# Effect of High-Oxygen and High-Carbon-Dioxide Atmospheres on Strawberry Flavor and Other Quality Traits

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The effect of high-oxygen atmospheres on strawberry flavor was studied. Strawberry fruits (*Fragaria* × *ananassa* Duch. cv. Camarosa) were stored at 8 °C in four different atmospheres: air, 5% $O_2$/20% $CO_2$, 80% $O_2$/20% $CO_2$, and 90% $O_2$/10% $CO_2$. Changes in several quality parameters were evaluated. Atmospheres combining high $O_2$ and high $CO_2$ were the most effective in preventing fungal growth and enhancing strawberry firmness. Other quality parameters such as color, titrable acidity, sugars and organic acids distribution, off-flavor development, and aroma were only mildly affected by superatmospheric $O_2$ levels. After one week of storage, unexpected high contents of off-flavor related compounds were found in the 80% $O_2$/20% $CO_2$ and 90% $O_2$/10% $CO_2$ atmospheres. Evidence of an altered ester biosynthesis was also found in fruits stored under these high-$O_2$ atmospheres. Data obtained suggest that stress induced by high $CO_2$ and stress induced by high $O_2$ have an additive effect on strawberry flavor alteration.

**Keywords:** *Strawberry; flavor; aroma; high-oxygen atmospheres; quality*

## INTRODUCTION

Strawberries are highly perishable fruits susceptible to mechanical injury, physiological deterioration, desiccation, and decay. At high temperatures, respiration increases, leading to a marked depletion of nutrient reserves so that fruit senescence is accelerated. Prompt cooling of strawberries to near 0 °C can slow undesirable quality changes and increase shelf life (*1*), but even under low temperature and high relative humidity storage life is only about 7 days.

Habitual postharvest practices used to control strawberry decay are based on the use of decreased temperatures (0−2 °C) and high $CO_2$ concentrations (up to 15−20%). Carbon-dioxide-treated strawberries are firmer and less susceptible to decay than air-stored fruits (*2*, *3*). However, the levels of $CO_2$ and $O_2$ recommended to maintain strawberry quality are often too close to the limits of tolerance for this fruit, and detrimental effects, mainly on strawberry flavor, have been reported (*4*). Continued presence of an elevated $CO_2$ environment induces a concomitant decrease in the pH of the fruit that leads to a deleterious aroma. Strawberry off-flavor has been associated with the accumulation of acetaldehyde, ethanol, and ethyl acetate formed through anaerobic respiration pathways (*5*). Nevertheless, Ke et al. (*6*) proposed that the aroma of controlled-atmosphere (CA)-stored strawberries was altered not only by overproduction of acetaldehyde and ethanol but also by reduced production of some volatile esters. The applications of novel gas mixtures (e.g., high $O_2$, argon, and nitrous oxide) are new approaches for designing modified atmospheres (MA) capable of overcoming the many disadvantages of the current high $CO_2$ and/or low $O_2$ in MAs and CAs (*7*). High-$O_2$ atmospheres have proved to be particularly effective at inhibiting enzymatic decoloration, preventing anaerobic fermentation reactions, and inhibiting microbial growth (*8*). Some responses of fresh fruits and vegetables to these superatmospheric $O_2$ concentrations, alone or in combination with elevated-$CO_2$ atmospheres, have been recently reviewed by Kader and Ben-Yehoshua (*9*). Stewart et al. (*10*) reported the effects of high-$O_2$ atmospheres on strawberry postharvest shelf life. In their study, strawberry fruits packed under 80% $O_2$ were found to be firmer and to have less fungal decay and better flavor than air- or low-$O_2$ packaged fruits. However, a recent study (*11*) points out the negative organoleptic properties of strawberries treated with high-$O_2$ atmospheres to control decay.

The aim of this work was to investigate the effect of atmospheres containing high $O_2$ and high $CO_2$ levels on strawberry organoleptic quality by means of determination of strawberry main flavor components: sugars, organic acids, and volatile compounds.

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## MATERIALS AND METHODS

**Material.** Camarosa strawberries (*Fragaria* × *ananassa* Duch.) were grown in San Bartolomé de la Torre (Huelva, Spain). Fruits were harvested, selecting for uniformity of size and color development. Berries were placed in 16-L containers (3 kg per container) and stored in cold rooms at 8 °C. The following controlled atmospheres were established inside the containers: air (21% $O_2$/0.03% $CO_2$), classical strawberry CA (5% $O_2$/20% $CO_2$), and two high oxygen atmospheres (80% $O_2$/20% $CO_2$ and 90% $O_2$/10% $O_2$). Gas mixtures were monitored by GC analysis and were maintained at ±3 kpa.

**Estimation of Fungal Decay**. Fungal infection was visually estimated during the course of the experiment. Strawberry fruits showing surface mycelial development were considered decayed. Fungal decay was expressed as percentage of decayed fruits. Commercial losses were calculated as the sum of mechanically damaged plus fungal-decayed fruits.

