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High Oxygen Treatment Increases Antioxidant Capacity and Postharvest Life of Strawberry Fruit

J. Fernando Ayala-Zavala[1,3], Shiow Y. Wang[2], Chien Y. Wang[1] and Gustavo A. González-Aguilar[3]*

[1]Produce Quality and Safety Laboratory, Plant Sciences Institute, Agricultural Research Service, US Department of Agriculture, Beltsville, MD 20705-2350, USA

[2]Fruit Laboratory, Plant Sciences Institute, Agricultural Research Service, US Department of Agriculture, Beltsville, MD 20705-2350, USA

[3]Centro de Investigación en Alimentación y Desarrollo (CIAD), AC, Carretera a la Victoria Km 0.6, La Victoria, Hermosillo, 83 000 Sonora, México

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Summary

The antioxidant capacity, total phenolics, volatile compounds, and postharvest quality of strawberry fruit were evaluated after storage in high oxygen atmospheres (40, 60, 80, and 100 kPa) during 14 days at 5 °C. Strawberries stored at high oxygen atmospheres (>40 kPa) showed higher antioxidant capacity, total phenolics, less decay, and longer postharvest life than those stored in air. Fruit stored under high oxygen atmospheres generally emitted lower levels of volatile compounds than those stored in air. However, individual volatile compounds were affected differently. While the emission of most volatiles decreased under high oxygen atmospheres during storage, the production of some volatile compounds such as methyl acetate and methyl hexanoate increased. In conclusion, strawberries stored under superatmospheric oxygen conditions maintained higher levels of antioxidant capacity but retained lower levels of volatile production than those stored in air.

Key words: antioxidant capacity, volatile compounds, high oxygen atmosphere, postharvest life, strawberry

Introduction

Strawberries are a good source of natural antioxidants (*1–3*). In addition to the usual nutrients, such as vitamins and minerals, strawberries are also rich in anthocyanins, flavonoids, and phenolic acids (*2,4*). Strawberries have shown a remarkably high scavenging activity toward chemically generated radicals, making them effective in inhibiting oxidation of human low-density lipoproteins (*4*). Previous studies (*3,5*) have shown that strawberries have high oxygen radical absorbance activity against peroxyl radicals ($ROO^{\bullet}$), superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$), and singlet oxygen ($^1O_2$); noting that antioxidant activities were different among varieties (*5*). There is a positive correlation between the antioxidant activity and total phenolic content (*1,3*).

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 postharvest storage and processing. Preharvest factors, such as genetic background and cultural practices, have the potential to influence antioxidant capacity in crops (*6*). Strawberry fruit from a hill

*Corresponding author; Phone: ++1 301 5045 981; Fax: ++1 301 5045 107; E-mail: ChienYi.Wang@ARS.USDA.GOV

plastic culture system has consistently had higher flavonoid content and antioxidant capacity than fruit from plants grown using the matted row system (*7*). Postharvest storage can also affect anthocyanin level, phenolic compound level and antioxidant capacity in fruits and vegetables. Controlled atmosphere (CA) storage of strawberry fruit did not affect anthocyanin content in external tissues but decreased anthocyanin content in internal tissues (*8*). Processing also has marked effects on phenolic content and antioxidant capacity in fruits. Strawberry processing to produce jams decreased the total ellagic acid content by 20 % and the flavonoids by 15–20 % (*9*). It has also been reported that the freezing process decreased both the total phenolic content and free radical scavenging capacity by 4–20 % in four cultivars of raspberries (*10*).

Several studies reported that high oxygen atmospheres delayed microbial growth (*11–13*). Caldwell (*11*) reported a total inhibition of different fungi and bacteria due to a high oxygen atmosphere. Bacteria continued growing normally after the exposure to normal oxygen concentration. On the other hand, fungi delayed their growth for a longer period after having been removed from the high oxygen atmosphere. Wszelaki and Mitcham (*12*) observed that atmospheres with 100 kPa oxygen concentration decreased fungal decay after 14 days at 5 °C. Therefore, the application of high oxygen atmospheres could be an effective way of preserving strawberry quality. An increase of the oxygen concentration in the internal and external fruit atmosphere could cause an increase in the free radical production that could damage the fruit tissue (*13*). Fruit sensibility to high oxygen concentrations can vary among species and developmental stages. Thus, the effect of high oxygen concentration needs to be evaluated for each commodity.

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. However, little information is available regarding the effects of storage conditions, such as exposure to high oxygen atmospheres, on changes of phenolic compounds, antioxidant capacity, and aroma compounds of strawberry fruit. This study has been undertaken to investigate the effects of high oxygen atmospheres on total phenolics and antioxidant capacity as well as the main aroma constituents and fruit quality in strawberry fruit during postharvest storage.

