

# Effects of superatmospheric oxygen on strawberry fruit quality and decay

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## Abstract

The effects of elevated O<sub>2</sub> alone or in combination with elevated CO<sub>2</sub> atmospheres for postharvest decay control on strawberry fruit (*Fragaria × ananassa* Duch.) were assessed. In vitro and in vivo growth of *Botrytis cinerea* Pers.:Fr. and the effects on fruit quality were determined under eight atmospheres: air, 40, 60, 80, 90 and 100 kPa O<sub>2</sub>, 40 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub> and air + 15 kPa CO<sub>2</sub>. The commercially-used CO<sub>2</sub> level of 15 kPa in air and its combination with 40 kPa O<sub>2</sub> were most effective in suppressing mycelial growth in vitro following 7 days at 5°C under the atmospheres. However, after 14 days of treatment at 5°C, 100 kPa O<sub>2</sub> inhibited mycelial growth more than either of the CO<sub>2</sub> treatments. In all treatments, the growth rate increased immediately upon removal from the atmosphere, indicating that there was no residual inhibitory effect. The 100 kPa O<sub>2</sub> treatment was also the most effective in controlling decay on the fruit during 14 days of storage. Although the quality parameters of respiration, ethylene production, firmness, soluble solids, titratable acidity and external color were only mildly affected by the superatmospheric O<sub>2</sub> treatments, volatile content (acetaldehyde, ethanol and ethyl acetate) increased greatly. While the fruit treated with 15 kPa CO<sub>2</sub> had the highest volatile concentrations after 14 days at 5°C, after an additional 2 days in air at 20°C, volatile concentrations in fruit treated with 100 kPa O<sub>2</sub> equaled or surpassed those of fruit treated with CO<sub>2</sub>. The volatile concentration in fruit treated with 15 kPa CO<sub>2</sub> generally decreased during 2 days in air, while the volatile content increased in fruit treated with high O<sub>2</sub>, with greater increases with increasing O<sub>2</sub> level. The fruit treated with 40 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub> achieved an intermediate level between the O<sub>2</sub> and CO<sub>2</sub> treatments. Although the 100 kPa O<sub>2</sub> treatment reduced decay, both in vitro and in vivo, increased production of fermentative metabolites that impart a negative organoleptic property to the fruit makes this a doubtful alternative for decay control on strawberry. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Controlled atmospheres; *Botrytis cinerea*; Grey mold; *Fragaria × ananassa* Duch

## 1. Introduction

With increasing consumer concern over pesticide residues on foods, along with pathogen resistance to many currently used pesticides, there is a

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need for decay-control alternatives. Grey mold, caused by *Botrytis cinerea* Pers.:Fr., is the most economically significant postharvest pathogen on strawberry fruit, causing up to 50% loss (Garcia et al., 1996). The current method of postharvest decay control for strawberry during storage and transport is low temperature augmented with a modified atmosphere (MA) of 15–20 kPa CO<sub>2</sub> (Mitchell, 1992). However, this CO<sub>2</sub> treatment is only fungistatic, rather than fungicidal. Hence, there is no residual decay protection once the commodity is removed from the atmosphere. Furthermore, atmospheres necessary for decay control are often close to the level of product tolerance. Improper MA can initiate or aggravate physiological disorders; cause irregular fruit ripening, off-flavors and odors; and even increase decay susceptibility (Kader, 1995).

Elevated O<sub>2</sub> atmospheres have been suggested as a viable decay control alternative to pesticides, as well as an improvement over the traditional MA treatments that use elevated CO<sub>2</sub> or reduced O<sub>2</sub> (Day, 1996). Day (1996) proposed that any level of elevated O<sub>2</sub> above ambient (21 kPa) would reduce decay, as anaerobes grow best under very low O<sub>2</sub> levels and aerobes grow best under atmospheric O<sub>2</sub>. Moreover, this technology has been thought to prevent anaerobic fermentation of the commodity in storage, which should prevent the development of off-flavors and odors due to fermentation.

While there have been several classic studies on the effects of elevated O<sub>2</sub> on ripening parameters, such as respiration rate, ethylene production and color formation on plums (Claypool and Allen, 1951), avocados (Biale, 1946) and potatoes (Frenkel and Garrison, 1976), none have focused on commodity decay control. Several studies have focused on the effects of elevated O<sub>2</sub> on the pathogens themselves, but these have been few and variable in their results (Caldwell, 1965; Robb, 1966; Amanatidou et al., 1999).

Caldwell (1965) completely suppressed the growth of bacteria and fungi by exposing them to 10-atm O<sub>2</sub>. Once returned to air, the bacteria resumed growth immediately, with one exception; there was a delay before the fungi resumed growth. It is noteworthy that this effect may not

have been solely due to elevated O<sub>2</sub> concentrations, but also to the increase in atmospheric pressure. Robb (1966) had similar results with 103 species of fungi, concluding that the delay before resumption of growth in air generally increased with increasing exposure times.

