# JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

# Controlled-Atmosphere Effects on Postharvest Quality and Antioxidant Activity of Cranberry Fruits

GURBUZ GUNES,<sup>†</sup> RUI HAI LIU,<sup>‡</sup> AND CHRISTOPHER B. WATKINS<sup>\*,§</sup>

Department of Food Engineering, Istanbul Technical University, Istanbul, Turkey, and Departments of Food Science and Horticulture, Cornell University, Ithaca, New York 14853

The effects of controlled-atmosphere (CA) storage on the firmness, respiration rate, quality, weight loss, total phenolics and flavonoids contents, and total antioxidant activities of the Pilgrim and Stevens cultivars of cranberries (Vaccinium macrocarpon Aiton) have been studied during storage in atmospheres of 2, 21, and 70% O<sub>2</sub> with 0, 15, and 30% CO<sub>2</sub> (balance N<sub>2</sub>); and 100% N<sub>2</sub> at 3 °C. Elevated CO<sub>2</sub> concentrations decreased bruising, physiological breakdown, and decay of berries, thereby reducing fruit losses. Respiration and weight loss of fruits decreased, but fruit softening increased, at higher CO<sub>2</sub> concentrations. Accumulations of acetaldehyde, ethanol, and ethyl acetate varied by cultivar and storage atmosphere but were generally highest in the 2 and 70% O<sub>2</sub> and 100% N<sub>2</sub> atmospheres and increased in response to elevated CO<sub>2</sub> concentrations. Overall, the 30% CO<sub>2</sub> plus 21% O<sub>2</sub> atmosphere appeared optimal for the storage of cranberries. Sensory analysis is required, however, to confirm that accumulations of fermentation products at this atmosphere are acceptable for consumers. Stevens fruits had a higher phenolics content and total antioxidant activity than Pilgrim fruits. The storage atmosphere did not affect the content of total phenolics or flavonoids. However, the total antioxidant activity of the fruits increased overall by about 45% in fruits stored in air. This increase was prevented by storage in 30% CO<sub>2</sub> plus 21% O<sub>2</sub>.

KEYWORDS: Cranberry; Vaccinium macrocarpon Aiton; controlled atmosphere; antioxidants; phenolics; flavonoids

# INTRODUCTION

Cranberry fruits are grown mainly for processing purposes, but in the U.S., strong markets for fresh fruit exist for the Thanksgiving and Christmas holiday seasons. In addition to the traditional aspects of cranberry consumption, health benefits of the fruit have become increasingly important. Fruits of Vaccinium sp. including cranberries are particularly rich in flavonoids and other phenolic compounds (1, 2). These compounds have health-promoting benefits as antioxidants and anticarcinogens (1, 3, 4). Wang and Stretch (2) found that the concentrations of antioxidants, anthocyanins, and total phenolics in 10 cranberry cultivars ranged from 8.6 to 14.1  $\mu$ mol of Trolox equivalents per gram, from 0.20 to 0.66 mg of cyanidin-3galactoside per gram of fresh fruits, and from 1.20 to 1.76 mg of gallic acid equivalents per gram of fresh fruits, respectively.

Cranberries are harvested by wet or dry methods. The most common method, wet-harvesting, involves scooping or raking of the fruit in flooded bogs with a motorized water reel. Dryharvesting involves hand picking, hand scooping, and machine scooping (5). Recently, a dry-harvester that works in a fashion similar to a vacuum cleaner has been developed in upstate New York (6). Harvest methods influence storage life through the different degrees of bruising and stress imposed on the fruits (7). Cranberries can be stored for 2-3 months with adequate cooling and ventilation, but quality losses can be high because of shrinkage due to moisture loss, physiological breakdown, end rot, and fungal diseases (8). Cultivar and fruit maturity can also affect storage quality (8). Optimum storage temperatures were reported to be 1.7-4.4 °C (9).

Controlled-atmosphere (CA) storage can extend the storage life of fruits and vegetables by decreasing metabolism and suppressing postharvest decay, but reports on the CA storage of cranberries are limited. Cranberries responded relatively poorly to CA conditions of 0, 5, 10% CO<sub>2</sub> with 3, 10, and 21%  $O_2$  (10), perhaps because of limited gas diffusion of the berry and naturally high internal  $CO_2$  concentrations (11). Initial treatments with N<sub>2</sub> gas have been shown to improve cranberry storage life by reducing fungal decay (12). These studies were carried out before the advent of rapid CA storage technology in which CA regimes are imposed within a few days of harvest, and in addition, further research is needed to identify potential benefits and limits of tolerance of cranberries to CO2 (13). Also, although Wszelaki and Mitcham (14) found that superatmo-

<sup>\*</sup> To whom correspondence should be addressed. Department of Horticulture, Cornell University, Ithaca, NY 14853. Telephone: (607) 255-1784. Fax: (607) 255-0599. E-mail: cbw3@cornell.edu.

<sup>&</sup>lt;sup>†</sup> Istanbul Technical University. <sup>‡</sup> Department of Food Science, Cornell University.

<sup>&</sup>lt;sup>§</sup> Department of Horticulture, Cornell University.

spheric O<sub>2</sub> reduced the decay of strawberries, no studies on the effect of this technology are available for cranberries.