Materials and Methods

Chemicals

R-phycoerythrin (R-PE) from *Porphydium cruentum* was purchased from Sigma (St. Louis, MO, USA). 2',2'--Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA, Inc. (Richmond, VA, USA), while 6-hydroxy-2,5,7,8-tetramethylchroman-2--carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee, WI, USA). Methyl jasmonate from jasmine (*Jasminum officinale* L.) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Plant material

Strawberry fruit (*Fragaria x ananassa* Duch. cv. Chandler) grown at Butler's Orchard in Germantown, MD, USA, was freshly harvested at a commercially mature stage, sorted to eliminate damaged, shriveled, and unripe fruit, and selected for uniform size and color. Three hundred berries were put into glass containers (three containers per treatment). Each jar was connected to a continuous flow (120 mL/min) of humidified air where oxygen concentration was adjusted to 20, 40, 60, 80 and 100 kPa. The oxygen and carbon dioxide concentrations were monitored daily during the storage period using an O_2/CO_2 analyzer (AMETEK, Pittsburgh, PA, USA) to verify the actual gas concentrations and maintained at ±2 kPa. After 4, 6, 10, 12, and 14 days at 5 °C, sixty fruits from each treatment were removed to evaluate their overall quality, fungal decay and color (L^*, C^*, and h). Fifteen fruits per treatment were used to measure the aroma compound emission. Afterwards, fruits were hand squeezed, and juice was frozen at –80 °C until assays of pH, titratable acidity, total soluble solids, antioxidant capacity, anthocyanins and phenolic compounds were performed.

Overall quality

Thirty fruits per treatment were used for each quality evaluation. Samples from each treatment were evaluated subjectively on the initial day and on days 5, 7, 11, and 13 during storage. Overall quality was evaluated on a 1 to 5 scale according to the overall condition of the fruit, where 1=unacceptable, 2=bad, 3=acceptable, 4=good, and 5=excellent. Results were expressed as an overall quality index.

Fungal decay index

Fungal decay was visually inspected during the course of the experiment. Strawberry fruits showing surface mycelial development were considered decayed. Fungal decay was evaluated on a 1 to 5 scale, where 1=normal (no decay), 2=trace (up to 5 % surface affected), 3= slight (5 to 20 % surface affected), 4=moderate (20 to 50 % surface affected), and 5=severe (>50 % surface affected). Results were expressed as overall decay index.

Total soluble solids and total titratable acidity

Twenty fruits from each replicate were wrapped in cheesecloth and squeezed with a hand press; the juice was analyzed in triplicate for total soluble solids (TSS) and titratable acidity (TA). TSS were determined at 20 °C on an Atago DBX-55 refractometer (Atago Co. Ltd., Tokyo, Japan). TA was determined by diluting each 5-mL aliquot of strawberry juice in 95 mL of distilled water and then titrated to pH=8.2 using NaOH (4 g/L).

Surface color measurement

Fruit surface color was measured on 10 fruits from each of three replicates using a chromameter (CR 200, Minolta, Ramsey, NJ, USA), 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 the hue degree (h=arctangent (b^*/a^*)), where 0°=red-purple; 90°=yellow; 180°==bluish green; and 270°=blue, and chroma ($C^*=(a^{*2}+b^{*2})^{1/2}$), which indicates the intensity or color saturation.

Total phenolic compound analysis

Total soluble phenolics in the fruit juice extracts were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (*14*) using gallic acid as a standard. Results were expressed as milligrams of gallic acid equivalent per 100 g of fresh mass (FM).

Oxygen radical absorbance capacity (ORAC) assay

The procedures for the ORAC assay on strawberries were modified from a previously described method by Cao *et al.* (*15*). This assay measures the effect of antioxidant components in fruit juices of strawberries on the decline in R-phycoerythrin (R-PE) fluorescence induced by a peroxyl radical generator, 2',2'-azobis (2-amidinopropane) dihydrochloride (AAPH). The reaction mixture contained 1.7 mL of phosphate buffer, $K_2HPO_4{\cdot}3H_2O$/$NaH_2PO_4{\cdot}H_2O$ (0.0172/0.01035 in g/mL; 61.6/38.4, by volume; pH=7.0), 100 μL of R-PE (3.4 mg/L), 100 μL of AAPH (86.78 g/L), and 100 μL of sample. Phosphate buffer was used as a blank, and 100 μL of Trolox (0.025 g/L), 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 the 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 absorption of 540 nm using a Shimadzu RF-Mini 150 recording fluorometer (Columbia, MD, USA) until the fluorescence of the last reading declined to less than 5 % of the first reading (approximately 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 mass basis (*15*):