**Firmness.** Twenty fruits per storage atmosphere and sampling day were measured for firmness, with two measurements taken per fruit. Firmness was measured as the penetra-

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tion force required to depress 2.4 mm into the equatorial zone of the fruit with a Zwick 3303 densimeter (Zwick Gmbh Co., Ulm, Germany), using a 5-mm plunger tip, and it is expressed as Newton (N)/cm$^2$.

**Total Soluble Solids**. Three replicates per treatment, of 10 berries each, were homogenized in an Omni-mixer (Sorvall/Kendro Laboratory Products, Newtown, CT) at high speed for 3 min. The homogenate was centrifuged at 27000*g* for 15 min, and the resulting supernatant was vacuum-filtered through Whatman filter paper No. 1 (Maidstone, UK). This supernatant was used for determination of total soluble solids (TSS) by means of an Atago DBX-55 refractometer (Atago Co. Ltd., Tokyo, Japan). Results were expressed in °Brix.

**Titrable Acidity.** Titrable acidity (TA) was measured three times on 1-mL aliquots of the previous supernatant diluted with 25 mL of distilled water with a Crison automatic titrator (Crison Instruments, S. A., Barcelona, Spain) that measures the volume of 0.1 N NaOH required to reach pH 8.1. Results were expressed as milligrams of citric acid in terms of fresh weight.

**Color.** Color was evaluated both colorimetrically and by quantification of total anthocyanins. A model CR-200 portable tristimulus colorimeter (Minolta Corp. , Ramsey, NJ), color space L*, a*, b* values, was used for colorimetry. Numerical values were converted into hue angle $\tan^{-1}$b*/a* (*12*) that represents the angle in a 360° color wheel where 0, 90, 180, and 270 represent red-purple, yellow, bluish-green, and blue, respectively. Two determinations at the strawberry equatorial zone were made on 20 fruits.

Anthocyanin quantification was performed by an 11-time dilution of the ethanolic extract, obtained for sugars and organic acids determination (see below), with ethanol containing 1.11% HCl and measuring its absorbance at 517 nm. Anthocyanin concentration was determined as pelargonidin 3-glucoside in a Beckman DU 640 spectrophotometer using a molar absorptivity coefficient of 36000. Four replicates were measured for each ethanolic extract (eight replicates in total).

**Sugars and Organic Acids Analysis.** Sixteen pieces from sixteen different fruits were blended in the dark with 95% ethanol for 3 min with an Omni-mixer (Sorvall/Kendro Laboratory Products, Newtown, CT). The homogenate was vacuum-filtered through Whatman No. 1 filter paper, and the residue was washed twice with 80% ethanol. The filtrates were combined and adjusted to 5 mL/g FW. Ten mL of this extract was evaporated in the dark to dryness at 50 °C. The dry residue was redissolved in 1 mL of 0.2 N $H_2SO_4$ with 0.05% EDTA, loaded onto a Sep-Pack C18 cartridge (Lida, Kenosha, WI), and eluted with up to 4 mL of the same solution. These extracts (two per treatment and day) containing sugars and organic acids were filtered through 0.45-$\mu$m Nylon filters before HPLC analysis.

Sugars and organic acids were analyzed in a Hewlett-Packard 1090 liquid chromatograph equipped with a photodiode array detector and a Waters 410 differential refractometer (Millipore) connected in series. Isocratic separations of the compounds were made on a stainless steel Ion-300 (300 mm × 7.8 mm, 10 mm) column, according to the method developed by Pérez et al. (*13*). Three analyses were completed for each extract.

**Off-Flavor Determination.** Acetaldehyde, ethanol, and ethyl acetate contents, indicators of off-flavor formation in fruits, were determined in strawberry purees. Sixteen portions of sixteen different strawberries were blended, and 3-mL aliquots of the obtained puree were placed in 11-mL headspace vials. The vial, after sealing, was transferred to an automatic headspace sampler (Hewlet-Packard 19395A) where a 15 min equilibrium time was set at 60 °C to allow the volatiles to enter the gas phase. Volatiles were analyzed by GLC in a gas chromatograph (Hewlett-Packard 5890A) equipped with a FID and a glass column (2 mm × 1.0 m) containing 5% Carbowax on 60/80 Carbopack as stationary phase. Oven temperature was held isothermally at 70 °C. Four vials per sample were analyzed.

**Analysis of Volatile Compounds**. Strawberry fruits (250−300 g) were placed in a hermetically closed container (600 mL) housed within a thermostated water bath (25 °C). After 10 min equilibrium time, volatile compounds were adsorbed on a SPME fiber (100 $\mu$m, poly(dimethylsiloxane)). Sampling time was 20 min. Two samples per day and 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−MS Fison series 8000 equipped with a fused silica capillary column DB-Wax (30 m × 0.25 mm). Oven temperature was initially held at 40 °C for 6 min and then a temperature ramp of 2 °C/min was programmed up to 190 °C. Compound identification was made by matching against the Wiley/NBS library and by GC retention time against standards. Quantification was performed using individual calibration curves for each identified compound.

