Effect of Different Atmospheres on Postharvest Decay and Quality of Fresh Strawberries

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ABSTRACT

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Effects of different atmospheres, ie, air + ethylene (C_2H_4) at 20 μl/L; air + 15% CO_2; air + 10% carbon monoxide (CO); a controlled atmosphere (CA) of 2.3% O_2 + 5% CO_2; CA + C_2H_4 (20 μl/L); and CA + 10% CO in addition to control (air), on the postharvest decay of strawberry fruits caused by *Botrytis cinerea* were studied with Aiko, G-3, and G-4 cultivars at 0.6 and 3.3 C for 21 days. Air + 15% CO_2 and CA + 10% CO were the most effective atmospheres in suppressing fruit rot. Presence of 20 μl of C_2H_4 per liter, added to either air or CA, resulted in more decay development than in other atmospheres, indicating that C_2H_4 might enhance disease development or fungal growth. Off-flavors were detected after treatment with air + 15% CO_2. Carbon monoxide added to CA during storage of strawberries at 0.6–3.3 C for up to three weeks may provide better results than the current practice of using high CO_2.

Strawberries (*Fragaria chiloensis* Duchesne var. *ananassa* Bailey) are highly perishable and suffer relatively high postharvest losses due to gray mold rot caused by *Botrytis cinerea* Pers. ex Fr. (6,11,17,18,23). Although elevated CO_2 atmospheres can be detrimental to many commodities, including strawberries, they have been used commercially to prevent decay during transit of strawberries (12). Tissue discoloration and off-flavors (8,20,25) result if critical limits of concentration or period of exposure are exceeded.

Dangers from excessive CO_2 can be reduced if artificial atmospheres are provided; these usually contain 2–4% O_2 and 5–10% CO_2 with the remainder N_2. The gas mixture is termed a controlled atmosphere (CA) if it is periodically or constantly adjusted to compensate for changes caused by fruit respiration, or a modified atmosphere if no adjustments are made.

El-Goorani and Sommer (8) studied the effect of CO added to air and to CA (2.3% O_2 + 5% CO_2) on Botrytis rot of strawberries and found that 9% CO + CA reduced the rate of rot development by 80–90%. They attributed the development of a slight off-flavor to low O_2 and elevated CO_2 and not to added CO. The biological effects of C_2H_4, such as hastening the ripening of climacteric fruits, has been reported to be mimicked by CO (9,14). In contrast, the nonclimacteric fruits, such as strawberries, produce very small amounts of C_2H_4 and exhibit less dramatic changes in their color and composition after harvest (19,21). Siriphanich (21) reported that strawberry quality was not influenced by exposure to exogenous C_2H_4 at 1, 10, or 100 ppm at 2.2 or 5 C for up to 14 days. He concluded that no advantage would be derived from removal of C_2H_4 from around strawberry fruits during postharvest handling.

The effect of C_2H_4 on fungal growth or disease development is still unclear. A retarding effect of C_2H_4 on disease development has been reported on sweet potatoes infected by *Ceratocystis fimbriata* (24) and on McIntosh apples infected by *Gloeosporium album* (16). On the other hand, C_2H_4 has been reported to stimulate disease development or spore germination of certain postharvest fungi (1,2,3,7,15).

The objectives of this study were to 1) compare the effect of elevated CO_2 atmospheres vs the effects of CO added to air or CA on decay control and quality of harvested strawberry fruits, ie, determine whether CO can be used as a substitute for elevated CO_2 to avoid possible CO_2 injury; 2) compare the effects of C_2H_4 and its mimic, CO, on disease development and quality of the strawberry; and 3) determine whether the postharvest life of strawberries could be extended to 21 days, a period possibly permitting transoceanic marine transport.

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MATERIALS AND METHODS

Fruits. Strawberry cultivars Aiko, G-3, and G-4 were obtained from Watsonville, CA. These fruits were forced-air cooled to near 0 C within a few hours of harvest, then transported to Davis in an insulated container within 3 hr. After the berries were held overnight at 0 C, they were sorted to eliminate defects. Matched samples of 20 fruits (about 450 g) per replicate were selected, and three replicates per treatment were used in all experiments.