$$\text{ORAC value (μmol TE/g FM)} = 20\ K(S_{\text{sample}}-S_{\text{blank}})/(S_{\text{Trolox}}-S_{\text{blank}}) \qquad /1/$$

where K is sample dilution factor and S is the area under the fluorescence decay curve of the sample, Trolox, or blank. S is calculated as follows:

$$S=(0.5+f_5/f_0+f_{10}/f_0+f_{15}/f_0+f_{20}/f_0+f_{25}/f_0+f_{30}/f_0+\ldots +f_{60}/f_0+f_{65}/f_0+f_{70}/f_0)\times 5 \qquad /2/$$

where f_0 is initial fluorescence at 0 min and f_i is fluorescence measurement at time i.

Analysis of volatile compounds

Strawberry fruit (100 g) was placed in a hermetically closed container (500 mL) housed within a thermostated water bath (25 °C). After a 10-minute equilibrium time period, volatile compounds were adsorbed on a SPME fiber (65 μm, poly(dimethylsiloxane)/DVB; Supelco, Bellefonte, PA, USA). Sampling time was 20 min. Two replicates per day per treatment were obtained with this procedure. Desorption of volatile compounds trapped in the SPME fiber was carried out directly into the GC injector. Volatiles were analyzed using a GC HP-6890 (Hewlett-Packard, Rockville, MD, USA) equipped with a fused silica capillary column 5-HP (30 m×0.25 mm). Oven temperature was initially held at 40 °C for 1.5 min and then a temperature ramp of 5 °C/min was programmed up to 250 °C. Authentic standards were used for identification of volatile compounds. Quantification was achieved by integrating the area under the curve of each identified compound (*16*).

Statistical analysis

Experiments were performed according to a completely randomized design. Analysis of variance (ANOVA) of the data for this experiment was performed using NCSS Statistical Analysis System (*17*). The effect of high oxygen atmospheres and storage time on fruit quality (decay, TSS, TA, fruit color, and aroma compounds) and the values of phenolics, and their antioxidant capacity were evaluated by the Fischer 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

Overall quality

Fig. 1 shows the effect of high oxygen atmospheres on the visual quality of strawberry fruit during storage at 5 °C. Fruits stored in high oxygen atmospheres showed the best overall quality, the higher the oxygen concentration, the better the overall quality. Thus, the fruits stored under 100 kPa of oxygen maintained an acceptable quality after 14 days at 5 °C. However, there were no significant differences ($p>0.05$) between those fruits stored under 20 and 40 kPa of oxygen. As can be seen in Fig. 1, the fruits stored under higher oxygen concentrations (60–100 kPa) maintained better quality than those stored under lower oxygen concentrations.

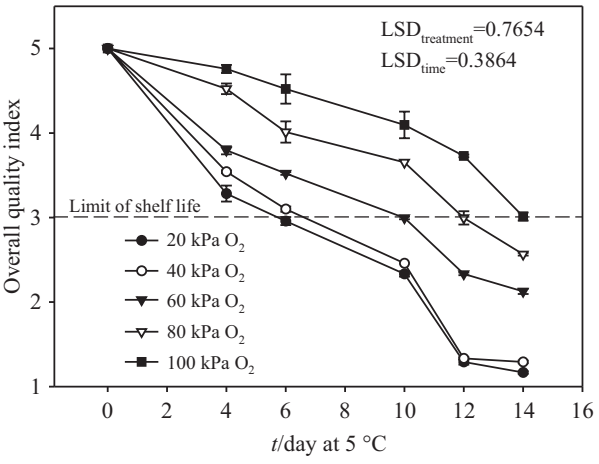

Fig. 1. Effect of high oxygen atmospheres on overall quality index of strawberries (cv. Chandler) after 14 days of storage at 5 °C. Data points are means of three replicates and LSD at 0.05 levels for treatment and time are shown

