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Changes in strawberry phenolics, anthocyanins, and antioxidant capacity in response to high oxygen treatments

Yonghua Zheng[a,1], Shiow Y. Wang[b], Chien Y. Wang[a,*], Wei Zheng[b,2]

[a]*Produce Quality and Safety Laboratory, Plant Sciences Institute, Beltsville Agricultural Research Center, Agricultural Research Service, US Department of Agriculture, Beltsville, MD 20705, USA*
[b]*Fruit Laboratory, Plant Sciences Institute, Beltsville Agricultural Research Center, Agricultural Research Service, US Department of Agriculture, Beltsville, MD 20705, USA*

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Abstract

Changes in fruit quality, decay, phenolic and anthocyanin content, and antioxidant capacity of strawberries (*Fragaria* × *ananassa* Duch. cv. Allstar) stored under air and high oxygen atmospheres at 5 °C were investigated. Freshly harvested strawberries were placed in jars and ventilated continuously with air or with 40, 60, 80, or 100 kPa O_2 at 5 °C for up to 14 days. Samples were taken initially, and after 3, 7, 10 and 14 days of storage. While fruit quality parameters such as titratable acidity, total soluble solids and surface color were only slightly affected by differing levels of O_2, the higher oxygen concentration treatments significantly reduced decay. Oxygen concentrations higher than 60 kPa also promoted increases in ORAC values, total phenolics and total anthocyanins as well as individual phenolic compounds analysed by HPLC during the initial 7 days of storage. However, this effect diminished with prolonged storage. No significant differences in ORAC values, total phenolics, total anthocyanins, or the individual phenolic compounds were observed among the high O_2 and air-stored fruits after 14 days of storage. These results indicate that high oxygen treatments exert the most effects on fruit quality and antioxidant capacity of strawberry fruit in the first 7 days of storage.

Keywords: Strawberry; High-oxygen atmospheres; Antioxidant; Phenolics; Anthocyanins

1. Introduction

Strawberries are highly perishable and susceptible to mechanical injury, physiological deterioration, water loss, and microbial decay. Carbon dioxide-enriched atmospheres have been used successfully to extend the storage life of strawberries by inhibiting fruit softening and decay (Gil, Holcroft, & Kader, 1997; Li & Kader, 1989). However, the high CO_2 concentrations recommended to maintain strawberry quality are often too close to the level of fruit tolerance, and some adverse effects have been reported (Ke, Goldstein, O'Mahony, & Kader, 1991; Shamaila, Powrie, & Skura, 1992). Recently, elevated O_2 atmospheres have been suggested as an alternative to the traditional low O_2 and high CO_2 modified atmosphere packaging (MAP) for minimally processed produce to maintain quality and safety (Day, 1996). High O_2 atmospheres have been proven to be particularly effective in preventing enzymatic browning, inhibiting microbial growth and decay incidence on a range of fresh and fresh-cut fruits and vegetables (Amanatidou, Slump, Smid, & Gorris, 1999; Amanatidou, Slump, & Smid, 2003; Amanatidou, Smid, & Gorris, 2000; Heimdal, Kuhn, Poll, & Larsen, 1995; Jacxsens, Devlieghere, Van der Steen, & Debevere, 2001; Jacxsens, Devlieghere, Van der Steen, Siro, & Debevere, 2003; Lu & Peter, 2000; Van der Steen,

Abbreviations: AAPH, 2′, 2′-azobis(2-amidinopropane) dihydrochloride; CA, controlled atmosphere; ORAC, oxygen radical absorbance capacity; R-PE, (R)-phycoerythrin; Trolox, 6-hy-droxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid; TE, Trolox equivalents

*Corresponding author. Tel.: +301 504 5981; fax: +301 504 5107.
E-mail address: wangc@ba.ars.usda.gov (C.Y. Wang).
[1]Visiting Scientist from College of Food Science and Technology, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China
[2]Visiting Scientist from Institute of Environmental Science, Zhejiang University at Yuquan, Hangzhou, Zhejiang 310027, China.

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Devlieghere, & Debevere, 2003). In strawberry, it was found that fruits held under high O_2 atmospheres were significantly firmer and had less fungal decay as compared to air or low-O_2-treated fruits (Pérez & Sanz, 2001; Stewart, 2003; Wszelaki & Mitcham, 2000).