Amanatidou et al. (1999) reported on the effects of elevated O<sub>2</sub> and CO<sub>2</sub> on the surface growth of various pathogens associated with vegetables. Generally, exposure to 80–90 kPa O<sub>2</sub> alone did not strongly inhibit microbial growth, but did cause a reduction in the growth rate of some of the microorganisms tested (i.e. *Escherichia coli* and *Salmonella enteritidis*). In contrast, growth of other microorganisms (i.e. *Listeria monocytogenes* and *Salmonella typhimurium*) was actually stimulated. Carbon dioxide alone at 10–20 kPa was much more effective in reducing the growth of *Pseudomonas fluorescens* and *S. enteritidis*. The combined application of 80–90 kPa O<sub>2</sub> together with either 10–20 kPa CO<sub>2</sub> had an inhibitory effect on the growth of all microorganisms tested and showed stronger and much more consistent inhibition than either gas alone.

Our objectives were to determine the effects of superatmospheric O<sub>2</sub> alone or in combination with elevated CO<sub>2</sub> atmospheres for postharvest decay control on strawberry fruit. Both in vitro and in vivo tests of *B. cinerea* growth were conducted, and the effects on objective fruit quality parameters were determined.

## 2. Methods

### 2.1. Controlled atmospheres

The following controlled atmospheres (CA) were established using micro-metering valves. The CA supplied to each 3.8 l treatment jar was at a constant flow rate of 150 ml min<sup>-1</sup> of the following compositions: air (~21 kPa O<sub>2</sub>, 0.03 kPa CO<sub>2</sub>), 40, 60, 80, 90 and 100 kPa O<sub>2</sub> (balance N<sub>2</sub>), 40 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub> (balance N<sub>2</sub>), and air + 15 kPa CO<sub>2</sub> (18 kPa O<sub>2</sub>, 67 kPa N<sub>2</sub>). All atmospheres were humidified to ~90% RH by bubbling through water. Gas mixtures were moni-

tored daily using rapid gas analyzers (model VIA-510 Infrared CO<sub>2</sub> Analyzer; Horiba Instruments, Irvine, CA & model S-3A O<sub>2</sub> Analyzer; Applied Electrochemistry, Inc., Sunnyvale, CA) and were maintained at  $\pm 2$  kPa for the duration of the experiment.

## 2.2. Mycelial growth *in vitro*

A non-sectoring strain of *B. cinerea* from our laboratory collection was grown on V8 agar (200 ml V8, 20 g agar, 800 ml water) for 3 days under fluorescent light at 23°C. Using a sterile cork borer, 4-mm mycelial plugs were aseptically transferred to the center of fresh potato dextrose agar (Difco Laboratories, Detroit, MI) plates amended with 100 mg/l streptomycin (Sigma, St. Louis, MO) (SPDA). Three replications of six plates each were placed under each CA treatment at 5°C. Mycelial growth (radial colony diameter) was measured immediately after 5, 7 and 14 days of CA treatment, and again after 1 and 3 days at 23°C in air for each treatment period.

## 2.3. Fruit decay and quality

Freshly-harvested 'Camarosa' strawberry fruit were sorted to remove injured berries and to obtain berries of uniform color prior to storage under each CA treatment. Fruit were stored at 5°C for 5 and 14 days. Three replicates of 20 fruit each were stored for each treatment, storage and evaluation time. Fruit quality was assessed prior to the start of the treatments, immediately after removal from the CA treatments and after an additional 2 days in air at 20°C to simulate retail market conditions.

Berry decay was evaluated subjectively and scored as none (no decay), slight (one to three small spots of decay), moderate (one-quarter to one-half of berry decayed) or severe (one-half to full berry rot). External berry color was measured with the Minolta Chromameter (model CR-300; Ramsey, NJ) in CIE L\*a\*b\* mode under CIE Standard Illuminant C. Changes in hue angle ( $h^\circ$ ) were calculated as  $h^\circ = \arctan b^*/a^*$  (deg) (McGuire, 1992). Two readings per fruit were taken on opposite cheeks of the berry for both