High oxygen concentrations have been particularly effective in decreasing the enzymatic discoloration, preventing anaerobic respiration, and decreasing the microbial growth (*18*). However, an increase in the oxygen concentration in the external or internal fruit atmosphere results in a high free radical production that could cause damage to the tissue (*19*). Still, the sensibility to reactive oxygen species could vary among species and developmental stage of the tissue (*6*). Wszelaki and Mitcham (*12*) reported that there was a decrease in strawberry fruit decay with an increase in oxygen concentration above 40 kPa. In longan fruit decay was also significantly reduced by 70 kPa O_2 in comparison with conventional modified atmosphere packaging (MAP) during 40 days of storage at 2 °C (*20*). Biale and Young (*19*) found that the change in color of lime fruit from green to yellow was affected noticeably during the exposure of the fruit to high oxygen concentrations. Ripe green tomatoes stored in atmospheres under 80 and 100 kPa of oxygen during 5 days exhibited skin browning; however, this problem depends on the exposure time (*21*). On the other hand, some studies have shown that the storage of apple fruit and lettuce under high oxygen atmosphere could be detrimental to the produce quality (*22,23*). The response of antioxidative systems to increasing oxidative stress during postharvest storage of fruits and vegetables does not appear to be consistent among species or cultivars/varieties within a species, underlying the complexity of antioxidant responses to oxidative stress (*6*).

Fungal decay index

Shelf life of strawberry fruit is mainly limited by fungal decay caused by *Botrytis cinerea*. Fungal decay was severely affected by the high oxygen atmospheres used during the strawberry storage (Fig. 2). Strawberry fruit stored under 20 kPa of oxygen showed the highest fungal decay index during storage period at 5 °C, compared to the fruit stored under higher oxygen concentrations (>20–100 kPa). It was observed that high oxygen atmospheres effectively inhibited fungal decay of blueberry fruit after 35 days of storage at 5 °C (*24*).

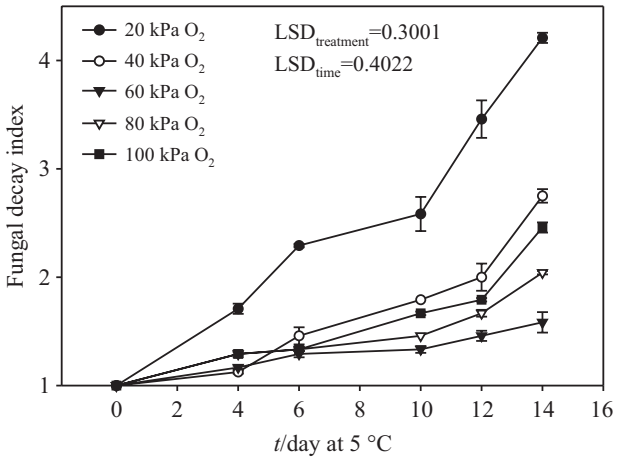

Fig. 2. Effect of high oxygen atmospheres on fungal decay index of strawberries (cv. Chandler) after 14 days of storage at 5 °C. Data points are means of three replicates and LSD at 0.05 levels for treatment and time are shown

High oxygen atmospheres alone or in conjunction with carbon dioxide have shown to be effective in inhibiting fungal decay of several produce. Similar to this work, Wszelaki and Mitcham (*12*) reported that strawberry fruit stored under 40, 90, and 100 kPa of oxygen showed low fungal decay after 14 days at 5 °C. Pérez and Sanz (*16*) found that high oxygen atmospheres (80–90 kPa) in conjunction with 10–20 kPa of carbon dioxide were more effective in controlling fungal decay in strawberry fruit than when stored in a normal atmosphere, during storage at 8 °C. The antimicrobial action of high oxygen atmospheres can be explained with the decrease in the growth of anaerobic or aerobic microorganisms. Anaerobic microorganisms grow best under low oxygen concentrations; therefore, they are inhibited by the high oxygen concentrations. On the other hand, aerobic microorganisms grow better in atmospheric oxygen concentration (approximately 21 kPa), and it has been proposed that reactive oxygen species could cause damage of vital macromolecules important in the development and reproduction of bacteria. Thus, high oxygen can inhibit microbial growth when oxidative stresses overpass the antioxidant protection systems of microorganisms (*25*).

pH, titratable acidity and total soluble solids

Fig. 3 shows the changes in pH and titratable acidity of strawberry fruit stored under high oxygen atmospheres during 14 days at 5 °C. pH values increased during storage period. Berries stored under high oxygen atmospheres (>40 kPa) had higher pH than berries stored