Strawberries contain high levels of antioxidant compounds such as anthocyanins, flavonoids, and phenolic acids, which provide protection against harmful free radicals. Antioxidants have also been associated with lower occurrences and mortality rates due to cancer and heart disease as well as offering a number of other health benefits (Ames, Shigena, & Hagen, 1993; Ascherio et al., 1992; Cao, Sofic, & Prior, 1996; Dragsted, Strube, & Larsen, 1993; Gey, 1990; Velioglu, Mazzam Gao, & Oomah, 1998; Wang, Cao, & Prior, 1996). Previous studies have shown that strawberries have high oxygen radical absorbance activity against peroxyl radicals, superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen, and their antioxidant activities were significantly affected by preharvest factors such as genotype, plant growth temperature, environmental CO_2 concentration and cultivation systems (Wang, Bunce, & Mass, 2003; Wang & Jiao, 2000; Wang & Lin, 2000; Wang & Zheng, 2001; Wang, Zheng, & Galletta, 2002). Little information is available on the effect of postharvest high oxygen treatment on the changes of phenolic compounds and antioxidant capacity in strawberries. The objective of this study was to investigate the effects of atmospheres containing high O_2 on total phenolics, total anthocyanins, and antioxidant capacity as well as the main phenolic constituents in strawberry fruit during postharvest storage at 5 °C.

2. Materials and methods

2.1. Chemicals

Kaempferol, (*R*)-phycoerythrin (R-PE) 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). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) 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 authentication standards were obtained from Sigma and Fisher Scientific (Pittsburgh, PA).

2.2. Plant materials and treatments

Strawberries (*Fragaria* × *ananassa* Duch. cv. Allstar) were hand-harvested at commercially mature stage from Butler's Orchard, MD, and sorted to eliminate damaged or unripe fruit, and selected for uniform size and color. Three kilogram sample was placed in each 18-l jar, with three jars per treatment. The jars were kept at 5 °C and provided with a continuous flow (120 ml/min) of either humidified air (control), 40, 60, 80, or 100 kPa O_2 (balanced with N_2 in all high O_2 treatments). The gases were checked regularly with an O_2/CO_2 analyser (AMETEK, Pittsburgh, PA) and maintained at ± 2 kPa for the duration of the experiment. Samples were taken initially and during storage for decay evaluation and other analyses.

2.3. Fruit decay

Fruit decay was visually evaluated during the course of the experiment. Any berries with visible mold growth were considered decayed. Fruit decay was expressed as a percentage of fruit showing decay symptoms.

2.4. Total soluble solids, total titratable acidity, and pH determinations

Ten fruit from each replicate were wrapped in cheesecloth and squeezed with a hand press, and the juice was analysed for total soluble solids (TSS), pH, and titratable acidity (TA). TSS was determined at 20 °C on a Bausch and Lomb refractometer. Juice pH was measured with a pH meter. TA was determined by diluting each 5 ml aliquot of strawberry juice in 50 ml distilled water and then titrating to pH 8.2 using 0.1 N NaOH.

2.5. Surface color measurement

Surface color of 10 fruit from each replicate was measured using a colorimeter (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 and negative b^* blue color. These values were then used to calculate hue degree (h° =arctangent $[b^*/a^*]$), where 0° = red-purple; 90° = yellow; 180° = bluish-green; and 270° = blue (McGuire, 1992), and Chroma ($C^* = [a^{*2} + b^{*2}]^{1/2}$), which indicates the intensity or color saturation.

2.6. Sample preparation for various assays

Ten berries from each replicate were cut into small slices and mixed. Batches of 5-g samples were stored at −80 °C until analysed. To prepare the fruit extracts, three 5-g samples of berries from each replicate were extracted twice with 15 ml of 80 percent acetone containing 0.2 percent formic acid using a Polytron (Brinkmann Instruments, Inc., Westbury, NY) for 1 min and then centrifuged at 20,000 *g* 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.

2.7. Total phenolics and anthocyanins analysis

Total phenolic content in the strawberry extracts were determined according to the Folin–Ciocalteu procedure (Slinkard & Singleton, 1977). Results are expressed as milligrams of gallic acid equivalent (GAE) per 100 g of fresh weight. Total anthocyanin content of the strawberry extracts were measured using the pH differential method (Cheng & Breen, 1991). Results are expressed as milligrams of pelargonidin 3-glucoside (P 3-G) equivalents per 100 g of fresh weight.

2.8. ORAC (oxygen radical absorbance capacity) assay

The procedures for the ORAC assay on strawberries were modified from the previously described method of Cao, Alessio, and Culter (1993). This assay measures the effectiveness of antioxidant components in strawberry 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 mmol/l phosphate buffer (pH 7.0), 100 μl of R-PE (3.4 mg/l), 100 μl of 320 mmol/l AAPH, and 100 μl of sample. Phosphate buffer was used as a blank and 1 μmol/l Trolox (a water-soluble α-tocopherol analogue) was used as a standard during each run. The final volume was 2 ml and this reaction mixture was placed 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 excitation of 540 mm using a Shimadzu RF-Mini 150 recording fluorometer (Columbia, MD) until the fluorescence of the last reading declined to <5 percent of the first reading ($\sim$70 min). One blank, one standard, and a maximum of 10 samples were analysed 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 values) were calculated and expressed using Trolox equivalents (TE) per gram on a fresh weight basis.