**Statistical Analysis.** ANOVA of data was performed for this study. Duncan's multiple range test (CoStat, CoHort Software) was applied to establish differences between means obtained for data from each parameter determined during the experiments. Significance was defined at $p \leq 0.05$.

**Table 1. Commercial Losses, Botrytis Decay, and Firmness in Strawberries Stored at 8 °C in Four Different Atmospheres: Air, 90/10, 80/20, and 5/20 (%$O_2$/%$CO_2$)**

| day and treatment | commercial loss (%) | Botrytis (%) | firmness (N/cm$^2$) |
|---|---|---|---|
| day 0 | 0 | 0 | 23.60[a] |
| day 2 air | 2.14 | 2.14 | 21.86 b |
| 90/10 | 0 | 0 | 25.50 a |
| 80/20 | 0 | 0 | 22.26 b |
| 5/20 | 0 | 0 | 24.51 a |
| day 4 air | 4.63 | 3.16 | 21.40 c |
| 90/10 | 0 | 0 | 25.60 a |
| 80/20 | 0 | 0 | 23.13 bc |
| 5/20 | 0 | 0 | 23.70 b |
| day 7 air | 34.40 | 19.00 | 16.21 c |
| 90/10 | 0 | 0 | 19.73 b |
| 80/20 | 0 | 0 | 14.06 c |
| 5/20 | 8.14 | 2.61 | 22.90 a |
| day 9 air | 62.57 | 62.57 | 14.45 b |
| 90/10 | 0 | 0 | 24.35 a |
| 80/20 | 0 | 0 | 23.21 a |
| 5/20 | 11.56 | 11.56 | 16.46 b |

[a] For each day, values with the same letter are not statistically different $P \leq 0.05$. Each value is the mean of 40 analyses.

## RESULTS AND DISCUSSION

**Fruit Decay.** Atmospheres combining high $O_2$ and high $CO_2$ were the most effective in preventing fungal growth during strawberry storage at 8 °C. Day (*14*) proposed that any level of elevated $O_2$ above ambient 21% $O_2$ would reduce decay, as anaerobes grow best under very low $O_2$ levels and aerobes grow best under atmospheric $O_2$. Amanatidou et al. (*15*) reported that the combined application of high $O_2$ together with high $CO_2$ had stronger and much more consistent inhibition of microbial growth. Fruits stored under 80/20 and 90/10 ($O_2$/$CO_2$) did not show any sign of fungal decay along the experiment with 0% commercial losses on day 9 (Table 1). Air-stored fruits showed slight Botrytis infection on days 2 and 4 and around 20% infected fruits on day 7. The final rate of decay for air-stored berries was higher than 60%. Fruits stored under current recommended CA conditions for strawberries, 5% $O_2$ and 20% $CO_2$ (*16*), showed visible fungal decay on day 7, increasing up to 11.5% at the end of the experiment. From these results it seems that 80/20 and 90/10 ($O_2$/$CO_2$) atmospheres are more effective in controlling fungal decay than low temperature and conventional CA (5/20). On the contrary, Wszelaki and Mitcham (*11*) found that storage at 100% $O_2$ was more effective in

**Table 2. Changes in Strawberry Color[a] and Anthocyanins[b] Content during Fruit Storage at 8 °C in Four Different Atmospheres: Air, 90/10, 80/20, and 5/20 (%$O_2$/%$CO_2$)**

| day and treatment | "L" | "a" | "b" | hue | anthocyanins nmol/g FW |
|---|---|---|---|---|---|
| day 0 | 34.43 | 32.35 | 19.74 | 30.93 | 951.28 |
| day 2 air | 34.91a[c] | 31.36b | 19.49ab | 30.99 ab | 891.35 b |
| 90/10 | 35.25a | 34.84a | 21.43ab | 30.83 ab | 763.29 c |
| 80/20 | 33.82a | 31.61b | 17.51b | 28.12 b | 997.60 a |
| 5/20 | 36.68a | 33.65a | 23.53a | 33.49 a | 727.20 c |
| day 4 air | 34.26a | 30.65a | 17.34a | 28.92 a | 887.41 b |
| 90/10 | 34.56a | 30.94a | 19.67a | 32.05 a | 698.54 c |
| 80/20 | 31.62b | 29.92a | 14.30b | 25.03 b | 970.50 a |
| 5/20 | 33.69a | 30.82a | 17.34a | 30.44 a | 804.21 c |
| day 7 air | 34.79a | 28.86a | 17.42a | 31.36 a | 1046.28 a |
| 90/10 | 33.30a | 26.84a | 14.56b | 27.91 a | 781.80 c |
| 80/20 | 34.67a | 29.05a | 16.67ab | 29.00 a | 905.46 b |
| 5/20 | 34.30a | 27.43a | 15.61ab | 28.98 a | 656.0 1d |
| day 9 air | 34.57a | 31.75ab | 22.17a | 34.11 a | 1040.82 a |
| 90/10 | 36.21a | 33.33a | 22.85a | 33.74 a | 590.23 c |
| 80/20 | 35.03a | 29.25c | 17.53b | 30.46 a | 722.21 b |
| 5/20 | 34.37a | 30.43bc | 18.51b | 30.54 a | 694.30 b |

