Elevated carbon dioxide affects fruit flavor in field-grown strawberries (Fragaria × ananassa Duch)†

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Abstract: The effect of elevated carbon dioxide on fruit quality and aroma volatile composition in field-grown strawberries (Fragaria × ananassa Duch) was studied. Elevating the ambient CO2 concentration (ambient + 300, and ambient + 600 µmol mol−1 CO2) resulted in high fruit dry matter, fructose, glucose and total sugar contents and low citric and malic acid contents. High CO2 growing conditions significantly enhanced the fruit content of ethyl hexanoate, ethyl butanoate, methyl hexanoate, methyl butanonate, hexyl acetate, hexyl hexanoate, furaneol, linalool and methyl octanoate. Thus, the total amounts of these compounds were higher in berries grown in CO2-enriched conditions than those grown in ambient conditions. The highest CO2 enrichment (600 µmol mol−1) condition yielded fruit with the highest levels of these aroma compounds.

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Keywords: CO2; sugars; organic acids; aroma compounds; Fragaria × ananassa Duch; strawberry

INTRODUCTION
The volatile compounds produced by strawberry (Fragaria × ananassa Duch) fruit create aroma and contribute to flavor, thus strongly affecting quality and influencing consumer acceptability. The soluble sugars and acids contribute directly to the perceived sweetness of the fruit and provide carbohydrates for other metabolic functions. Sugars and acids also potentiate the effect of volatile aroma compounds that impart the flavor of fruit.

Strawberries are good sources of natural antioxidants.1–3 In addition to the usual nutrients such as vitamins and minerals, strawberries are rich in anthocyanins, flavonoids and phenolic acids.1,2 These compounds have health functional properties that may protect humans from cardiovascular disease, certain cancers, infection and other diet-related chronic diseases.1–9 Previous research10 found that strawberry plants grown under CO2-enriched conditions had increased phenonutrient content and antioxidant capacity in the fruit. Increased CO2 concentrations resulted in increases in ascorbic acid (AsA) and glutathione (GSH), and in the ratios of AsA to dehydroascorbic acid (DHAAsA) and GSH to oxidized glutathione (GSSG). Meanwhile, high CO2 concentrations decreased DHAAsA in strawberry fruit. Growing strawberry plants under CO2-enriched conditions also significantly enhanced the levels of anthocyanins, total phenolics and flavonoids in the fruit.10 Fruit of strawberry plants grown in CO2-enriched conditions also had high oxygen radical absorbance activity against active oxygen species. However, there is no information available on the effect of CO2 concentration on sugars, acids and the composition of aromatic volatiles of field-grown strawberry fruit; this study was performed to evaluate the effect of elevated carbon dioxide on these factors.

MATERIALS AND METHODS
Plant materials and experimental plans
Fragaria × ananassa Duchesne cv Honeoye, obtained as rooted runners from Miller Nurseries, Canandaigua, NY, USA were planted into field plots at the Beltsville Agricultural Research Center in March 1998. Sixteen plants were transplanted into each of six open-topped clear acrylic chambers, each of which covered 1.1 m² of ground. The chambers were 1.8 m high. A blower pulled air out of each chamber at the base, at a rate of 6 m³ min⁻¹. Carbon dioxide was introduced into four

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of the chambers at the inlets of mixing fans positioned above the canopies. Flow rates of CO₂ were such that two chambers had a [CO₂] of 300 ± 50 µmol mol⁻¹ above that of the outside air, and two chambers had a [CO₂] of 600 ± 50 µmol mol⁻¹ above that of the outside air, while two chambers received no supplemental CO₂. Samples of air from each [CO₂] treatment were pumped sequentially through an absolute infrared analyzer in an adjacent air-conditioned shelter, and [CO₂], air temperature and photosynthetic photon flux density (PPFD) were logged every 5 min. The chambers transmitted 90% of the PPFD, and had air temperatures which averaged 1 °C above those of the outside air. The mean daytime [CO₂] of ambient air was 353 µmol mol⁻¹, with concentrations at night of 400–600 µmol mol⁻¹. The duration of the entire experiment was 28 months (from early spring of 1998 continuing until the end of fruiting in June 2000). Full details of this experimental plan were described in a previous publication.¹¹

Fruit sample preparation
Strawberry fruits were harvested at the commercially ripe stage. The ripeness of fruit was determined by color. Fruits were collected over 2 years (2000 and 2001) and in two replicated chambers per CO₂ treatment (ambient, ambient + CO₂, and ambient + 2001 CO₂). Three samplings (four fruit per sampling) were taken from each chamber to determine fruit weight (fresh and dry weight) and for chemical analyses. The fruits were weighed for fresh weight immediately after harvest and for dry weight after being lyophilized.