2.9. HPLC analysis of strawberry 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 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 10 ml of acidified water (3 percent formic acid), and then passed through a C18 Sep-Pak cartridge (Waters Corp., Milford, MA), which was previously activated with methanol followed by water and then 3 percent aqueous formic acid. Anthocyanins and other phenolics were absorbed onto the column, while sugars, acids, and other water-soluble compounds were eluted with 10 ml of 3 percent formic acid. Anthocyanins and other phenolics were then recovered with 2.0 ml of acidified methanol containing 3 percent formic acid. The methanol extract was passed through a 0.45 μm membrane filter (Millipore, MSI, Westboro, MA), and 20 μl was analysed by HPLC. The samples were determined using a Waters Corp. 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 C18 column ($150 \times 3.9\,mm^2$, particle size 4 μm) with a guard column (NOVA-PAK C18, 20×3.9 mm, particle size 4 μm) (Waters Corp.). The mobile phase consisted of 2.5 percent aqueous formic acid (A) and HPLC grade acetonitrile (B). The mobile phase was acidified water containing 2.5 percent formic acid (A) and acetonitrile (B) in a linear gradient from 5 to 20 percent B in the first 15 min, followed by a linear gradient from 20 to 30 percent B for 5 min, then an isocratic mixture for 5 min, followed by a linear gradient from 30 to 90 percent B for 5 min, and then an isocratic mixture for 2 min before returning to the initial conditions. The flow rate was 1.0 ml/min, and the wavelengths of detection were set at 320, 350, and 510 nm. Scanning between 240 and 550 nm was performed, and data were collected by the Waters 990 3D chromatography data system. Retention times and spectra were compared to those of authentication standards.

3. Results and discussion

3.1. Fruit decay

The primary cause of reduced strawberry shelf-life is decay caused by *Botrytis cinerea* infections. Fruit decay was markedly affected by different O_2 treatments. Strawberries stored under air started to develop decay on day 3 but reached 37.23 percent decay on day 14 during storage at 5 °C (Table 1). Treatment with 40 kPa O_2 had little effect on strawberry decay. However, atmospheres enriched with $O_2 \geqslant 60$ kPa were effective in inhibiting strawberry fruit decay during storage. The higher the O_2 concentration, the greater the inhibition and 100 kPa O_2 was the most effective in suppressing fruit decay among all of the treatments, with only 8.33 percent of fruit decayed after 14 days of storage (Table 1). A similar pattern of decay suppression by various high O_2 levels during cold storage at 5 °C was also observed in blueberries (Zheng, Wang, Wang, & Zheng, 2003). High O_2 atmospheres, alone or in combination with high CO_2, have also been shown to inhibit fungal growth and decay incidence in strawberries in previous studies. Wszelaki and Mitcham (2000) reported that there was a decrease in strawberry fruit decay with an increase in oxygen concentration above 40 kPa. The 90 kPa O_2 and 100 kPa O_2 treatments had significantly less fruit decay than either the 15 kPa CO_2 alone or in combination with 40 kPa O_2 after 14 days of storage at 5 °C. Pérez and

Table 1
Changes in strawberry fruit decay, pH, titratable acidity (TA), and total soluble solids (TSS) during storage at 5 °C in air or high O_2 atmospheres[a]