color and firmness data. Firmness was measured with a penetrometer (Ametek, Largo, FL), using a 3-mm tip. Ethylene production and respiration (CO<sub>2</sub> production) rates were determined daily. Forty berries for each of three replications were placed in 3.8-l jars under a constant flow of humidified air (150 ml min<sup>-1</sup>). One-millilitre samples of the outlet gas were analyzed for ethylene by gas chromatography using flame ionization detection (model 211; Carle Analytical Gas Chromatograph, Tulsa, OK), and CO<sub>2</sub> was measured with an infrared CO<sub>2</sub> analyzer (model PIR-2000R; Horiba Instruments, Irvine, CA). Juice was extracted from a composite of five berries per replicate for determination of soluble solids (SS), titratable acidity (TA) and volatile compounds. Soluble solids were assessed using a temperature-compensating digital refractometer (Abbe model 10450; American Optical, Buffalo, NY). Titratable acidity was determined by automatic titration and calculated using citric acid to determine acid equivalents (PHM85 Precision, ABU80; Radiometer, Copenhagen, Denmark). Fermentative volatile content was determined by the method of Ke et al. (1991). Five millilitres of juice were incubated at 65°C for 1 h in a septum-capped tube. A 1-ml sample of headspace gas was analyzed for acetaldehyde, ethanol and ethyl acetate concentration using a gas chromatograph (GC-9AM; Shimadzu Scientific Instruments, Columbia, MD) with flame ionization detection (250°C) and a 5% carbowax on 60/80 Carbopack column (Supelco, Bellefonte, PA). Volatiles were quantified by comparison to known standards.

## 3. Results

### 3.1. Mycelial growth *in vitro*

Mycelial radial growth rate decreased with increasing O<sub>2</sub> concentration. After 7 days at 5°C, mycelial growth was significantly reduced in all CA treatments compared to air (Fig. 1). However, the treatments with elevated CO<sub>2</sub>, either alone or in combination with 40 kPa O<sub>2</sub>, were more effective in suppressing mycelial growth, with the combination being most effective. After 7 days under

CA at 5°C plus 1 day in air at 23°C, growth showed a similar pattern among treatments, but overall fungal colony diameter had increased, indicating that none of these treatments provided residual inhibition after removal from CA. After 7 days under CA at 5°C plus 3 days in air at 23°C, all but the two CO<sub>2</sub> treatments had reached the edge of the Petri plates.

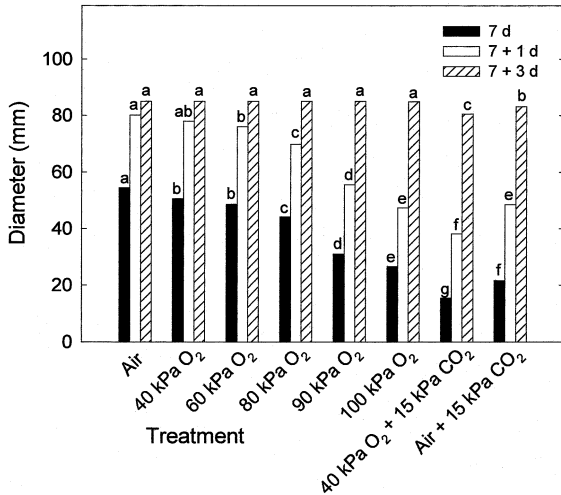


Fig. 1. In vitro mycelial growth of *B. cinerea* under controlled atmosphere treatments after 7 days at 5°C and after another 1 or 3 days in air at 23°C. Each column represents the average of three replications of six plates each. Mean separation within storage period by LSD test. Means within storage period followed by the same letter are not significantly different ( $P \leq 0.05$ ).

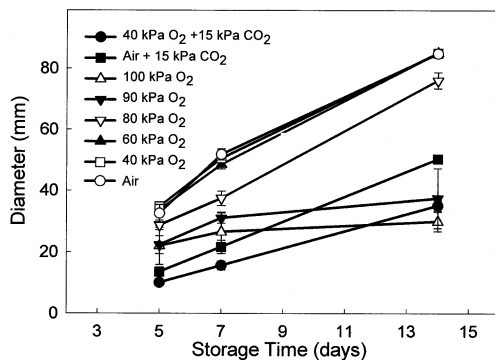


Fig. 2. In vitro mycelial growth of *B. cinerea* under controlled atmosphere treatments after 5, 7 and 14 days at 5°C. Each data point represents the average of three replications of six plates each. Error bars indicate one standard deviation.

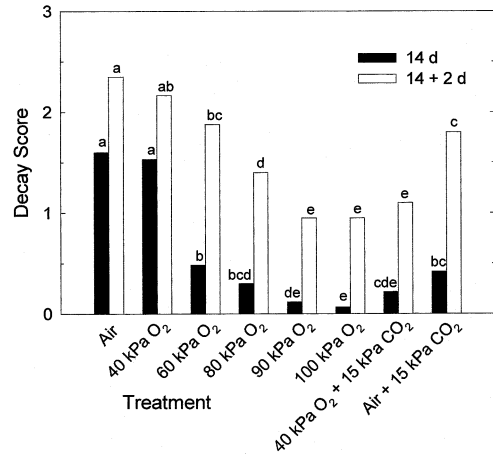


Fig. 3. Decay severity on 'Camarosa' strawberry after 14-days exposure to controlled atmosphere treatments at 5°C, and after an additional 2 days in air at 20°C. Decay score: 0 = none, 1 = slight, 2 = moderate, 3 = severe. Mean separation within the same storage period by LSD test. Means within storage period followed by the same letter are not significantly different ( $P \leq 0.05$ ).