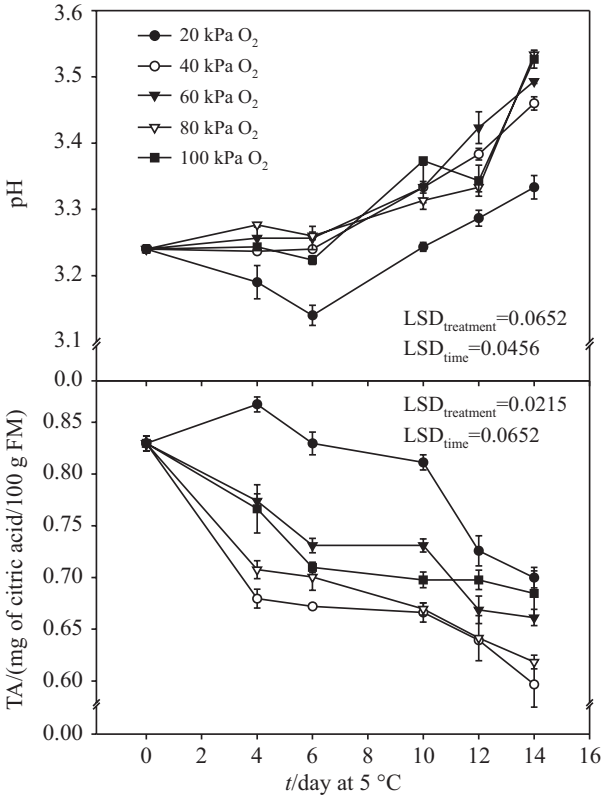

Fig. 3. Effect of high oxygen atmospheres on pH and titratable acidity of strawberries (cv. Chandler) after 14 days of storage at 5 °C. Data points are means of three replicates and LSD at 0.05 levels for treatment and time are shown

under 20 kPa of oxygen. The increase in pH values seems to be normal during the postharvest life of strawberry fruit. A decrease was observed in titratable acidity proportional to the increase in pH values, however, at the end of storage period no significant differences were observed among 20, 60, and 100 kPa. Pérez and Sanz (*16*) also reported that titratable acidity was only mildly affected by super atmospheric oxygen levels.

Fig. 4 shows the changes in the total soluble solids content during storage of strawberries under high oxygen atmospheres. There were significant differences depending on the different atmospheres and the storage period. The fruit stored in air showed a continuous increase in the soluble solids content until day 12, then decreased at the end of the storage period. An increase in the respiration rate could cause the decrease in TSS in strawberry fruits stored under high oxygen atmospheres (*12,16*). However, it has been reported that in other fruits high oxygen atmospheres could increase, decrease, or have no effect on the respiration rate, depending on the species, variety, ripening stage, oxygen concentration, storage period and temperature (*16*). Wszelaki and Mitcham (*12*) found a relation between high respiration rates and a decrease of the TSS content in strawberry fruit under 90 and 100 kPa of oxygen during 14 days at 8 °C.

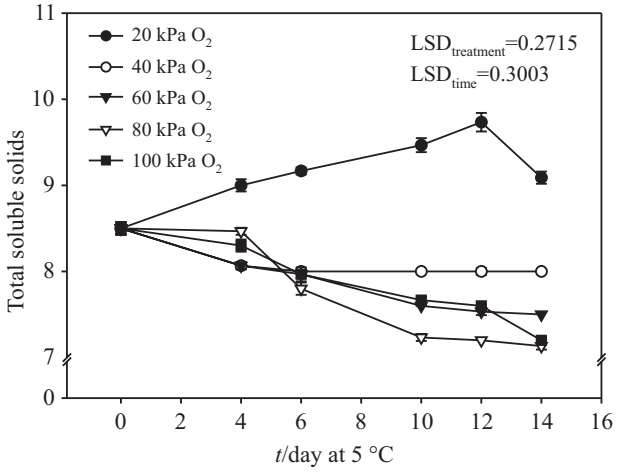

Fig. 4. Effect of high oxygen atmospheres on total soluble solids content of strawberries (cv. Chandler) after 14 days of storage at 5 °C. Data points are means of three replicates and LSD at 0.05 levels for treatment and time are shown

The changes observed in pH, titratable acidity, and the TSS content could be associated with the effect of high oxygen on the respiration of the fruit. Being the principal energetic substrates of the plant metabolism, carbohydrates and organic acids tend to decrease during the postharvest life of the fruit.

Color

Table 1 shows the effect of high oxygen atmospheres on the color of strawberry fruit. As can be observed, when stored under high oxygen atmospheres (80–100 kPa), it showed a more vivid color (chroma) and a higher L^* compared with the fruit stored in air. In other cases, a continuous decrease in L^* values for all the treatments during storage period is observed; which is a normal pattern

Table 1. Effect of high oxygen atmospheres on color values of strawberries (cv. Chandler) after 14 days of storage at 5 °C

Treatment/kPa	L^*	C^*	h
20	31.51b**	29.55c	28.30abc
40	32.01b	34.32a	26.84c
60	32.29ab	34.17ab	27.92abc
80	32.87a	33.18b	27.29abc
100	32.85a	33.25ab	28.70a

**Different letters among means indicate significant difference (p=0.05)

during the postharvest life of several fruits. L^* values of blueberries stored under high oxygen atmospheres decreased during a storage period of 35 days, but there were no differences among the treatments (*24*). Pérez and Sanz (*16*) found no significant effect on strawberry color under high oxygen concentrations during 9 days at 8 °C.