Day and treatment		Decay (%)	pH	TA (%)	TSS (%)
Day 0		0.00±0.00	3.84±0.02	0.74±0.03	8.1±0.2
Day 3	Air	1.67±0.72a	3.91±0.05a	0.71±0.01a	7.7±0.2a
	40 kPa O_2	0.42±0.72a	3.93±0.05a	0.69±0.03a	7.6±0.2a
	60 kPa O_2	0.00±0.00a	3.89±0.02a	0.70±0.06a	7.6±0.2a
	80 kPa O_2	0.00±0.00a	3.90±0.02a	0.68±0.03a	7.6±0.2a
	100 kPa O_2	0.00±0.00a	3.89±0.04a	0.69±0.03a	7.4±0.2a
Day 7	Air	17.08± ±2.60a	3.94±0.01a	0.58±0.02a	6.9±0.4a
	40 kPa O_2	18.75±3.31a	3.95±0.06a	0.60±0.01a	6.8±0.4a
	60 kPa O_2	8.75±1.25b	3.94±0.08a	0.59±0.03a	6.7±0.2a
	80 kPa O_2	7.08±1.91bc	3.93±0.05a	0.61±0.03a	6.8±0.3a
	100 kPa O_2	4.58±1.91c	3.92±0.03a	0.61±0.03a	6.8±0.4a
Day 10	Air	23.75±1.25a	4.01±0.05a	0.59±0.04a	6.4±0.2a
	40 kPa O_2	22.17±2.57a	4.03±0.02a	0.58±0.04a	6.3±0.2a
	60 kPa O_2	11.67±0.72b	3.97±0.05a	0.61±0.04a	6.4±0.6a
	80 kPa O_2	9.58±1.91bc	3.99±0.06a	0.57±0.01a	6.5±0.2a
	100 kPa O_2	6.67±0.72c	4.01±0.02a	0.58±0.07a	6.7±0.3a
Day 14	Air	37.23±2.89a	4.03±0.02a	0.56±0.03a	6.1±0.2bc
	40 kPa O_2	35.54±3.82a	4.02±0.03a	0.55±0.03a	5.9±0.2c
	60 kPa O_2	15.17±1.91b	4.00±0.09a	0.57±0.04a	6.0±0.1c
	80 kPa O_2	12.21±1.25b	4.01±0.04a	0.59±0.04a	6.5±0.2ab
	100 kPa O_2	8.33±0.72c	4.00±0.03a	0.57±0.02a	6.6±0.2a
$LSD_{0.05}$		3.07	0.07	0.06	0.5

[a]Data are expressed as mean ±SE of triplicate assays. Values in the same column having the same letter for each day are not significantly different at $P \leqslant 0.05$.

Sanz (2001) found that 80 kPa O_2 in combination with 20 kPa CO_2 and 100 kPa O_2 alone were more effective in controlling fungal decay than conventional CA during storage of strawberries at 8 °C. Mold development and yeast growth on the berries were also significantly reduced by high oxygen MAP (95 kPa O_2) in comparison with conventional MAP during 10 days of storage at 7 °C (Jacxsens et al., 2001). Allende, Luo, McEvoy, Artés, and Wang (2004) reported that baby spinach leaves stored in barrier film packaging containing 100 kPa O_2 showed a significant reduction in aerobic mesophilic growth compared to those stored in perforated film packaging. However, the mechanisms by which high O_2 atmospheres inhibit decay are yet unclear. It is possible that atmospheres containing higher than 40 kPa oxygen are toxic to decay microorganisms. The inhibition of decay could be due to the unfavorable effects of high oxygen on the oxidation–reduction potential in the microbial system. The accumulation of injurious reactive oxygen species and the oxidation of certain enzymes especially those having sulfhydryl groups may be detrimental to the survival of microorganisms (Fridovitch, 1975).

3.2. Fruit quality

Strawberry fruit TSS decreased slightly during 14 days of storage. No significant differences in TSS content were observed among high O_2 and air-treated berries during the 14 days of storage at 5 °C (Table 1). However, significantly lower TSS values in high O_2-treated strawberries than in air-stored fruit during the later period of storage at 5 °C were reported in earlier studies (Pérez & Sanz, 2001; Wszelaki & Mitcham, 2000). The pH of strawberry juice increased slightly during storage, corresponding to a decrease in TA in all treatments (Table 1). There were no significant differences in pH and TA among all the treatments during 14 days of storage. Little differences in TA content were also observed among high O_2 and air-treated strawberries during 14 days of storage (Wszelaki & Mitcham, 2000). However, Pérez and Sanz (2001) found significantly higher TA content before day 4 and lower TA content after day 7 in strawberry fruit exposed to 90 kPa O_2 + 10 kPa CO_2 than fruit held in air during 9 days of storage at 8 °C. Sugars and acids are utilized as the main substrates of respiratory metabolism, causing corresponding changes in TSS, TA, and pH during storage. Exposure of fruits and vegetables to superatmospheric O_2 levels may have different effects on respiration, depending on the commodity, maturity stage, time and temperature of storage, and O_2, CO_2 and ethylene concentrations (Kader & Ben-Yehoshua, 2000). The differences in pH, TA, and TSS results among different studies may be related to differing effects of elevated O_2 on the respiratory rates of the commodities.

3.3. Fruit color

Strawberry fruit surface color did not change significantly during storage (Table 2). Comparable L values were found in all treatments throughout the experiment. Similarly, no significant differences were observed in fruit color of strawberries stored under high O_2 atmospheres or air (Pérez & Sanz, 2001; Wszelaki & Mitcham, 2000). The hue angle decreased during the first 7–10 days and then increased again. Comparable hue angle values were found in all treatments on days 3 and 14. However, significantly lower hue angle values, indicating more red color, were detected in fruits stored at 100 percent O_2 on day 7 in comparison with the other high oxygen treatments and air control. Conversely, significantly lower hue angle value was found in fruits stored in air compared to all high oxygen treatments on day 10 of storage. Pérez and Sanz (2001) also reported significantly lower hue angle values in strawberries held in 80 kPa O_2 + 20 kPa CO_2 during the initial 2 and 4 days of storage. The changes in hue angle values found in high oxygen-treated berries or air-stored berries during storage might be a result of ripening process affected by the treatments.