Although information about the effects of air storage on antioxidant capacity and associated antioxidant compounds of strawberries, raspberries, and blueberries is becoming available (15), little is known about the effects of postharvest treatments, especially CA storage, on the antioxidant and phenolics contents of fruit. Wang and Stretch (2) found that the antioxidant activities and anthocyanin and phenolic contents of cranberries increased during storage, with maximum increases occurring at 15 °C compared with 0, 5, 10, and 20 °C.

The objective of this research was to determine the effect of a wider range of  $CO_2$  and  $O_2$  concentrations, including superatmospheric  $O_2$ , than those previously studied on the quality, storage life, total phenolic and flavonoid contents, and total antioxidant activities of cranberries.

#### MATERIALS AND METHODS

**Reagents.** Folin–Ciocalteu's phenol reagent, (+)-catechin, sodium nitrite (NaNO<sub>2</sub>), and acetaldehyde were obtained from Sigma (St. Louis, MO). Gallic acid, sodium carbonate, aluminum chloride, ethyl acetate, and sodium hydroxide were purchased from Fisher Scientific (Pittsburgh, PA). Acetone and ethanol were obtained from Mallinckrodt (Paris, Kentucky) and Pharmcoproducts Inc. (Brookfield, CT), respectively.

**Fruit Material.** Cranberries were purchased from the Oswego Cranberry Company LLC (Pulaski, NY). The berries from each cultivar were dry-harvested by vacuum on two occasions: Pilgrim cultivar cranberries were harvested in 2000 on October 3 (harvest 1) and October 16 (harvest 2), and Stevens cultivar cranberries were harvested on October 25 (harvest 1) and November 7 (harvest 2). The berries were sorted and packaged in clamshell containers (each approximately 450 g) and transported to our laboratory on the day of harvest in cardboard boxes.

**CA Storage.** Berries were weighed (500 g) in half-gallon mason jars and placed in a flow-though system kept at 3 °C. The gases ( $O_2$ ,  $CO_2$ , and  $N_2$ ) were mixed in a main flowboard to obtain the desired atmospheres of 0%  $O_2$  and 0%  $CO_2$ , and 2, 21, and 70%  $O_2$  with 0, 15, and 30%  $CO_2$ . Each gas mixture was distributed to the jars (75 mL/min) through smaller flowboards, each with 12 outlets. Atmospheres were verified daily by gas chromatography (model 1200 gas partitioner, Fisher Scientific, Springfield, NJ) Three replicates of fruits (three jars) from each treatment were evaluated at each sampling time.

**Determination of Weight Loss and Fruit Losses.** The fruit weight was recorded, and the percentage weight loss from harvest was calculated. The fruits were inspected to sort out the sound berries from decayed/spoiled berries. Unsound berries were those with mechanical damage, poppers (turgid berries with internal tissue breakdown), and fungal and physiological breakdown. Both the sound and the unsound berries were weighed, and the percentage of unsound berries was calculated. Only sound fruits were used for subsequent measurements.

Respiration Rate and Ethylene Production Rate. Respiration rates of fruits from harvest 1 were measured by a closed system. Fruits (100 g), removed from CA storage, were placed in a 500-mL jar and kept overnight in air at 3 °C to equilibrate, and then the jar was sealed with a cap containing a rubber septum for headspace gas sampling. The initial and final (after 3-4 h storage at 3 °C) headspace gas compositions were analyzed using a gas chromatograph with a thermal conductivity detector (Fisher Gas Partitioner, model 1200, Fisher Scientific, Springfield, NJ). The gases were separated in a dual-column configuration consisting of a 1.8 m  $\times$  0.32 cm stainless steel column packed with 80/100 mesh poropak Q and a 3.3 m  $\times$  0.48 cm stainless steel column packed with 60/80 mesh molecular sieve 13X (Supelco, Bellefonte, PA). The column and injector temperatures were 90 and 135 °C, respectively. Ethylene concentrations were determined by headspace sampling using a gas chromatograph equipped with a flame ionization detector and a 1 m  $\times$  3 mm stainless steel column packed with 80/100 Alumina F-1. (Hewlett-Packard 5890, Wilmington, DE). The column, detector, and injector temperatures were 190, 240, and 220  $^{\circ}\mathrm{C},$  respectively.

**Firmness Measurement.** Firmness of the berries was measured by puncture test using an Instron Universal Testing Machine (model 1122, Instron Corp., Canton, MA.). The puncture test was carried out by a flat-point probe (4-mm diameter) installed on the Instron with a cross-head speed of 50 mm/min. The maximum force to punch a hole through a fruit was recorded in newtons. Ten fruits randomly selected from each treatment replicate were measured.

Volatile Analysis. Selected berries (50 g) were frozen in liquid N<sub>2</sub> and kept at -80 °C prior to analysis. The acetaldehyde, ethanol, and ethyl acetate concentrations in the tissues were measured using a modification of the procedure described by Gunes et al. (16). Five grams of frozen powdered fruits was placed in each 20-mL glass vial (Fisher Scientific, Pittsburgh, PA), which was then sealed with Teflon-lined septum caps. The vials were heated in a water bath (Precision Scientific Co., Chicago, IL) at 70 °C for 25 min. A 0.1-mL-headspace gas sample was injected into a gas chromatograph with a FID (HP 5890, Hewlett-Packard, Wilmington, DE). The volatiles were separated in a 15 m  $\times$ 0.53 mm Stabilwax wide-bore capillary column with 1.0  $\mu$ m coating (Restek Corp., Bellefonte, PA). The column temperature was set to 40 °C for the initial 4 min and then increased to 220 °C at a 20 °C/min rate and held at that temperature for 10 min. The areas of the peaks corresponding to acetaldehyde, ethyl acetate and ethanol were quantified with an integrator (HP 3394, Hewlett-Packard, Wilmington, DE). Standard curves were obtained using authentic compounds.

