

## Effect of High-Oxygen Atmospheres on Blueberry Phenolics, Anthocyanins, and Antioxidant Capacity

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The influence of high oxygen concentrations on total phenolic, total anthocyanin, individual phenolic compounds, and antioxidant capacity (measured as oxygen radical absorbance capacity, ORAC) in highbush blueberry fruit (*Vaccinium corymbosum* L. cv. Duke) was investigated. Freshly harvested blueberries were placed in jars ventilated continuously with air or with 40, 60, 80, or 100% O<sub>2</sub> at 5 °C for up to 35 days. Samples were taken initially and at 7-day intervals during storage. Whereas the quality parameters of titratable acidity, total soluble solids, and surface color were only slightly affected by the superatmospheric O<sub>2</sub> treatments, the antioxidant levels were markedly increased by 60–100% O<sub>2</sub> treatments as compared with 40% O<sub>2</sub> treatment or air control during 35 days of storage. Elevated O<sub>2</sub> between 60 and 100% also promoted increases of total phenolics and total anthocyanins as well as the individual phenolic compounds analyzed by HPLC. Fruit treated with O<sub>2</sub> concentrations of ≥60% also exhibited significantly less decay. Data obtained in this study suggest that high-oxygen treatments may improve the antioxidant capacity of blueberry fruit. Furthermore, antioxidant capacity may be correlated with total phenolic and anthocyanin contents in blueberries.

**KEYWORDS:** Blueberry; high-oxygen atmospheres; antioxidant; phenolics; anthocyanins

### INTRODUCTION

Fruits and vegetables are rich in phenolic compounds such as anthocyanins, flavonols, flavones, isoflavones, flavonones, and catechins. Many of these compounds exhibit a wide range of biological effects, including antioxidant (1–6), antifungal (7), and anticarcinogenic properties (8). There is convincing epidemiological evidence showing that dietary antioxidant flavonoids may provide protection against coronary heart disease (9, 10), stroke (11), and lung cancer (12). In recent years there have been an increasing number of studies that have quantified the total antioxidant capacity in fruits and vegetables. A high scavenging activity of berry extracts toward chemically generated active oxygen species has been shown in several studies (13–15). In a previous study, phenolic compounds from berry extracts inhibited human low-density lipoprotein (LDL) and liposome oxidation (14). Thus, high fruit consumption may significantly reduce the incidence and mortality rates of cancer, cardiovascular disorders, and other degenerative diseases caused by oxidative stress (16).

Interest in the role of antioxidants in human health has promoted research in the field of horticulture and food science to evaluate fruit and vegetable antioxidants and to determine how their content and activity can be maintained or even improved through crop breeding, cultural practices, and post-harvest storage and processing. Preharvest factors, such as genetic background and cultural practices, have the potential to influence antioxidant capacity in crops. Strawberry fruit from a hill plasticulture system consistently had higher flavonoid content and antioxidant capacity than fruit from plants grown in the row system (17). Postharvest storage can also affect phenolic compound levels and antioxidant capacity in fruits and vegetables. In cranberries, storage temperature between 0 and 15 °C increased antioxidant capacity and total anthocyanin and total phenolic contents. Strawberries and raspberries stored at temperatures >0 °C also resulted in an increase in antioxidant capacity (18). Controlled atmosphere (CA) storage of strawberry fruit did not affect anthocyanin content in external tissues but decreased anthocyanin content in internal tissues (19). Cv. Delicious and Granny Smith apples in the first 2 months of storage at –1 °C had 10-fold increases in the antioxidant content, whereas antioxidant content significantly decreased during the following 4 months of storage (20). No significant changes in flavonoid concentration and antioxidant activity were found in cv. Jonagold and Elstar apples during long-term storage under regular and CA conditions (21, 22). A decrease in the total antioxidant activity in fresh-cut spinach was also observed during storage, but total flavonoid content remained stable both

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in air and in modified-atmosphere packaging (MAP) (23). Processing also has marked effects on phenolic content and antioxidant capacity in fruits. It has been shown that industrial processing of pomegranates to obtain juice increased their phenolic content and antioxidant capacity (24), whereas strawberry processing to produce jams decreased the total ellagic acid content by 20% (25) and the flavonoids by 15–20% (26). It has also been reported that a freezing process slightly decreased the total phenolic content and produced a decrease of free radical scavenging capacity ranging between 4 and 20% in four cultivars of raspberries (27). However, no significant changes in either total phenolics or free radical scavenging capacity were observed during the following 12 months of frozen storage.

As antioxidant content is becoming an increasingly important parameter with respect to fruit and vegetable quality, it is of great interest to evaluate changes in the antioxidant status during postharvest storage of horticultural crops. Recently, blueberries have become of special interest to researchers studying antioxidants because of their high phenolic content and antioxidant capacity (3, 28). However, little information is available regarding the effects of storage conditions, such as CA, on the changes of phenolic compounds and antioxidant capacity in blueberries. The aim of this work was to investigate the effects of atmospheres containing high O<sub>2</sub> on total phenolics, total anthocyanins, and antioxidant capacity as well as the main phenolic constituents in blueberry fruit during postharvest storage at 5 °C.

## MATERIALS AND METHODS

**Chemicals.** Kaempferol, (*R*)-phycoerythrin (R-PE) from *Prophydium cruentum*, and quercetin were purchased from Sigma Chemical Co. (St. Louis, MO). 2',2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA Inc. (Richmond VA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Aldrich (Milwaukee, WI). Acetonitrile, methanol, acetone, and water were of HPLC grade and were purchased from Baxter (Muskegon, MI). All anthocyanins and their aglycons were obtained from Indofine Chemical Co., Inc. (Somerville, NJ). Other authentic standards were obtained from Sigma and Fisher Scientific (Pittsburgh, PA).

**Plant Materials and Treatments.** Highbush blueberries (*Vaccinium corymbosum* L. cv. Duke) were hand-harvested at commercially mature stage from Butler's orchard, Maryland, sorted to eliminate damaged, shriveled, and unripened fruit, and selected for uniform size and color. Five hundred grams of sample was placed in each 18-L jar, and three jars were used for each treatment. The jars were placed at 5 °C and connected to a continuous flow (120 mL/min) of humidified air (control) and 40, 60, 80, or 100% O<sub>2</sub> (balance N<sub>2</sub> in all high-O<sub>2</sub> treatments). The gases were checked regularly with an O<sub>2</sub>/CO<sub>2</sub> analyzer (AMETEK, Pittsburgh, PA) and maintained at ±2% for the duration of the experiment. Samples were taken initially and at 7-day intervals during storage for decay evaluation and other analysis.

**Fruit Decay.** Fruit decay was visually estimated during the course of the experiment. Any berries with visible mold growth were considered to be decayed. Fruit decay was expressed as percentage of fruit showing decay symptoms.