Sugar analysis
Fruit tissue (0.2 g of composite lyophilized fruit tissues from eight berries) was extracted with 800 ml⁻¹ aqueous ethanol, and then centrifuged at 5000 × g for 5 min. The residue was re-extracted, washed twice by centrifugation, and then re-suspended in 800 ml⁻¹ ethanol. The supernatants were combined and an aliquot of the extract was concentrated to dryness in vacuo and then centrifuged at 5000 × g for 5 min. An aliquot of the extract was concentrated to dryness under vacuum. Derivatization of the sugars was performed according to procedures described by Wang et al.¹² Derivatized sugars were compared with derivatized sugar standards for qualitative and quantitative determination. A known amount of β-phenyl-D-glucopyranoside was included in all samples as an internal standard.

Organic acid analysis
A Baker 10-extraction system (JT Baker, Phillipsburg, NJ, USA) was used for purification of the organic acids in the fruit tissue. One gram of composite lyophilized fruit tissue from 10 berries was extracted with 100 ml of 20 mM imidazole buffer (pH 7.0). A 40 ml aliquot was then placed onto a 3 ml quaternary amine column that had been previously conditioned with hexane and methanol. The organic acids were eluted with 6 ml 0.1 N HCl. The eluate was concentrated to dryness under vacuum. Derivatization and determination of organic acids were similar to those for non-structural carbohydrates except that the initial oven temperature was reduced to 100 °C and held for 3 min; the temperature program rate was 4 °C min⁻¹, and final oven temperature was 230 °C. Separation of organic acids was compared with derivatized organic acid standards for qualitative and quantitative determination. Sugars and organic acids were quantified by comparing peak areas with those of standards.

Aroma constituent analysis
Strawberry fruits (0.2 g of composite lyophilized fruit tissue from eight berries) were placed into a closed sampling chamber (25 ml) within a thermostatic water bath (25 °C). After 10 min equilibrium time, the volatile compounds were desorbed onto a SPME fiber (65 µm, poly (dimethylsiloxane)/DBD) (Supelco Inc, Bellefonte, PA, USA). Sampling time was 20 min. The volatiles were desorbed from the SPME fiber and carried directly into the glass-lined, splitless injector port of a GC (HP-6890, Hewlett Packard). Volatiles were analyzed using a GC HP-6890 equipped with a fused silica capillary column (5-HP, 30 m × 0.32 mm × 0.25 thickness; Hewlett Packard). The carrier gas was ultra purified helium (99.999%) at a flow rate 0.5 ml min⁻¹. Injector temperature was 220 °C, column oven temperature was initially held at 40 °C for 1.5 min and then a temperature ramp of 5 °C min⁻¹ was programmed up to 250 °C. All compounds were identified according to the procedure of Pérez et al.¹³ Compound identifications were also performed by computer data searches and coelution with authentic compounds on the column and comparing retention time with those of authentic compounds. Quantification of volatile peaks was done using a range of external standards. Peak areas were expressed as ng volatile g⁻¹ dry weight.

Statistical analysis
Analyses were replicated three times for samples grown under the control and CO₂ enhancement conditions. The experiments were replicated for
Strawberry fruits high in sugars and acids are considered to have the most fruity flavor. In strawberry fruit, soluble sugars such as glucose, fructose and sucrose are the early products of photosynthesis and are transported throughout the plant, including to the fruit. Elevating CO₂ concentrations increased glucose, fructose and sucrose levels (Table 2). The proportions of fructose, glucose and sucrose are important in the perception of fruit quality, since fructose is 1.8 times sweeter than sucrose, and sucrose is about 1.7 times sweeter than glucose.

Strawberry fruit relies on imported sugars to increase sweetness. Little starch is present in ripe fruit and thus harvested fruit must use sugars, acids or lipids for respiration and other metabolic processes after harvest. Sugar metabolism is necessary for the synthesis of some aromatic volatile compounds, such as the furanones that give the strawberry its characteristic flavor.

The increase in soluble sugars in the fruit arising from elevated CO₂ treatment may therefore result in an increase in the availability of precursors able to produce aromatic compounds.

The predominant organic acids found in strawberry fruit are citric and malic acids, which contribute to the perceived sourness of strawberries. Changes in the levels of these organic acids can also alter color by affecting cellular pH and anthocyanin structure. CO₂ enhancement increased sugar, glucose, fructose and sucrose contents but decreased organic acid, citric, malic, quinic and total organic acid contents. The ratio of total sugars to organic acids also increased from 9.31 to 13.0 (Tables 2 and 3). These results indicate that CO₂ has an effect on carbohydrate and organic acid metabolism. Since organic acids and carbohydrates can be interconverted, high CO₂ may promote the conversion of organic acids into carbohydrates.

