Effects of Short-term Exposure to Low O₂ and High CO₂ Atmospheres on Quality Attributes of Strawberries

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ABSTRACT

Strawberries (Fragaria ananassa Duch., cv. 'Selva') were stored 10 days in 1.0%, 0.5%, or 0.25% O₂ or air + 20% CO₂ or 6 days in air + 50% or 80% CO₂ at 0 or 5°C without detrimental effects on quality. Decay and softening were reduced by treatments. An untrained taste panel, under ordinary eating conditions, did not consistently differentiate 'Pajaro' strawberries kept in 0.25% O₂ from those stored in air. A trained taste panel, under controlled conditions, perceived slight off-flavor in 'G3' strawberries kept in 0.25% or 0% O₂. This correlated with ethanol, ethyl acetate, and acetaldehyde in juice. The 50% or 80% CO₂ treatments caused injury after 8 to 10 days, while 20% CO₂ treatments did not. All high CO₂ treatments caused increase in pH of juice.

INTRODUCTION

SHORT-TERM EXPOSURES to controlled atmospheres (CA) with very low O₂ and/or very high CO₂ levels have been studied for postharvest insect control to meet quarantine requirements for various commodities including strawberries (Aharoni et al., 1979), apples (Lidster et al., 1981, 1984), and dried fruits (Soderstrom et al., 1986). Exposures to O₂ levels between 0.5% and 1.0% or CO₂ levels between 5% and 20% were reported to have some fungistatic effects on pathogens of strawberries (Couey et al., 1966; Couey and Wells 1970; El-Kazzaz et al., 1985; Harris and Harvey, 1973; Harvey, 1982; Harvey et al., 1966, 1968; Prasad and Stadelbacher, 1974; Shaw, 1969; Smith, 1957; Sommer et al., 1973; Wells, 1970; Woodward and Topping, 1972).

Storing strawberries in low O₂ and/or high CO₂ atmospheres reduced rates of respiration (Li and Kader, 1989; Siriphanich, 1980; Tomalin and Robinson, 1971; Woodward and Topping, 1972) and ethylene production (El-Kazzaz et al., 1983; Li and Kader, 1989; Siriphanich, 1980). Several researchers (Li and Kader, 1989; Prasad and Stadelbacher, 1974; Shaw, 1969; Smith, 1957; Woodward and Topping, 1972) reported the accumulation of certain volatile compounds in strawberries treated with low O₂ and/or high CO₂. El-Kazzaz et al. (1983) showed that strawberries stored in CA conditions were firmer than those stored in air. Siriphanich (1980) reported that soluble solids content and pH of strawberries treated with 20% CO₂-enriched air were higher than those kept in air. However, El-Kazzaz et al. (1983) did not find significant differences in soluble solids and titratable acidity between fruits treated with CA (air + 15% CO₂ or 2.3% O₂ + 5% CO₂) and those in air storage.

Postharvest insect control requires exposures to very low O₂ and/or very high CO₂ concentrations. Thus, it was necessary to investigate the tolerance of fresh commodities to short-term exposures to such stress conditions. CA treatments can be used for postharvest quarantine procedures only when they can destroy the insects of concern without detrimental effects on quality attributes of the fresh commodities. The objective of our research, was to determine the effects of short-term exposures to O₂ levels at or below 1% or CO₂ levels at or above 20% on quality attributes of strawberries.

MATERIALS & METHODS

Materials and handling

Fruits of 'Selva', 'Pajaro', and 'G3' strawberries were obtained on the day of harvest from commercial shippers in Watsonville, Calif. and transported in an air-conditioned auto to our laboratory at Davis where they were kept overnight at 0°C. Damaged and nonuniform fruits were removed and good fruits were matched by color to remove unripe and overripe fruits. Twenty selected fruits were placed in a 2-L glass jar as one replicate and three replicates were used per treatment (with the exception that in taste panel experiments, more jars were used so that enough fruits were available for sensory evaluation). The jars were placed in a 0 or 5°C room and ventilated with humidified air or other gas mixtures at a continuous 55 or 83 mL/min, respectively. Flow boards and capillary tubing were used for flow control.

CA treatments

For one set of experiments, fruit samples were kept in air and in 1.0%, 0.5%, 0.25%, or 0% O₂ (balance was nitrogen). In another set of experiments, fruits were kept in air and in 20%, 50%, or 80% CO₂ (balance was air). The fruit samples were kept in these atmospheres 3 to 10 days at 0 or 5°C before they were used for final evaluation. The required O₂ or CO₂ concentrations of all gas mixtures were verified each day by removing a 10-mL gas sample and analyzing it by a gas chromatograph (GC) equipped with a thermal conductivity detector.

