Firmness and Aroma Composition of Strawberries following Short-term High Carbon Dioxide Treatments

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Abstract. Firmness and aroma composition of strawberry fruit (Fragaria ×ananassa Duch. cv. Pajaro) stored in air or treated with 20% CO₂ for up to 12 days at 0°C were analyzed upon removal from storage. Fruit firmness increased after 2 days in CO₂, while the composition of aroma compounds in the fruit was unaffected at this time. Ethanol and ethyl hexanoate accumulated after 3 days during high CO₂ treatment, but these compounds usually decreased during subsequent cold storage in air. Ethyl butanoate and ethyl acetate also accumulated but continued to increase after 6 and 9 days of CO₂ storage, respectively. This study suggests that treatment of strawberry fruit with CO₂ after harvest, followed by air storage at 0°C, can maintain firmness while minimizing off-flavor development.

Storing strawberries at high CO₂ concentrations (15% to 20%) maintains firmness and reduces decay of the fruit (Harris and Harvey, 1973; Li and Kader, 1989; Plocharski et al., 1978), and pallet shroud systems for transporting fruit under such conditions are available commercially (Mitchell, 1992). Some studies also show that high CO₂ can increase rather than just maintain firmness of strawberries in storage (Plocharski, 1982; Smith, 1992; Smith and Skog, 1992). Residual effects of controlled-atmosphere (CA) storage on fruit quality have been reported; when strawberries were stored for up to 4 days in 20% CO₂ followed by storage in air for a total storage time of 8 days, fruit treated with high CO₂ were firmer than controls, and fruit treated with CO₂ for 3 or 4 days were firmer than fruit treated for 1 or 2 days (Li and Kader, 1989).

High concentrations of CO₂ in the storage atmosphere also favor the accumulation of several volatiles (Guichard et al., 1992; Ke et al., 1991; Li and Kader, 1989; Ueda and Bai, 1993). Some of these volatiles, acetaldehyde, ethyl acetate, and ethanol, correlate with off-flavors (Ke et al., 1991), although Larsen and Watkins (1995) found that off-flavors in CA-stored strawberries were related to increases in ethyl acetate and ethanol concentrations, but not acetaldehyde. Ueda and Bai (1993) studied the effects of short-term CO₂ treatments and found an immediate increase in the production of ethyl acetate and ethyl butanoate associated with an "unnatural" aroma. Guichard et al. (1992) studied the aroma composition of strawberries after storage at high CO₂ concentrations and found that the level of all ethyl esters increased strongly under these conditions. However, the relationships between the various aroma compounds in response to CO₂ are not clear, and yet these may greatly influence the quality of the fruit. For example, ethyl acetate is associated with the formation of an anaerobic off-flavor (Larsen, 1994), but ethyl butanoate and ethyl hexanoate have pleasant, fruity flavors. Our objective was to investigate the effects of high CO₂ treatment on firmness and aroma composition of 'Pajaro' strawberries, either held in this treatment or in subsequent air storage.

Materials and Methods

‘Pajaro’ strawberries were obtained from a commercial grower in the Auckland area. Damaged, unripe (<50% red surface), and overripe fruit were discarded. Fruit were allocated randomly to 1-kg samples (>50 berries) and stored at 0°C in 27-liter chambers, each holding two samples. Precision needle valves were used to mix CO₂ and air to produce a 20% CO₂ atmosphere. Control samples were flushed with air. The gas mixture and air were bubbled through water at 0°C to obtain vapor saturation, and passed through each chamber at 200 ml-min⁻¹. Carbon dioxide concentrations in the chambers were verified by gas chromatography.

Samples were stored for 1, 2, 4, 6, 9, and 12 days. Upon removal from chambers, one sample from each chamber was transferred to air at 20°C and analyzed after 4 h of temperature equilibration. The other sample was transferred to air at 0°C and analyzed after 12 days of storage.

Firmness was assessed on two replicates of eight fruit from each treatment by measuring the maximum shear force required for tissue failure. Horizontal slices 6 mm thick were cut from the broadest part of the fruit, and one-quarter of each of these eight slices was placed in a Kramer shear cell of an Instron model 4301 materials testing machine (Instron, Canton, Mass.) fitted with a 5-kN load cell. The crosshead speed was 100 mm-min⁻¹. Two measurements were carried out per replicate. Firmness values were divided by the tissue weight to correct for differences in the area of tissue bisection by the plates of the shear cell.

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Fig. 1. Firmness (N) of ‘Pajaro’ strawberries stored in air or in (©) 20% CO₂ for up to 12 days.
For analysis of aroma compounds, 300 g of fruit from each sample was homogenized in a Waring blender for 30 sec (low speed). Duplicate 5-g samples of homogenate were transferred to 20-ml vials with septum lids and stored at –20°C. For subsequent analysis, samples were incubated at 40°C for 20 min; then, 1-ml headspace samples (in triplicate) were analyzed at 50°C in a HP5890 gas chromatograph (Hewlett-Packard, Delaware) equipped with a DBWAX column (30 m × 0.33 mm × 1 μm) and a flame ionization detector.