Total phenolic compounds

Fig. 5 shows the changes of total phenolic compounds, which were significantly affected ($p<0.05$) by the

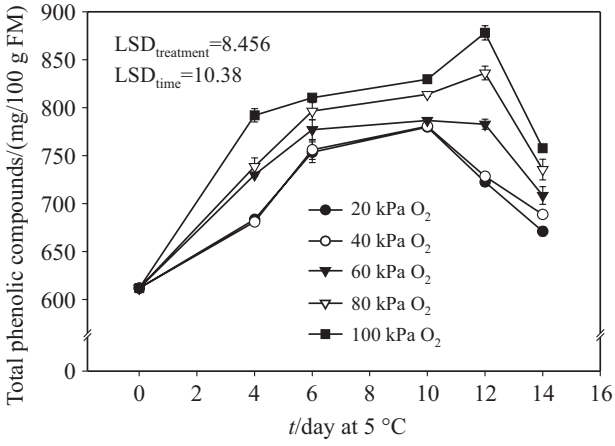

Fig. 5. Effect of high oxygen atmospheres on total phenolic compounds of strawberries (cv. Chandler) after 14 days of storage at 5 °C. Data points are means of three replicates and LSD at 0.05 levels for treatment and time are shown

high oxygen atmosphere and by the storage period. The phenolic content of the fruit stored under lower concentrations of oxygen (20–40 kPa) was affected to a lesser extent. There was a correlation between high oxygen concentration (>21 kPa) and high levels of phenolic compounds. An increase of phenolic compounds was observed for all the treatments during storage period, but their levels decreased at the end. High oxygen atmospheres also increased the phenolic content of blueberries during storage (*24*). The increase of the total phenolic compounds could be a response to the oxidative stress caused by high oxygen concentrations (*25*).

Antioxidant capacity (ORAC)

The antioxidant capacity showed a similar pattern to that of the total phenolic compounds, increasing with the oxygen atmosphere concentration (Fig. 6). Fruit stored

under 100, 80, and 60 kPa of oxygen showed a similar response, at different magnitudes. Antioxidant capacity continued to increase during storage and reached maximum values of 21.5, 19, and 18.2 μmol TE/g on the tenth day, for the fruit stored under 100, 80, and 60 kPa of oxygen, respectively. However, no noticeable changes were observed in the fruit stored under 20 or 40 kPa of oxygen, with the exception of a little increase on the fifth day of storage at 5 °C. It seems that the effect of high oxygen concentrations on the phenolic content and antioxidant capacity could vary depending on the product, oxygen concentration, storage period, and temperature. Previous studies indicate a linear correlation between phenolic content and antioxidant capacity of several berries (*26–28*). High values of antioxidant capacity observed in the berries stored under high oxygen atmospheres could be attributed to the high level of phenolic compounds.

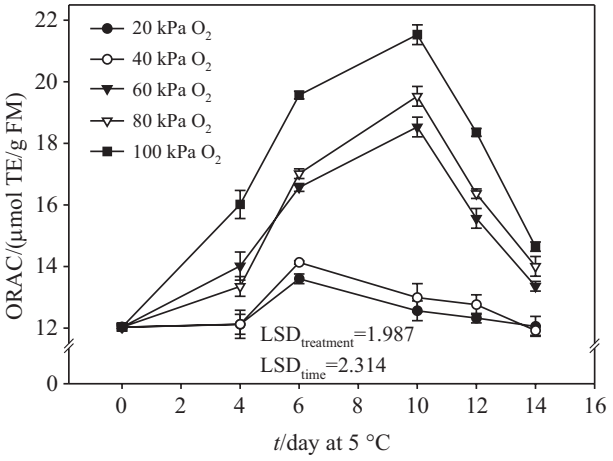

Fig. 6. Effect of high oxygen atmospheres on the antioxidant capacity of strawberries (cv. Chandler) after 14 days of storage at 5 °C. Data points are means of three replicates and LSD at 0.05 levels for treatment and time are shown