Objective evaluation of quality attributes

Three initial samples of 10 fruits each were evaluated for skin and flesh color, firmness, soluble solids content, pH, titratable acidity, and volatile contents. Similar evaluations were made after 4, 6, 8, and 10 days under the various storage conditions. Color was measured with a Gardner XL-23 Tristimulus colorimeter, using the "a", "b", and "L" values. A greater "a" value indicated a redder color, a smaller "b" value indicated a bluer color, and a lower "L" value indicated a darker color. Firmness was measured as penetration force with a U.C. fruit firmness tester, using a 1.5 mm plunger tip. Fruit juice was extracted with a hand-press juicer. Soluble solids content of the juice was measured with an Abbe refractometer. pH and titratable acidity were measured with an automatic titrator with a PHM85 precision pH meter, an ABU80 autoburette, a PRS12 Alpha printer, and a SAC80 sample changer. Contents of volatiles (such as acetaldehyde, ethanol, ethyl acetate, and methanol) were measured by injection of diluted and filtered juice samples into a HP5880A GC with a flame ionization detector (at 250°C) and a glass column (2 mm x 1.0m) containing 5% Carbowax on 60/80 Carbopack as stationary phase (at 85°C).

Untrained taste panel evaluation

About 100 untrained judges (about half male and half female, age 18 to 40 years) were selected at random to test whether the 'Pajaro' strawberries treated with 0.25% O₂ and those exposed to air at 5°C could be distinguished by sensory evaluation. Comparisons were made with some groups of the judges after 3 to 6 days of storage. Judges made evaluations in a conference room under ordinary eating conditions. The judges performed dual standard sensory difference tests, modified to incorporate preference judgments. The dual standard test
required the judge to taste two standard stimuli (A and B) and then match two unknown stimuli (one A and one B) to them by tasting. If a judge could match the stimuli (A to A and B to B), then they had performed the test correctly. The number of judges in the group who performed the test correctly was noted to determine whether judges were performing above chance levels.

'Pejaro' strawberries were presented in 4 oz plastic cups from which judges sampled strawberries by hand. Cups holding the standards contained three strawberries each, while the test sample cups held two strawberries each. Each cup was coded with a three digit random number. The two standards were presented to the left and to the right of the judge, while the two test samples to be matched were presented in the center, one near and one further from the judge. The positioning of each standard and test sample was randomized. Judges sampled strawberries (by chewing and swallowing) from the two standard cups first and tried to determine any difference between the two. Next, they sampled the test strawberries and attempted to match them to the standards. Having matched the test strawberries to the standards, the judge then recorded which of the standards was preferred. The judge reported in writing by indicating the code numbers of samples in appropriate places on a response sheet. Judgements were made under ambient, fluorescent lighting. Water was available for rinsing between tastings as desired.

### Trained taste panel evaluation

Four trained judges (2 male, 2 female, age 21 to 41 years) assessed 'G3' strawberries that had been held in air and in 0.25% or 0% O2. They were selected on the basis of their performance; the judges had performed two prior experiments and could recognize and were familiar with the low O2-induced off-flavor. As a check, each judge was required to perform six triangle tests without error to ensure that they could distinguish the 0% O2-treated strawberries from the control. The judges were then given a category scale ranging from 0 (no off-flavor) to 10 (strong off-flavor). The strength of the off-flavor they had perceived in the difference test was arbitrarily given a value of 2. The limitation of numerical estimation for category scales was noted.

The 'G3' strawberries had been held in air and in 0.25% or 0% O2 at 2°C for 3 to 7 days. The strawberries were presented in 4 oz plastic cups from which judges sampled strawberries by hand. Each cup was coded with a three digit random number. The judges tasted in random order, three strawberries from each of the air control, 0.25% and 0% O2 treatments, without knowing their identities. The judges rated the degree of off-flavor of each on the category scale. The tasting was performed under red light to mask visual cues. Judges rinsed ad-lib between tastings developing their own compromise between reduction of orientation effects (by cleaning the mouth of residual strawberry samples) and forgetting flavors of prior samples. Volatile contents in the juice of strawberries from each treatment were measured by GC.