The relative rates of mycelial growth among the CA treatments were similar after 5 and 7 days at 5°C; however, between 7 and 14 days the efficacy of the treatments shifted (Fig. 2). After 14 days, the 100 kPa O<sub>2</sub> treatment had the greatest effect on reducing mycelial growth, surpassing the CO<sub>2</sub> treatments, and the 90 kPa O<sub>2</sub> treatment showed significantly less mycelial growth than the treatment with 15 kPa CO<sub>2</sub> alone.

### 3.2. Fruit decay

After 5 days at 5°C, fruit decay was minimal for strawberries from all treatments and only slight decay was observed after an additional 2 days in air at 20°C (data not shown). After 14 days in the CA treatments, decay was reduced with increasing O<sub>2</sub> concentrations and with 15 kPa CO<sub>2</sub>, except that 40 kPa O<sub>2</sub> was not significantly different from air-stored fruit (Fig. 3). The decay score was lowest for fruit stored under 100 kPa O<sub>2</sub>, although there was no statistical difference in fruit decay between fruit stored under 90 and 100 kPa O<sub>2</sub> or 40 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub>. The differences in fruit decay among the treatments were maintained after an additional 2 days in air

at 20°C, except there was significantly less decay in the 40 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub> treatment as compared to 15 kPa CO<sub>2</sub> alone.

### 3.3. Fruit quality

Berry firmness changed slightly during 14 days of storage. Only fruit held in air or 40 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub> exhibited significantly lower firmness than the initial value (Table 1). Fruit stored in 40 or 80 kPa O<sub>2</sub> were significantly more firm than fruit stored 14 days in air. However, after 14 days at 5°C under CA treatments plus 2 days in air at 20°C, fruit exposed to 40 and 100 kPa O<sub>2</sub>, 40 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub> or Air + 15% kPa CO<sub>2</sub> were firmer than the fruit stored in air (Table 1).

Soluble solids decreased in the fruit exposed for 14 days to 90 and 100 kPa O<sub>2</sub>, and 40 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub> (Table 1). After an additional 2 days in air at 20°C, soluble solids content was significantly lower in all stored fruit, but the soluble solids content was again lowest in fruit exposed to 90 and 100 kPa O<sub>2</sub>, and 40 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub>. Titratable acidity did not differ

significantly among the treatments (data not shown).

After 14 days of storage at 5°C, fruit hue angle was significantly lower, indicating more red color, in fruit stored in air, 60 and 80 kPa O<sub>2</sub> and air + 15 kPa CO<sub>2</sub> as compared to the initial sample (Table 1). Fruit stored in 90 and 100 kPa O<sub>2</sub> had significantly lighter skin color (L value) than the initial or air-stored fruit, while fruit stored in 60 and 80 kPa O<sub>2</sub> were similar to air-stored fruit and significantly darker than the initial fruit (Table 1). However, after 14 d at 5°C plus an additional 2 days in air at 20°C, fruit from all treatments were significantly more red and darker than the initial fruit, except for fruit treated with 40 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub>. Fruit stored in 90 kPa O<sub>2</sub> and 40 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub> remained less red than fruit stored in air, while fruit stored in 60 kPa O<sub>2</sub> were more red (Table 1).

The respiration rate was initially suppressed by the high O<sub>2</sub> treatments; however, between 3 and 5 days of treatment the fruit in the high O<sub>2</sub> treatments began to respire more rapidly (Fig. 4). By 7 days of treatment, fruit from all treatments with ≥ 40 kPa O<sub>2</sub> had a greater respiration rate than

Table 1

Quality of 'Camarosa' strawberries after 14-days exposure to controlled atmosphere treatments at 5°C, and after an additional 2 days in air at 20°C