**Extraction of Phenolics and Flavonoids.** Frozen berries (only the first harvest from both cultivars) were crushed into coarse pieces, and 30 g of fruit was homogenized with 60 mL of 80% acetone using a Warring blender for 3 min. The samples were further homogenized using a homogenizer (Ultra-Turrax T25, IKA Works, Inc., Wilmington, NC) for an additional 2 min and filtered through #2 Whatman paper. The filtrate was recovered and the acetone was evaporated off by a rotary evaporator (model 78820-00, Labconco, Kansas City, MO) at 45 °C for 30 min. The samples were then brought to the desired volume (50 mL) with deionized water and kept frozen at -80 °C prior to analysis.

**Determination of Total Phenolics.** The total phenolic contents of the berries were measured using a modified Folin–Ciocalteu colorimetric method (17). A 0.2-mL sample of fruit extract was added to a 25-mL flask containing 5 mL of deionized water. One milliliter of Folin–Ciocalteu reagent was added to the mixture, and the flask was stoppered and allowed to stand at room temperature for 5 min. Ten milliliters of 7% Na<sub>2</sub>CO<sub>3</sub> was added and the solution was diluted to the desired volume (25 mL) with deionized water. Absorbance was measured at 750 nm versus a blank after 2 h at room temperature. The results are expressed as gallic acid (GA) equivalents using the standard curve (absorbance versus concentration) prepared from authentic gallic acid.

**Determination of Total Flavonoids.** The total flavonoid content of the berries was determined by a colorimetric method developed by Jia et al. (18). A 0.3-mL fruit extract was added to a 10-mL flask containing 5 mL of deionized water. A 0.3-mL portion of 5% NaNO<sub>2</sub> was added to this mixture, which was allowed to stand for 5 min at room temperature. A 0.3-mL portion of 10% AlCl<sub>3</sub>•6H<sub>2</sub>O was added, and the mixture was allowed to stand for 6 min at room temperature. Two milliliters of 1 M NaOH was added, and the solution was diluted to the desired volume (10 mL) with deionized water. The absorbance of the solution versus a blank at 510 nm was measured immediately. The results are expressed as catechin equivalents using a standard curve (absorbance versus concentration) prepared from authentic catechin.

**Quantification of Total Antioxidant Activity.** The total antioxidant activity of the cranberry extracts (at harvest and after storage for 2 months under air and 21%  $O_2$  plus 30%  $CO_2$ ) was measured by the total oxyradical scavenging capacity (TOSC) assay (19). Antioxidant activity was assessed at four different time points (15, 30, 45, and 60 min) and six concentrations to determine the TOSC value. The TOSC value was quantified from the integration of the area under the kinetics

Table 1. Firmness (N) of Pilgrim and Stevens Cranberries Stored under Different Combinations of  $O_2$  and  $CO_2$  at 3 °C for 2 Months<sup>a</sup>

		Pilgrim			Stevens		
		CO <sub>2</sub> (%)			CO <sub>2</sub> (%)		
O <sub>2</sub> (%)	0	15	30	0	15	30	
0	13.3	-	_	13.5	_	-	
2	14.9	12.7	12.9	14.4	12.3	11.7	
21	14.1	12.7	12.8	14.1	12.6	11.6	
70	14.6	12.9	12.1	13.6	11.9	12.9	

<sup>a</sup> Data for both harvests have been combined. Pooled standard deviations are 2.69 and 2.34 for Pilgrim and Stevens, respectively. Firmness at harvest was 13.3 and 12.6 N for Pilgrim and Stevens, respectively.

curve according to the equation

$$TOSC = 100 - (\int SA / \int CA) \times 100$$

where  $\int SA$  and  $\int CA$  are the integrated areas from the sample and the control reaction, respectively. Total antioxidant activity is expressed as the median effective dose (EC<sub>50</sub> in micrograms of tissue per milliliter) for the samples.

**Statistical Analysis.** An analysis of the variance was performed using the general linear model procedure to determine the main effects and interactions (Minitab Release 13.1, State College, PA). Multiple comparisons of individual treatments were also performed by Fisher's least significant difference (LSD) procedure in one-way analysis of variance.

#### RESULTS

**Fruit Firmness.** The firmness of the cranberries was found to be greater after storage in 0% CO<sub>2</sub> than at harvest, but overall, firmness was not affected by cultivar or O<sub>2</sub> in the storage atmosphere (Table 1). However, fruits stored in 15 and 30% CO<sub>2</sub> were softer in both cultivars compared with fruits from CO<sub>2</sub>-free atmospheres. The overall firmness readings of fruits stored under 0, 15, and 30% CO<sub>2</sub> were 14.2, 12.8, and 12.6 N for Pilgrim and 13.9, 12.3, and 12.1 N for Stevens, respectively. Thus, 15 and 30% CO<sub>2</sub> levels resulted in up to 10 and 14% reductions in firmness compared to 0% CO<sub>2</sub>. Differences in firmness between fruits kept at 15 and 30% CO<sub>2</sub> were not significant.