3.4. Total phenolics, total anthocyanins, and ORAC

The changes in total phenolic content, total anthocyanins, and antioxidant capacity (expressed as an ORAC value) of strawberry fruits stored in air or at 60 and 100 kPa O_2 atmospheres are shown in Table 3. The total phenolic contents in high O_2-treated and control fruit increased during the first 7 and 10 days, respectively, of storage, thereafter they decreased gradually during the remainder of storage. No significant differences in total phenolic content were found among all high O_2-treated and air control fruit throughout the experimental period.

The total anthocyanins and ORAC value showed a similar pattern of change as did total phenolics during storage in response to high O_2 treatments (Table 3). The total anthocyanin content and ORAC value in high O_2-treated fruit increased by an average of 1.1- and 1.2-fold, respectively, after 7 days of storage. While both the total anthocyanin content and ORAC value in control fruit exhibited a 1.2-fold increase after 10 days of storage, high O_2-treated fruit tended to have higher total anthocyanin content and ORAC value as compared to air-treated fruits after the first 7 days of storage. Significantly higher levels of total anthocyanin content and ORAC values were detected in 100 kPa O_2-treated fruit when compared to 60 kPa O_2 and air-treated fruit on day 7 of storage. The control fruit exhibited higher levels of total anthocyanin content and ORAC values over the high O_2-treated fruit during the later storage period. Significantly higher total anthocyanin content was observed in control fruit in comparison with high O_2-treated fruit on day 10 of storage. However, no significant differences in total anthocyanin

Table 2
Changes in strawberry fruit color during storage at 5 °C in air or high O_2 atmospheres[a]

Day and treatment		L^*	a^*	b^*	Chroma value	Hue angle
Day 0		38.33±0.96	37.36±1.30	26.04±1.02	45.56±1.51	34.80±0.83
Day 3	Air	38.24±0.92a	36.34±1.36a	25.04±1.93a	41.13±1.49b	34.53±1.16a
	40 kPa O_2	37.56±1.33a	36.73±1.66a	24.86±1.86a	44.07±2.26a	34.07±1.37a
	60 kPa O_2	36.78±0.63a	36.11±0.51a	23.96±0.67a	43.34±1.39a	33.56±0.98a
	80 kPa O_2	36.56±0.27a	36.21±0.57a	24.17±1.50a	43.54±1.30a	33.73±1.25a
	100 kPa O_2	36.98± ±2.37a	35.25±1.40a	23.26±1.43a	42.25±0.69ab	33.44±2.07a
Day 7	Air	38.24±0.39a	33.04±1.55b	22.55±0.75a	40.01±1.36a	34.34±1.51a
	40 kPa O_2	37.67±1.16a	34.04±1.34ab	22.45±1.21a	40.78±0.91a	33.42±2.19ab
	60 kPa O_2	37.12±1.72a	34.91±0.52ab	22.23±0.99a	41.40±0.41a	32.48±1.44ab
	80 kPa O_2	36.96±1.74a	34.95±1.63ab	21.69±1.20a	41.13±1.48a	31.84±1.92ab
	100 kPa O_2	36.52±0.96a	35.26±1.72a	21.46±0.85a	41.28±1.52a	31.35±1.80b
Day 10	Air	36.89±1.55a	35.60±0.56a	21.68±1.42b	41.69±1.10b	31.32±1.45b
	40 kPa O_2	37.38±0.49a	35.85±0.60a	24.26±0.76a	43.29±0.61ab	34.08±0.90a
	60 kPa O_2	38.22±1.96a	36.21±1.13a	25.25±1.57a	44.15± ±1.23a	34.89±2.51a
	80 kPa O_2	38.16±1.27a	36.73±1.59a	24.86±1.19a	44.36±1.14a	34.11±2.10a
	100 kPa O_2	38.64±1.34a	36.91±1.19a	25.17±0.94a	44.68±1.16a	34.29±1.26a
Day 14	Air	37.69±1.72a	36.45±0.98ab	24.39±1.09a	43.86±0.40ab	33.79±1.84a
	40 kPa O_2	37.94±1.23a	34.75±1.44b	24.31±0.64a	42.41±1.12b	34.97±1.51a
	60 kPa O_2	38.63±0.43a	36.98±1.06a	25.11±1.16a	44.70±1.34a	34.18±1.05a
	80 kPa O_2	38.48±0.99a	37.01±1.45a	25.16±1.06a	44.76±1.66a	34.22±0.89a
	100 kPa O_2	38.94±1.53a	37.66±1.07a	26.23±1.01a	45.95±1.29a	34.96±0.88a
$LSD_{0.05}$		2.03	2.00	2.01	2.06	2.60

[a]Data are expressed as means ± SE of 30 assays. Values in the same column having the same letter for each day are not significantly different at $P \leqslant 0.05$.