Treatment	Firmness (N) <sup>a</sup>		Soluble solids (%) <sup>b</sup>		Hue angle (h°) <sup>a,c</sup>		Lightness (L*) <sup>d</sup>	
	Days							
	14	14+2	14	14+2	14	14+2	14	14+2
Initial	3.7 ab <sup>d</sup>	3.7 ab	8.2 a	8.2 a	26.6 ab	26.6 a	35.9 cd	35.9 a
Air	3.4 cd	3.0 d	8.2 a	7.7 b	24.8 cd	23.4 de	34.9 e	33.3 d
40 kPa O <sub>2</sub>	3.8 a	3.5 abc	8.1 a	7.3 c	25.6 bc	24.6 bcd	35.2 de	34.8 b
60 kPa O <sub>2</sub>	3.5 abc	3.0 d	8.3 a	7.7 b	23.9 d	21.4 f	34.8 e	32.8 d
80 kPa O <sub>2</sub>	3.8 a	3.1 d	8.3 a	7.8 b	24.0 d	22.3 ef	34.5 e	33.5 cd
90 kPa O <sub>2</sub>	3.7 abc	3.3 cd	7.3 b	7.0 d	27.1 a	25.0 bc	37.7 a	34.6 b
100 kPa O <sub>2</sub>	3.4 bcd	3.4 bc	7.1 b	7.0 d	26.3 ab	24.2 bcd	37.2 ab	34.2 bc
40 kPa O <sub>2</sub> + 15 kPa CO <sub>2</sub>	3.2 d	3.5 abc	7.2 b	7.0 d	26.7 ab	25.4 ab	36.8 bc	35.0 ab
Air + 15 kPa CO <sub>2</sub>	3.7 abc	3.8 a	8.3 a	7.9 b	24.9 cd	23.6 cd	35.2 de	34.3 bc
LSD	0.3	0.4	0.3	0.3	1.3	1.4	0.9	0.9

<sup>a</sup> Average of 60 berries.

<sup>b</sup> Average of 12 samples of five berries each.

<sup>c</sup> Angle attributed to colors classed as red (0°), yellow (90°), green (180°), blue (270°), or an intermediate between any adjacent pair of these colors.

<sup>d</sup> Mean separation by LSD within columns,  $P \leq 0.05$ .

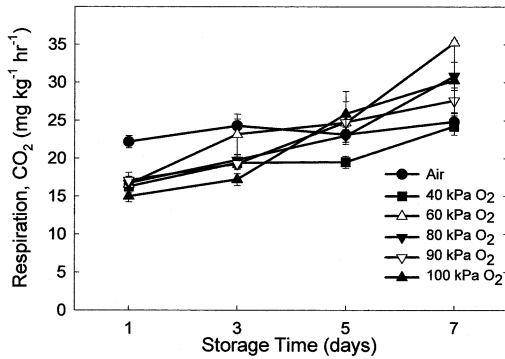


Fig. 4. Respiration rate of 'Camarosa' strawberry under air and elevated O<sub>2</sub> atmospheres during the first 7 days of treatment. Each data point represents the average CO<sub>2</sub> concentration from three jars of 40 fruit. Error bars indicate one standard error.

fruit stored in air. After 7 days, fungal growth began to interfere with the respiration measurements and they were terminated. The respiration rate of fruit treated with high CO<sub>2</sub> was not measured. Ethylene production did not differ significantly among treatments (data not shown).

After 5 days of exposure to the treatment atmospheres, there was a minimal effect on fruit acetaldehyde, ethanol or ethyl acetate content (data not shown). After 14 days of exposure, volatile content significantly increased in fruit treated with  $\geq 60$  kPa O<sub>2</sub> over that found in air-stored fruit (Fig. 5). For acetaldehyde, the combination of 40 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub> did not differ significantly from air, while the 15 kPa CO<sub>2</sub> alone was significantly higher than all other treatments (Fig. 5A). After a 14-days exposure at 5°C plus 2 days in air at 20°C, the same patterns were observed with fruit from the O<sub>2</sub> treatments and the concentrations did not change greatly. However, the acetaldehyde concentration in the air + 15 kPa CO<sub>2</sub> treatment had declined by 67%, and was similar to that of fruit from the O<sub>2</sub> treatments (Fig. 5A).

After 14 days at 5°C, ethanol concentrations were significantly higher for all fruit treated with  $\geq 60$  kPa O<sub>2</sub> and for fruit from both of the CO<sub>2</sub> treatments as compared to the air-stored fruit (Fig. 5B). As with acetaldehyde, the ethanol content of fruit from the air + 15 kPa CO<sub>2</sub> treatment was higher than that of fruit from the other

treatments after 14 days of treatment, but declined by almost half during 2 days in air at 20°C. Yet, unlike acetaldehyde, the ethanol content of fruit treated with O<sub>2</sub> concentrations  $\geq 60$  kPa continued to increase during 2 days in air at 20°C, more than doubling in the 90 and 100 kPa O<sub>2</sub> treatments. The ethanol content of the combination treatment of 40 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub> was similar to the high O<sub>2</sub> treatments after 14 days, but remained at the same level after 2 d in air, rather than increasing like the O<sub>2</sub> treatments or decreasing like the 15 kPa CO<sub>2</sub> treatment.