**Total Soluble Solids, Total Titratable Acidity, and pH Determinations.** Twenty pieces of fruit from each replicate were wrapped in cheesecloth and squeezed with a hand press, and the juice was analyzed for total soluble solids (TSS), pH, and titratable acidity (TA). TSS was determined at 20 °C on a Bausch and Lomb refractometer. pH was measured with a pH-meter. TA was determined by diluting each 5 mL aliquot of blueberry juice in 50 mL of distilled water and then titrating to pH 8.2 using 0.1 N NaOH.

**Surface Color Measurement.** Because the stem end of blueberry fruit is the last to develop color (29), fruit surface color was measured on this part of 10 fruits from each replicate using a chromameter (CR

200, Minolta, Ramsey, NJ), which provided CIE *L*\*, *a*\*, and *b*\* values. Negative *a*\* values indicate green and higher positive *a*\* values red color. Higher positive *b*\* values indicate a more yellow skin color. These values were then used to calculate hue degree ( $h^\circ = \arctangent-[b^*/a^*]$ ), where 0° = red-purple; 90° = yellow; 180° = bluish green; and 270° = blue (30), and chroma ( $C^* = [a^{*2} + b^{*2}]^{1/2}$ ), which indicates the intensity or color saturation.

**Sample Preparation for Total Phenolics, Total Anthocyanins, and ORAC Assays.** Twenty berries from each replicate were cut into small slices, mixed, and stored at -80 °C until analyzed. To prepare the fruit extracts, three 5-g samples of berries from each replicate were extracted twice with 10 mL of 80% acetone containing 0.2% formic acid using a Polytron (Brinkmann Instruments, Inc., Westbury, NY) for 2 min and then centrifuged at 20000g for 20 min. The supernatants were combined and transferred to vials, stored at -80 °C, and then used for analysis of total phenolics, total anthocyanins, and ORAC.

**Total Phenolics and Anthocyanins Analysis.** Total phenolic contents in blueberry extracts were determined according to the Folin-Ciocalteu procedure (31), and the results are expressed as milligrams of gallic acid equivalent (GAE) per gram of fresh weight. Total anthocyanin contents of blueberry extracts were measured using the pH differential method (32). Results are expressed as milligrams of cyanidin 3-glucoside (C 3-G) equivalents per gram of fresh weight.

**ORAC Assay.** The procedures for the ORAC assay on strawberries were modified from the previously described method of Cao et al. (33). This assay measures the effect of antioxidant components in blueberry extracts to inhibit the decline of R-PE fluorescence induced by a peroxyl radical generator, AAPH. The reaction mixture contained 1.7 mL of 75 mM phosphate buffer (pH 7.0), 100 μL of R-PE (3.4 mg/L), 100 μL of 320 mM AAPH, and 100 μL of sample. Phosphate buffer was used as a blank, and 1 μM Trolox (a water-soluble α-tocopherol analogue) was used as a standard during each run. The final volume of 2 mL was used in a 10-mm-wide fluorometer cuvette. R-PE, phosphate buffer, and samples were preincubated at 37 °C for 15 min. The reaction was started by the addition of AAPH. Fluorescence was measured and recorded every 5 min at the emission of 570 nm and the excitation of 540 nm using a Shimadzu RF-Mini 150 recording fluorometer (Columbia, MD) until the fluorescence of the last reading declined to <5% of the first reading (~70 min). One blank, one standard, and a maximum of 10 samples were analyzed at the same time. Each sample was repeated three times. The ORAC value refers to the net protection area under the quenching curve of R-PE in the presence of an antioxidant. The final results (ORAC value) were calculated and expressed using Trolox equivalents (TE) per gram on a fresh weight basis.

**HPLC Analysis of Anthocyanins and Phenolic Compounds.** High-performance liquid chromatography (HPLC) was used to separate and determine individual anthocyanins and phenolic compounds in berry tissue samples. The supernatants (18 mL) from the extractions described above were concentrated to dryness using a Buchler Evapomix (Fort Lee, NJ) in a water bath at 35 °C, dissolved in 4 mL of acidified water (3% formic acid), and then passed through a C18 Sep-Pak cartridge (Waters), which was previously activated with methanol followed by water and then 3% aqueous formic acid. Anthocyanins and other phenolics were adsorbed onto the column, while sugars, acids, and other water-soluble compounds were eluted with 10 mL of 3% formic acid. Anthocyanins and other phenolics were then recovered with 2.0 mL of acidified methanol containing 3% formic acid. The methanol extract was passed through a 0.45 μm membrane filter (Millipore, MSI, Westboro, MA), and 20 μL was analyzed by HPLC. The samples were determined using a Waters Corp. (Milford, MA) HPLC system coupled with a photodiode array detector (Waters 990 series) and equipped with two pumps (600E system controller). Samples were injected at ambient temperature (20 °C) into a reversed-phase Nova-Pak C<sub>18</sub> column (150 × 3.9 mm, particle size = 4 μm) with a guard column (Nova-Pak C<sub>18</sub>, 20 × 3.9 mm, particle size = 4 μm) (Waters). The mobile phase consisted of 5% aqueous formic acid (A) and HPLC grade acetonitrile (B). The flow rate was 1 mL/min, with a gradient profile consisting of A with the following proportions (v/v) of B: 0–1 min, 4% B; 1–10 min, 4–6% B; 10–15 min, 6% B; 15–35 min, 6–18% B; 35–40 min, 18–20% B; 40–42 min, 20–45% B; 42–45 min, 45–100% B; 45–

**Table 1.** Changes in Blueberry Fruit Decay, pH, Titratable Acidity (TA), and Total Soluble Solids (TSS) during Storage at 5 °C in Air or High-O<sub>2</sub> Atmospheres<sup>a</sup>