Table 3
Changes in strawberry fruit total phenolics, anthocyanins and oxygen radical absorbance capacity (ORAC) during storage at 5 °C in air or high O_2 atmosphere[a]

Day and treatment		Total phenolics[b] (mg /100 g)	Total anthocyanin[c] (mg /100 g)	ORAC[d] (μmol of TE/g)
Day 0		102±5	20.07±1.13	10.63±0.23
Day 3	Air	108±7a	20.18±0.45a	11.62±0.92a
	60 kPa O_2	113±9a	21.67±0.46a	12.80±0.82a
	100 kPa O_2	115±2a	22.34±1.53a	12.96±0.35a
Day 7	Air	110±8a	20.70±1.71b	11.92±0.60b
	60 kPa O_2	116±8a	22.13±0.38ab	13.06±0.92b
	100 kPa O_2	117±3a	24.23±1.57a	13.41±0.40a
Day 10	Air	114±6a	23.68±1.01a	12.33±1.18a
	60 kPa O_2	112±11a	20.49±2.56b	12.07±0.34a
	100 kPa O_2	109±10a	20.08±2.28b	11.88±0.33a
Day 14	Air	112±7a	20.12±1.85a	12.17±1.48a
	60 kPa O_2	107±7a	18.52±0.57a	11.61±0.77a
	100 kPa O_2	106±7a	18.97±1.67a	10.92±0.72a
$LSD_{0.05}$		12	2.56	1.37

Values in the same column having the same letter for each day are not significantly different at $P \leqslant 0.05$.
[a]Data expressed as mean ± SE of triplicate assays.
[b]Data expressed as milligrams of gallic acid equivalents per 100 g of fresh weight.
[c]Data expressed as milligrams of pelargonidin 3-glucoside equivalents per 100 g of fresh weight.
[d]Data expressed as micromoles of Trolox equivalents per g of fresh weight.

content or ORAC value were found among the two high O_2 treatments and air control after 14 days of storage. High O_2 has also been shown to affect changes in anthocyanin content and antioxidant capacity in strawberries in other studies. It was reported that strawberry fruit stored under elevated O_2 levels exhibited good antioxidant capacity over the first 4 days of storage but that this declined with prolonged storage (Stewart, Oparka, Johnstone, Iannetta, & Davies, 1999). Pérez and Sanz (2001) found that, in comparison with fruits stored in air, strawberries held in 80 kPa O_2 + 20 kPa CO_2 had significantly higher levels of total anthocyanins during the first 4 days of storage but significantly lower levels of total anthocyanins were observed at the end of storage. However, it was also reported that the total anthocyanins, total phenolic contents as well as antioxidant capacity in blueberry fruit were markedly increased by 60–100 kPa O_2 treatments during 35 days of storage (Zheng, et al., 2003). These results, together with the findings in this study, indicate that the effect of high O_2 on total phenolics, total anthocyanins, and ORAC values may vary depending on the commodity, O_2 concentration, storage time and temperature. In strawberry, high O_2 may induce the accumulation of phenolic compounds during the initial treatment period, but it may also promote the oxidation of the phenolic compounds after prolonged treatment.

Previous research shows a linear relationship between total phenolic or anthocyanin content and ORAC values in some berry crops (Kalt, Forney, Martin, & Prior, 1999; Prior et al., 1998; Zheng & Wang, 2003). In general, the correlation coefficient for phenolic content and the ORAC value is higher than for anthocyanin content and the ORAC value (Prior et al., 1998; Zheng & Wang, 2003). In the present study, the correlation coefficient for phenolic content (x) and ORAC value (y) was 0.964 ($y = 0.187x - 8.612$, $r = 0.964$) and that for anthocyanins versus ORAC value was 0.778 ($y = 0.339x + 4.939$, $r = 0.778$). It has been shown that phenolic compounds are strong antioxidants (Rice-Evans & Miller, 1996; Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995). Increasing ORAC values during the initial 7 days of storage, in this experiment with strawberry fruits subjected to high O_2 treatments, may be attributed to the increase in total phenolics as well as total anthocyanins contents.