The volatile compounds produced by strawberry fruit create aroma and contribute to flavor, thus strongly affecting quality, value and consumer acceptability. Strawberry aroma is mainly determined by a complex mixture of esters, alcohols, aldehydes and sulfur compounds. More than 100 ester types have been detected in strawberry. The composition of strawberry aroma is influenced by cultivar, maturity, preharvest and postharvest changes, and the relative importance of aroma compounds to the flavor of strawberries has been studied.

**RESULTS AND DISCUSSION**

Strawberry plants grown in higher CO₂ concentration environments resulted in increased fruit dry weight (Table 1). The average berry, expressed on the dry weight basis, was heavier and contained more dry matter than fruit grown under ambient conditions without supplemental CO₂. This was probably due to an increase in net photosynthetic rate of the plant by decreasing the oxygen inhibition of photosynthesis and transpiration. Chen et al. showed that CO₂ enrichment in the greenhouse can promote carbohydrate production and accumulation in strawberry plants and may consequently enlarge the carbohydrate reservoir. However, our data showed that the fruit fresh weights per chamber for all harvests (yield) were not significantly different among the CO₂ treatments.

Sugars are among the main soluble components in strawberry tissue and provide energy for metabolic changes while also acting as precursors of flavor compounds. Sugars and organic acids also have an important impact on the sensory quality of strawberry fruit. The predominant sugars in strawberries are glucose, fructose and sucrose and these three sugars account for more than 990 g kg⁻¹ of the total sugars in ripe strawberries. These soluble sugars contribute directly to the perceived sweetness of the fruit and provide carbohydrates for other metabolic functions.

### Table 1. Effect of elevated carbon dioxide treatments on dry weight of strawberry fruit

<table>
<thead>
<tr>
<th>CO₂ treatment (µmol mol⁻¹)</th>
<th>Dry weight (g fruit⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient (350)</td>
<td>2.05</td>
</tr>
<tr>
<td>Ambient + 300</td>
<td>2.41</td>
</tr>
<tr>
<td>Ambient + 600</td>
<td>2.84</td>
</tr>
<tr>
<td>Significance *</td>
<td></td>
</tr>
</tbody>
</table>

* Significant at p ≤ 0.05.

<table>
<thead>
<tr>
<th>CO₂ treatment (µmol mol⁻¹)</th>
<th>Fructose</th>
<th>Glucose</th>
<th>Sucrose</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient (350)</td>
<td>313.8 ± 2.72</td>
<td>243.4 ± 3.29</td>
<td>5.87 ± 0.15</td>
<td>563.1 ± 2.69</td>
</tr>
<tr>
<td>Ambient + 300</td>
<td>364.4 ± 0.83</td>
<td>259.7 ± 3.17</td>
<td>6.43 ± 0.32</td>
<td>630.5 ± 1.73</td>
</tr>
<tr>
<td>Ambient + 600</td>
<td>393.0 ± 3.01</td>
<td>274.9 ± 4.80</td>
<td>7.39 ± 0.27</td>
<td>675.3 ± 3.05</td>
</tr>
</tbody>
</table>

**Table 2. Effect of elevated carbon dioxide treatments on sugar content of strawberry fruit**

* Data expressed as means ± SEM.

* Significant at p ≤ 0.05.
Carbon dioxide and aroma volatile composition of strawberries

Table 3. Effect of elevated carbon dioxide treatments on organic acid content of strawberry fruit

<table>
<thead>
<tr>
<th>CO2 treatment (µmol mol⁻¹)</th>
<th>Malic acid (mg g⁻¹ dry weight)</th>
<th>Citric acid (mg g⁻¹ dry weight)</th>
<th>Quinic acid (mg g⁻¹ dry weight)</th>
<th>Total (mg g⁻¹ dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient (350)</td>
<td>8.64 ± 0.11</td>
<td>46.8 ± 1.18</td>
<td>5.06 ± 0.29</td>
<td>60.5 ± 0.88</td>
</tr>
<tr>
<td>Ambient + 300</td>
<td>8.19 ± 0.23</td>
<td>43.7 ± 0.54</td>
<td>3.54 ± 0.47</td>
<td>55.4 ± 0.17</td>
</tr>
<tr>
<td>Ambient + 600</td>
<td>7.39 ± 0.34</td>
<td>39.9 ± 1.53</td>
<td>2.66 ± 0.19</td>
<td>50.0 ± 0.82</td>
</tr>
</tbody>
</table>

Data expressed as means ± SEM.
* Significant at p ≤ 0.05.