treatment	decay (%)	pH	TA (%)	TSS (%)
day 0	0.00	3.43 ± 0.02	0.82 ± 0.05	9.8 ± 0.2
day 7, air	0.00	3.63 ± 0.03	0.55 ± 0.14	9.8 ± 0.2
40% O <sub>2</sub>	0.00	3.65 ± 0.10	0.53 ± 0.08	9.8 ± 0.2
60% O <sub>2</sub>	0.00	3.59 ± 0.05	0.61 ± 0.06	10.1 ± 0.4
80% O <sub>2</sub>	0.00	3.60 ± 0.05	0.61 ± 0.13	10.2 ± 0.6
100% O <sub>2</sub>	0.00	3.60 ± 0.02	0.61 ± 0.15	10.0 ± 0.3
day 14, air	2.02 ± 0.8	3.72 ± 0.03	0.50 ± 0.04	9.5 ± 0.3
40% O <sub>2</sub>	1.90 ± 0.9	3.73 ± 0.04	0.50 ± 0.06	9.4 ± 0.6
60% O <sub>2</sub>	0.00	3.67 ± 0.02	0.51 ± 0.03	9.5 ± 0.1
80% O <sub>2</sub>	0.00	3.70 ± 0.03	0.52 ± 0.07	9.5 ± 0.1
100% O <sub>2</sub>	0.00	3.71 ± 0.02	0.52 ± 0.08	9.8 ± 0.5
day 21, air	6.02 ± 2.5	3.73 ± 0.04	0.49 ± 0.05	9.0 ± 0.2
40% O <sub>2</sub>	5.12 ± 0.7	3.82 ± 0.04	0.39 ± 0.01	9.1 ± 0.1
60% O <sub>2</sub>	2.18 ± 0.9	3.69 ± 0.03	0.48 ± 0.01	9.4 ± 0.2
80% O <sub>2</sub>	1.10 ± 0.9	3.74 ± 0.05	0.49 ± 0.03	9.4 ± 0.3
100% O <sub>2</sub>	0.00	3.71 ± 0.03	0.54 ± 0.03	9.5 ± 0.1
day 28, air	20.36 ± 1.2	3.85 ± 0.04	0.48 ± 0.01	8.8 ± 0.4
40% O <sub>2</sub>	18.93 ± 3.8	3.85 ± 0.04	0.38 ± 0.03	8.7 ± 0.2
60% O <sub>2</sub>	6.50 ± 11.1	3.79 ± 0.03	0.45 ± 0.03	9.4 ± 0.2
80% O <sub>2</sub>	2.06 ± 0.9	3.82 ± 0.04	0.48 ± 0.01	9.6 ± 0.1
100% O <sub>2</sub>	1.52 ± 0.1	3.82 ± 0.03	0.48 ± 0.04	9.7 ± 0.2
day 35, air	40.27 ± 2.9	3.93 ± 0.04	0.44 ± 0.03	8.5 ± 0.3
40% O <sub>2</sub>	39.20 ± 2.5	3.94 ± 0.06	0.41 ± 0.05	8.3 ± 0.5
60% O <sub>2</sub>	19.48 ± 1.2	3.89 ± 0.02	0.43 ± 0.04	9.0 ± 0.1
80% O <sub>2</sub>	6.66 ± 1.3	3.90 ± 0.02	0.44 ± 0.01	9.2 ± 0.1
100% O <sub>2</sub>	4.49 ± 0.4	3.90 ± 0.02	0.45 ± 0.03	9.5 ± 0.2
LSD <sub>0.05</sub>	1.08	0.15	0.12	0.52
significance <sup>b</sup>				
treatment (T)	sig	ns	ns	sig
duration (D)	sig	sig	sig	sig
T × D	sig	ns	ns	sig

<sup>a</sup> Data expressed as mean ± SE of triplicate assays. <sup>b</sup> ns = nonsignificant; sig = significant at  $p \leq 0.05$ .

50 min, 100% B. The phenolic compounds in fruit extracts were identified by their UV spectra, recorded with a diode array detector, and by chromatographic comparison with authentic markers (34–36). Individual flavonols and anthocyanins were quantified by comparison with an external standard of myricetin, quercetin, kaempferol, and cyanidin 3-glucoside. Scanning between 250 and 550 nm was performed, and data were collected using the Waters 990 3D chromatography data system.

**Statistical Analysis.** ANOVA of data was performed for this experiment. The effect of high-oxygen treatment and duration on fruit quality (decay, TSS, TA, pH, and fruit color) and the values of phenolic, anthocyanin, and flavonoid concentrations in blueberry fruit extract and their antioxidant capacity were evaluated by the Tukey–Kramer multiple-comparison test. Differences between means of data were compared by least significant difference (LSD). Differences at  $p \leq 0.05$  were considered to be significant.

## RESULTS AND DISCUSSION

**Fruit Decay.** Blueberry shelf life is limited by fruit decay primarily due to *Botrytis cinerea* infections at the stem scar. Fruit decay was markedly affected by different high-O<sub>2</sub> levels in storage (**Table 1**). Blueberries stored under air showed slight decay on day 14 but 40% decay on day 35 during storage at 5 °C. Treatment with 40% O<sub>2</sub> had little effect on blueberry decay compared to fruits held in air. Atmospheres enriched with ≥60% O<sub>2</sub> were effective in inhibiting blueberry fruit decay during storage, and improved decay control was obtained with increased O<sub>2</sub> concentration. One hundred percent O<sub>2</sub> was the most

effective in suppressing fruit decay among all of the treatments, with only 4.49% fruit decayed after 35 days of storage. However, no significant difference was noted in fruit decay between 80% O<sub>2</sub> and 100% O<sub>2</sub> treatments after 28 days of storage (**Table 1**). High-O<sub>2</sub> atmospheres, alone or in combination with high CO<sub>2</sub>, have also been shown to inhibit fungal growth and decay incidence in other studies. In strawberry, Wszelaki and Mitcham (37) reported that there was a decrease in fruit decay with an increase in oxygen concentration above 40%. The 90% and 100% O<sub>2</sub> treatments had significantly less fruit decay than either the 15% CO<sub>2</sub> itself or in combination with 40% O<sub>2</sub> after 14 days of storage at 5 °C. Pérez and Sanz (38) found that 80% and 100% O<sub>2</sub>, both in combination with 20% CO<sub>2</sub>, were more effective in controlling fungal decay than the conventional CA during storage at 8 °C. In longan, fruit decay was also significantly reduced by 70% O<sub>2</sub> in comparison with conventional MAP during 40 days of storage at 2 °C (39). However, the mechanisms by which high-O<sub>2</sub> atmospheres inhibit fruit decay is yet unclear. It is possible that atmospheres containing >40% oxygen are not suitable for the growth of decay microorganisms.

**pH, TA, and TSS.** The pH of blueberry juice increased slightly during storage, corresponding to a decrease in TA in all treatments (**Table 1**). Fruit exposed to high-O<sub>2</sub> levels tended to have lower pH values and higher TA contents. However, no significant differences were observed among all of the treatments on all sampling days. Little differences in TA content were also observed among high-O<sub>2</sub>- and air-treated strawberries during 14 days of storage (37). However, Pérez and Sanz (38) found significantly higher TA content before day 4 and lower TA content after day 7 in strawberry fruit exposed to 90% O<sub>2</sub> + 10% CO<sub>2</sub> than in fruit held in air during 9 days of storage at 8 °C. Longan fruit kept in 70% O<sub>2</sub> also showed significantly lower pH than fruit stored in conventional low-O<sub>2</sub> and high-CO<sub>2</sub> atmospheres during 40 days of storage (39). TSS decreased during storage in all air-stored blueberry fruit. High-O<sub>2</sub> levels ≥60% tended to maintain higher TSS values than did 40% O<sub>2</sub> and air. After prolonged storage (28 days and longer), significantly higher values of TSS in three higher level O<sub>2</sub>-treated fruit were detected in comparison with 40% O<sub>2</sub> and air treatments (**Table 1**). However, no significant differences were observed in TSS among the three higher level O<sub>2</sub> treatments. On the contrary, significantly lower TSS values in high-O<sub>2</sub>-treated strawberries than in air-stored fruit during the later period of storage at 5 °C were reported in earlier studies (37, 38). As the main substrates of respiratory metabolism, sugars and acids are depleted, causing corresponding changes in TSS, TA, and pH during storage. It has been shown that exposure of harvested horticultural crops to superatmospheric O<sub>2</sub> levels may stimulate, have no effect on, or reduce rates of respiration, depending on the commodity, maturity stage, time and temperature of storage, and O<sub>2</sub>, CO<sub>2</sub>, and ethylene concentrations (40). The different change patterns of pH, TA, and TSS in different studies seem to be associated with the different effects of elevated O<sub>2</sub> on commodity respiratory rate.

