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

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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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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

to fresh PDA and incubated as described above. Sporulated cultures were identified at the genus level after macroscopic and microscopic observation of the morphological characteristics of the isolates.

2.5. 'Off-flavor' detection

Sensory evaluation to detect 'off-flavors' was performed after 7, 14, 21, 28, or 35 d cold storage at 1 °C on the fruit of the eight blueberry cultivars. Only berries that had not been treated with SO_2 were used, since SO_2 is not legally registered for use on blueberries. Five trained and experienced panelists, recruited at Kearney Agricultural Center, received instructions regarding the evaluation procedure, differences in flavor, overall quality, and texture, and recognition of 'off-flavors' in the berries. Each panelist was served with three coded clear cups containing three blueberries that had been stored in regular atmosphere (air) or one of four CO_2-enriched atmospheres (3% O_2 plus 3, 6, 12 or 24% CO_2). Samples were equilibrated to ambient temperature before evaluation and presentation was randomized. Evaluations were done under natural light. Water was provided for rinsing between samples. 'Off-flavor' data was expressed as the percentage of panelists detecting off flavors in the sample for each cultivar-treatment combination. Arcsine-transformation of the data was performed prior to the analysis of variance.

2.6. Phytochemical analysis

Based on data regarding fruit quality and resistance to fungal decay, fruit from the most effective CA treatments (3% O_2 + 6 or 12% CO_2), with regular atmosphere (air) as a control, were further used for phytochemical analysis. Immediately after harvest or after removal from cold storage, three 5-g replicates from each of the eight cultivars were frozen in liquid nitrogen and stored at −80 °C until needed.

Blueberries (~2 g) were homogenized in 20 mL ethanol/water 1:1 (v/v) acidified with 50 mmol L^{-1} H_3PO_4 and placed in darkness at 4 °C for 20 h. Subsequently, samples were centrifuged at 12,000 × *g* and 4 °C for 10 min and supernatant aliquots were stored at −80 °C for further phytochemical analysis as elsewhere described (Olsson et al., 2004).

The determination of different polyphenolic classes was performed using a spectrophotometric assay. Briefly, 1 mL of each blueberry extract was mixed with 1 mL 0.1% HCl–ethanol solution (0.1 mL HCl in 100 mL 95% ethanol) and 8 mL 2% HCl–ethanol solution in a 10-mL volumetric flask. The absorbance was measured at 280, 320, 360, and 520 nm to evaluate total phenolics (TPC), hydroxycinnamic acid derivatives (THA), total flavonols (TF), and total anthocyanins (TAn), respectively. The corresponding standard curves were prepared using ethanol solutions of gallic acid, caffeic acid, rutin, and cyanidin-3-O-glucoside chloride (CyGC), respectively (Goulas and Manganaris, 2011).

The antioxidant activity (AA) of blueberry extracts was evaluated using a ferric reducing/antioxidant power (FRAP) assay as described by Pantelidis et al. (2007). Briefly, a 3-mL sample of freshly prepared FRAP solution [0.3 mol L^{-1} acetate buffer (pH 3.6) containing 10 mmol L^{-1} TPTZ (2,4,6-tripyridyl-1,3,6-triazine) and 40 mmol L^{-1} $FeCl_3 \cdot 10\ H_2O$] and 100 μL of blueberry extract were incubated at 37 °C for 4 min and the absorbance was measured at 593 nm. The absorbance change was converted into a FRAP measure by relating the change of absorbance at 593 nm of the test sample to that of a standard solution of ascorbic acid (AsA) and results were expressed as quantity of AsA per fresh weight mass of berries, mol kg^{-1}.

2.7. Statistical analysis

Homogeneity of variances was determined using Levene's test. Data analyses were performed by ANOVA (analysis of variance) and GLM (general linear model) using SPSS statistical software 19.0 (SPSS Inc., Chicago, IL). For data expressed as percentage derived from counts ('off-flavor' and decay), arcsine-transformation was performed prior to the analysis of variance. Mean separations among treatments with significant differences tested in ANOVA were conducted using the LSD test ($p \leq 0.05$). Non-transformed means are presented in the tables.

3. Results and discussion

3.1. Assessment of ($C \times t$) product

Excessive concentrations of SO_2 can cause undesirable effects on fruit quality (Cantín et al., 2011; Crisosto and Mitchell, 2002; Zoffoli et al., 2008). None of the treatments applied in the current study [7, 14, 28 or 194 nL s^{-1} L^{-1} SO_2 ($C \times t$) products] induced bleaching or skin discoloration. The 28 nL s^{-1} L^{-1} SO_2 treatment best controlled decay on fresh blueberries and therefore was selected for the current study. To ensure that this SO_2 ($C \times t$) product was innocuous for the berries and would not cause skin discoloration or bleaching, a test with 194 nL s^{-1} L^{-1} was also carried out and no bleaching was detected.