Other strawberry aroma compounds were extracted from duplicate 20-g samples of homogenate with 20 ml diethyl ether and concentrated as described by Larsen and Poll (1990). An internal standard of 400 μl of 50 ppm 4-methyl-1-pentanol solution was added to the homogenate. Concentrated extracts were analyzed by gas chromatography as above using the following temperature program: 50°C, for 5 min; 10°C/min until 200°C; 200°C, for 25 min. All compounds were identified on the basis of their retention times, and amounts relative to the internal standard were quantified by using a range of standards of the compounds (in water).

Differences in aroma composition at harvest and after storage in air or CO₂ for 12 days were assessed using least significant differences based on Tukey’s Studentized range or w statistic (Steel and Torrie, 1981). Concentrations of the compounds were transformed to logarithms to reduce variance before analysis. Values for ethyl butanoate were analyzed without transformation because transformation increased variance differences. For the remaining compounds, 0.01 was added before taking logarithms, to accommodate zeros. A consequence of logarithm use is that the least significant differences (LSD) gave a ratio of larger to smaller values. Where the ratio was greater than the LSD, values were significantly different. Orthogonal polynomials were used to study changes with time, partitioning the sums of squares into components that were associated, successively, with linear, quadratic, and cubic terms (Steel and Torrie, 1981).

**Results and Discussion**

The firmness of strawberries from 20% CO₂ was higher than that of the air control fruit (Fig. 1), the average benefit of CO₂ treatment over air treatment being 0.23 N during the 12 days of storage. The relationship of the CO₂ effect against time was quadratic (P < 0.05), and the maximum effect of CO₂ on enhancing firmness was obtained within 2 days of treatment. Fruit held in air, without CO₂ treatment, appeared to be initially firmer after 2 days than at harvest, but the effect was not statistically significant. Fruit transferred from CO₂ treatment to air after 2, 4, 6, and 9 days did not change in firmness after a total storage period of 12 days (data not shown), providing further evidence of the residual effect of CA storage as suggested by Li and Kader (1989).

Concentrations of acetaldehyde, ethyl acetate, ethanol, ethyl butanoate, and ethyl hexanoate, and acetic acid increased substantially during treatment with CO₂. Acetic acid concentrations also increased during air storage. Trans-2-hexenal, γ-decalactone, and butanoic acid remained constant during either air or CO₂ storage, while hexyl acetate, methlyhexanoate, 2-methyl propanoic acid, 2-methyl butanoic acid, and hexanoic acid decreased during treatment with CO₂ (Table 1). 2,5-Dimethyl-4-furanone and 2,5-dimethyl-4-hydroxy-3(2H)-furanone increased during both air and CO₂ storage.

**Concentrations of ethyl acetate, ethyl butanoate, ethyl hexanoate, and ethanol**

Concentrations of ethyl acetate, ethyl butanoate, ethyl hexanoate, and ethanol began to increase appreciably after 4 days or more of storage (Table 2), in a similar pattern to that reported by Guichard et al. (1992). After transfer to air storage, the concentrations of ethyl acetate, ethyl hexanoate, and ethanol in the fruit generally decreased, whereas those of ethyl butanoate generally increased (Table 2). However, ethyl acetate increased in air after an initial 9 days of CO₂ treatment. Ueda and Bai (1993) reported high accumulations of ethyl acetate.