**Fruit Color.** There was a decrease in the measurement of surface color lightness (*L* value) after harvest in all treatment groups (**Table 2**), which indicates the fruit became darker with storage. Comparable *L* values were found among different high-O<sub>2</sub> treatment groups and the air control group throughout the experiment. Similarly, no significant differences were observed in fruit color of strawberries stored under high-O<sub>2</sub> atmospheres compared to air-stored fruit (37, 38). The hue angle, which gives a better idea of blueberry color, increased with storage time.

**Table 2.** Changes in Blueberry Fruit Color during Storage at 5 °C in Air or High-O<sub>2</sub> Atmospheres<sup>a</sup>

treatment	L*	a*	b*	chroma value	hue degree
day 0	32.0 ± 0.5	4.68 ± 0.4	0.46 ± 0.1	4.71 ± 0.4	305.7 ± 2.21
day 7, air	30.5 ± 0.3	1.27 ± 0.3	-1.85 ± 0.7	2.30 ± 0.4	306.4 ± 15.6
40% O <sub>2</sub>	30.1 ± 0.5	1.25 ± 0.2	-2.04 ± 0.1	2.40 ± 0.1	301.3 ± 5.3
60% O <sub>2</sub>	31.5 ± 0.6	2.20 ± 0.3	-1.80 ± 0.1	2.85 ± 0.2	320.3 ± 5.3
80% O <sub>2</sub>	30.7 ± 0.6	2.46 ± 0.7	-1.64 ± 0.5	3.03 ± 0.3	325.1 ± 15.3
100% O <sub>2</sub>	30.9 ± 1.1	2.36 ± 0.3	-1.28 ± 0.2	2.87 ± 0.1	331.2 ± 8.2
day 14, air	30.2 ± 0.5	1.86 ± 0.2	-1.62 ± 0.3	2.48 ± 0.1	318.9 ± 8.1
40% O <sub>2</sub>	30.2 ± 0.9	1.58 ± 0.5	-2.15 ± 0.2	2.71 ± 0.2	305.9 ± 12.7
60% O <sub>2</sub>	31.5 ± 0.5	2.86 ± 0.3	-1.60 ± 0.4	3.30 ± 0.2	330.7 ± 8.4
80% O <sub>2</sub>	30.8 ± 0.4	1.79 ± 0.8	-2.06 ± 0.3	2.81 ± 0.3	309.9 ± 16.8
100% O <sub>2</sub>	30.9 ± 0.2	2.93 ± 0.1	-1.37 ± 0.2	3.24 ± 0.1	334.9 ± 4.1
day 21, air	30.7 ± 0.6	2.49 ± 0.6	-2.00 ± 0.4	3.25 ± 0.2	320.5 ± 13.2
40% O <sub>2</sub>	30.1 ± 0.5	2.62 ± 0.3	-1.37 ± 0.1	2.96 ± 0.3	332.2 ± 3.7
60% O <sub>2</sub>	31.0 ± 0.9	2.64 ± 0.9	-2.19 ± 0.5	3.53 ± 0.3	319.2 ± 17.1
80% O <sub>2</sub>	30.8 ± 0.3	3.14 ± 0.5	-1.98 ± 0.6	3.77 ± 0.3	327.6 ± 11.9
100% O <sub>2</sub>	31.0 ± 0.5	3.44 ± 0.4	-1.93 ± 0.2	3.95 ± 0.4	330.6 ± 4.7
day 28, air	30.8 ± 0.5	1.38 ± 0.3	-2.27 ± 0.1	2.76 ± 0.1	301.2 ± 7.5
40% O <sub>2</sub>	31.6 ± 0.7	1.89 ± 0.3	-2.78 ± 0.6	3.07 ± 0.4	309.2 ± 10.4
60% O <sub>2</sub>	30.3 ± 1.0	3.14 ± 0.1	-1.76 ± 0.1	3.60 ± 0.1	330.7 ± 3.2
80% O <sub>2</sub>	30.9 ± 0.1	3.30 ± 0.2	-1.66 ± 0.3	3.70 ± 0.3	333.4 ± 4.3
100% O <sub>2</sub>	30.3 ± 0.5	3.90 ± 0.3	-1.03 ± 0.2	4.13 ± 0.3	337.2 ± 9.1
day 35, air	30.4 ± 0.6	1.63 ± 0.3	-2.06 ± 0.2	2.63 ± 0.3	308.5 ± 5.1
40% O <sub>2</sub>	30.4 ± 0.2	2.01 ± 0.7	-2.21 ± 0.4	3.07 ± 0.2	311.6 ± 15.0
60% O <sub>2</sub>	30.7 ± 0.7	3.07 ± 0.7	-1.44 ± 0.2	3.42 ± 0.5	333.9 ± 9.1
80% O <sub>2</sub>	30.4 ± 0.2	3.80 ± 1.1	-1.31 ± 0.6	4.06 ± 1.2	340.3 ± 8.9
100% O <sub>2</sub>	30.7 ± 0.5	4.20 ± 0.7	-1.38 ± 1.1	4.53 ± 0.6	341.2 ± 15.1
LSD <sub>0.05</sub>	0.73	0.56	0.69	0.43	12.38
significance <sup>b</sup>					
treatment (T)	ns	ns	ns	ns	sig
duration (D)	sig	sig	sig	sig	sig
T × D	ns	ns	ns	ns	sig

<sup>a</sup> Data expressed as mean ± SE of 30 assays. <sup>b</sup> ns = nonsignificant; sig = significant at  $p \leq 0.05$ .

Comparable hue angle values were found in all treatments during the first 21 days of storage. However, significantly higher hue angle values, indicating more blue color, were detected in fruits stored at 60–100% O<sub>2</sub> after 28 and 35 days of storage. On the contrary, Pérez and Sanz (38) reported significantly lower hue angle values in strawberries held in 80% O<sub>2</sub> + 20% CO<sub>2</sub> after 2 and 4 days of storage. This was perhaps due to differences in the colors of strawberries and blueberries. The ripe strawberries tend to have more red color and lower hue values because the hue angle starts at the +a\* (red) axis, whereas mature blueberries have more blue color and higher hue angle values.