Previous studies described the harmful effect of excessive SO_2 on postharvest quality of table grapes (Zoffoli et al., 2008), litchi (Sivakumar et al., 2010) and figs (Cantín et al., 2011). In table grapes, excess SO_2 caused bleaching on the berry surface, premature stem browning, or hairline splits (Harvey et al., 1988; Crisosto and Mitchell, 2002; Zoffoli et al., 2008). Negative effects on litchi, including taste alteration, weight loss, and micro-cracking of the pericarp, have also been reported (Sivakumar et al., 2010). However, no damage was observed in the blueberries after fumigation with SO_2, possibly due to their relatively thick, waxy cuticles.

3.2. Effect of SO_2 treatments and CA on berry quality

Berries of the eight cultivars had significant differences in quality traits at harvest, including weight (1.3–2.5 g), firmness (7.0–16.8 N), SSC (11.3–18.1%), and TA (0.6–0.9% citric acid) (Supplementary data, SD 1).

No statistically significant differences in fruit weight loss were found among the examined CA storage treatments during 35 d of cold storage at 1 °C (data not shown), in accordance with previous studies (Duarte et al., 2009). However, fruit weight loss varied by cultivar, as reported for other highbush blueberry cultivars (Alsmairat et al., 2011). 'Snow' and 'Star' fruit tended to lose more weight during storage (6–7% weight loss after 35 d cold storage), while 'Emerald' and 'Jewel' fruit lost only ~3–4%. This may reflect differences in cuticle permeability to water vapor, stem scar morphology, and/or the surface-to-volume ratios. The maximum weight loss before blueberries become non-marketable is approximately 5–8% (Wills et al., 1998).

Soluble solids content and titratable acidity did not show consistent differences among storage treatments for any cultivar (data not shown). SSC values generally remained invariable throughout storage period, as described (Almenar et al., 2008; Chiabrando and Giacalone, 2011). SO_2, CA, or their interaction (SO_2 and CA) did not affect the SSC, which is similar to SO_2 fumigation treatments applied to figs (Cantín et al., 2011).

The pH and TA of the blueberries remained generally unaffected throughout the 35 d cold storage (data not shown), independent of the initial TA for each cultivar. Sulfur dioxide (SO_2) and CA had

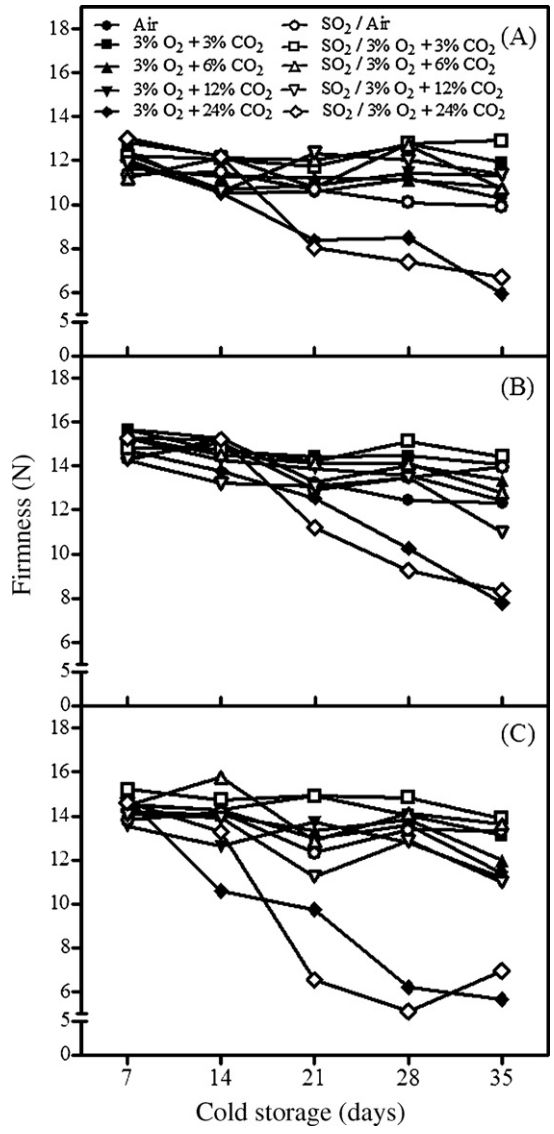

Fig. 1. Effect of different storage treatments (controlled atmosphere alone or after SO_2 fumigation) on the firmness of 'Emerald' (A), 'Reveille' (B) and 'Star' (C) blueberries after 7, 14, 21, 28, or 35 d of cold storage at 1 °C. Sulfur dioxide (SO_2) was applied at 28 nL s^{-1} L^{-1}. Controlled atmosphere storage regimes were 3% O_2 plus 3, 6, 12, or 24% CO_2.

significant effects on fruit pH, usually a slight increase, while pH was not affected by the interaction SO_2 and CA. No significant effect for SO_2, CA, or the interaction between them on fruit TA was found.