[a] Color values are the mean of 40 analyses. [b] Anthocyanins values are the mean of 8 determinations. [c] For each day, values with the same letter are not statistically different $P \leq 0.05$

controlling strawberry fungal decay than high $O_2$ combined with high $CO_2$. Atmospheres of 80/20 and 90/10 also had some protective effect against physical deterioration of the fruits as can be deduced from differences in total commercial losses (Table 1).

**Firmness.** Strawberries stored under high $O_2$/high $CO_2$ atmospheres (80/20 and 90/10) were the firmest (Table 1) on all sampling days, except on day 7. The effectiveness of this high-$O_2$ storage is quite similar to that achieved by using current recommended CA conditions for strawberries (5/20). The increase, rather than simply maintenance, of strawberry firmness when fruit are exposed to high levels of $CO_2$ during cold storage has been reported by several authors (*3*, *5*). More recently, Harker et al. (*17*) have speculated that the mechanism for $CO_2$-induced firmness enhancement in strawberry is due to changes in the pH of the apoplast that could promote precipitation of soluble pectins. The mechanism by which high $O_2$ atmospheres promote fruit firmness has been also studied (*10*) and the maintenance of constant activity of cell wall hydrolyzing enzymes has been proposed as the key factor explaining firmness retention in high-$O_2$-stored berries. Wszelaki and Mitcham (*11*) found that strawberries stored under 80% $O_2$ were significantly firmer than fruit stored 14 days at 5 °C in air, but after two additional days at 20 °C combinations of high $O_2$ or air plus 15% $CO_2$ gave rise to the highest firmness values.

**Fruit Color.** Data from color evaluation by colorimeter and anthocyanin quantification are presented in Table 2. Non-significant differences ($p \leq 0.05$) were obtained in the measurement of skin color lightness ("L" value). Wszelaki and Mitcham (*11*) found also mild differences among high-$O_2$- and air-stored fruits in relation to this parameter. Color values a* and b* were converted to hue angle, which gives a better idea of strawberry color change along the experiments. After 2 and 4 days of storage, strawberries hue angle was significantly lower ($p \leq 0.05$) in fruits stored under an 80/20 atmosphere. No significant differences were found after one week. The lower hue value for 80/20-stored strawberries, indicating more red color, is in good agreement with the higher anthocyanins content found in these fruits on days 2 and 4. The effect of the three CA atmospheres used to maintain fruit quality is clearly seen on days 7 and 9 of storage. During this period of time, anthocyanins biosynthesis increased in air-stored fruits, but it remained stable or even decreased in high $O_2$ and conventional CA (5/20) atmospheres. These data could be explained as a retardation of the fruit ripening process caused by the 5/20, 80/20, and 90/10 atmospheres.

**Sugars and Organic Acids Content.** An equilibrated balance between main sugars and organic acids determines strawberry taste. Sucrose, glucose, fructose, citric acid, and malic acid are among the most important flavor components in strawberry. Besides these compounds, vitamin C (ascorbic acid) gives additional value to this fruit because of its important nutritional implications. In this study, several parameters were selected to evaluate taste changes induced by high-$O_2$-storage of strawberries. Table 3 shows TSS, TA, and contents of sucrose, glucose, fructose, citric acid, malic acid, and vitamin C. Although some authors have found very poor correlation values for TSS and total sugars in strawberry (*13*, *18*), TSS is routinely taken as an index of sugar content. In this study we have found that at the end of the experiments, on days 7 and 9, the TSS values of high-$O_2$-stored strawberries are significantly lower ($p \leq 0.05$) than air or 5/20 ($CO_2$/$O_2$) treated fruits. This could be explained by a higher respiratory activity of these fruits that causes a depletion of sugars. Exposure to superatmospheric $O_2$ levels may stimulate, have no effect on, or reduce rates of respiration, depending on the commodity, maturity stage, $O_2$ concentration, time and temperature of storage, and $CO_2$ and ethylene concentrations (*9*). In a recent study, Wszelaki and Mitcham (*11*) also reported higher respiration rates and a parallel TSS decrease in berries exposed for 14 days to high $O_2$ (90%, 100% $O_2$, and 40% $O_2$ plus 15% $CO_2$). On the other hand, as shown in Table 3, variations in TA along storage time do not seem to be associated with different treatments.