3.5. Phenolic compounds

HPLC analysis of strawberry extracts showed that, in addition to anthocyanins, other phenolic compounds were present in significant amounts (Tables 4 and 5). Compounds such as ellagic acid, ellagic acid glucoside, *p*-coumaroylglucose, quercetin3-glucoside, quercetin3-glucuronide, kaempferol 3-glucoside, and kaempferol 3-glucuronide were detected (Table 4). Ellagic acid glucoside and *p*-coumaroylglucose were the predominant phenolic compounds with initial concentrations of 20.3 and 19.3 μg/g fresh wt, respectively, and quercetin and the kaempferol-based flavonols occurred only in small amounts in strawberry cv 'Allstar' in the present study. Considerable variation was found in flavonoid content of strawberries stored at different concentrations of high O_2. Ellagic acid, ellagic acid glucoside, and *p*-coumaroylglucose contents in

Table 4
Changes of ellagic acid, ellagic acid glucoside, *p*-coumaroyl glucose, quercetin3-glucoside and 3-glucuronide, kaempferol 3-glucoside, and kaempferol 3-glucuronide contents in strawberry fruit during storage in air or high O_2 atmospheres[a]

Day and treatment		Ellagic acid	Ellagic acid glucoside[b]	*p*-coumaroyl glucose[c]	Quercetin3-glucoside & 3-glucuronide[d]	Kaempferol 3-glucoside[d]	kaempferol 3-glucuronide[d]
Day 0		6.1±0.3	20.3±1.2	19.3±0.9	8.8±0.4	1.7±0.1	1.2±0.1
Day 3	Air	8.3±0.3b	24.2±1.5a	20.6±0.5c	10.2±0.3a	1.5±0.2b	1.3±0.1b
	60 kPa O_2	10.5±0.5a	26.4±2.2a	24.3±1.1b	10.4±1.0a	1.9±0.2a	1.5±0.1a
	100 kPa O_2	10.8±0.5a	26.9±1.5a	27.2±1.4a	11.2±0.4a	1.6±0.0b	1.6±0.1a
Day 7	Air	9.2±0.8b	19.9±1.7b	21.8±2.0c	11.4±0.8a	1.8±0.0b	1.5±0.1a
	60 kPa O_2	10.8±0.8a	30.9±2.9a	28.1±0.9b	12.6±0.5a	2.0±0.1a	1.4±0.1a
	100 kPa O_2	11.5±0.9a	31.9±2.3a	30.9±1.4a	12.4±0.7a	1.7±0.1b	1.5±0.2a
Day 10	Air	8.0±0.5b	17.3±2.4b	24.1±0.6b	13.4±1.1b	1.8±0.1a	1.4±0.1ab
	60 kPa O_2	9.9±0.6a	26.9±1.8a	26.9±2.2a	15.1±1.0a	1.9±0.2a	1.3±0.1b
	100 kPa O_2	10.1±0.9a	27.9±1.7a	27.1±1.5a	15.4±1.3a	2.0±0.2a	1.5±0.2a
Day 14	Air	6.3±0.5b	17.0±1.8b	18.9±1.6b	13.8±1.0b	1.7±0.1b	1.3±0.2a
	60 kPa O_2	8.3±0.7a	26.8±1.7a	22.2±0.7a	16.0±0.6a	2.1±0.1a	1.3±0.0a
	100 kPa O_2	8.4±0.7a	27.2±1.5a	22.8±1.9a	15.5±0.8a	1.9±0.1b	1.4±0.1a
$LSD_{0.05}$		1.2	3.3	2.4	1.4	0.2	0.2

Values in the same column having the same letter for each day are not significantly different at $P \leqslant 0.05$.
[a]Data expressed as mean±SE of triplicate assays.
[b]Data expressed as micrograms of ellagic acid equivalents per g of fresh weight.
[c]Data expressed as micrograms of *p*-coumaric acid equivalents per g of fresh weight.
[d]Data expressed as micrograms of quercetin3-glucoside equivalents per g of fresh weight.

Table 5
Changes of cyanidin 3-glucoside, cyanidin 3-glucoside-succinate, pelargonidin 3-glucoside, and pelargonidin 3-glucoside-succinate contents in strawberry fruit during storage in air or high O_2 atmospheres[a,b]