No significant variations in SSC or TA have been reported in either blueberries (Beaudry et al., 1998) or strawberries (Gil et al., 1997) stored under CO_2-enriched atmospheres. However, higher acidity in blueberries stored under controlled atmospheres has been reported (Duarte et al., 2009). Here, a slightly increased pH was observed under CA storage, as previously reported in strawberries (Gil et al., 1997).

Firmness is a major quality indicator in blueberries for fruit marketability. CA and SO_2 storage had a significant effect on berry firmness, although their interaction was non-significant. Fruit from all eight cultivars lost more firmness during storage under 24% CO_2, with or without SO_2 fumigation, than under any other storage treatment (Fig. 1). These results agree with data reported for fig (Cantín et al., 2011). Decreased firmness of different berries stored under high-CO_2 atmospheres has been reported (Alsmairat et al., 2011; Duarte et al., 2009; Schotsmans et al., 2007).

A subjective estimate of tissue firmness was also made using touch firmness. As with the mechanical determination of firmness, storage treatment affected berry touch firmness after 28 and 35 d cold storage for most cultivars (data not shown).

No pronounced differences among treatments for shrivel development were found after 28 and 35 d cold storage (Table 1). However, more shriveling was normally detected in fruit stored under regular atmosphere (air), with or without SO_2. For some cultivars ('Jewel', 'Snow', and 'Star'), significant differences were found after 35 d cold storage. Some cultivars also had different susceptibility to shriveling. 'South Moon' had the highest shriveling rate after 35 d storage, while 'Emerald' had the lowest (Table 1). Controlled atmosphere (CA), SO_2, and their interaction significantly reduced fruit moisture loss. Shrivel development correlated positively with fruit weight loss, while there was no significant correlation between shrivel development and touch firmness (Supplementary data, SD 3).

3.3. 'Off-flavors' detection

There were significant variations among the different atmosphere treatments in the percentage of 'off-flavor' fruit detected after 35 d cold storage (Fig. 2). Fruit of all cultivars stored under 24% CO_2 had the highest percentage of 'off-flavors'. The least 'off-flavor' fruit was found with 3, 6, or 12% CO_2-enriched atmosphere storage. Blueberry cultivars exhibited different susceptibility to 'off-flavor' development in high-CO_2 atmospheres. Almost all 'Star' fruit stored under 24% CO_2 was described as 'off-flavor' by the panelists, but only 60% of 'Emerald' and 'Reveille' fruit were of unacceptable flavor. 'Jewel' and 'Legacy' fruits were less susceptible to developing 'off-flavor' after storage under high-CO_2 atmospheres.

The high percentages of 'off-flavor' fruit found under the regular atmosphere (air) in the cultivars 'Snow' and 'Misty' were described by the panelists as 'decayed flavor', and were probably due to the existence of decay microorganisms, although decay was not visible macroscopically.

Although 24% CO_2 suppressed decay, it resulted in 'off-flavor' development. SO_2 enhanced decay control at each CO_2 concentration without inducing 'off-flavors'. Therefore, a lower CO_2 concentration (3, 6, or 12%) is recommended for fresh blueberries to avoid 'off-flavor' problems. 'Off-flavor' development in atmospheres with either low O_2 or high CO_2 has already been documented (Beaudry et al., 1998). On the other hand, susceptibility to the development of 'off-flavors' under high-CO_2 atmospheres varied greatly among cultivars; 'Star', 'Emerald' and 'Reveille' fruit were most sensitive.

3.4. Decay and microbiological studies

Decay, usually caused by fungi, is a major cause of postharvest losses in fresh blueberries and is a major problem in the blueberry industry. CO_2, SO_2, and their interaction all significantly controlled berry decay. Significant differences were found among storage treatments for all cultivars after 28 or 35 d of cold storage (Table 2). SO_2 fumigation after harvest combined with enriched-CO_2 atmosphere during storage (3% O_2 + 6, 12 or 24% CO_2) effectively reduced the incidence of decay in all cultivars compared to untreated fruit (Fig. 3). SO_2-treated fruit stored under regular atmosphere (air) also had less decay than SO_2-untreated fruit stored under CA. However, the higher CO_2 concentrations provided relatively little additional benefit to decay control in SO_2-treated fruit (Fig. 3). In general, slightly higher percentages of decay were found when fruit were stored under 3% O_2 + 3% CO_2 than under any other combination of gases applied (Table 2, Fig. 3). The CA treatments by themselves also controlled fruit decay, but the effect was more

Table 1
Effect of controlled atmosphere, alone or after SO_2 fumigation, on berry shrivel development of eight different blueberry cultivars after 28 and 35 d cold storage at 1 °C plus an additional 3 d period at 20 °C to simulate retail holding conditions. Shrivel development was scored on a scale of 0–2: 0, no shrivel; 1, intermediate shrivel; 2, severe shrivel. Sulfur dioxide (SO_2) was applied at 28 nL s^{-1} L^{-1}.