Fruit treated with  $\geq 60$  kPa O<sub>2</sub> had a significantly higher ethyl acetate concentration than air-stored fruit after 14 days, yet both CO<sub>2</sub> treatments

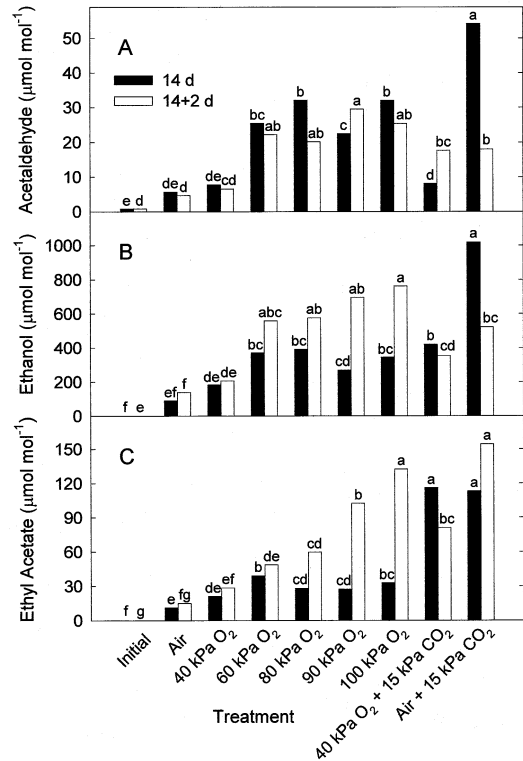


Fig. 5. Concentration of fermentative metabolites (A, acetaldehyde; B, ethanol; C, ethyl acetate) of 'Camarosa' strawberry after 14 days exposure to controlled atmosphere treatments at 5°C and after another 2 days in air at 20°C. Each column is the average of 12 samples of five berries each. Mean separation within the same storage period by LSD test. Means within storage period followed by the same letter are not significantly different ( $P \leq 0.05$ ).

were significantly higher than the O<sub>2</sub> treatments (Fig. 5C). During the 2 days in air at 20°C after treatment, ethyl acetate concentration continued to increase in fruit stored in high O<sub>2</sub> and increased more with increasing O<sub>2</sub> concentration ( $\geq 60$  kPa O<sub>2</sub>). After 2 days in air, ethyl acetate content in fruit stored in 40 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub> decreased, while it increased in fruit stored in air + 15 kPa CO<sub>2</sub>.

## 4. Discussion

### 4.1. Mycelial growth in vitro

Mycelial growth rate decreased with increasing O<sub>2</sub> levels. However, the treatments with 15 kPa CO<sub>2</sub>, either alone or in combination, were the most effective inhibitors of mycelial growth. Upon removal from the atmospheres, there was no residual inhibition. This lack of residual inhibition was expected for the CO<sub>2</sub> treatments, as CO<sub>2</sub> is generally known to be fungistatic rather than fungicidal. However, contrary to our findings with high O<sub>2</sub> atmospheres, Robb (1966) found that *B. cinerea* exposed to 10-atm pressure of O<sub>2</sub> did not resume growth upon removal from the atmosphere, indicating the high O<sub>2</sub> may have been fungicidal. Furthermore, Caldwell (1965) found that treatment of bacteria and fungi with 10-atm O<sub>2</sub> suppressed their growth completely both during and after exposure to the atmosphere. The difference between our results and those of Robb and of Caldwell was most likely due to the 10-atm pressure used in the earlier studies or the use of closed systems that may have accumulated inhibitory levels of CO<sub>2</sub>.

That the efficacy of the 100 kPa O<sub>2</sub> treatment surpassed that of the CO<sub>2</sub> treatments between 7 and 14 days of exposure at 5°C indicates that there may be an effect of exposure time on the activity of high O<sub>2</sub> atmospheres. This is in accordance with the findings of Robb (1966) that the lag time for resumption of fungal growth generally increased with increasing exposure times to elevated O<sub>2</sub>. This phenomenon deserves further investigation.

### 4.2. Fruit decay

The effect of high O<sub>2</sub> atmospheres on strawberry fruit decay showed a similar pattern to the in vitro mycelial growth. After 14 days at 5°C, the 100 kPa O<sub>2</sub> treatment was the most effective in inhibiting fruit decay and mycelial growth, and both 90 and 100 kPa O<sub>2</sub> were more effective against fruit decay than the standard 15 kPa CO<sub>2</sub> treatment. However, there was a much greater difference between the efficacy of 40 and 60 kPa O<sub>2</sub> on fruit decay than on in vitro mycelial growth. The higher O<sub>2</sub> may have had additional effects on the fruit as well as on the pathogen resulting in enhanced decay control.