**Total Phenolics, Total Anthocyanins, and ORAC.** The changes in total phenolic content, total anthocyanins, and antioxidant capacity (expressed as an ORAC value) of blueberry fruits stored at different high-O<sub>2</sub> atmospheres are shown in **Table 3**. The total phenolic content in control fruit exhibited a slight increase during the first 7 days of storage; thereafter, it remained steady during the remainder of storage. Whereas the phenolic contents in all high-O<sub>2</sub>-treated fruit increased gradually throughout the experimental period, an average of 1.2-fold increase in total phenolic content in high-O<sub>2</sub> fruit was observed after 35 days. There was an increase in total phenolic content with an increase in O<sub>2</sub> concentrations. No significant differences in total phenolic content were found during the first 2 weeks of storage in all treatments. However, there were significantly higher levels of total phenolic content in fruits treated with ≥60% O<sub>2</sub> during the following 3 weeks of storage. The total phenolic content did not differ significantly among the fruits held in atmospheres with >60% O<sub>2</sub>. There was also no difference between 40% O<sub>2</sub> treatment and air control.

The total anthocyanins and ORAC value showed a similar

change pattern as did total phenolics during storage in response to high-O<sub>2</sub> treatments (**Table 3**). The total anthocyanin content and ORAC value in fruits treated with ≥60% O<sub>2</sub> increased by averages of 1.2- and 1.5-fold, respectively, after 35 days of storage. Fruits stored in ≥60% O<sub>2</sub> exhibited significantly higher total anthocyanins and ORAC value compared to 40% O<sub>2</sub>- and air-treated fruits at the end of the experiment. Increases in total phenolic and total anthocyanin content were also observed during postharvest storage in various berry fruits including highbush blueberry cv. Bluecrop (18), lowbush blueberries (41), rabbiteye blueberries (42), strawberries (43), and cranberries (44). Kalt et al. (18) showed that the contents of total anthocyanins and total phenolics increased substantially in raspberries and strawberries stored at temperatures >0 °C, and this increase was accompanied by an increase in total antioxidant capacity. It was also reported that fruit stored under elevated O<sub>2</sub> levels exhibited good antioxidant capacity over the first 4 days of storage, but this declined with prolonged storage, possibly due to O<sub>2</sub>-promoted oxidation of the main antioxidants including anthocyanins and other phenolic compounds (45). This was confirmed by Pérez and Sanz (38), who found that, in comparison with fruits stored in air, strawberries held in 80% O<sub>2</sub> + 20% CO<sub>2</sub> had significantly higher levels of total anthocyanins during the first 4 days of storage but significantly lower levels of total anthocyanins at the end of storage.

In the present study, high-O<sub>2</sub> treatments had little effect on total phenolics, total anthocyanins, and ORAC in blueberry fruits over the first 14 days of storage. However, blueberries stored at 60% O<sub>2</sub> and above exhibited significantly higher levels of total phenolics, total anthocyanins, and ORAC values during the following 3 weeks of storage compared to 40% O<sub>2</sub>- and

**Table 3.** Changes in Blueberry Fruit Total Phenolics, Anthocyanins, and Oxygen Radical Absorbance Capacity (ORAC) during Storage at 5 °C in Air or High-O<sub>2</sub> Atmospheres<sup>a</sup>

treatment	total phenolics <sup>b</sup> (mg/100g)	total anthocyanins <sup>c</sup> (mg/100 g)	ORAC <sup>d</sup> ( $\mu$ mol of TE/g)
day 0	313 $\pm$ 8	182 $\pm$ 9	13.1 $\pm$ 0.5
day 7, air	327 $\pm$ 14	188 $\pm$ 10	14.3 $\pm$ 0.9
40% O <sub>2</sub>	323 $\pm$ 28	204 $\pm$ 8	15.4 $\pm$ 0.8
60% O <sub>2</sub>	351 $\pm$ 15	203 $\pm$ 11	15.5 $\pm$ 1.3
80% O <sub>2</sub>	330 $\pm$ 7	360 $\pm$ 5	15.2 $\pm$ 0.4
100% O <sub>2</sub>	338 $\pm$ 15	193 $\pm$ 11	15.7 $\pm$ 0.3
day 14, air	331 $\pm$ 15	199 $\pm$ 11	15.5 $\pm$ 0.6
40% O <sub>2</sub>	329 $\pm$ 22	193 $\pm$ 38	14.9 $\pm$ 0.4
60% O <sub>2</sub>	354 $\pm$ 17	217 $\pm$ 16	17.5 $\pm$ 1.5
80% O <sub>2</sub>	357 $\pm$ 24	218 $\pm$ 20	17.3 $\pm$ 2.2
100% O <sub>2</sub>	348 $\pm$ 15	213 $\pm$ 8	17.6 $\pm$ 1.8
day 21, air	324 $\pm$ 15	189 $\pm$ 23	13.4 $\pm$ 0.2
40% O <sub>2</sub>	330 $\pm$ 9	181 $\pm$ 9	14.5 $\pm$ 0.6
60% O <sub>2</sub>	357 $\pm$ 13	204 $\pm$ 12	18.5 $\pm$ 1.1
80% O <sub>2</sub>	358 $\pm$ 26	206 $\pm$ 7	18.5 $\pm$ 1.3
100% O <sub>2</sub>	360 $\pm$ 12	207 $\pm$ 13	18.3 $\pm$ 0.8
day 28, air	323 $\pm$ 4	185 $\pm$ 13	13.8 $\pm$ 1.8
40% O <sub>2</sub>	344 $\pm$ 8	183 $\pm$ 13	14.2 $\pm$ 2.5
60% O <sub>2</sub>	363 $\pm$ 24	224 $\pm$ 22	18.0 $\pm$ 2.6
80% O <sub>2</sub>	378 $\pm$ 21	216 $\pm$ 13	20.6 $\pm$ 3.8
100% O <sub>2</sub>	386 $\pm$ 49	228 $\pm$ 45	19.5 $\pm$ 2.4
day 35, air	315 $\pm$ 18	160 $\pm$ 20	11.9 $\pm$ 1.5
40% O <sub>2</sub>	343 $\pm$ 2	180 $\pm$ 25	12.1 $\pm$ 3.1
60% O <sub>2</sub>	362 $\pm$ 14	200 $\pm$ 9	19.7 $\pm$ 3.0
80% O <sub>2</sub>	382 $\pm$ 27	206 $\pm$ 12	19.8 $\pm$ 1.4
100% O <sub>2</sub>	390 $\pm$ 12	217 $\pm$ 9	20.8 $\pm$ 1.2
LSD <sub>0.05</sub> significance <sup>e</sup>	18	15	3.31
treatment (T)	sig	sig	sig
duration (D)	ns	ns	ns
T $\times$ D	sig	sig	sig

<sup>a</sup>Data expressed as mean  $\pm$  SE of triplicate assays. <sup>b</sup>Data expressed as milligrams of gallic acid equivalents per 100 g of fresh weight. <sup>c</sup>Data expressed as milligrams of cyanidin 3-glucoside equivalents per 100 g of fresh weight. <sup>d</sup>Data expressed as micromoles of Trolox equivalents per gram of fresh weight. <sup>e</sup>ns = nonsignificant; sig = significant at  $p \leq 0.05$ .