Treatment	Cultivar															
	Emerald		Jewel		Legacy		Misty		Reveille		Snow		South Moon		Star	
28 d (1 °C)																
Air	1.1	a	1.3	a	1.5	a	1.0	ab	1.3	ab	1.5	ab	1.7	a	0.8	bc
3% O_2 + 3% CO_2	0.6	bc	1.5	a	1.0	bc	1.2	a	1.3	a	1.5	ab	1.0	b	1.2	a
6% O_2 + 3% CO_2	0.8	ab	0.9	b	1.1	b	1.0	ab	0.9	abcd	1.4	ab	0.9	bc	0.8	bc
12% O_2 + 3% CO_2	0.6	bc	0.6	bcd	1.0	bc	0.5	c	0.9	abcd	1.2	ab	0.9	bc	0.7	bc
24% O_2 + 3% CO_2	0.5	bc	0.5	cd	0.6	d	0.9	ab	0.8	bcd	1.8	a	0.6	c	0.9	abc
SO_2/Air	0.2	c	0.9	b	1.1	b	0.9	ab	1.1	ab	0.8	b	1.6	a	0.5	c
SO_2/3% O_2 + 3% CO_2	0.7	bc	0.8	bc	0.9	bc	0.6	bc	1.0	abc	1.3	ab	0.8	cd	1.1	ab
SO_2/3% O_2 + 6% CO_2	0.7	ab	0.7	bcd	0.9	c	0.9	ab	0.9	abcd	1.1	ab	0.9	bc	0.8	bc
SO_2/3% O_2 + 12% CO_2	0.3	c	0.6	bcd	0.9	bc	0.9	ab	0.6	cd	1.1	ab	0.9	bc	0.8	bc
SO_2/3% O_2 + 24% CO_2	0.5	bc	0.4	d	0.9	bc	0.7	bc	0.5	d	1.1	ab	0.9	bc	0.6	c
35 d (1 °C)																
Air	1.1	b	1.5	a	1.1	ef	1.3	a	1.5	a	1.9	a	1.8	a	1.4	a
3% O_2 + 3% CO_2	0.9	bc	1.1	bc	1.1	f	1.3	a	1.1	abcd	1.4	c	1.6	a	1.3	ab
6% O_2 + 3% CO_2	1.5	a	1.3	ab	1.3	de	1.2	a	1.4	ab	1.6	bc	1.7	a	1.3	ab
12% O_2 + 3% CO_2	0.9	bc	1.0	bc	1.7	b	1.1	a	1.1	bcd	1.4	c	1.8	a	0.9	c
24% O_2 + 3% CO_2	0.4	d	0.5	e	1.7	b	0.8	bc	0.9	d	1.0	de	1.8	a	1.0	bc
SO_2/Air	1.5	a	1.2	ab	1.6	bc	0.7	c	1.3	abc	1.8	ab	1.8	a	1.4	a
SO_2/3% O_2 + 3% CO_2	0.9	bc	0.6	de	1.4	cde	0.7	c	1.0	cd	1.2	d	1.7	a	1.0	bc
SO_2/3% O_2 + 6% CO_2	0.9	bc	1.0	bcd	1.5	bcd	1.1	ab	1.1	bcd	1.0	d	1.8	a	0.8	c
SO_2/3% O_2 + 12% CO_2	0.6	cd	0.8	cde	1.6	b	1.0	abc	0.8	d	1.0	de	1.8	a	0.9	c
SO_2/3% O_2 + 24% CO_2	0.4	d	0.8	cde	2.0	a	1.1	ab	0.9	d	0.9	e	1.8	a	1.1	abc

For each cultivar, means followed by the same letter in each column are not significantly different at $p \leq 0.05$, according to the LSD test.

pronounced when combined with SO_2 fumigation. Differences among treatments regarding decay control were apparent, as exemplified by the response of 'Reveille' fruit (Fig. 4). Accordingly, combining SO_2 fumigation with elevated-CO_2 controlled atmosphere (3% O_2 + 6, 12, or 24%) during cold storage was the most appropriate treatment for decay control. Previous studies detailed the positive effect of CA on decay control in blueberries (Alsmairat et al., 2011; Beaudry et al., 1998; Schotsmans et al., 2007).

