (Chen et al., 2004). However, to our knowledge, the efficacy of SO_2 as a postharvest treatment on quality attributes of fresh blueberries has not been previously reported.

Because of varietal differences within the species, we investigated the potential of SO_2 fumigation followed by different CO_2-enriched controlled atmospheres as a useful, low-cost tool to reduce postharvest losses and extend market life in eight commercial highbush blueberry cultivars. Fruit quality and sensory attributes, the phytochemical content, and the antioxidant activity of the blueberry fruit were also determined.

2. Materials and methods

2.1. Fruit material

Highbush blueberries of eight commercial cultivars ('Emerald', 'Jewel', 'Legacy', 'Misty', 'Reveille', 'Snow', 'South Moon', and 'Star') grown in the San Joaquin Valley (CA, USA) were harvested early in the morning and transported to Kearney Agricultural Center (Parlier, CA, USA). After elimination of defective fruit (crushed, cracked, or immature), berries of each cultivar with uniform size and color were selected for further analysis. Initially, three replicates of ten berries per cultivar were used to determine fruit weight, firmness, soluble solids, titratable acidity, and pH at harvest as measures of initial fruit quality. Subsequently, approximately 200 g of blueberries from each cultivar were placed in small plastic baskets and then in vented plastic trays (40 cm × 60 cm × 12.5 cm) before receiving any storage treatment.

2.2. Assessment of SO_2 concentration × time ($C \times t$) products

Preliminary experiments were carried out to select the most appropriate SO_2 dose (7, 14, 28, or 194 nL s^{-1} L^{-1}). Fumigation treatments were conducted on four blueberry cultivars ('Emerald', 'Jewel', 'Misty', and 'Star') in 330-L steel chambers with two 10-cm-diameter fans mixing the inner air to ensure homogeneous distribution of SO_2 as described elsewhere (Cantín et al., 2011). The gas was released into the container through a 1.5-cm diameter polyvinyl chloride tube connected directly to the sulfur dioxide cylinder (Praxair, Los Angeles, CA). The concentration of SO_2 inside the tank was continuously monitored with a gas-sampling pump (model 8014-400A, SE certified model 42CFR84; Matheson Kitagawa, East Rutherford, NJ), using an SO_2 meter/data logger (model Z1300XP, Environmental Sensors Co., Boca Raton, FL). The SO_2 meter was connected to a computer that performed real-time calculations of the SO_2 dose applied, allowing application of very low and precise amounts of SO_2. The SO_2 dose applied during the fumigation was verified at the end of the fumigation with passive dosimeter tubes (5D tubes with detection limits of 0.05–28 nL s^{-1} L^{-1} SO_2, Gastec Corporation, Ayase-Shi, Kanagawa) placed into each tray before fumigation. Fumigations were conducted at 20 °C based on previous results of assays on pathogen survival rates performed at different fumigation temperatures (Cantín et al., 2011). Five trays of 12 baskets (~200 g) of fruit (15 baskets per cultivar) were used for each SO_2 treatment. After fumigation, the blueberries were covered with paper produce pads to avoid dehydration and stored at 1 °C and 95% RH. After 14, 28, and 42 d of cold storage, touch firmness, shrivel incidence, decay, and bleaching were monitored in three replicates of 25 berries for each cultivar-treatment combination considered.

2.3. SO_2 treatments and controlled atmosphere (CA)

Immediately after harvest, ~200 g of fruit from each of the eight cultivars were transferred to 60 small, vented commercial strawberry baskets (480 baskets total). Thirty baskets per cultivar were placed in vented plastic trays (40 cm × 60 cm × 12.5 cm) to receive SO_2 at 28 nL s^{-1} L^{-1}. Subsequently, fruit were stored in 330-L aluminum tanks at 1 °C in regular atmosphere (air) and four different CA treatments (3% O_2 + 3, 6, 12, or 24% CO_2). All tanks had a flow-through system to ensure comparable relative humidity and driving force for water loss. The flow rate entering into the tanks was calculated to change the atmosphere inside the tanks every 8 h (12 mL s^{-1}). For each cultivar, six SO_2-treated and six non-treated baskets were placed in each atmosphere while still inside the vented plastic trays. Fruit quality and phytochemical attributes were determined after 7, 14, 21, 28, or 35 d cold storage at 1 °C.

2.4. Fruit quality evaluation and decay incidence

Immediately after harvest, three replicates of 10 berries per cultivar were used to measure fruit weight, tissue firmness, soluble solids content (SSC), and titratable acidity (TA). Three baskets of each cultivar-treatment combination were weighed at the beginning of the trial and used to measure weight loss (%) during the entire storage time.

After 7, 14, 21, 28, or 35 d of cold storage at 1 °C under different storage conditions, five replicates of 10 berries were randomly selected from each cultivar-treatment combination to measure tissue and touch firmness, shrivel development, SSC, TA, and decay incidence.

Tissue firmness was measured by compression of each fruit with a fruit texture analyzer (FTA, model GS, Güss Manufacturing Ltd., Strand, South Africa) with a 2.5 cm flat tip at a speed of 5 mm s^{-1} to a depth of 4 mm; the maximum force was recorded and expressed in Newtons (N). Additionally, touch firmness and shrivel development were assessed. Touch firmness is a rating used by the industry and is measured by slightly squeezing the berry and assigning a rating of 1, very firm; 2, slightly less firm; or 3, not firm enough for marketing. Shrivel development was estimated visually and rated as 0, no shrivel; 1, intermediate shrivel; or 2, severe shrivel.

Ten berries per replicate were wrapped together in two layers of cheesecloth and squeezed with a hand-pressed juicer to obtain a composite juice sample. The juice was used for determination of SSC with a temperature-compensated refractometer (Atago Co., Tokyo, Japan). The juice was also used to determine initial pH and TA with an automatic titrator (TIM850 auto-titrator, Radiometer Analytical, Lyon, France). TA was calculated as % of citric acid per volume of juice. The ratio SSC/TA was also calculated.

External fungal development was visually determined on each individual fruit at every evaluation time. Any blueberry with visible mold growth was considered decayed. Results were expressed as percentage of decayed fruit. Arcsine-transformation of the data was performed prior to analysis of variance.

To identify the contaminating microorganisms living on the surface and inside of the blueberries, decayed fruit from different treatments and cultivars were analyzed after 35 d of cold storage at 1 °C plus 3 d at 20 °C to simulate retail holding conditions. On the evaluation day, a piece of the fruit skin (1 cm^2) was collected from each berry. The pieces were immersed in 1 mL sterile water in a 2-mL Eppendorf tube and vortexed for 30 s. To identify the microorganisms that entered the fruit tissues, a previous harsh surface sterilization [10% household bleach (of 5.25% sodium hypochlorite) solution for 4 min] was performed before isolating from decayed tissues. One hundred microliters of the shaken water suspension was placed in a Petri dish containing PDA medium. Petri dishes were incubated at 25 °C under a 12-h photoperiod of cold fluorescent light (350 μmol m^{-2} s^{-1}) until microorganisms were examined. To obtain pure cultures for identification, hyphal tips from the colonies of each growing species were transferred