Among individual sugars, sucrose content decreased with storage time from an initial value of 12.96 mg/g FW at day 0 to less than 35% of initial value at the end of the experiment in all stored strawberries. Concomitant with the decrease in sucrose content an increase in glucose and fructose levels was observed. Similarly to what happened with TSS, on days 4, 7, and 9 sucrose content was lower ($p \leq 0.05$) in fruits stored under the 90/10 atmosphere. However, this lower content of sucrose in high-$O_2$-stored fruits was not correlated in all cases with a higher accumulation of glucose and fructose.

Levels of malic and citric acid could give an indication of degree of ripeness. Reyes et al. (*19*) reported that, as with sucrose, malic acid content decreases sharply during strawberry ripening whereas citric acid content decreases only slightly. Thus, malic acid content could give an idea of the degree of fruit ripeness. In this sense, although malic acid content in the 80/20 and 90/10 atmospheres was significantly lower on days 4 and 7, nonsignificant differences ($p \leq 0.05$) were found between the malic acid contents of air- and high-$O_2$-stored strawberries at the end of the experiment. On the contrary, at the end of storage (days 7 and 9) citric acid content in all CA-stored strawberries was significantly ($p \leq 0.05$) lower than that in air-stored fruits.

As antioxidant content is becoming an increasingly important parameter with respect to fruits and veg-

**Table 3. Changes in TSS[a] (Brix°), TA[a] (mg of citric acid per g of FW) Major Sugars, and Organic Acids[b] (mg/g of FW) during Strawberry Storage at 8 °C in Four Different Atmospheres: Air, 90/10, 80/20, and 5/20 (% $O_2$/% $CO_2$)**

| day/treatment | TSS (Brix°) | TA | sucrose | glucose | fructose | citric acid | malic acid | ascorbic acid |
|---|---|---|---|---|---|---|---|---|
| day 0 | 7.0 | 9.15 | 12.96 | 16.75 | 16.94 | 8.48 | 1.58 | 0.24 |
| day 2 air | 7.9 b[c] | 8.96 c | 11.88 b | 17.47 c | 18.62 b | 7.79 b | 1.96 a | 0.25 c |
| 90/10 | 7.6 c | 9.61 a | 12.27 b | 19.66 b | 19.10 b | 8.53 a | 1.66 b | 0.27 b |
| 80/20 | 8.5 a | 8.51 d | 15.46 a | 23.76 a | 23.22 a | 7.42 b | 1.55 bc | 0.31 a |
| 5/20 | 7.0 d | 9.34 b | 10.33 c | 18.72 bc | 18.38 b | 8.75 a | 1.42 c | 0.28 b |
| day 4 air | 6.0 b | 9.47 b | 6.51 c | 15.81 c | 16.72 c | 8.90 b | 1.51 a | 0.26 a |
| 90/10 | 6.0 b | 9.98 a | 5.19 d | 15.43 c | 16.19 c | 9.50 a | 1.30 b | 0.24 a |
| 80/20 | 6.7 a | 7.68 d | 9.12 b | 19.56 a | 20.26 a | 7.35 c | 1.17 b | 0.24 a |
| 5/20 | 6.7 a | 9.28 c | 9.70 a | 18.84 b | 19.23 b | 8.71 b | 1.50 a | 0.24 a |
| day 7 air | 6.6 a | 11.02 a | 5.26 a | 16.73 a | 18.65 a | 10.34 a | 1.49 a | 0.29 a |
| 90/10 | 6.3 c | 9.92 b | 3.77 b | 14.54 b | 15.97 c | 7.92 c | 1.15 b | 0.24 b |
| 80/20 | 6.3 c | 10.11 b | 5.06 a | 16.45 a | 17.86 b | 8.17 c | 1.11 b | 0.20 c |
| 5/20 | 6.2 b | 9.53 c | 4.15 b | 14.88 b | 16.26 c | 9.17 b | 1.43 a | 0.23 b |
| day 9 air | 6.8 a | 10.68 a | 4.31 b | 14.95 b | 17.09 b | 10.57 a | 1.30 a | 0.29 a |
| 90/10 | 6.1 c | 10.17 b | 4.27 b | 14.45 b | 16.50 b | 9.85 b | 1.29 a | 0.21 b |
| 80/20 | 5.2 d | 7.16 d | 4.09 b | 13.83 c | 15.68 c | 7.16 c | 1.16 ab | 0.20 b |
| 5/20 | 6.6 b | 8.32 c | 5.67 a | 16.49 a | 17.94 a | 7.47 c | 1.03 b | 0.20 b |

[a] SS and TA values are mean of 3 determinations. [b] Sugars and organic acid values are mean of 6 determinations. [c] For each day, values with the same letter are not statistically different $P \leq 0.05$.