The efficacy of treatments in delaying mold growth varied among cultivars and was more evident in the most susceptible cultivars ('Jewel', 'Misty' and 'Reveille') (Table 2). This variability in treatment efficacy against decay has been previously described (Alsmairat et al., 2011; Beaudry et al., 1998; Milholla and Jones, 1972; Smith et al., 1996). Differences among cultivars of the same species have also been reported for litchi, where fumigation rates vary greatly among cultivars due to the different thickness, texture, and concentration of anthocyanin in the skin (Sivakumar et al., 2010). Those differences were also noticeable when all blueberry cultivars were analyzed at the same time (Fig. 3).

When the fungi on the fruit surface were plated aseptically on agar media, 57% of the visibly affected fruit were infected by *Alternaria* spp., 43% by *Botrytis* spp., and 14% by *Cladosporium* spp. after 35 d cold storage plus an additional 3 d at 20 °C to simulate retail holding conditions. These data agree with previous results for highbush blueberry, where decay was primarily caused by *Alternaria* and *Botrytis* spp. (Smith et al., 1996; Wang et al., 2010). However, when the interior of the berries was isolated, 45% of the decayed fruit were infected by *B. cinerea* and 36% by *Aureobasidium pullulans*. No *Alternaria* or *Cladosporium* spp. was isolated from the berry interiors. Although ripe rot (*Colletotrichum acutatum*) was reported as one of the most common fruit rots in fresh blueberries (Wang et al., 2010), this fungal pathogen was not detected in the present study.

Differences in the pathogenic microorganisms contaminating the berries were also observed among cultivars. *B. cinerea* was identified in all cultivars except 'South Moon', and it was the main pathogen found in decayed 'Emerald', 'Jewel', 'Misty', 'Snow', and 'Star' berries. *Alternaria* spp. were found in 'Legacy', 'Reveille', and 'Snow', and were the main pathogen found in decayed 'South Moon' fruit. *Cladosporium* spp. were found in a small percentage of fruit

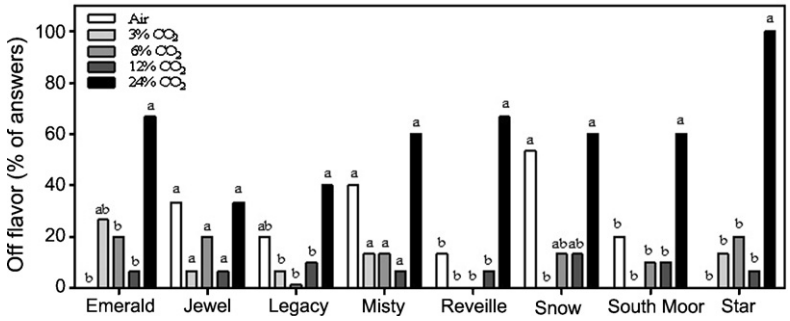

Fig. 2. Effect of different controlled atmosphere regimes (3% O_2 plus 3, 6, 12, or 24% CO_2) on the percentage of 'off-flavor' fruits of eight blueberry cultivars after 35 d cold storage at 1 °C. For each cultivar, bars with the same letter are not significantly different according to the LSD test ($p \leq 0.05$). Statistical analysis was performed with arcsine-transformed data; values presented are non-transformed means.

Table 2
Effect of controlled atmosphere, alone or after SO_2 fumigation, on the percentage of decayed fruit of eight different blueberry cultivars after 28 and 35 d cold storage at 1 °C and an additional three-day period at 20 °C to simulate retail holding conditions. Sulfur dioxide (SO_2) was applied at 28 nL s^{-1} L^{-1}.