Table 4. Effect of elevated carbon dioxide treatments on aroma volatile composition of strawberry fruit

<table>
<thead>
<tr>
<th>Aroma compound (ng g⁻¹ dry weight)</th>
<th>CO2 treatment (µmol mol⁻¹)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ambient (350)</td>
<td>Ambient + 300</td>
</tr>
<tr>
<td>Ethyl hexanoate</td>
<td>651.3 ± 25.2</td>
<td>814.8 ± 32.2</td>
</tr>
<tr>
<td>Ethyl butanoate</td>
<td>530.6 ± 17.2</td>
<td>637.1 ± 21.3</td>
</tr>
<tr>
<td>Methyl hexanoate</td>
<td>294.1 ± 12.6</td>
<td>364.8 ± 15.2</td>
</tr>
<tr>
<td>Methyl butanoate</td>
<td>219.4 ± 23.4</td>
<td>298.5 ± 12.3</td>
</tr>
<tr>
<td>Hexyl acetate</td>
<td>168.6 ± 17.3</td>
<td>262.7 ± 11.5</td>
</tr>
<tr>
<td>Hexyl hexanoate</td>
<td>125.1 ± 13.4</td>
<td>267.5 ± 17.5</td>
</tr>
<tr>
<td>Methyl acetate</td>
<td>141.4 ± 9.8</td>
<td>130.2 ± 7.4</td>
</tr>
<tr>
<td>Butyl acetate</td>
<td>124.2 ± 7.5</td>
<td>76.7 ± 8.2</td>
</tr>
<tr>
<td>Methyl acetate</td>
<td>53.1 ± 6.1</td>
<td>58.9 ± 5.7</td>
</tr>
<tr>
<td>Furaneol</td>
<td>55.1 ± 4.3</td>
<td>89.7 ± 8.5</td>
</tr>
<tr>
<td>Linalool</td>
<td>57.9 ± 6.4</td>
<td>77.9 ± 11.2</td>
</tr>
<tr>
<td>Methyl octanoate</td>
<td>49.2 ± 5.7</td>
<td>63.2 ± 6.5</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM.
* Significant NS, non-significant at p ≤ 0.05.

Ethyl hexanoate, ethyl butanoate, methyl hexanoate, methyl butanoate, hexyl acetate and hexyl hexanoate are the most important volatiles in Honeoye strawberries and may be the major contributors to its aroma, although the possibility of an underlying minor component strongly influencing the sensory results can never be ruled out. Larsen and Poll showed that ethyl butanoate, furaneol, ethyl hexanoate and methyl hexanoate contributed most to the aroma in strawberries. High CO₂ growing conditions significantly enhanced the strawberry fruit content of methyl butanoate, ethyl butanoate, methyl hexanoate, ethyl hexanoate, hexyl hexanoate, hexyl acetate, furaneol, linalool and methyl octanoate (Table 4). The highest CO₂ enrichment (600 µmol mol⁻¹) condition yielded fruit with the highest levels of these compounds, with the exception of methyl metanoate, butyl acetate and methyl acetate. Elevating CO₂ concentration decreased butyl acetate and methyl metanoate concentrations, but showed no significant effect on the methyl acetate level. Total amounts of aroma compounds were also higher in berries grown in CO₂-enriched conditions than those grown in ambient conditions, and this would probably improve perceived fruit quality.

Amino acids, sugars and lipids can all act as precursors for aroma substrates. Substrate availability may also play a major role in the composition of aroma produced by strawberry. Although we did not determine amino acids and lipids in this study, we found that elevating CO₂ concentrations increased glucose, fructose, sucrose, total sugars and aroma compounds in the fruit. Normal aerobic metabolism of sugars can produce precursors for aroma ester production. Furanones also make an important contribution to the aroma and flavor of strawberry fruit. Sugars have been suggested as precursors for furanone synthesis, with fructose being the most likely candidate. Sanz et al suggested that furanone could be synthesized from an intermediate of the pentose phosphate cycle and that fructose-6-phosphate could be its precursor. Adding 6-deoxy-D-fructose to tissue-cultured strawberry cells stimulated the production of furanone-glucoside, suggesting its role as a precursor of furanone synthesis. Elevating CO₂ concentration resulted in enhancing furanone concentration in strawberries (Table 4). The results of this study indicate that enhancing CO₂ concentration in the growing atmosphere would probably improve fruit quality by increasing fruit dry weight, sugar and aroma concentration and decreasing acid content.

REFERENCES