No other studies have examined elevated O<sub>2</sub> as a decay control measure on fresh produce, except for a preliminary experiment on grapefruit by Kader and Ben-Yehoshua (2000). They investigated the effects of various concentrations of O<sub>2</sub> with and without 15 kPa CO<sub>2</sub> on the natural incidence of grapefruit decay, which was mostly caused by *Penicillium digitatum*. An effective reduction of decay occurred with 80 kPa O<sub>2</sub> and its combination with 15 kPa CO<sub>2</sub>, but not with 40 kPa O<sub>2</sub>, or its combination with 15 kPa CO<sub>2</sub>. It is interesting that 100 kPa O<sub>2</sub>, which was the most effective treatment in our study, actually enhanced *Penicillium* decay on grapefruit. The differing results in these studies could be due to the use of different pathogens, to differing substrate availability to the pathogens, or to differences in commodity response to high O<sub>2</sub>.

### 4.3. Fruit quality

The fruit treated with 40, 80, 90 and 100 kPa O<sub>2</sub> and 15 kPa CO<sub>2</sub> tended to be more firm with less red color development after 14 days of cold storage. These data indicate a reduction in fruit ripening rate. Storage in 15 kPa CO<sub>2</sub> has been shown to slow fruit ripening (Kader, 1995). However, others have found that elevated O<sub>2</sub> atmospheres actually accelerate ripening and color development, as well as ethylene production and respiration in commodities such as plums (Claypool and Allen, 1951), avocado (Biale, 1946) and oranges (Aharoni and Houck, 1980). Our data

indicate higher respiration rates in the fruit treated with high O<sub>2</sub>, and this may be the reason for the lower soluble solids content in fruit treated with 90 and 100 kPa O<sub>2</sub>. These data indicate that very high O<sub>2</sub> atmospheres caused a stress response in the strawberry fruit.

The pattern of accumulation of fermentative metabolites in strawberries during and after exposure to elevated O<sub>2</sub> atmospheres was often different than that observed in fruit exposed to 15 kPa CO<sub>2</sub>. In all cases, the 15 kPa CO<sub>2</sub> treatment showed significantly higher volatile concentrations compared to the rest of the treatments after 14 days under the atmospheres. This is in agreement with the hypothesis of Day (1996) that under high O<sub>2</sub> atmospheres there would be less fermentative metabolites than under high CO<sub>2</sub>. However, after returning to air, the high O<sub>2</sub> treatments continued to accumulate fermentative metabolites while the levels in high CO<sub>2</sub> treatments declined. This decrease in fermentative volatiles after removal from elevated CO<sub>2</sub> treatments has been well documented by others (Saltveit and Ballinger, 1983; DePooter et al., 1987; Mattheis et al., 1991; Ahumada et al., 1996). Aldehydes are reduced to ethanol. This ethanol can be lost via evaporation and can also be further metabolized into organic acids, amino acids, lipids and carbohydrates (Mattheis et al., 1991).

There are several possible explanations for the continued increase in ethanol content in fruit from the elevated O<sub>2</sub> treatments 2 days after removal from the atmospheres. First, the cells may have adjusted to the higher O<sub>2</sub> concentration and, upon removal, the ambient environment was seen as anaerobic. Secondly, some of the fruit cells may have died as a result of the high O<sub>2</sub> stress, though we did not observe any tissue browning or other evidence of necrosis. The most accepted explanation for oxygen toxicity is the formation of superoxide radicals (O<sub>2</sub><sup>-</sup>), which are destructive to some aspects of cell metabolism (Fridovitch, 1975). Lastly, several studies have shown that under elevated O<sub>2</sub>, cells shift to the alternate oxidase respiratory pathway (Rychter et al., 1978; Theologis and Laties, 1982). This pathway can be induced by exposure

to environmental stresses and by ripening. Since electron transport through this path does not develop a proton gradient across the membrane, it is nonphosphorylating. Therefore, electron transport through this path is not restricted by the availability of ADP and can occur with high levels of ATP (Purvis, 1997). Upon transfer of the fruit to air from the high O<sub>2</sub> atmospheres and return to the cytochrome pathway, decarboxylation of pyruvate to acetaldehyde occurs. As acetaldehyde is further reduced to ethanol, this could explain the decrease in acetaldehyde concentration in most of the high O<sub>2</sub> treatments and an increase in ethanol concentration after 2 days in air.