Treatment	Cultivar															
	Emerald		Jewel		Legacy		Misty		Reveille		Snow		South Moon		Star	
28 d (1 °C)																
Air	13.3	b	70.0	a	50.0	a	53.3	ab	73.3	a	63.3	a	3.3	b	23.3	abc
3% O_2 + 3% CO_2	10.0	b	53.3	a	6.7	b	56.7	a	60.0	ab	46.7	abc	20.0	a	43.3	a
6% O_2 + 3% CO_2	33.3	a	63.3	a	26.7	b	43.3	ab	63.3	ab	40.0	abcd	10.0	ab	33.3	a
12% O_2 + 3% CO_2	6.7	b	50.0	ab	26.7	b	30.0	b	66.7	a	30.0	bcde	10.0	ab	30.0	ab
24% O_2 + 3% CO_2	10.0	b	26.7	bc	20.0	b	36.7	ab	50.0	b	20.0	cde	13.3	ab	43.3	a
SO_2/Air	10.0	b	16.7	c	23.3	b	33.3	ab	3.3	c	53.3	ab	6.7	b	6.7	bc
SO_2/3% O_2 + 3% CO_2	0.0	b	13.3	c	23.3	b	0.0	c	3.3	c	16.7	de	10.0	ab	3.3	bc
SO_2/3% O_2 + 6% CO_2	6.7	b	6.7	c	16.7	b	6.7	c	0.0	c	10.0	de	3.3	b	0.0	c
SO_2/3% O_2 + 12% CO_2	0.0	b	0.0	c	23.0	b	0.0	c	6.7	c	6.7	e	3.3	b	3.3	bc
SO_2/3% O_2 + 24% CO_2	0.0	b	6.7	c	6.7	b	0.0	c	3.3	c	20.0	cde	0.0	b	3.3	bc
35 d (1 °C)																
Air	20.0	ab	46.7	a	40.0	a	40.0	ab	76.7	a	56.7	a	23.3	b	23.3	bc
3% O_2 + 3% CO_2	30.0	a	43.3	a	30.0	ab	53.3	a	63.3	a	46.7	ab	43.3	a	50.0	a
6% O_2 + 3% CO_2	13.3	ab	50.0	a	26.7	ab	23.3	bcd	66.7	a	36.7	bc	30.0	b	33.3	ab
12% O_2 + 3% CO_2	10.0	ab	16.7	a	26.7	ab	26.7	bc	63.3	a	30.0	cd	16.7	b	33.3	ab
24% O_2 + 3% CO_2	10.0	ab	16.7	b	6.7	b	40.0	ab	16.7	b	3.3	e	16.7	b	13.3	bc
SO_2/Air	13.3	ab	13.3	b	23.3	ab	0.0	e	0.0	b	36.7	bc	20.0	b	3.3	c
SO_2/3% O_2 + 3% CO_2	13.3	ab	0.0	b	26.7	ab	3.3	de	3.3	b	30.0	cd	0.0	c	0.0	c
SO_2/3% O_2 + 6% CO_2	3.3	b	16.7	b	23.3	ab	6.7	cde	6.7	b	20.0	cde	0.0	c	6.7	bc
SO_2/3% O_2 + 12% CO_2	0.0	b	3.3	b	23.3	ab	13.3	cd	0.0	b	10.0	e	0.0	c	0.0	c
SO_2/3% O_2 + 24% CO_2	0.0	b	0.0	b	10.0	b	3.3	de	3.3	b	13.3	de	10.0	b	0.0	c

For each cultivar and storage time, means followed by the same letter in each column are not significantly different at $p \leq 0.05$, according to the LSD test. Statistical analysis was performed with arcsine-transformed data; values presented are non-transformed means.

from 'Misty' and 'Snow' (17% and 10%, respectively), and *Penicillium* spp. were only found in a small percentage (13%) of decayed berries from 'South Moon' (Supplementary data, SD 2). However, *Penicillium* spp. can become a major problem in litchi fruit after SO_2 fumigation for export markets (Jiang et al., 2003). SO_2 fumigation affects the natural ecological balance and enhances decay due to saprophytic postharvest colonization of *Penicillium* spp. (Sivakumar et al., 2010).

On the other hand, the percentage of decayed fruit was positively correlated with shrivel development, indicating that fruit with higher shrivel development could be more susceptible to decay. No correlations between decay and weight loss or decay and touch firmness were found.

3.5. Phytochemical analysis

Blueberries are a rich source of natural polyphenolics and are recognized as functional foods (Castrejon et al., 2008). It is well known that phenolic compounds are critical for both organoleptic and health-promoting properties of fruits and vegetables. The polyphenolic content and antioxidant activity of blueberries at harvest varied greatly among cultivars. The highest total phenolics at harvest was found in 'Jewel', 'Reveille', and 'Emerald' fruit (>1.5 g gallic acid kg^{-1}, fresh weight basis) (Table 3), while the phenolic content of the other cultivars ranged between 1.10 and 1.45 g gallic acid kg^{-1} fresh weight basis (data not shown). 'Jewel', 'South Moon', and 'Star' fruit had the highest concentrations of THA (>1.5 caffeic acid g kg^{-1}, fresh weight basis), while the highest TF concentrations were found in 'Jewel', 'Misty', and 'South Moon'. 'Jewel' and 'South Moon' fruit had the highest TAn content and the most potent antioxidant potential (AsA content 0.90 and 0.88 mol/kg fresh weight basis, respectively). Our results demonstrated that blueberry cultivars presented a significant diversity in phytochemical content and antioxidant potential at harvest, corroborating the important role of genotype in phenolic content (Howard et al., 2003; Kalt et al., 2001). The phytochemical contents reported here are consistent with previous studies (Perkins-Veazie et al., 2008; Dragovic-Uzelac et al., 2010; Wang et al., 2010).