### Estimation of CO2 injury

CO2 injury was visually estimated using the following subjective scale for evaluating fresh color of fruit longitudinal sections: 1 = no injury, red flesh; 2 = slight injury, slightly blue-red flesh; 3 = moderate injury, slightly dark-blue red flesh; 4 = severe injury, dark blue-red flesh; 5 = very severe injury, very dark blue-red and water-soaked flesh. Again, the limitation of this scale was noted. Three samples of 10 fruits each were used in each treatment for this evaluation. The judgments were under ambient, fluorescent lighting by the principal investigator who had prior experience in CO2 injury. A photograph showing symptoms of slight to severe CO2 injury of strawberries was used as reference for the judgements.

### Statistical analysis

Data were treated for multiple comparisons by analysis of variance with least significant difference (LSD) between means determined at the 5% level. Binomial analysis was used to compare ratios in the dual standard sensory difference tests. A computer curve fit program (Cricket Graph, Macinosh SE, Apple Computer, Inc., Cupertino, Calif.) was used to analyze the regression and correlation between contents of volatile compounds and off-flavor scores. For the sensory evaluations, additional nonparametric ranked tests (O'Mahony, 1986) were also done, when appropriate.

### Table 1—Effects of reduced O2 or elevated CO2 concentrations on some quality and physical attributes of 'Selva' strawberries after 10 days storage

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>CA composition decay (%)</th>
<th>Skin color (CDM &quot;a&quot; value)</th>
<th>Flesh color (CDM &quot;a&quot; value)</th>
<th>Firmness (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Air</td>
<td>4</td>
<td>48</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>1.0% O2</td>
<td>3</td>
<td>47</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>0.5% O2</td>
<td>4</td>
<td>47</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>0.25% O2</td>
<td>0</td>
<td>46</td>
<td>44</td>
</tr>
<tr>
<td>5</td>
<td>Air</td>
<td>23</td>
<td>48</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>1.0% O2</td>
<td>13</td>
<td>46</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>0.5% O2</td>
<td>10</td>
<td>46</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>0.25% O2</td>
<td>9</td>
<td>47</td>
<td>44</td>
</tr>
<tr>
<td>LSD at 5%</td>
<td></td>
<td>6</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

*CDM = Color Difference Meter; greater "a" value indicates more red color.

### Table 2—Number of judges who distinguished 'Pejaro' strawberries stored in air from those kept in 0.25% O2 at 5°C (dual standard test) and treatments preferred (paired preference test)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of judges</td>
<td>103</td>
<td>104</td>
<td>104</td>
<td>104</td>
</tr>
<tr>
<td>Number that could distinguish the two treatments</td>
<td>62</td>
<td>61</td>
<td>62</td>
<td>67</td>
</tr>
</tbody>
</table>

### RESULTS & DISCUSSION

#### Effects of exposure to reduced O2 atmospheres

One important effect of keeping 'Selva' strawberries in low O2 atmospheres was a reduction in decay incidence (Table 1). The effect of 0.25% O2 was more pronounced. Generally, decay incidence was higher on fruits kept at 5°C than on those stored at 0°C, especially with the air control strawberries. Decay is an important problem during the storage and transport of strawberries. Therefore, a reduction in decay incidence by CA treatments would be very beneficial. Couey et al. (1966) and Couey and Wells (1970) also found that low O2 could control postharvest decay of strawberries.

Strawberries are nonclimacteric fruits and must have at least 75% of their surface showing red color at harvest time. The red color development after harvest is slight while loss in green color continues. Keeping 'Selva' strawberries in 1.0%, 0.5%, or 0.25% O2 had a slight or no effect on skin and flesh red color development (Table 1). These treatments had more pronounced effects on retarding softening. The low O2 treatments did not significantly affect soluble solids content, pH, or titratable acidity (data not shown). No visual injury was observed in strawberries treated with the low O2 atmospheres for up to 10 days storage.

An untrained taste panel evaluation was performed to test...
whether the 'Pajaro' strawberries kept in 0.25% O$_2$ and those kept in air at 5°C could be distinguished by sensory judgement. After 3 to 6 days storage, about 60% of the untrained judges in the groups could match the two treatments (Table 2). Binomial tests indicated that after 3, 4, 5 and 6 days, the majority was significant at $p = 0.049, 0.09, 0.06$, and 0.003 respectively. Of the judges who could distinguish the two treatments, the preference was split equally until the last day when the proportion of judges who preferred the air treatment was a significant majority over those who preferred the low O$_2$ treatment or had no preference (binomial $p = 0.002$). Patterns were similar for the total group of judges and for the judges who could not distinguish the two treatments. Note that, because only one dual standard test was given to each judge, it was difficult to assess which individuals were true discriminators and thus indicate only their preferences. Data from those who evaluated the dual standard test correctly and from the total number of judges gave the best estimates of preference and in both cases the trend was evident.