**Table 1. Aroma composition (mg-kg⁻¹) of strawberries at harvest and after 12 days of storage in air or 20% CO₂ at OC.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>At harvest</th>
<th>Stored 12 days</th>
<th>LSD</th>
<th>120% CO₂</th>
<th>20% CO₂</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaldehyde</td>
<td>2.1 b</td>
<td>3.0 b</td>
<td>13.8 a</td>
<td>1.80</td>
<td>3.0 b</td>
<td>13.8 a</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.14 c</td>
<td>1.0 b</td>
<td>84.0 a</td>
<td>1.68</td>
<td>0.14 c</td>
<td>1.0 b</td>
</tr>
<tr>
<td>Ethanol</td>
<td>11.0b</td>
<td>19.0 b</td>
<td>366.0 a</td>
<td>2.04</td>
<td>11.0b</td>
<td>19.0 b</td>
</tr>
<tr>
<td>Ethyl butanoate</td>
<td>0.1 b</td>
<td>0.5 b</td>
<td>10.3 a</td>
<td>2.40</td>
<td>0.1 b</td>
<td>0.5 b</td>
</tr>
<tr>
<td>Hexyl acetate</td>
<td>0.2 a</td>
<td>0.00 b</td>
<td>0.15 a</td>
<td>1.32</td>
<td>0.2 a</td>
<td>0.00 b</td>
</tr>
<tr>
<td>Methyl hexanoate</td>
<td>0.37 a</td>
<td>0.00 b</td>
<td>0.15 a</td>
<td>1.32</td>
<td>0.37 a</td>
<td>0.00 b</td>
</tr>
<tr>
<td>Trans-2-hexenal</td>
<td>10.5 a</td>
<td>9.7 a</td>
<td>9.2 a</td>
<td>1.44</td>
<td>10.5 a</td>
<td>9.7 a</td>
</tr>
<tr>
<td>γ-decalactone</td>
<td>0.34 a</td>
<td>0.24 a</td>
<td>0.17 a</td>
<td>1.47</td>
<td>0.34 a</td>
<td>0.24 a</td>
</tr>
<tr>
<td>2,5-Dimethyl-4-furanone</td>
<td>2.6 b</td>
<td>10.7 a</td>
<td>15.2 a</td>
<td>1.79</td>
<td>2.6 b</td>
<td>10.7 a</td>
</tr>
<tr>
<td>2,5-Dimethyl-4-hydroxy-3(2H)-furanone</td>
<td>3.9 b</td>
<td>21.1 a</td>
<td>13.9 a</td>
<td>1.64</td>
<td>3.9 b</td>
<td>21.1 a</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>22.1 c</td>
<td>48.3 b</td>
<td>251 a</td>
<td>1.54</td>
<td>22.1 c</td>
<td>48.3 b</td>
</tr>
<tr>
<td>2-Methyl propanoic acid</td>
<td>0.93 a</td>
<td>0.66 ab</td>
<td>0.42 b</td>
<td>1.57</td>
<td>0.93 a</td>
<td>0.66 ab</td>
</tr>
<tr>
<td>2-Methyl butanoic acid</td>
<td>3.9 a</td>
<td>2.8 a</td>
<td>1.7 a</td>
<td>3.00</td>
<td>3.9 a</td>
<td>2.8 a</td>
</tr>
<tr>
<td>Butanoic acid</td>
<td>0.21 a</td>
<td>0.14 a</td>
<td>0.15 a</td>
<td>2.26</td>
<td>0.21 a</td>
<td>0.14 a</td>
</tr>
<tr>
<td>Hexanoic acid</td>
<td>1.0 a</td>
<td>1.1 a</td>
<td>0.5 b</td>
<td>1.36</td>
<td>1.0 a</td>
<td>1.1 a</td>
</tr>
</tbody>
</table>

- LSD: Least significant difference (LSD) test, P = 0.05.
- LSD’s are ratios of larger mean to smaller mean (P = 0.05).
- Mean separation within a row by Tukey’s Studentized range test on the transformed values (P = 0.05).
- LSD for comparing means under CO₂ storage only with CO₂ plus air storage. Except for ethyl butanoate, LSD’s are ratios of larger mean to smaller mean (P = 0.05).
- LSD for comparing means under CO₂ storage only with CO₂ plus air storage. Except for ethyl butanoate, LSD’s are ratios of larger mean to smaller mean (P = 0.05).
Acetate and ethyl butanoate in fruit stored in 20% CO₂ at 1°C for 12 h. They also noted that the ethyl ester continued to accumulate in fruit stored for 24 h in 20% CO₂ and then transferred to air.

Accumulation of individual ethyl esters was variable under the same conditions, and such changes may affect the flavor of the fruit. Informal taste tests indicated that the presence of off-flavors was consistent with changes in aroma compound concentrations. Off-flavors developed after 4 days of high CO₂ storage, but they disappeared when fruit was subsequently held in air for a few days. Only slight off-flavors were detected in fruit stored for 6 days at high CO₂ plus 6 days in air, although the ethyl acetate content in this fruit was similar to that of fruit evaluated after 4 days of high CO₂ storage. However, the ethyl butanoate content had increased markedly in samples stored for 6 days under high CO₂ and 6 days in air. This compound has a pleasant, fruity flavor and a low odor threshold and is one of the characteristic compounds of some strong-flavored strawberry cultivars, whereas it is scarce or absent in more weakly flavored cultivars (Larsen et al., 1992). As the threshold values of ethyl butanoate and ethyl hexanoate are very low compared to the threshold value of ethyl acetate (Larsen and Poll, 1992), formation of smaller amounts of ethyl butanoate and ethyl hexanoate might improve flavor quality. Thus, in our experiments, the higher ethyl butanoate content may have contributed to pleasant flavors that masked the off-flavor due to ethyl acetate in the fruit stored in air for 6 days after 6 days of CO₂ treatment. Variation in production of individual ethyl esters under the same treatment conditions may improve flavor quality. This study suggests that treatment of strawberry fruit with CO₂ after harvest, followed by air storage at 0°C, can maintain flesh firmness while minimizing off-flavor development. Further research is needed to determine the CA conditions that result in optimally flavored fruit.

Literature Cited