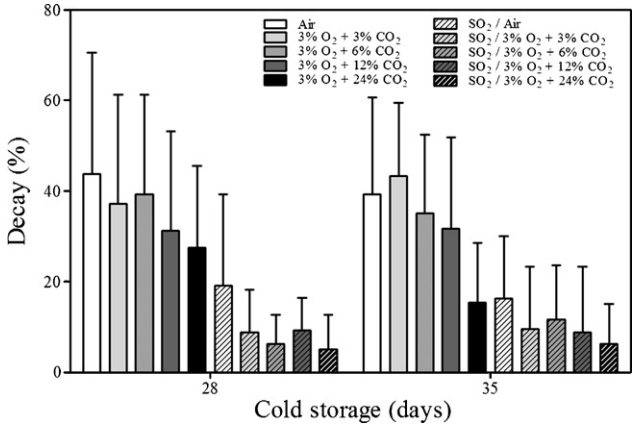

Fig. 3. Effect of different storage treatments (controlled atmosphere alone or in combination with SO_2 fumigation) on the percentage of decayed blueberry fruit after 28 and 35 d of cold storage at 1 °C and an additional three days at 20 °C (in simulation of retail holding conditions). Values presented are means for the eight cultivars studied; vertical bars represent standard deviation.

The selection of postharvest techniques used to reduce decay in berries is crucial to preserve the antioxidant potential of fruits during storage. Fruit postharvest storage can have an important impact on phenolic content and enzymes involved in phenolic metabolism, leading to quality changes (Tomás-Barberán and Espín, 2001). No consistent effect of storage treatment was observed on the phytochemical content and antioxidant activity of fruit for any cultivar tested (Table 3). Previous studies showed that as a secondary response some postharvest treatments could induce mechanisms that affect the metabolic activity of the treated produce (Gonzalez-Aguilar et al., 2010). Anthocyanin and other phenolics are decreased by CO_2-enriched atmospheres in strawberry (Gil et al., 1997; Holcroft and Kader, 1999), while the phenolic content of blueberries may be enhanced by the use of UV-C radiation (Wang et al., 2009) or natural antifungal agents like allyl isothiocyanate and essential oils (Wang et al., 2008, 2010). In our work, no significant effect of SO_2 fumigation and/or high CO_2 atmosphere on the polyphenolic content or total antioxidant activity of the examined blueberry

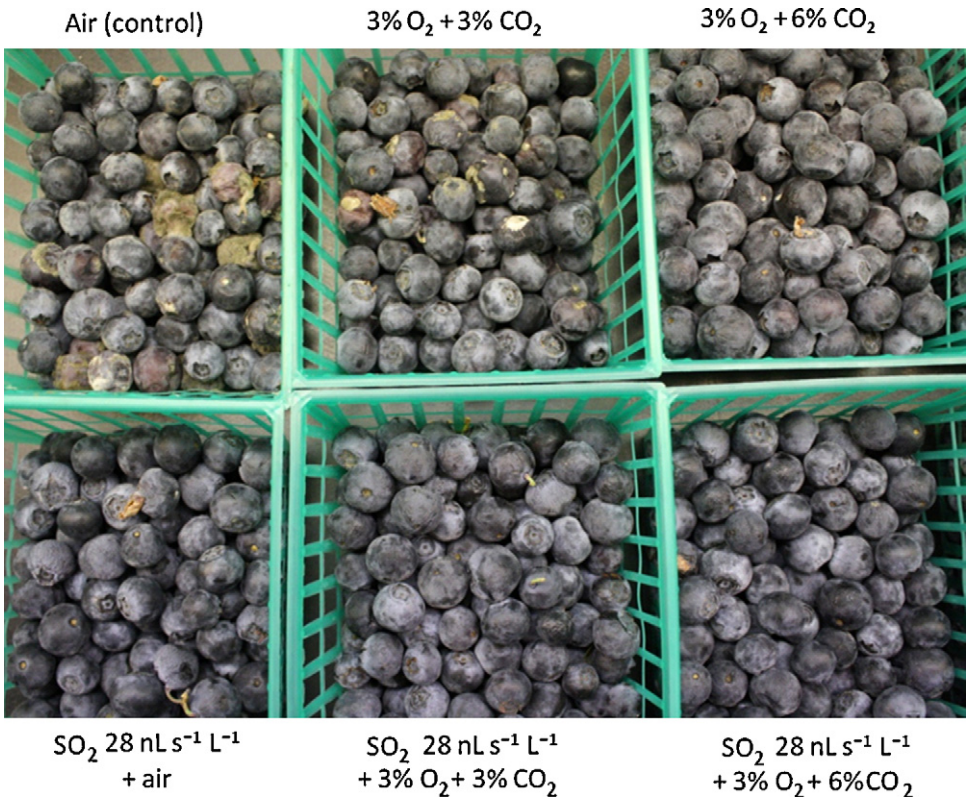

Fig. 4. Effect of different storage treatments (controlled atmosphere alone or after SO_2 fumigation) on fresh 'Reveille' blueberries after 35 d cold storage at 1 °C and an additional 3 d at 20 °C (in simulation of retail holding conditions).