On the last day when the effect was strongest, 52% of the judges who preferred the air treatment said they did so because of an off-flavor in the low O$_2$-treated fruits; while 70% of the judges who preferred the low O$_2$ treatment said they did so because those strawberries had a sweeter taste. These results indicate that preference for the two treatments varied with judges. For the fruits stored in air and those kept in 0.25% O$_2$, the nature of any slight differences in sensory characteristics that were detected in the dual standard tests were probably not easy to describe.

In order to create a stronger off-flavor, lower O$_2$ levels (0.25% to 0%) were used in a subsequent experiment. A trained taste panel consisting of four judges was selected to estimate the degree of off-flavor in 'G3' strawberries kept in air, 0.25% O$_2$, or 0% O$_2$ for 3 to 7 days at 2°C. A category scale ranging from 0 (no off-flavor) to 10 (strong off-flavor) was used. The mean off-flavor scores of the strawberries stored in air, 0.25% O$_2$, or 0% O$_2$ for 3 to 7 days ranged from 0.3 to 0.6, 0.6 to 2.6, and 1.9 to 3.0, respectively (Table 3). Additional non-parametric ranked testing (Newell and Macfarlane, 1987) was done to justify that there were significant differences among the off-flavor scores of the fruits from the three treatments (data not shown).

The research by Shaw (1969) suggested that accumulation of volatile compounds might be responsible for off-flavor development in strawberries. In our study, the correlations between estimated off-flavor scores and measured volatile contents in the fruit juice were analyzed (Fig. 1) to determine which volatile compounds might be associated with development of off-flavor. A computer curve fit program (including linear, polynomial, logarithmic, and exponential regressions) was used for analysis and the best curve fits (i.e., reasonable and logical correlations with the highest $r$ values) were obtained from the logarithmic regression. The correlation between ethanol content and off-flavor score was best ($r = 0.966, p < 0.001$); the correlation between ethyl acetate content and off-flavor score ($r = 0.904, p < 0.001$) and that between acetaldehyde content and off-flavor score ($r = 0.899, p < 0.001$) were also very good; although correlation between methanol content and off-flavor score was statistically significant ($r = 0.723, p < 0.01$) due to the relatively large number of data points (higher degree of freedom), the coefficient of determination ($R^2 = 0.523$) indicated that only half of the variance in the flavor score could be attributed to variance in methanol concentration. Similar correlation coefficients were obtained by using Spearman's ranked correlation analysis (data not shown). The contents of other volatiles (isopropyl acetate, 2-pentanone, propyl acetate, isobutyl acetate, methyl 2-methylbutyrate, ethyl butyrate, and n-butyl acetate) were very low or not detected with the GC method used and their correlations with off-flavor scores were not analyzed.

The estimated off-flavor scores varied considerably among the judges and among the fruits within the same treatment. This could be due to the subjective nature of the category scale used. From the regression formula, the concentrations of volatiles corresponding to an off-flavor score of 1.5 (the value of LSD at 5%) were 8.1, 23, 63, and 0.66 μL-1 for acetaldehyde, ethanol, ethyl acetate, and methanol, respectively. While the accumulation of acetaldehyde, ethanol, and ethyl acetate may be responsible, at least in part, for development of off-flavor, methanol probably did not play an important role since its content was much lower and its correlation with off-flavor score was poorer. We should point out that some other unknown volatiles may also contribute to off-flavor development.

While 1.0% or 0.5% O$_2$ did not have significant effects, 0.25% O$_2$ increased ethanol content of 'Selva' strawberries (Fig. 2). The effect of 0.25% O$_2$ was greater at 5°C than at 0°C. The same pattern was observed with acetaldehyde content. The low O$_2$ treatments also had similar effects on ethyl acetate content. For example, after 10 days of storage at 0°C, ethyl acetate contents were 0.7 ± 0.2, 0.6 ± 0.2, 2.6 ± 1.7, and 30.9 ± 5.7 μL-1 for air, 1.0%, 0.5%, and 0.25% O$_2$ treatments, respectively; while at 5°C, the corresponding values were 1.4 ± 0.3, 1.6 ± 0.6, 2.5 ± 1.4, and 66.8 ± 12.0 μL-1, respectively.
mulation of volatile compounds was the direct effect on decay control, of high CO₂ treatments, since that effect was reduced by removal of the CO₂-induced volatiles. Aharoni et al. (1979) found that acetaldehyde, 50% CO₂, or 1% O₂ could control western flower thrips on harvested strawberries.