Respiration and Ethylene Production. The respiration of the cranberries, in terms of both CO<sub>2</sub> production rates (RCO<sub>2</sub>) and O<sub>2</sub> consumption rates (RO<sub>2</sub>), was measured at only one time point after removals of the berries from CA storage, and therefore, dynamic changes in fruit after CA treatments over time were not taken into account. However, respiration was affected by cultivar and CO<sub>2</sub> and O<sub>2</sub> concentrations in the storage atmosphere (Table 2). Both RCO<sub>2</sub> and RO<sub>2</sub> were lower for the 30% CO<sub>2</sub> atmosphere than for the the 0 and 15% CO<sub>2</sub> atmospheres ( $P \leq 0.05$ ), but there was no difference between the 0 and 15% CO<sub>2</sub> treatments. The overall mean RCO<sub>2</sub> values of fruits stored at 0, 15, and 30% CO2 were 5.0, 4.7, and 4.8 mL of CO<sub>2</sub>/kg/h, respectively, for Pilgrim and 2.8, 2.8, and 2.12 mL of CO<sub>2</sub>/kg/h, respectively, for Stevens. The overall mean RO<sub>2</sub> values of fruits stored at 0, 15 and 30% CO<sub>2</sub>, respectively, were 3.4, 2.2 and 1.9 mL of O<sub>2</sub>/kg/h for Pilgrim and 2.1, 1.3, and 1.5 mL of O<sub>2</sub>/kg/h for Stevens. Storage of fruits at 2% O<sub>2</sub> resulted in a lower respiration rate than the ambient and superatmospheric  $O_2$  storage ( $P \le 0.05$ ). However, the respiration rates were similar at 21 and 70% O<sub>2</sub>. Overall, the mean RCO<sub>2</sub> values for 2% O<sub>2</sub> were 4.2 and 2.2 mL of CO<sub>2</sub>/kg/h for Pilgrim and Stevens, respectively, whereas for 21 and 70%  $O_2$ , they were 4.9 and 5.4 mL of CO<sub>2</sub>/kg/h, respectively, for Pilgrim

**Table 2.** Rates of  $CO_2$  Production ( $RCO_2^a$ ) and  $O_2$  Consumption ( $RO_2^b$ ) of Pilgrim and Stevens Cranberries Stored under Different Combinations of  $O_2$  and  $CO_2$  at 3 °C for 2 Months<sup>*c*</sup>

Pilgrim				Stevens				
CO <sub>2</sub> (%)				CO <sub>2</sub> (%)				
02 (%)	0	15	30	0	15	30		
			RCO <sub>2</sub>					
0	5.03	_	_	2.20	_	_		
2	4.09	4.40	4.14	2.89	2.86	2.38		
21	4.27	5.23	5.35	3.13	3.01	2.61		
70	6.64	4.54	4.91	2.83	2.60	1.37		
			RO <sub>2</sub>					
0	3.44	-	_	0.86	-	_		
2	3.18	3.06	2.75	2.52	2.35	2.28		
21	2.91	3.38	2.59	2.84	1.53	2.47		
70	4.09	0.10	0.34	1.94	0.05	0.00		

<sup>*a*</sup> RCO<sub>2</sub> in milliliters of CO<sub>2</sub> per kilogram per hour. <sup>*b*</sup> RO<sub>2</sub> in milliliters of O<sub>2</sub> per kilogram per hour. <sup>*c*</sup> Rates were determined on the day after removal from CA storage. Pooled standard deviations for RCO<sub>2</sub> are 0.32 and 0.46 and for RO<sub>2</sub> are 0.28 and 0.45 for Pilgrim and Stevens, respectively. At harvest, RCO<sub>2</sub> values were 6.08 and 4.66 mL of CO<sub>2</sub>/kg/h and RO<sub>2</sub> values were 5.50 and 3.92 mL of O<sub>2</sub>/kg/h for Pilgrim and Stevens, respectively.



**Figure 1.** Percentage of unsound cranberries with bruising, decay, and physiological breakdown as affected by storage in various  $O_2$  and  $CO_2$  combinations for 2 months at 3 °C for 0% (white bars), 15% (shaded bars), and 30%  $CO_2$  (black bars): (A) Pilgrim harvest 1, (B) Pilgrim harvest 2, (C) Stevens harvest 1, and (D) Stevens harvest 2. Bars with different letters are significantly different from each other ( $P \le 0.05$ ).

and 2.9 and 2.3 mL of CO<sub>2</sub>/kg/h, respectively,for Stevens. Similar differences due to O<sub>2</sub> concentration in the atmosphere were detected for RO<sub>2</sub>. Pilgrim had higher respiration rates than Stevens, with overall respiration rates of 4.8 mL of CO<sub>2</sub>/kg/h and 2.5 mL of O<sub>2</sub>/kg/h for Pilgrim and 2.6 mL of CO<sub>2</sub>/kg/h and 1.6 mL of O<sub>2</sub>/kg/h for Stevens ( $P \le 0.05$ ). The ethylene production of the fruits was less than 0.10  $\mu$ L/kg/h irrespective of cultivar, harvest time, and storage atmosphere (data not shown).

Fruit Bruising and Physiological and Fungal Breakdown. In general, losses of fruits due to damage, whether from the presence of bruising or physiological or fungal breakdown, decreased as the CO<sub>2</sub> concentration in the 2 and 21% O<sub>2</sub> atmospheres increased (**Figure 1A–D**). However, the reduced losses associated with the CO<sub>2</sub> atmospheres were lower in fruits from the second harvest. Superatmospheric O<sub>2</sub> increased the



**Figure 2.** Acetaldehyde concentrations of (A) Pilgrim and (B) Stevens cranberries as affected by  $O_2$  and  $CO_2$  after 2 months storage at 3 °C for 0% (white bars), 15% (shaded bars), and 30%  $CO_2$  (black bars). Bars with different letters are significantly different from each other ( $P \le 0.05$ ).