Table 3
Effect of different storage treatments (controlled atmosphere alone or after SO_2 fumigation) on total phenolic compounds (TPC), total hydroxycinnamic acids (THA), total flavonoids (TF), total anthocyanins (TAn), and total antioxidant capacity (FRAP) of 'Emerald', 'Reveille', and 'South Moon' blueberries after 35 d of cold storage at 1 °C. Sulfur dioxide (SO_2) was applied at 28 nL s^{-1} L^{-1}.

Cultivar/Treatment	TPC (g GA/kg FW)		THA (g caffeic acid/kg FW)		TF (g rutin/kg FW)		TAn (g CyCC/kg FW)		FRAP (mol AsA/kg FW)	
Emerald										
Air	1.92	a	1.85	a	0.34	ab	0.54	c	0.80	bc
3%O_2 + 6%CO_2	1.45	a	1.19	d	0.31	bc	0.66	bc	0.67	c
3%O_2 + 12%CO_2	1.55	a	1.37	cd	0.38	a	0.75	ab	0.72	c
SO_2/3%O_2 + 6%CO_2	1.75	a	1.74	ab	0.27	c	0.83	a	1.10	a
SO_2/3%O_2 + 6%CO_2	1.60	a	1.54	bc	0.27	c	0.82	a	0.85	ab
Reveille										
Air	1.62	b	1.29	c	0.31	a	0.71	a	0.63	b
3%O_2 + 6%CO_2	2.05	a	1.82	a	0.33	a	0.75	a	0.84	a
3%O_2 + 12%CO_2	1.42	b	1.39	bc	0.21	b	0.39	c	0.64	b
SO_2/3%O_2 + 6%CO_2	1.70	b	1.69	a	0.32	a	0.44	c	0.74	ab
SO_2/3%O_2 + 6%CO_2	1.56	b	1.50	b	0.30	a	0.61	b	0.64	b
South Moon										
Air	1.55	a	1.60	b	0.55	a	1.04	a	0.82	b
3%O_2 + 6%CO_2	1.57	a	2.37	a	0.43	b	1.09	a	1.11	a
3%O_2 + 12%CO_2	1.44	a	1.64	b	0.47	ab	0.84	b	0.94	ab
SO_2/3%O_2 + 6%CO_2	1.09	b	1.52	b	0.40	b	0.65	c	0.88	ab
SO_2/3%O_2 + 6%CO_2	1.51	a	1.68	b	0.48	ab	0.93	ab	1.01	ab

Abbreviations: GA, gallic acid; FW, fresh weight; CyGC, cyanidin-3-O-glucoside chloride; AsA, ascorbic acid.
For each cultivar and storage time, means followed by the same letter in each column are not significantly different at $p \leq 0.05$, according to the LSD test.

cultivars was observed. These findings are consistent with previous data that reported no effect of CA on the anthocyanins content of blueberries (Duarte et al., 2009).

Storage temperature is also a key factor affecting stability of fruit phenolic compounds during postharvest storage. The storage temperature used in this work (1 °C) may prevent postharvest biosynthesis and/or degradation of phenolic compounds, preventing changes in antioxidant activity during storage (Jin et al., 2011). The unchanged antioxidant potential after 35 d cold storage demonstrated that the blueberries continued to satisfy consumer demands for fruit with high nutritional value.

4. Conclusions

SO_2 fumigation after harvest followed by controlled atmosphere storage (3% O_2 + 6 or 12% CO_2) is a promising postharvest strategy to reduce decay and to extend the market life of fresh blueberries. Very high concentrations (24%) of CO_2 should be avoided to prevent softening and/or 'off-flavor'. SO_2 fumigation at 28 nL s^{-1} L^{-1} followed by CA storage (3% O_2 plus 6% or 12% CO_2) reduced decay without affecting berry quality. SO_2 fumigation also reduced the pathogenic fungi found on the surface of the berries but did not affect quality attributes and/or polyphenolic content of the fruit.

To our knowledge, the use of SO_2 to extend the market life of fresh blueberries has never been thoroughly investigated; so future efforts should be in this direction. Recently, research on SO_2 usage is included in the US Federal IR-4 program and studies on gas residues are ongoing to develop a protocol for future registration of this technology on fresh blueberries.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.postharvbio.2011.12.006.

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