'Selva' strawberries treated with 50% or 80% CO₂ had much higher ethanol and acetaldehyde contents than those of air control fruits, whereas 20% CO₂ had only a slight effect (Fig. 3). In general, the effects of 50% or 80% CO₂ were greater when the fruits were kept at 5°C than at 0°C. The high CO₂ treatments also increased ethyl acetate content (2 to 15 μL·L⁻¹) over that of air controls (0.3 to 1.2 μL·L⁻¹) after 10 days storage.

Associated with accumulation of volatile compounds, some undesirable odor could be detected from the strawberries treated with 50% or 80% CO₂ at 0 or 5°C for 10 days. In strawberries treated with 50% or 80% CO₂ for 4 to 10 days, ethanol contents were in the range of 220 to 460 μL·L⁻¹ and acetaldehyde contents were in the range of 36 to 120 μL·L⁻¹, respectively (Fig. 3). These values were several times higher than the levels (23 and 8.1 μL·L⁻¹ for ethanol and acetaldehyde, respectively) detected by the taste panel that corresponded to an off-flavor score of 1.5 (the value of LSD at 5%) in the low O₂-treated strawberries (Fig. 1). While the 20% CO₂-treated strawberries tasted very good, the high levels of ethanol and acetaldehyde contents may have caused off-flavor in the fruits treated with 50% or 80% CO₂. A possible solution for this problem would be to store strawberries in 50% or 80% CO₂ for less than 8 days (before injury occurs). This may be long enough for insect control, and then transfer the fruits to air at 0°C for several days before marketing to consumers. By this approach, the levels of ethanol and acetaldehyde may gradually drop during the holding period in air and at consumption the concentrations of the volatile compounds may be below the threshold levels. Li and Kader (1989) showed that if 'Selva' strawberries were stored in high CO₂ for 7 days followed by transfer to air at 2°C for another 7 days, ethanol content dropped to much lower level than that at transfer.

While 20% CO₂ did not significantly affect skin color, 50% or 80% CO₂ slightly reduced the redness of 'Selva' strawberries (Table 1). Fruits treated with 20% CO₂ were firmer and appeared fresher than those exposed to air. After 6 days storage at 0 or 5°C, no visual injury was observed in the strawberries treated with 50% or 80% CO₂, the fruits appeared firmer and were firmer than those kept in air. For example, firmness was 1.1, 1.4, and 1.4 newtons for fruits kept 6 days at 5°C in air, 50% CO₂, and 80% CO₂, respectively. However, after 8 days storage, CO₂ injury was observed in fruits treated with 50% or 80% CO₂ at 0 or 5°C (Table 4). The CO₂ injury in 'Selva' strawberries started with a change in skin and/or flesh color from red to blue-red. In severe cases, the flesh became very dark blue-red and water-soaked. Associated with the injury, the fruits became soft so that after 10 days firmness of the...
strawberries treated with 50% or 80% CO₂ dropped to the same level as, or lower than, that of those exposed to air (Table 1). While 20% CO₂ did not cause visual injury, 50% or 80% CO₂ caused moderate to severe injury in 'Selva' strawberries after 10 days storage at 0 or 5°C (Table 4). Additional nonparametric test (Kruskal-Wallis ranked analysis) also indicated that significant differences existed among treatments, e.g., the injury scores of 50% or 80% CO₂-treated strawberries were significantly higher than those of air control or 20% CO₂-treated fruits.

The high CO₂ treatments did not significantly affect soluble solids content and titratable acidity of 'Selva' strawberries (data not shown) but resulted in higher pH values than those of the air control fruits immediately after they were transferred to air at 20°C for evaluation (Table 4).

Results from our study indicated strawberries could be treated with 1.0%, 0.5%, or 0.25% O₂ (balance nitrogen), or air + 20% CO₂ at 0 or 5°C for 10 days without detrimental effects on their quality attributes. Strawberries could also be treated with 0% O₂ or 50% or 80% CO₂ for up to 6 days without visual injury. If these treatments were followed by transferring the fruits to air at 0°C for several days to reduce ethanol and acetaldehyde levels, there would probably be no detrimental effect on their final sensory quality. Once entomologists have determined which treatments can effectively control specific insects, such treatments may be approved by quarantine authorities for commercial use. In addition, the treatments could effectively control decay and reduce deterioration rate and therefore extend the postharvest life of strawberries for export and domestic consumption.

REFERENCES


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