**Table 3.** Analysis of Variance<sup>*a*</sup> for Acetaldehyde, Ethanol, and Ethyl Acetate in Pilgrim and Stevens Cranberries Stored under Different Combinations of  $O_2$  and  $CO_2$  at 3 °C for 2 Months

source	DF <sup>a</sup>	acetaldehyde <sup>b</sup>	ethanol <sup>b</sup>	ethyl acetate <sup>b,c</sup>
02	2	***	***	***
CO <sub>2</sub>	2	***	***	***
cultivar (C)	1	NS	NS	NS
O <sub>2</sub> *CO <sub>2</sub>	4	*	*	***
0 <sub>2</sub> *C	2	*	*	NS
CO <sub>2</sub> *C	2	NS	*	NS
02*CO2*C	4	*	*	**

<sup>*a*</sup> DF, degrees of freedom. <sup>*b*</sup> NS, \*, \*\*, and \*\*\* indicate nonsignificant and significant at  $P \leq 0.05$ , 0.01, and 0.001, respectively. <sup>*c*</sup> Natural logarithm of the transformed data was used to satisfy the assumptions of the model.

loss of fruits compared with the 2 and 21%  $O_2$  atmospheres. The fruits from both cultivars were damaged extensively by the end of the fourth month of CA storage (>97% unsound fruit; data not shown).

Among the atmospheres tested, 21% O<sub>2</sub> plus 30% CO<sub>2</sub> resulted in the least loss of quality. The incidence of unsound fruits in this atmosphere was about 15% in contrast to 75% in air for Stevens (**Figure 1C**). An atmosphere of 2% O<sub>2</sub> plus 30% CO<sub>2</sub> also resulted in better-quality fruits than did air storage, but it was not as beneficial as 21% O<sub>2</sub> plus 30% CO<sub>2</sub>.

Weight Loss. The weight loss of the fruits was less than 1% for all atmospheres (data not shown), probably because of the high relative humidity (>95%) in the storage compartments. However, fruits stored in 30% CO<sub>2</sub> atmospheres had up to 30% less weight loss compared to those stored in CO<sub>2</sub>-free atmospheres ( $P \le 0.05$ ), and overall, Pilgrim had a greater weight loss (0.85%) than Stevens (0.70%).

**Fermentation Products.** Accumulations of acetaldehyde, ethanol, and ethyl acetate were affected by atmosphere, as well as interactions between cultivar and atmosphere (**Figures 2–4A,B; Table 3**). These fermentation products accumulated to similar extents in both cultivars. Because no harvest time



**Figure 3.** Ethanol concentration of (A) Pilgrim and (B) Stevens cranberries as affected by O<sub>2</sub> and CO<sub>2</sub> after 2 months storage at 3 °C for 0% (white bars), 15% (shaded bars), and 30% CO<sub>2</sub> (black bars). Bars with different letters are significantly different from each other ( $P \le 0.05$ ).

effect was detected, data for both harvests were combined within each cultivar. Accumulation of acetaldehyde increased under elevated CO<sub>2</sub> conditions in 70% O<sub>2</sub> (**Figure 2A,B**). However, there was no additional effect of CO<sub>2</sub> at 2% and 21% O<sub>2</sub>. Fruits kept in 0% O<sub>2</sub> also accumulated large concentrations of acetaldehyde.

Accumulation of ethanol showed patterns similar to those found for acetaldehyde, with a significant atmosphere effect (**Figure 3A,B**). Greater concentrations of ethanol were found in fruits stored in 15 and 30% CO<sub>2</sub> atmospheres compared with 0% CO<sub>2</sub> for Pilgrim, but no significant effect of CO<sub>2</sub> was detected for Stevens. The ethanol concentrations for the 15 and 30% CO<sub>2</sub> levels were similar. Superatmospheric O<sub>2</sub> resulted in higher ethanol accumulations than did 2 and 21% O<sub>2</sub> for both cultivars ( $P \le 0.05$ ). The accumulation of ethanol was greatest under 0% O<sub>2</sub> for both Pilgrim and Stevens (**Figure 3A,B**).

Ethyl acetate showed little accumulation at 0, 2 or 21%  $O_2$ , irrespective of the CO<sub>2</sub> concentration, but it did accumulate in 70%  $O_2$  (**Figure 4A,B**). For Pilgrim, but not Stevens, increasing CO<sub>2</sub> concentrations in 70%  $O_2$  resulted in higher ethyl acetate accumulations.

**Total Phenolic and Flavonoid Contents.** Stevens cranberries had a slightly higher total phenolic content than Pilgrim fruits (**Table 4**), the average values over all storage conditions being 3.45 and 3.27 mg/g of tissue, respectively. The total phenolic contents of the fruits were not affected by CA storage conditions.

The storage atmosphere affected the content of flavonoids in the cranberries. Flavonoid contents were lower in 15% CO<sub>2</sub> than in 0 and 30% CO<sub>2</sub> ( $P \le 0.05$ ), but the magnitude of the overall difference was very small (0.09 mg/g). Neither the O<sub>2</sub> level nor its interactions with the CO<sub>2</sub> level affected the flavonoid content. The total flavonoids content of Pilgrim did not change over 2 months of CA storage, but that of Stevens increased slightly in some atmospheres, such as 2% O<sub>2</sub> with 0 and 30% CO<sub>2</sub>, air, and 70% O<sub>2</sub> with 0 and 15% CO<sub>2</sub>. Overall, the flavonoid contents of Pilgrim and Stevens were similar.