Aroma compounds

High oxygen atmospheres could affect the synthesis and accumulation of aroma compounds associated with the respiratory metabolism, including the metabolite products of the anaerobic respiration such as acetaldehyde and ethanol (*29,30*). Volatile constituents of the aroma compounds of strawberry fruit were significantly affected ($p<0.05$) by the application of high oxygen atmospheres (Fig. 7). The higher the oxygen concentration, the lower the emission of aroma compounds. However, high oxygen had a different effect on each compound analyzed. Methyl acetate, methyl metanoate, ethyl butanoate, butyl acetate, methyl hexanoate, ethyl hexanoate, and hexyl acetate were the compounds most affected by high oxygen atmospheres. A continuous decrease of 3-hexenyl acetate for all the treatments was detected. High oxygen atmospheres reduced the production of methyl me-

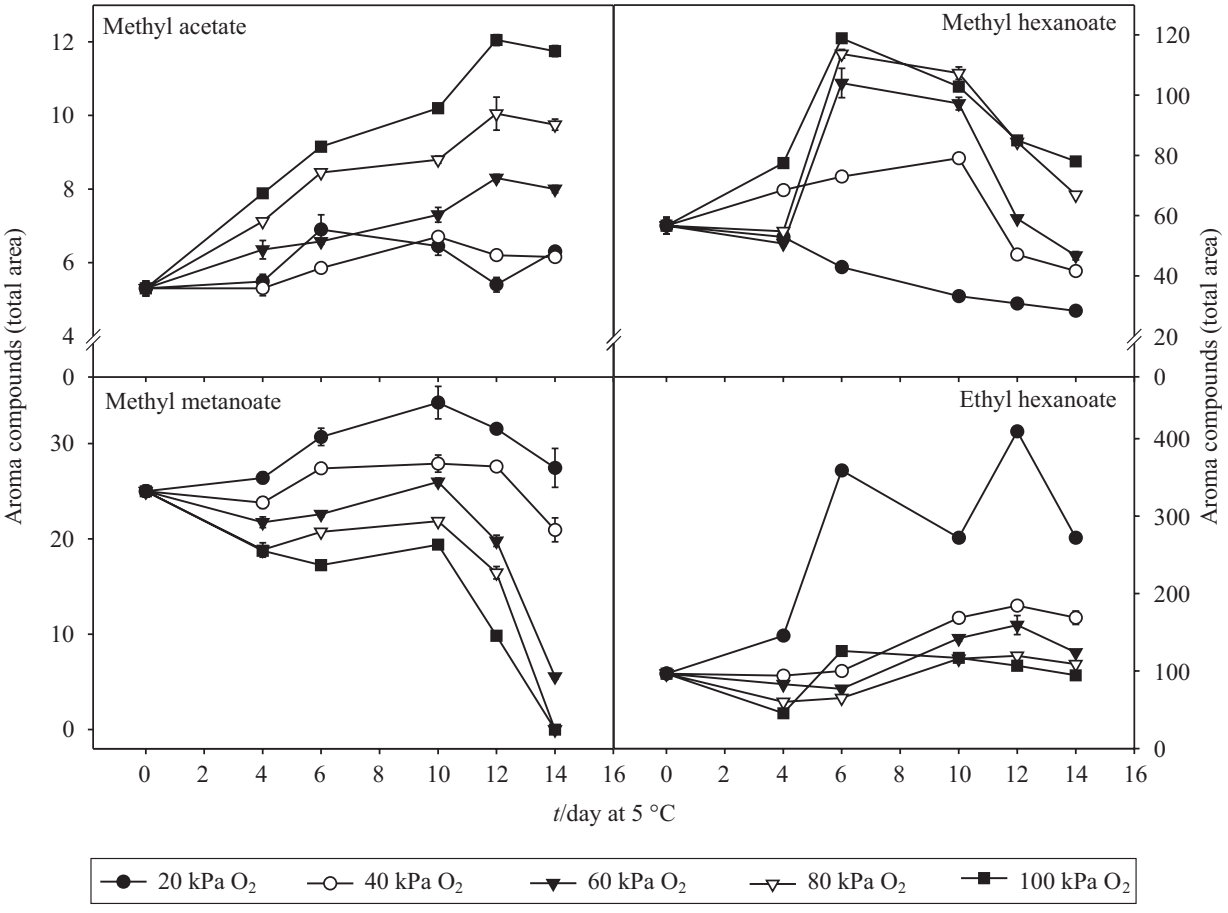

Fig. 7. Effect of high oxygen atmospheres on the aroma compounds of strawberries (cv. Chandler) after 14 days of storage at 5 °C. Data points are means of three replicates and LSD at 0.05 levels for treatment and time are shown