Fungal spore preparation and inoculation of fruits. Fungal cultures of *B. cinerea* used in this study were provided by the second author. Cultures were routinely stored at about 4 C on potato dextrose agar slants. Pathogenicity of the fungus was verified by fruit inoculation before use in this study. Spores were produced on V-8 juice agar in 300-ml Erlenmeyer flasks at about 21–24 C. Seven-day-old cultures were flooded with 20 ml of Tween-80 solution (1 drop of Tween-80 in 100 ml of distilled water), and the resulting conidial suspension was filtered through two layers of sterile cheesecloth. Conidia were washed twice by centrifugation and discarding of the supernatant, which was replaced by fresh Tween-80 solution. A concentration of 1×10^6 conidia per milliliter was established by relating absorption at 490 nm in a B & L Spectronic 20 colorimeter to a concentration curve previously established with a hemacytometer for a similar spore suspension. Fruits were wound-inoculated (approximately 0.05 ml per wound) using a needle (2 mm long) previously dipped in the *B. cinerea* suspension. The effect of atmospheres on mycelial infection and "nesting" was determined in one test by surrounding a diseased berry that was almost completely covered with mycelium of *B. cinerea* with 20 sound G-3 berries in a basket.

Test fruits were placed in a 11.5-L glass jar through which atmospheres were metered at a rate of 160 ml/min using the capillary flow meters described by Claypool and Keefer (5). Atmospheres were air (20.8% O_2, 0.03% CO_2), air + C_2H_4 (20 μl/L), air + 15% CO_2, air + 10% CO, CA (2.3% O_2 + 5% CO_2), CA + C_2H_4

(20 μl/L), and CA + 10% CO. The difference in the sums of gases indicated for each atmosphere and 100% consisted of N_2. Uninoculated control fruits were included in separate jars provided with the same array of atmospheres. Atmospheres were periodically verified by gas chromatography and determined to be accurate to ±5% of the desired O_2, CO_2, CO, and C_2H_4 concentrations. Carbon-dioxide and C_2H_4 evolution by uninoculated fruits was measured by gas chromatographic methods. Production rates were calculated from the known weight of strawberries, the flow rate, and the CO_2 or C_2H_4 concentrations in exhausting gases.

In the first experiment, 'Aiko' fruits were held at 0.6 ± 0.5 C for 21 days, followed by 2 days in air at 20 C. In the second and third experiments, G-3 and G-4 fruits were held at 3.3 C for 21 days. After the required period, fruits were examined for rot development by measuring the diameter of the rot lesions in needle-inoculated fruits.

Quality evaluation. Quality of fruits of each cultivar was evaluated initially and after 7 and 21 days of storage unless otherwise indicated. Twenty-four uninoculated fruits were taken at random from those stored under each of the seven atmospheres for the following evaluations:

1. Firmness: measured using the University of California fruit firmness tester (penetrometer; Ametek, Inc., Hunter Spring Div., Hatfield, PA 19440) with a 3-mm plunger tip (4).
2. Titratable acidity: determined by titrating 6 g of juice (squeezed from 24 fruits) added to 50 ml of distilled water with 0.1 NaOH and calculated as percent citric acid.
3. Soluble solids content: determined using a hand refractometer.
4. Sensory evaluation: performed by 12 trained judges for firmness, sweetness, sourness, strawberry flavor intensity, and off-flavor after 7 days for the G-4 cultivar and 21 days for both G-3 and G-4 cultivars. The judges scored uninoculated fruits for each of the sensory characteristics by use of a 0 (lowest) to 10 (highest) rating scale. Judges received three portions of sliced fruit per sample and evaluated seven samples per day. Samples at room temperature were presented in random order to judges in individual booths illuminated by red light to mask color differences among samples. Judges were instructed to rinse their mouths with distilled water between samples and to expectorate samples.

Sensory evaluation data were computed and analysis of variance was applied to all quantitative analyses to test the significance of differences. Other data were evaluated by analysis of variance, and means were compared using Duncan's new multiple range test.