**Total Antioxidant Activity.** We selected the optimum atmosphere of 21% O<sub>2</sub> plus 30% CO<sub>2</sub> indicated from the above



**Figure 4.** Ethyl acetate concentration of (A) Pilgrim and (B) Stevens cranberries as affected by  $O_2$  and  $CO_2$  after 2 months storage at 3 °C for 0% (white bars), 15% (shaded bars), and 30%  $CO_2$  (black bars). Bars with different letters are significantly different from each other ( $P \le 0.05$ ).

Table 4. Total Phenolic and Flavonoid Concentrations (mg/g) of Pilgrim and Stevens Cranberries Stored under Different Combinations of  $O_2$  and  $CO_2$  at 3 °C for 2 Months<sup>a</sup>

		Pilgrim			Stevens		
		CO <sub>2</sub> (%)			CO <sub>2</sub> (%)		
O <sub>2</sub> (%)	0	15	30	0	15	30	
Phenolics <sup>a-c</sup> (mq/q)							
0	3.21	-		3.33	-	-	
2	3.36	3.18	3.34	3.50	3.27	3.39	
21	3.35	3.02	3.37	3.39	3.39	3.36	
70	3.42	3.14	3.27	3.61	3.68	3.58	
Flavonoids <sup><math>a-c</math></sup> (mg/g)							
0	0.94	-	-	0.86	-	-	
2	0.99	0.94	0.97	0.98	0.92	0.97	
21	0.91	0.79	1.00	1.02	0.82	0.92	
70	1.06	0.95	0.93	0.98	1.03	0.95	

<sup>*a*</sup> Pooled standard deviations for phenolics are 0.15 and 0.29 and for flavonoids are 0.06 and 0.11 for Pilgrim and Stevens, respectively. <sup>*b*</sup> At harvest, the concentrations of total phenolics and flavonoids were 3.33 and 1.11 mg/g, respectively, for Pilgrim and 3.45 and 0.78 mg/g, respectively, for Stevens. <sup>*c*</sup> For phenolics, only the cultivar effect was significant at  $P \le 0.05$ , whereas for flavonoids, only the CO<sub>2</sub> effect was significant at  $P \le 0.05$ .

results for a comparison of the changes in total antioxidant activity in fruits under CA and air storage. The total antioxidant activity of the fruits was expressed as the median effective dose (EC<sub>50</sub> in micrograms of tissue per milliliter), where lower EC<sub>50</sub> values indicate higher antioxidant activities. Stevens fruits had higher total antioxidant activity than Pilgrim fruits, with EC<sub>50</sub> averaging 0.94 and 1.26  $\mu$ g of tissue/mL, respectively (**Figure 5**). The antioxidant activities of the fruits increased by 36 and 50% from the initial values in Stevens and Pilgrim, respectively, during 2 months of air storage. CA storage conditions prevented this increase.

# DISCUSSION

Our results indicate that CA atmospheres can beneficially affect cranberry fruit quality. Among the atmospheres tested, 30% CO<sub>2</sub> in 21% O<sub>2</sub>, and to a lesser extent 30% CO<sub>2</sub> with 2%



**Figure 5.** EC<sub>50</sub> of total antioxidant activity of the extracts of Pilgrim and Stevens cranberries: at harvest (black bars); after 2 months storage in 21% O<sub>2</sub> plus 30% CO<sub>2</sub> (shaded bars); and after 2 months storage in air (white bars). Bars with different letters are significantly different from each other ( $P \le 0.05$ ).

O<sub>2</sub>, resulted in the most sound fruits. A reduction in firmness due to elevated CO<sub>2</sub>, although significant, did not appear to affect fruit quality. These results contrast with those of Anderson et al. (10), who tested CO<sub>2</sub> atmospheres in a more restricted range of 0-10% (in 1, 3, and 21% O<sub>2</sub>). They found that increasing CO2 resulted in increased decay at 3 °C and that 1% O<sub>2</sub> resulted in greater decay than higher O<sub>2</sub> levels. One factor might be our use of rapid CA conditions, whereas the rate of CA application was much slower in the study of Anderson et al. (10). Also, the benefit of the higher  $CO_2$  concentrations on the quality of cranberries in our study might be associated with its inhibitory effect on the respiration rate, as well as its known inhibitory effect on the growth of mold and some spoilage bacteria (21). Levine at al. (22) reported that lower internal  $CO_2/$ O2 ratios in cranberries were associated with reduced decay, but they obtained these ratios by storing fruits at different temperatures: lower temperatures resulted in lower ratios. Thus, the effects of temperature and CO<sub>2</sub>/O<sub>2</sub> ratios were confounded in their study.

Superatmospheric  $O_2$  did not improve the storability of cranberries and resulted in greater fruit loss, especially when combined with high  $CO_2$  levels. Fruits from these treatments were mostly poppers that were rotted cranberries with soft skin and either fully intact (turgid) or not. The poppers released extensive red-colored liquid, and the flesh was dark red colored due to diffusion of pigments through the flesh. In intact fruits, the flesh was mostly white, and the pigment was confined to the skin. Because of the nature of the damaged berries, it seemed that superatmospheric  $O_2$  caused physiological breakdown of the tissues. Therefore, the beneficial effects of this atmosphere in reducing decay and inhibiting fungal growth of strawberries (14) were not found for cranberries.