## 5. Conclusion

While these treatments have shown some promise for decay control, the practicality of implementation must be addressed. It appears that the O<sub>2</sub> atmospheres that are most effective for decay control are those close to 100 kPa or those in combination with CO<sub>2</sub>. Near 100 kPa O<sub>2</sub> atmospheres could be difficult to maintain either in a package or on a larger scale, as well as perilous in a commercial situation due to flammability. Furthermore, the decay control afforded by high O<sub>2</sub> was not greatly superior to the currently used treatment of 15–20 kPa CO<sub>2</sub> in air. Moreover, another benefit originally expected from these high O<sub>2</sub> atmospheres was decay control without off-flavor development. We have found that these treatments actually enhanced fermentative metabolites upon removal from the atmospheres. For strawberry, our results indicate that the benefits of high O<sub>2</sub> for control of *B. cinerea* are minor and outweighed by the detrimental effects on flavor quality.

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## References

- Aharoni, Y., Houck, L.G., 1980. Improvement of internal color of oranges stored in oxygen-enriched atmospheres. *Sci. Hort.* 13, 331–338.
- Ahumada, M.H., Mitcham, E.J., Moore, D.G., 1996. Postharvest quality of 'Thompson Seedless' grapes after insecticidal controlled-atmosphere treatments. *HortScience* 31, 833–836.
- Amanatidou, A., Smid, E.J., Gorris, L.G.M., 1999. Effect of elevated oxygen and carbon dioxide on the surface growth of vegetable associated microorganisms. *J. Appl. Microbiol.* 86, 429–438.
- Biale, J.B., 1946. Effect of oxygen concentration on respiration on the Fuerte avocado fruit. *Am. J. Bot.* 33, 363–373.
- Caldwell, J., 1965. Effects of high partial pressures of oxygen on fungi and bacteria. *Nature* 206, 321–323.
- Claypool, L.L., Allen, F.W., 1951. The influence of temperature and oxygen level on the respiration and ripening of Wickson plums. *Hilgardia* 21, 129–160.
- Day, B.P.F., 1996. High oxygen modified atmosphere packaging for fresh prepared produce. *Postharvest News Inform.* 7, 31N–34N.
- DePooter, H.L., VanAcker, M.R., Schamp, N.M., 1987. Aldehyde metabolism and the aroma quality of stored 'Golden Delicious' apples. *Phytochemistry* 26, 89–92.
- Frenkel, C., Garrison, S.A., 1976. Initiation of lycopene synthesis in the tomato mutant rin as influenced by oxygen and ethylene interactions. *HortScience* 11, 20–21.
- Fridovitch, I., 1975. Superoxide dismutase. *Annu. Rev. Biochem.* 44, 147–159.
- Garcia, J.M., Aguilera, C., Jimenez, A.M., 1996. Gray mold in and quality of strawberry fruit following postharvest heat treatment. *HortScience* 31, 255–257.
- Kader, A.A., 1995. Regulation of fruit physiology by controlled/modified atmospheres. *Acta Hort.* 398, 59–70.
- Kader, A.A., Ben-Yehoshua, S., 2000. Effects of superatmospheric oxygen levels on postharvest physiology and quality of fresh fruits and vegetables. *Postharvest Biol. Technol.* 20, 1–13.
- Ke, D., Rodriguez-Sinobas, L., Kader, A.A., 1991. Physiological responses and quality attributes of peaches in low oxygen atmospheres. *Sci. Hort.* 47, 295–303.
- Mattheis, J.P., Buchanan, D.A., Fellman, J.K., 1991. Change in apple fruit volatiles after storage in atmospheres inducing anaerobic metabolism. *J. Agric. Food Chem.* 39, 1602–1605.
- McGuire, R.G., 1992. Reporting of objective color measurements. *HortScience* 27, 1254–1255.
- Mitchell, F.G., 1992. Postharvest handling systems: small fruits. In: Kader, A.A. (Ed.), *Postharvest Technology of Horticultural Crops*. Publication 3311, University of California Division of Agriculture and Natural Resources, Oakland, CA, pp. 223–231.
- Purvis, A.C., 1997. The role of adaptive enzymes in carbohydrate oxidation by stressed and senescing plant tissues. *HortScience* 32, 1165–1168.
- Robb, S.M., 1966. Reactions of fungi to exposure to 10 atmospheres pressure of oxygen. *J. Gen. Microbiol.* 45, 17–29.
- Rychter, A., Janes, H.W., Frenkel, C., 1978. Cyanide-resistant respiration in freshly cut potato slices. *Plant Physiol.* 61, 667–668.
- Saltveit, M.E., Ballinger, W.E., 1983. Effects of anaerobic nitrogen and carbon dioxide atmospheres on ethanol production and postharvest quality of 'Carlos' grapes. *J. Am. Soc. Hort. Sci.* 108, 462–465.
- Theologis, A., Laties, G.G., 1982. Selective enhancement of alternative pathway capacity in plant storage organs in response to ethylene plus oxygen: a comparative study. *Plant Physiol.* 69, 1036–1039.