RESULTS

The development of Botrytis gray mold lesions in strawberry fruits in storage was suppressed by elevated CO_2 and CO treatments in this study (Table 1). The addition of 20 μl of C_2H_4 per liter to air or CA (Table 1) resulted in more rapid lesion development than occurred in the same atmospheres without C_2H_4. Contrary to expectation, the rate of lesion development in CA was significantly greater than that in air in the case of Aiko fruits.

"Nesting" (the growth of mycelium from a rotting berry to infect nearby sound fruits in the container) was strongly suppressed under atmospheres of air + 15% CO_2 or CA + 10% CO (Table 2). Other atmospheres affected "nesting" little or not at all.

The addition of CO to air significantly decreased the respiration rate of Aiko strawberries held at 0.6 C for 16 days (Fig. 1). When 20 μl of C_2H_4 per liter was added, the respiration rate was not affected until the second week of storage, when it slightly increased in comparison to fruits held in air.

The effect of adding CO or CO_2 to air on C_2H_4 production was determined with Aiko strawberries (Fig. 2). A near fourfold increase in C_2H_4 evolution was caused by the addition of 10% CO to air. By contrast, the addition of 15% CO_2 to air resulted in the complete absence of detectable C_2H_4 evolution during the first 9 days and suppression of the rate thereafter.

Sensory evaluations (Table 3) and objective measurements with a firmness tester generally showed that fruits at 0.6 C in air + 15% CO_2 were firmest. For example, G-4 berries after 7 days in air + 15% CO_2 were judged to be significantly firmer than berries in all other treatments (Table 3). After 21 days, G-3 berries were firmer in air + 15% CO_2 than similar fruits in air or mixtures including C_2H_4. Objective measures of firmness of Aiko fruits were not significantly different regardless of the atmosphere except that berries held in air + 20 μl of C_2H_4 per liter for 21 days were significantly softer than berries held in CA. No significant differences in soluble solid contents or titratable acidity were noted among fruits of any treatment.

TABLE 1. Mean diameter of lesions in Aiko, G-3, and G-4 cultivars of strawberry fruits as influenced by different atmospheres[a]

Treatment	Cultivar[b]		
	Aiko	G-3	G-4
Air	7.5 c[a]	62.7 e	45.0 c
Air + C_2H_4 (20 μl/L)	39.1 f	86.8 f	61.3 d
Air + 15% CO_2	2.0 a	2.6 a	2.9 a
Air + 10% CO	3.6 b	44.8 d	33.3 b
CA (2.3% O_2 + 5% CO_2)	11.8 d	21.8 b	27.7 b
CA + C_2H_4 (20 μl/L)	16.6 e	30.2 c	31.0 b
CA + 10% CO	0.6 a	1.6 a	2.2 a

[a] Each berry was needle-inoculated with *Botrytis cinerea* before storage for 21 days.
[b] Aiko was stored at 0.6 C followed by 2 days at 20 C. Others were stored continuously at 3.3 C.
[c] Mean diameter of lesions in millimeters of three replicates per treatment. Within each column, means with a common letter are not significantly different according to Duncan's new multiple range test ($P = 0.05$).

TABLE 2. Effect of different atmospheres on mycelial infection and "nesting" of strawberry fruits by *Botrytis cinerea*

Treatment	Mean number of rotted berries[a] (20 fruits)
Air	19.3 e[b]
Air + C_2H_4 (20 μl/L)	20.0 e
Air + 15% CO_2	0.15 a
Air + 10% CO	16.0 c
CA (2.3% O_2 + 5% CO_2)	14.0 b
CA + C_2H_4 (20 μl/L)	17.6 d
CA + 10% CO	0.15 a

[a] Data indicate rotted G-3 berries surrounding a diseased berry after 21 days at 3.3 C.
[b] Mean of three replicates per treatment. Means with a common letter are not significantly different according to Duncan's new multiple range test ($P = 0.05$).