air-treated fruit (**Table 3**). It seems that the effect of high O<sub>2</sub> on total phenolics, total anthocyanins, and ORAC value may vary depending on the commodity, O<sub>2</sub> concentration, and storage time and temperature. Our results suggest that storage at atmospheres enriched with O<sub>2</sub> at  $\geq 60\%$  will improve the health benefit of blueberry fruit by positively affecting phenolic metabolism to enhance the antioxidant capacity. Previous research shows a linear relationship between total phenolic or anthocyanin content and ORAC in some berry crops (3, 18, 46). In general, the correlation coefficient for phenolic content and ORAC is higher than that for anthocyanin content and ORAC value (3, 46). It has been shown that phenolic compounds are strong antioxidants (2, 47, 48). In the present study, the correlation coefficient for phenolic content ( $x$ ) and ORAC ( $y$ ) was 0.883 ( $y = 10.151x - 18.827$ ,  $r = 0.883$ ) and that for anthocyanins versus ORAC was 0.826 ( $y = 18.291x - 7.951$ ,  $r = 0.826$ ). The increase of ORAC values observed in this experiment in blueberry fruits subjected to high-O<sub>2</sub> treatments may be attributed to the increase of total phenolic as well as total anthocyanin contents.

**Phenolic Compounds.** HPLC analysis of blueberry extracts showed that, in addition to anthocyanins, other phenolic compounds were present in significant amounts (**Tables 4** and **5**). Compounds such as chlorogenic acid, myricetin 3-arabino-

side, quercetin 3-galactoside, quercetin 3-arabinoside, and kaempferol were detected (**Table 4**). Chlorogenic acid was found to be the major phenolic compound with a high initial concentration of 254.2  $\mu$ g/g of fresh weight in blueberry cv. Duke in the present study. Chlorogenic acid was also found in large amounts in other blueberry cultivars (36, 46). The chlorogenic acid content fluctuated during storage and was not consistently affected by high-O<sub>2</sub> treatments. No significant differences in chlorogenic acid were observed among all of the treatments throughout the experiment. Considerable variation was found in flavonols of blueberries stored at different concentrations of high O<sub>2</sub>. In general, myricetin 3-arabinoside and quercetin-based flavonol contents in blueberries held in  $\geq 60\%$  O<sub>2</sub> atmospheres increased slightly with storage duration, whereas those in blueberry fruit stored in 40% O<sub>2</sub> and air decreased steadily with storage time. There were significantly higher levels of myricetin 3-arabinoside and quercetin-based flavonols in fruits stored at 100% O<sub>2</sub> than in fruits held in air after 35 days of storage. Kaempferol contents in all fruits increased steadily during storage. At the end of storage, significantly higher levels of kaempferol were detected in fruits treated with  $\geq 60\%$  O<sub>2</sub> compared to other treatments. Flavonols are effective antioxidants (49). Quercetin and other polyphenols have been shown to play a protective role in carcinogenesis by reducing the bioavailability of carcinogens (50). Quercetin, with 3',4'-dihydroxy substitution in the B-ring and conjugation between the A- and B-rings, has a high antioxidant potential (51). Kaempferol has a low antioxidant capacity against peroxy radicals. The antioxidant capacities measured by the ORAC assay for quercetin and kaempferol are 3.29 and 2.67, respectively (52). Clegg and Morton (53) reported that quercetin had the greatest antioxidant activity, followed by dihydroquercetin > kaempferol > quercitrin > chlorogenic acid. The observed higher content of flavonols, especially quercetin-based flavonols, may have partly contributed to the increased ORAC values in blueberries stored under high-O<sub>2</sub> atmospheres (**Table 3**).

**Anthocyanins.** Duke blueberries contained nine anthocyanins: delphinidin 3-galactoside, delphinidin 3-glucoside, cyanidin 3-galactoside, cyanidin 3-glucoside, petunidin 3-galactoside, petunidin 3-arabinoside, malvidin 3-galactoside, malvidin 3-glucoside, and malvidin 3-arabinoside (**Table 5**). Malvidin 3-galactoside, malvidin 3-arabinoside, petunidin 3-galactoside, and delphinidin 3-galactoside were the predominant anthocyanins, with malvidin 3-galactoside being the most dominant anthocyanin with an initial content of 554.01  $\mu$ g/g of fresh weight at harvest. All nine anthocyanins were significantly affected by high-O<sub>2</sub> treatments. In general, the nine anthocyanins in air control fruit increased during the first 2–3 weeks of storage; thereafter, all of these anthocyanins except for malvidin 3-arabinoside decreased steadily, reaching much lower levels at the end of the experiment compared to the initial harvest contents. All of the anthocyanins in fruits held in  $\geq 60\%$  O<sub>2</sub> kept increasing or maintained the same levels during the remainder of storage. After 5 weeks of storage, blueberries stored in  $\geq 60\%$  O<sub>2</sub> had significantly higher anthocyanins content than those kept in air and 40% O<sub>2</sub>. It has been shown that anthocyanins are strong antioxidants with free radical scavenging properties attributed to the phenolic hydroxyl groups attached to ring structures. Different hydroxylation and glycosylation may modulate their antioxidative properties (2, 47, 48). The anthocyanin cyanidin possesses a high antioxidant activity (51) and has an antioxidant potential 4 times that of Trolox (48). The total antioxidant capacity measured by ORAC assay for 11 anthocyanins identified in Sierra blueberries was 12.83  $\mu$ mol

**Table 4.** Changes of Chlorogenic Acid and Individual Flavonols in Blueberry Fruit during Storage in Air or High-O<sub>2</sub> Atmospheres<sup>a</sup>