etables, it is of great interest to evaluate changes in the antioxidant status during fruit storage. It was initially reported that high-$O_2$ modified-atmosphere packaging (MAP) had beneficial effects on the retention of vitamin C (*7*). On the other hand, Stewart et al. (*10*) found that fruit stored under elevated $O_2$ exhibited good antioxidant capacity over the first 4 days of storage but this declined with prolonged storage, possibly due to $O_2$-promoted oxidation of the main antioxidants (vitamin C, anthocyanins, and phenolic compounds). In this study, on day 2 of storage the vitamin C levels are higher in the 5/20, 80/20, and 90/10 atmospheres than those in air, with the maximum content in 80/20 strawberries. From this date to the end of the experiment, vitamin C levels decrease in all controlled atmospheres while a minor increase in ascorbic acid content is observed in air-stored fruits. In a previous study (*20*), we found a similar response in strawberry fruits exposed to ozonated atmospheres, with an initial increase in vitamin C content as a probable physiological response to oxidative stress and a further depletion of this antioxidant compound. As shown in Table 3, on day 9 vitamin C levels are significantly lower in fruits stored in high-$O_2$ atmospheres than in air-stored fruits. In a similar way, levels of anthocyanins, which also have antioxidant activity, were also lower at the end of the experiment in 80/20- and 90/10- than in air-stored berries (Table 2).

**Off-Flavor Development and Aroma Composition.** Elevated $O_2$ atmospheres may affect synthesis and accumulation of some volatile compounds associated with respiratory metabolism such as acetaldehyde, ethanol, and ethyl acetate (*21*, *22*), and could also interfere with some oxidative reactions included in aroma biosynthesis pathways. The accumulation of acetaldehyde, ethanol, and ethyl acetate is the primary cause of off-flavor development originated by anaerobic respiration induced when either too-low-$O_2$ or too-high-$CO_2$ atmospheres are used during storage of strawberries (*4*, *23*, *24*). In fact, one of the theoretical advantages of high-$O_2$ modified atmospheres is to avoid formation of these compounds by preventing anaerobic respiration (*7*).

In this work, we evaluated the effects of high-$O_2$ atmospheres on strawberry aroma by quantification of off-flavor related compounds and by analyzing the full aroma profile of stored fruits. Table 4 presents the contents of acetaldehyde, ethanol, and ethyl acetate

**Table 4. Changes in Acetaldehyde, Ethanol, and Ethyl Acetate Concentrations[a] ($\mu$L/kg of FW) in Strawberries during Storage at 8 °C in Four Different Atmospheres: Air, 90/10, 80/20, and 5/20 (% $O_2$/% $CO_2$)**

| day and treatment | acetaldehyde | ethanol | ethyl acetate |
|---|---|---|---|
| day 0 | 0.8 | 1.5 | 2.1 |
| day 2 air | 0.9a[b] | 2.1b | 1.9a |
| 90/10 | 2.1a | 2.5b | 2.0a |
| 80/20 | 2.2a | 5.2a | 2.0a |
| 5/20 | 0.9a | 7.1a | 1.5a |
| day 4 air | 2.1a | 197.6d | 2.0c |
| 90/10 | 5.0a | 256.6c | 5.3c |
| 80/20 | 3.3a | 360.0a | 9.0b |
| 5/20 | 3.6a | 334.3b | 131.2a |
| day 7 air | 3.3c | 231.3d | 5.2d |
| 90/10 | 15.0a | 414.3b | 127.6c |
| 80/20 | 3.1c | 722.0a | 298.1a |
| 5/20 | 7.0b | 390.3c | 146.6b |
| day 9 air | 8.6d | 386.6d | 14.0d |
| 90/10 | 15.3b | 412.0c | 120.3c |
| 80/20 | 28.0a | 942.0a | 525.3a |
| 5/20 | 13.0c | 532.6b | 273.3b |

[b] For each day, values with the same letter are not statistically different $P \leq 0.05$. [a] Values are mean of 4 determinations.

determined in air- and CA-stored strawberries. Larsen and Watkins (*5*) reported that off-flavors in strawberry were related to ethyl acetate and ethanol but not to acetaldehyde. Those two compounds exhibited clear increases during storage time. On day 4, strawberries kept in the 5/20 atmosphere developed a slightly detectable off-flavor that could be partially explained by the amounts of ethanol and ethyl acetate, 334.3 and 131.2 $\mu$L/kg, respectively, found in these fruits. On days 7 and 9, 5/20-, 80/10-, and 90/10-stored strawberries had ethanol and ethyl acetate levels above limits established as weak or medium off-flavor (*5*, *6*). Among them, 80/20 strawberries exhibited significantly higher contents of ethanol and ethyl acetate than was found in the other CA-stored and air-stored berries. It is especially remarkable that atmospheres with high $CO_2$ combined with high $O_2$ apparently caused a stronger off-flavor development than atmospheres containing high $CO_2$ combined with low $O_2$. Thus, on days 7 and 9, strawberries stored under the 80/20 atmosphere had almost double concentrations of ethanol and ethyl acetate than the 5/20-stored fruits. High $O_2$ does not alleviate off-flavor problems originated in classical high $CO_2$/low $O_2$ atmospheres, on the contrary, it seems that stress