Day and treatment		Cyanidin 3-glucoside	Cyanidin 3-glucoside-succinate	Pelargonidin 3-glucoside	Pelargonidin 3-glucoside-succinate
Day 0		16.2±0.5	14.2±1.0	112.2±2.5	27.8±1.3
Day 3	Air	16.7±0.3c	15.0±0.9b	112.9±4.6b	27.0±1.7b
	60 kPa O_2	18.5±0.6b	16.3±1.1ab	116.7±4.8ab	28.9±2.2ab
	100 kPa O_2	20.7±0.8a	18.1±1.0a	122.8±7.5a	31.0±1.5a
Day 7	Air	19.3±1.6b	18.3±0.9b	114.8±3.1b	30.4±1.3b
	60 kPa O_2	21.9±1.0a	19.4±0.4b	121.4±3.0ab	32.2±2.3ab
	100 kPa O_2	22.8±0.5a	22.1±1.4a	127.4±3.5a	35.1±0.4a
Day 10	Air	22.4±1.5a	19.6±0.6a	121.2±3.9a	37.9±1.5a
	60 kPa O_2	17.3±1.2b	15.8±0.4b	109.6±4.5b	34.9±0.9b
	100 kPa O_2	16.4±0.7b	16.1±0.9b	112.9±4.9b	34.1±3.4b
Day14	Air	16.3±0.5a	18.5±0.4a	111.0±5.7a	24.7±0.8a
	60 kPa O_2	14.8±1.1a	14.3±0.4b	108.1±2.7a	21.2±1.3b
	100 kPa O_2	15.0±0.6a	14.4±0.5b	107.8±5.7a	21.5±1.9b
$LSD_{0.05}$		1.6	1.4	7.9	3.0

Values in the same column having the same letter for each day are not significantly different at $P \leqslant 0.05$.
[a]Data expressed as mean±SE of triplicate assays.
[b]Data of anthocyanidin expressed as micrograms of cyanidin 3-glucoside equivalents per g of fresh weight.

strawberry increased substantially after the initial 7 days of storage and then slightly decreased during the remainder of storage. Significantly higher levels of ellagic acid, ellagic acid glucoside and *p*-coumaroylglucose contents were observed in fruits stored at 60 and 100 kPa O_2 when compared to fruits held in air for 14 days of storage except on day 3 for ellagic acid. Quercetin-based flavonol content kept increasing throughout the experimental period. There were significantly higher levels of quercetin-based flavonols in fruits stored at 60 and 100 kPa O_2 than fruits held in air

after 10 and 14 days of storage. Kaempferol-based flavonol contents fluctuated during storage and were not consistently affected by high O_2 treatments.

3.6. Anthocyanins

'Allstar' strawberries contained four major anthocyanins: cyanidin 3-glucoside, cyanidin 3-glucoside-succinate, pelargonidin 3-glucoside, and pelargonidin 3-glucoside-succinate. Pelargonidin 3-glucoside was the predominant anthocyanin, with an initial content of 111.2 μg/g fresh wt at harvest (Table 5). All four anthocyanins were markedly affected by high O_2 treatments. In general, the four anthocyanins contents in high O_2-treated and control fruit increased during the first 7 and 10 days of storage; thereafter, they decreased gradually during the remainder of storage. Significantly higher levels of cyanidin 3-glucoside, cyanidin 3-glucoside-succinate, pelargonidin 3-glucoside, and pelargonidin 3-glucoside-succinate contents were detected in 100 kPa O_2-treated fruit as compared to air-treated fruit on day 7 in storage, but the control fruit exhibited significantly higher levels of the four anthocyanins over the high O_2-treated fruit by day 10 of storage. However, no significant differences in cyanidin 3-glucoside and pelargonidin 3-glucoside contents were found among the two high O_2 treatments and air control after 14 days of storage. It has been shown that anthocyanins are strong antioxidants with free radical scavenging properties attributed to the phenolic hydroxyl groups found attached to ring structures. Different hydroxylation and glycosylation may modulate their antioxidative properties (Rice-Evans & Miller, 1996; Wang, Cao, & Prior, 1997). The anthocyanin cyanidin possesses a high antioxidant activity (Ratty & Das, 1988) and has antioxidant potentials 4 times that of Trolox (Rice-Evans et al., 1995). The total antioxidant capacity measured by ORAC assay for the 11 anthocyanins identified in 'Sierra' blueberries was 12.83 μmol of TE/g fresh wt and accounted for 56.3 percent of the total ORAC value in fruit extracts, which indicated that anthocyanins showed significant contribution to antioxidant activity in blueberry (Zheng & Wang, 2003). Therefore, the significant differences in ORAC values between high O_2 and air-treated fruits on days 7 and 10 may be largely ascribed to the significant differences in the four anthocyanins contents, in particular, pelargonidin-based anthocyanins, due to their high concentrations in the same fruit.

In summary, the data presented in this paper indicate that strawberries treated with high O_2 levels between 60 and 100 kPa generally had higher ORAC values, total phenolic and total anthocyanin content as well as individual phenolic compounds and less decay than the air-stored fruit during the initial stage of storage at 5 °C.

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