A major factor restricting the storability of cranberry fruits in these experiments was the high incidence of losses due to bruising and physiological and fungal breakdown. Damaged fruits had extremely soft and degraded tissues. It has been proposed that the activity of polygalacturonase, a cell-walldegrading enzyme, is triggered by bruising, resulting in extensive softening and tissue breakdown (20). Losses of fruits were greatest in the later harvests of both cultivars, possibly because of the riper fruits in those harvests, which were more prone to bruising during harvesting and handling. Differences in respiration rate that were detected for each cultivar might have been associated with differences in the ripeness and degree of bruising and stress encountered during harvesting and sorting. Most losses of berries by the end of fourth month of storage were due to bruising. This might be due to the new dry-harvest method, which employs vacuum technology to collect the fruits (6). The extent of bruising associated with this method compared with alternative methods has not been studied. In addition, the berries might have been bruised during subsequent sorting operations employed in the elevator, bounce board, and conveyor belt used for grading. It has been reported that cranberries stored in chaff undergo less spoilage than those that are cleaned or sorted before storage (10). An additional factor in the high losses found in our experiments might have been the high relative humidity under CA conditions. Anderson et al. (10) found that CA storage was only as good as air storage for maintaining fruit quality when the humidity and the  $O_2$  and  $CO_2$  concentrations were regulated.

The effects of fermentation product accumulation at 21% O<sub>2</sub> and 30% CO<sub>2</sub> on fruit acceptability was not tested by formal sensory analysis in this study. Informal assessment indicated that flavor was not detrimentally affected; the acetaldehyde, ethyl acetate, and ethanol concentrations were less than 6, 0.30, and 161 mg/kg, respectively, and close to the olfactory threshold concentrations of 5, 0.015, and 100  $\mu$ L/L for acetaldehyde, ethyl acetate, and ethanol, respectively, in apple essence for human perception (23). However, although the concentrations of these compounds at which the fruits become unacceptable can be expected to be much higher than the threshold concentrations, the effects of the fermentation product accumulation on fruit acceptability remain to be confirmed.

The total phenolic contents of the Pilgrim and Stevens cranberries at harvest were 3.33 and 3.45 mg/g, respectively, and therefore much higher than the values of 1.20 and 1.26 mg/g of fruits, respectively, reported by Wang and Stretch (2). The higher values might be due to inclusion of seeds in our study or to environmental influences.

The total phenolic contents of either cranberry cultivar did not increase during storage. Wang and Stretch (2) also found little increase in phenolic concentrations at 0 °C, but at 5 °C and above, increases were substantial. Our storage temperature of 3 °C was intermediate between these temperatures and indicates that storage temperature might be an important factor in phenolic metabolism.

Total antioxidant activity in Pilgrim and Stevens increased by 50 and 36%, respectively, during air storage for 2 months. Changes in total antioxidant activity are also affected by temperature (2). They were lowest at lower storage temperatures but increased by about 20% in both cultivars at 0 °C during three months of air storage. The correlations between antioxidant activity and concentrations of phenolics and anthocyanins were lowest at the lower storage temperatures (2).

The mechanism by which CA storage prevented the increase in total antioxidant activity that was found in air-stored fruits is not clear, but CA conditions might affect the release of bound phytochemicals that contribute to antioxidant activity. Heatprocessed tomatoes have higher bioaccessible lycopene, apparently because thermal processing disrupts cell membranes and cell walls to release lycopene from the cell matrix and insoluble fibers (24). Although elevated CO<sub>2</sub> concentrations in the storage atmosphere did not maintain berry firmness relative to low CO<sub>2</sub> atmospheres, maintenance of cellular integrity by CA storage conditions might prevent the release of bound phenolics and flavonoids from the cell matrix of cranberry fruits and maintain lower antioxidant activities.

Overall, our data indicate that CA storage might have benefits for storage of cranberry fruits that are greater than previously realized. The storage life of cranberries can be extended using a CA of 21%  $O_2$  plus 30%  $CO_2$  through reductions in decay and respiration rate. This increase in storage life could provide extended availability of fresh cranberries with premium quality in the market, provided that the storability of unsorted fruits is greater than that shown for sorted and packed fruits and that accumulations of fermentation products do not reach unacceptable levels. The concentrations of phytochemicals and total antioxidant activity in cranberries were maintained at harvest levels during storage in this atmosphere.

### ABBREVIATIONS USED

CA, controlled atmosphere; CO<sub>2</sub>, carbon dioxide; O<sub>2</sub>, oxygen; N<sub>2</sub>, nitrogen; RCO<sub>2</sub>, CO<sub>2</sub> production rate; RO<sub>2</sub>, O<sub>2</sub> consumption rate; RQ, respiratory quotient; P, probability; EC<sub>50</sub>, median effective dose; NaNO<sub>2</sub>, sodium nitrite, AlCl<sub>3</sub>•6H<sub>2</sub>O, aluminum chloride; NaOH, sodium hydroxide; KOH, potassium hydroxide; L, liter; mL, milliliter;  $\mu$ L, microliter; min, minute; h, hour; kg, kilogram; g, gram; °C, degrees Celsius; nm, nanometer; M, molar; N, Newton.