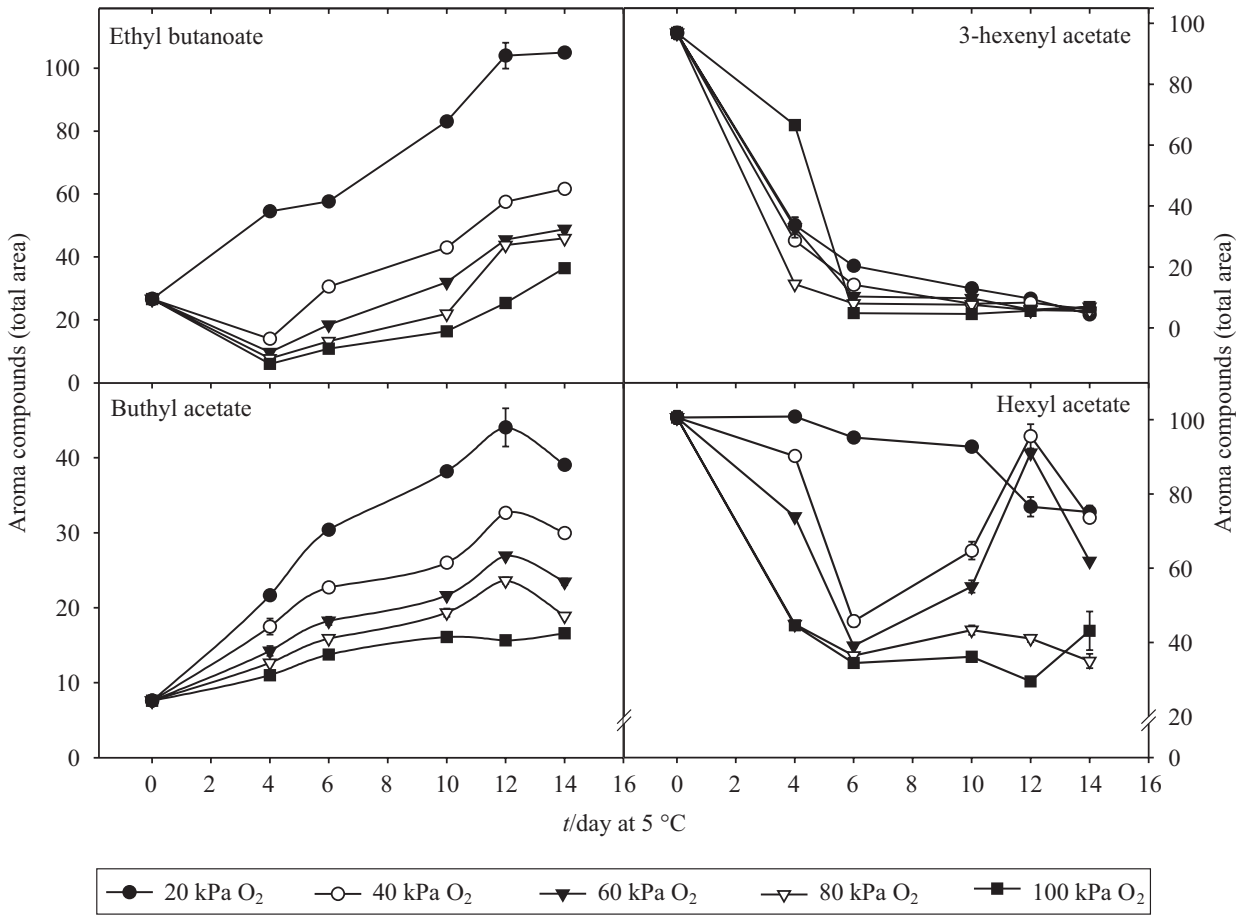

Fig. 7. (continued) Effect of high oxygen atmospheres on the aroma compounds of strawberries (cv. Chandler) after 14 days of storage at 5 °C. Data points are means of three replicates and LSD at 0.05 levels for treatment and time are shown

tanoate, ethyl butanoate, butyl acetate, ethyl hexanoate, and hexyl acetate. Methyl acetate showed higher values in strawberry fruit stored under high oxygen atmospheres (>60 kPa). The present work shows that storage under high oxygen atmospheres markedly influenced the emission of aroma compounds from strawberry fruit. The normal pattern of aroma emission during ripening of fruit can be modified by the application of high oxygen atmospheres.

Conclusions

The storage of strawberry fruit under high oxygen concentrations significantly affected the antioxidant capacity, phenolic content, aroma compounds, and overall quality. Data presented in this study suggest that even when overall quality and antioxidant capacity of strawberry fruit were positively affected by high oxygen atmospheres, the use of this technology significantly decreased the aroma compounds of the fruit.

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