treatment	chlorogenic acid	myricetin 3-arabinoside <sup>b</sup>	quercetin 3-galactoside <sup>c</sup>	quercetin 3-arabinoside <sup>c</sup>	quercetin derivative <sup>c</sup>	kaempferol derivative <sup>d</sup>
day 0	254.2 ± 12.3	9.7 ± 0.7	97.8 ± 9.9	8.1 ± 2.7	15.9 ± 0.2	10.2 ± 0.5
day 8, air	248.1 ± 23.0	10.9 ± 0.8	100.7 ± 14.4	6.8 ± 0.5	17.8 ± 0.5	11.8 ± 3.3
40% O <sub>2</sub>	225.3 ± 24.6	9.3 ± 0.4	87.4 ± 8.8	6.9 ± 0.1	15.1 ± 1.0	12.7 ± 1.9
60% O <sub>2</sub>	239.6 ± 3.2	11.6 ± 2.0	89.9 ± 8.5	7.9 ± 0.4	15.8 ± 0.4	12.3 ± 1.5
80% O <sub>2</sub>	229.4 ± 28.2	10.6 ± 0.3	114.6 ± 10.4	6.0 ± 0.3	20.1 ± 3.8	10.1 ± 0.7
100% O <sub>2</sub>	274.5 ± 23.7	13.3 ± 0.6	131.1 ± 17.3	7.6 ± 0.4	24.2 ± 2.9	11.9 ± 0.5
day 14, air	240.1 ± 26.6	10.6 ± 1.3	95.6 ± 13.3	7.0 ± 0.1	16.1 ± 1.9	12.7 ± 0.2
40% O <sub>2</sub>	257.0 ± 16.5	8.9 ± 1.0	62.8 ± 17.9	5.4 ± 1.5	13.9 ± 2.5	11.9 ± 0.2
60% O <sub>2</sub>	277.7 ± 29.9	12.7 ± 1.2	100.6 ± 13.0	7.7 ± 1.2	20.6 ± 2.1	13.4 ± 0.7
80% O <sub>2</sub>	246.3 ± 5.9	8.9 ± 0.9	91.8 ± 13.0	5.8 ± 0.7	18.3 ± 6.8	13.9 ± 0.9
100% O <sub>2</sub>	265.1 ± 12.0	12.3 ± 1.2	97.3 ± 15.3	7.3 ± 1.9	18.8 ± 0.6	13.5 ± 0.5
day 21, air	297.1 ± 26.3	8.2 ± 1.7	75.8 ± 13.6	5.3 ± 1.3	15.1 ± 2.8	10.2 ± 0.7
40% O <sub>2</sub>	264.2 ± 29.7	7.2 ± 1.0	91.6 ± 12.4	5.4 ± 0.4	16.7 ± 0.8	13.0 ± 0.7
60% O <sub>2</sub>	280.3 ± 20.8	10.7 ± 2.3	98.3 ± 15.0	7.0 ± 1.0	18.0 ± 1.0	13.3 ± 1.2
80% O <sub>2</sub>	296.7 ± 30.2	11.2 ± 1.91	112.5 ± 19.2	10.4 ± 1.5	20.4 ± 1.6	13.2 ± 1.2
100% O <sub>2</sub>	311.5 ± 25.9	13.8 ± 2.1	101.9 ± 15.1	8.2 ± 0.8	20.5 ± 4.7	14.6 ± 1.5
day 28, air	255.2 ± 27.6	7.6 ± 0.8	89.1 ± 11.8	5.2 ± 0.6	16.4 ± 1.4	11.4 ± 0.8
40% O <sub>2</sub>	281.4 ± 3.5	8.3 ± 1.4	101.0 ± 12.7	5.5 ± 0.2	19.4 ± 2.3	11.1 ± 0.9
60% O <sub>2</sub>	268.8 ± 27.1	9.6 ± 0.5	107.0 ± 5.3	6.9 ± 0.3	17.6 ± 1.1	13.3 ± 0.4
80% O <sub>2</sub>	309.8 ± 13.0	13.0 ± 3.1	117.4 ± 19.0	7.8 ± 1.3	22.1 ± 5.1	13.4 ± 0.1
100% O <sub>2</sub>	273.6 ± 13.5	16.1 ± 0.7	116.9 ± 12.6	9.9 ± 0.9	20.6 ± 2.4	17.7 ± 3.6
day 35, air	280.1 ± 16.7	6.6 ± 3.2	71.3 ± 11.2	4.8 ± 1.5	13.4 ± 1.3	11.2 ± 1.8
40% O <sub>2</sub>	267.3 ± 23.8	6.7 ± 2.8	90.0 ± 9.2	4.8 ± 0.2	14.8 ± 3.6	11.3 ± 2.5
60% O <sub>2</sub>	279.3 ± 29.4	10.0 ± 1.9	101.1 ± 16.3	6.7 ± 1.0	17.9 ± 4.7	13.2 ± 2.2
80% O <sub>2</sub>	248.7 ± 21.3	11.2 ± 1.0	107.8 ± 13.7	6.9 ± 1.0	18.7 ± 3.5	14.9 ± 1.4
100% O <sub>2</sub>	251.9 ± 31.3	11.1 ± 0.9	125.1 ± 9.4	7.1 ± 1.2	21.1 ± 2.0	16.0 ± 1.8
LSD <sub>0.05</sub> significance <sup>e</sup>	44.7	1.4	15.2	1.3	1.7	0.8
treatment (T)	ns	sig	sig	sig	sig	sig
duration (D)	ns	sig	sig	sig	sig	sig
T × D	ns	sig	sig	sig	sig	sig

<sup>a</sup> Data expressed as mean ± SE of triplicate assays. <sup>b</sup> Data of myricetin 3-arabinoside expressed as micrograms of myricetin equivalents per gram of fresh weight. <sup>c</sup> Data of quercetin aglycons expressed as micrograms of quercetin equivalents per gram of fresh weight. <sup>d</sup> Data of kaempferol derivative expressed as micrograms of kaempferol equivalents per gram of fresh weight. <sup>e</sup> ns = nonsignificant; sig = significant at  $p \leq 0.05$ .

**Table 5.** Changes of Main Individual Anthocyanins in Blueberry Fruit during Storage in Air or High-O<sub>2</sub> Atmospheres<sup>a</sup>