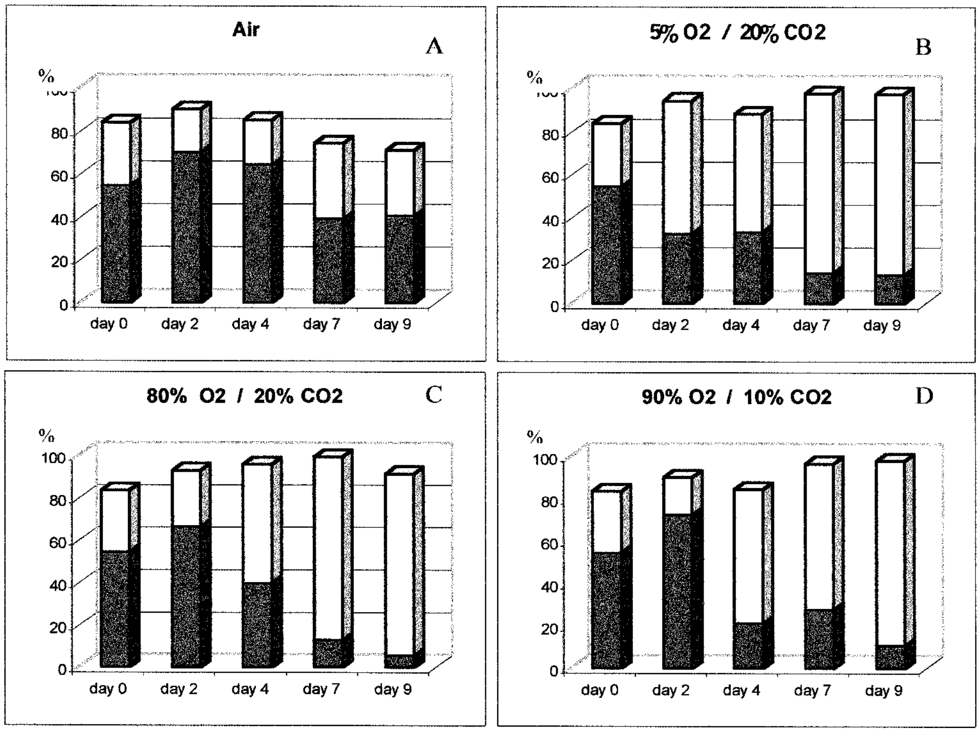


**Figure 1.** Methyl (solid bars) and ethyl (open bars) esters distribution (% of total volatile compounds) in the aroma of strawberries stored at 8 °C in four different atmospheres: air (A), 5/20 (B), 80/20 (C), and 90/10 (D) (%$O_2$/%$CO_2$).

**Table 5. Changes in Main Volatile Constituents of Strawberry Aroma after 7 and 9 days of Storage at 8 °C in Four Different Atmospheres: Air, 90/10, 80/20, and 5/20 (%$O_2$/%$CO_2$) Compared to Fruit in Origin (day 0)**