#### ACKNOWLEDGMENT

This project was funded by a New York Department of Agriculture and Markets special grant. We thank Jackie Nock and Karen Scott for assistance.

#### LITERATURE CITED

- Prior, R. L.; Cao, G. Antioxidant phytochemicals in fruits and vegetables: Diet and health implications. *HortScience* 2000, *35*, 588–592.
- (2) Wang, S. Y.; Stretch, A. W. Antioxidant capacity in cranberry is influenced by cultivar and storage temperature. J. Agric. Food Chem. 2001, 49, 969–974.
- (3) Ames, B. M.; Shigena, M. K.; Haggen, T. M. Oxidants, antioxidants and the degenerative diseases of aging. *Proc. Natl. Acad. Sci.* **1993**, *90*, 7915–7922.
- (4) Hakkinen, S. H.; Karenlampi, S. O.; Heinonen, I. M.; Mykkanen, H. M.; Torronen, A. R. Contents of the flavonols quercetin, myricetin, and kaempferol in 25 edible berries. *J. Agric. Food Chem.* **1999**, *47*, 2274–2279.
- (5) Eck, P. *The American Cranberry*; Rutgers University Press: New Brunswick, NJ, 1990; pp 283–311.
- (6) Bailey, L. Oswego cranberry harvesting first commercial crop. *Queen Central News*, Camden, NY, 2000, Vol. 26, No. 25, p 1.
- (7) Davis, D. C.; Shawa, A. Y. Reducing injury during mechanical harvesting of cranberries. J. Am. Soc. Hortic. Sci. 1983, 108, 444–447.
- (8) Stretch, A. W.; Ceponis, M. J. Fungal and physiological breakdown in six cranberry cultivars following water harvesting and cold storage. *HortScience* **1986**, *21*, 265–267.
- (9) Swanson, B. G.; Weckel, K. G. Refrigerated storage of fresh cranberries. J. Food Sci. 1975, 40, 259–261.
- (10) Anderson, R. C.; Hardenburg, R. E.; Vaught, H. C. Controlled atmosphere storage studies with cranberries. *Proc. Am. Soc. Hortic. Sci.* **1963**, 83, 416–422.
- (11) Forsyth, F. R.; Hall, I. V.; Lightfoot, H. J. Diffusion of CO<sub>2</sub>, O<sub>2</sub>, and ethylene in cranberry fruit. *HortScience* **1973**, 8, 45– 46.
- (12) Lockhart, C. L.; Forsyth, F. R.; Stark, R.; Hall, I. V. Nitrogen gas suppresses microorganisms on cranberries in short-term storage. *Phytopathol. Notes* **1971**, *61*, 335.
- (13) Kader, A. A. A summary of CA requirements and recommendations for fruits other than apples and pears. In *Proceedings of CA* '97; Postharvest Horticultural Series No. 17; University of California at Davis: Davis, CA, 1997; Vol. 3, pp 1–34.

- (14) Wszelaki, A. L.; Mitcham, E. J. Effects of superatmospheric oxygen on strawberry fruit quality and decay. *Postharvest Biol. Technol.* 2000, 20, 125–133.
- (15) Kalt, W.; Forney C. F.; Martin, A.; Rrior, R. L. Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruit. J. Agric. Food Chem. 1999, 47, 4638– 4644.
- (16) Gunes, G.; Watkins, C. B.; Hotchkiss, J. H. Physiological responses of fresh-cut apple slices under high CO<sub>2</sub> and low O<sub>2</sub> partial pressures. *Postharvest Biol. Technol.* 2001, 22, 197– 204.
- (17) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* **1999**, 299, 152–178.
- (18) Jia, Z.; Tang, M.; Wu. J. The determination of flavonoid contents in mulberry and their scavenging effects on superoxides radicals. *Food Chem.* **1998**, *64*, 555–559.
- (19) Winston, G. W.; Regoli, F.; Dugas, A. J.; Fong, J. H.; Blanchard, K. A. A rapid gas chromatographic assay for determining oxyradical scavenging capacity of antioxidants and biological fluids. *Free Radical Biol. Med.* **1998**, *24*, 480–493.

- (20) Patterson, M. E.; Doughty, C. C.; Graham, S. O.; Allan, B. Effect of bruising on postharvest softening, color changes and detection of polygalacturonase enzyme in cranberries. *Proc. Am. Soc. Hortic. Sci.* **1967**, *90*, 498–514.
- (21) Philips, C. A. Review: Modified atmosphere packaging and its effects on the microbiological quality and safety of produce. *Int. J. Food Sci. Technol.* **1996**, *31*, 463–479.
- (22) Levine, A. S.; Fellers, C. R.; Gunness, C. I. Carbon dioxideoxygen and storage relationships in cranberries. *Proc. Am. Soc. Hortic. Sci.* **1940**, *38*, 239–242.
- (23) Flath, R. A.; Black, D. R.; Guadagni, D. G.; McFadden, W. H.; Schultz, T. H. Identification and organoleptic evaluation of compounds in Delicious apple essence. J. Agric. Food Chem. 1967, 15, 29–35.
- (24) Dewanto, V.; Wu, X.; Adom, K. K.; Liu, R. H. Thermal processing enhances the nutritional value of tomatoes by increasing antioxidant activity. *J. Agric. Food Chem.* **2002**, *50*, 3010–3014.

Received for review April 1, 2002. Revised manuscript received August 1, 2002. Accepted August 1, 2002.

JF025572C