treatment	delphinidin 3-galactoside <sup>b</sup>	delphinidin 3-glucoside	cyanidin 3-galactoside	cyanidin 3-glucoside	petunidin 3-galactoside	petunidin 3-arabinoside	malvidin 3-galactoside	malvidin 3-glucoside	malvidin 3-arabinoside
day 0	203.7 ± 14.7	11.5 ± 2.5	65.2 ± 8.9	88.6 ± 6.4	214.5 ± 14.8	131.8 ± 8.8	554.0 ± 17.6	7.4 ± 5.3	247.8 ± 16.9
day 8, air	226.3 ± 15.9	12.9 ± 2.9	69.4 ± 1.5	97.1 ± 17.1	234.9 ± 22.3	148.9 ± 1.2	632.5 ± 29.3	13.9 ± 6.8	285.1 ± 28.5
40% O <sub>2</sub>	202.5 ± 14.8	9.1 ± 1.8	64.2 ± 12.1	93.0 ± 14.4	223.6 ± 13.6	151.0 ± 5.2	684.1 ± 28.9	13.3 ± 4.7	313.1 ± 21.9
60% O <sub>2</sub>	223.3 ± 25.8	12.8 ± 2.7	72.3 ± 9.2	99.0 ± 9.6	242.6 ± 27.2	145.7 ± 0.3	634.5 ± 13.0	10.7 ± 3.8	284.7 ± 29.3
80% O <sub>2</sub>	217.6 ± 2.2	10.7 ± 6.0	78.5 ± 4.1	96.1 ± 5.1	230.4 ± 7.0	137.4 ± 3.5	533.4 ± 20.9	5.8 ± 1.8	262.6 ± 21.7
100% O <sub>2</sub>	252.0 ± 8.6	14.9 ± 6.2	90.8 ± 7.2	110.7 ± 3.5	261.4 ± 6.8	163.6 ± 14.5	620.3 ± 21.7	13.4 ± 5.7	290.7 ± 10.8
day 14, air	220.7 ± 23.0	12.4 ± 1.9	72.3 ± 5.8	97.8 ± 9.1	238.7 ± 19.5	151.8 ± 6.0	681.2 ± 23.7	13.9 ± 5.1	311.6 ± 14.7
40% O <sub>2</sub>	177.1 ± 5.3	10.1 ± 0.9	58.4 ± 3.6	78.9 ± 5.2	197.0 ± 5.6	123.5 ± 4.6	600.2 ± 22.9	8.2 ± 5.1	277.7 ± 4.1
60% O <sub>2</sub>	256.6 ± 12.3	18.3 ± 1.2	85.8 ± 4.4	120.4 ± 6.4	270.3 ± 10.6	184.0 ± 2.8	720.5 ± 20.9	17.2 ± 2.0	349.6 ± 6.9
80% O <sub>2</sub>	183.4 ± 11.9	10.1 ± 2.7	55.7 ± 3.5	76.6 ± 2.0	195.6 ± 16.9	143.3 ± 4.9	622.2 ± 23.3	15.5 ± 2.1	317.7 ± 4.0
100% O <sub>2</sub>	256.3 ± 8.6	13.0 ± 1.7	90.9 ± 10.1	118.4 ± 2.5	280.6 ± 14.9	173.0 ± 4.3	737.5 ± 32.8	15.8 ± 5.6	345.2 ± 12.5
day 21, air	169.8 ± 13.0	10.4 ± 1.1	55.6 ± 4.4	82.5 ± 5.5	179.5 ± 14.7	129.8 ± 13.1	530.1 ± 34.9	9.6 ± 6.4	273.5 ± 23.6
40% O <sub>2</sub>	170.3 ± 12.1	11.1 ± 2.6	59.7 ± 2.1	77.6 ± 6.4	190.3 ± 13.6	136.7 ± 10.1	578.7 ± 20.6	12.0 ± 4.3	289.8 ± 10.1
60% O <sub>2</sub>	232.5 ± 4.2	13.2 ± 1.9	79.5 ± 5.3	109.0 ± 1.7	246.9 ± 11.4	150.1 ± 6.9	650.2 ± 22.6	10.2 ± 5.2	321.5 ± 12.7
80% O <sub>2</sub>	230.8 ± 11.1	13.2 ± 3.9	80.0 ± 24.9	112.3 ± 31.1	235.2 ± 18.3	140.5 ± 0.7	588.4 ± 20.8	10.4 ± 6.0	295.9 ± 7.6
100% O <sub>2</sub>	253.5 ± 21.9	10.0 ± 2.8	81.9 ± 35.0	123.1 ± 37.1	254.4 ± 34.4	175.4 ± 5.9	696.9 ± 26.8	10.7 ± 4.1	355.4 ± 13.9
day 28, air	170.5 ± 7.3	8.6 ± 0.7	60.6 ± 4.6	85.6 ± 6.4	183.1 ± 9.8	125.7 ± 3.0	508.9 ± 33.6	7.0 ± 3.6	299.1 ± 5.4
40% O <sub>2</sub>	173.2 ± 17.7	10.4 ± 3.6	62.3 ± 12.7	89.2 ± 13.2	189.7 ± 12.5	134.8 ± 3.3	510.1 ± 35.6	4.8 ± 3.2	303.2 ± 15.1
60% O <sub>2</sub>	201.4 ± 6.4b	10.4 ± 0.8	63.7 ± 0.7	88.3 ± 1.2	218.1 ± 4.8	156.0 ± 7.6	654.7 ± 32.7	15.6 ± 1.9	320.2 ± 16.3
80% O <sub>2</sub>	229.1 ± 12.5	14.2 ± 4.6	74.3 ± 11.3	104.8 ± 13.0	227.6 ± 11.3	165.2 ± 3.4	609.6 ± 35.9	10.5 ± 3.5	331.8 ± 2.8
100% O <sub>2</sub>	251.7 ± 13.3	14.8 ± 1.7	87.7 ± 12.6	114.8 ± 6.0	278.6 ± 24.5	176.2 ± 22.8	762.9 ± 45.0	18.2 ± 7.2	360.3 ± 54.0
day 35, air	111.3 ± 15.4	5.4 ± 0.7	47.3 ± 11.9	60.3 ± 8.5	140.1 ± 1.3	107.3 ± 4.2	454.3 ± 29.9	6.1 ± 3.1	274.8 ± 4.0
40% O <sub>2</sub>	138.1 ± 12.9	8.4 ± 3.3	53.5 ± 6.06	74.9 ± 17.4	164.2 ± 22.5	113.9 ± 3.9	455.7 ± 27.7	4.4 ± 4.1	274.1 ± 0.6
60% O <sub>2</sub>	186.2 ± 16.2	12.1 ± 3.3	68.9 ± 12.4	98.0 ± 14.3	213.0 ± 3.8	150.4 ± 2.8	587.1 ± 20.3	11.1 ± 6.8	321.1 ± 12.6
80% O <sub>2</sub>	222.4 ± 11.5	13.2 ± 2.2	79.1 ± 11.5	102.7 ± 3.5	241.3 ± 3.4	146.6 ± 4.9	625.7 ± 28.9	9.9 ± 4.4	317.9 ± 14.3
100% O <sub>2</sub>	242.8 ± 20.1	15.7 ± 7.2	83.9 ± 6.1	122.3 ± 17.1	259.4 ± 18.3	167.4 ± 4.7	663.3 ± 23.2	17.6 ± 2.6	356.2 ± 9.6
LSD <sub>0.05</sub> significance <sup>c</sup>	20.4	2.1	14.3	17.5	19.4	11.7	63.8	2.3	21.6
treatment (T)	sig	sig	sig	sig	sig	sig	sig	sig	sig
duration (D)	sig	sig	sig	sig	sig	sig	sig	sig	sig
T × D	sig	sig	sig	sig	sig	sig	sig	sig	sig

<sup>a</sup> Data expressed as mean ± SE of triplicate assays. <sup>b</sup> Data of anthocyanidin expressed as micrograms of cyanidin 3-glucoside equivalents per gram of fresh weight. <sup>c</sup> ns = nonsignificant; sig = significant at  $p \leq 0.05$ .

of TE/g of fresh weight and accounted for 56.3% of the total ORAC value in fruit extracts, which indicated that anthocyanins showed significant contribution to antioxidant activity in blueberry (46). Therefore, the higher ORAC values in blueberries stored at high-O<sub>2</sub> levels between 60 and 100% may be largely ascribed to the higher contents of nine anthocyanins, in particular, malvidin-based anthocyanins, due to their high concentrations in the same fruit.

In summary, the data presented in this paper indicate that high-O<sub>2</sub> treatments significantly affect blueberry decay, total and individual phenolic compounds, and ORAC value. High-O<sub>2</sub> levels between 60 and 100% generally had significantly less decay rate and higher ORAC value and total phenolic and total anthocyanins contents after 5 weeks of storage.

#### ABBREVIATIONS USED

AAPH, 2',2'-azobis(2-amidinopropane) dihydrochloride; ORAC, oxygen radical absorbance capacity; R-PE, (R)-phycoerythrin; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TE, Trolox equivalents.

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