| | | day 7 | | | | day 9 | | | |
|---|---|---|---|---|---|---|---|---|---|
| compound % | day 0 | air | 5/20 | 80/20 | 90/10 | air | 5/20 | 80/20 | 90/10 |
| methyl acetate | 40.14 | 11.80 | 13.47 | 12.36 | 26.37 | 21.22 | 12.51 | 5.17 | 9.59 |
| ethyl acetate | 15.09 | 27.79 | 82.29 | 84.12 | 65.70 | 20.86 | 82.43 | 87.07 | 82.94 |
| ethanol | 6.28 | 1.81 | 0.13 | 4.89 | 2.28 | 0.00 | 1.64 | 1.41 | 0.08 |
| methyl butanonate | 12.63 | 21.63 | 0.51 | 0.24 | 0.84 | 12.81 | 0.16 | 0.18 | 0.69 |
| isobutyl acetate | 0.00 | 0.00 | 0.52 | 0.06 | 0.13 | 0.00 | 0.06 | 0.00 | 0.09 |
| ethyl butanoate | 7.92 | 7.20 | 0.99 | 1.29 | 2.16 | 7.29 | 1.16 | 2.49 | 3.15 |
| ethyl 2-methylbutanoate | 1.48 | 0.60 | 0.06 | 0.78 | 0.81 | 0.60 | 0.66 | 1.72 | 0.80 |
| ethyl pentanoate | 0.45 | 0.06 | 0.22 | 0.07 | 0.06 | 0.00 | 0.08 | 0.16 | 0.11 |
| butyl acetate | 1.02 | 5.61 | 0.00 | 0.15 | 0.29 | 7.35 | 0.20 | 0.30 | 0.50 |
| hexanal | 0.02 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| isoamyl acetate | 0.62 | 0.69 | 0.60 | 0.10 | 0.15 | 0.97 | 0.29 | 0.37 | 0.33 |
| amyl acetate | 0.02 | 0.11 | 0.03 | 0.01 | 0.01 | 0.14 | 0.01 | 0.00 | 0.00 |
| methyl hexanoate | 1.56 | 5.43 | 0.05 | 0.01 | 0.07 | 6.18 | 0.03 | 0.02 | 0.09 |
| E,2 hexenal | 5.16 | 6.54 | 0.06 | 0.00 | 0.04 | 5.81 | 0.02 | 0.00 | 0.06 |
| ethyl hexanoate | 4.34 | 1.38 | 0.04 | 0.29 | 0.37 | 1.96 | 0.15 | 0.29 | 0.49 |
| hexyl acetate | 0.80 | 4.89 | 0.19 | 0.10 | 0.10 | 9.29 | 0.11 | 0.19 | 0.23 |
| 3-hexenyl acetate | 0.30 | 1.12 | 0.38 | 0.10 | 0.24 | 1.52 | 0.10 | 0.11 | 0.38 |
| 2-hexenyl acetate | 0.57 | 0.68 | 0.13 | 0.12 | 0.05 | 0.69 | 0.09 | 0.13 | 0.15 |
| octyl acetate | 0.21 | 0.45 | 0.01 | 0.01 | 0.04 | 0.66 | 0.02 | 0.03 | 0.04 |
| linalool | 0.35 | 0.44 | 0.14 | 0.01 | 0.02 | 0.57 | 0.02 | 0.04 | 0.04 |
| mesifurane | 1.13 | 1.83 | 0.11 | 0.08 | 0.19 | 1.22 | 0.17 | 0.24 | 0.16 |

induced by high $CO_2$ and stress induced by high $O_2$ have an additive effect on off-flavor development. Wszelaki and Mitcham, (*11*) found that after 14 days of storage in $O_2$ concentrations higher than 60% and no $CO_2$ strawberries had significantly higher ($p \leq 0.05$) ethanol and ethyl acetate concentrations than air-stored fruits, and these contents continued to increase when fruits were moved to air for two more days. In fact, these authors concluded that the benefits of high $O_2$ for control of *Botrytis cinerea* were clearly outweighed by detrimental effects on flavor quality.

Aroma of CA-stored fruits can be altered not only by accumulation of acetaldehyde, ethanol, and ethyl acetate, but also by reduced or altered biosynthesis of other volatile compounds (*6, 25*). One of the main objectives of this study was to determine whether high-$O_2$-/high-$CO_2$-storage affects volatile composition of strawberry fruits. Using the SPME system the main volatile constituents of Camarosa strawberry aroma were analyzed during storage. Table 5 presents volatile composition of air-, 5/20-, 80/20-, and 90/10-stored strawberries on days 7 and 9 compared to volatile component distribution in fresh Camarosa strawberries (day 0). On days 2 and 4 some incipient differences among fruit aroma profiles were observed (data not shown) but it was after one week of storage when each atmosphere originated a characteristic aroma pattern.

On day 0 the typical volatile composition of Camarosa strawberries was determined. Methyl and ethyl esters could be considered the most significant components of the Camarosa profile. As we have reported previously, the ratio of methyl to ethyl esters is characteristic of each strawberry cultivar (*20, 26*) being about 2 for Camarosa fruits. Medium-chain esters such as methyl and ethyl butanoate, ethyl 2-methylbutanoate, ethyl hexanoate, and other compounds such as mesifurane, are the most important volatile components in Camarosa aroma. Clear differences in volatile composition were observed between air- and CA-stored strawberries on days 7 and 9 (Table 5). Although certain alteration in volatile distribution is detected in air-stored berries, methyl esters are always more abundant than ethyl esters and the relative amounts of key volatile compounds such as methyl butanoate, ethyl butanoate, and mesifurane were maintained through storage. On the contrary, the aroma pattern of CA-stored strawberries changed dramatically, especially on days 7 and 9. In berries stored under the 5/20, 80/20, and 90/10 atmospheres, levels of ethyl acetate higher than 80% total volatiles were detected while a significant reduction in the amount of medium esters and mesifurane was observed. Similar to what happened in the off-flavor analysis, the alteration of the aroma of 5/20 and 80/20 strawberries was quite similar, whereas 90/10 fruits showed a slightly less altered volatile composition. Thus the modification of aroma composition along different storage conditions can be evaluated by simple comparison of methyl/ethyl esters distribution. Figure 1 graphically shows how methyl/ethyl esters ratios change during storage. After 2 days, in all studied CAs this ratio was below 0.6 reaching a minimum value of 0.06 for 80/20-stored fruits on day 9 that indicates the extreme aroma alteration of these strawberries stored under a high-$O_2$ atmosphere.

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