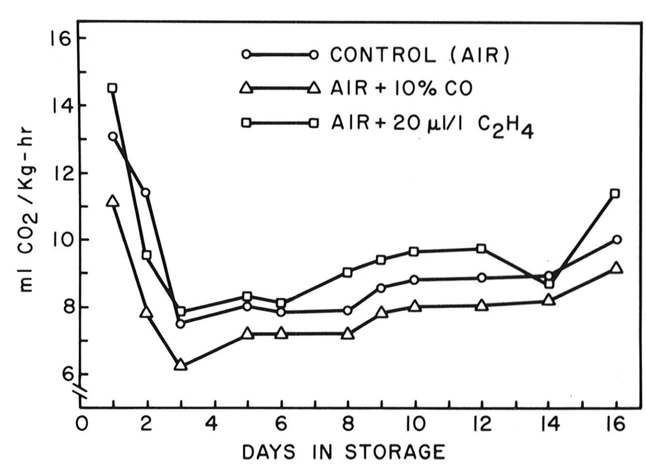

Fig. 1. Effect of CO and C_2H_4 on respiration rate of cultivar Aiko strawberry fruits held at 0.6 ± 0.5 C under indicated atmospheres.

Neither sweetness, sourness, nor flavor intensity was markedly affected by the atmosphere in which the fruits were held (Table 3). Similarly, no large differences were found in panelists' perception of off-flavors in G-3 after 21 days except that fruits held in air + 15% CO_2 or air + C_2H_4 were scored higher in off-flavor than fruits from other treatments.

Fruits of G-3 and G-4 held at 3.3 C for 7 or 21 days varied in firmness (Table 4), with the firmest berries being those held in air + 15% CO_2. Berries held in air or atmospheres containing C_2H_4 were softer after 21 days than fruits from other atmospheres. Differences among treatments in soluble solid contents and titratable acidity were not significant.

Differences between fruits held for 7 and 21 days were compared by sensory evaluation. G-4 berries after 7 days were slightly sweeter, sourer, and had higher flavor intensity than similar berries held for 21 days. In the same comparison, differences in firmness and off-flavors after 21 days of storage were not significantly different from those determined after 7 days.

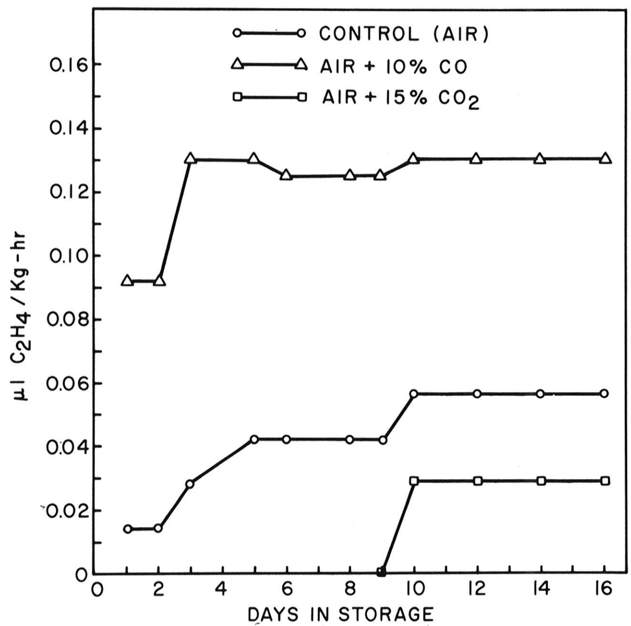

Fig. 2. Effect of CO and CO_2 concentrations on ethylene production by cultivar Aiko strawberry fruits held at 0.6 ± 0.5 C.

TABLE 3. Effect of different atmosphere treatments on sensory characteristics of G-3 and G-4 strawberry cultivars held at 3.3 C for 7 and 21 days, respectively

Cultivar	Days in storage	Treatment	Sensory evaluation criteria scored: Firmness	Sweetness	Sourness	Flavor intensity	Off-flavor
G-4	7	Air (control)	4.7[a]	5.1	5.0	5.3	2.1
		Air + C_2H_4 (20 μl/L)	4.7	4.8	5.1	4.9	2.7
		Air + 15% CO_2	5.6	5.2	4.9	5.4	2.1
		Air + 10% CO	4.9	5.2	5.1	5.4	1.9
		CA (2.3% O_2 + 5% CO_2)	4.8	4.9	5.1	4.9	1.9
		CA + C_2H_4 (20 μl/L)	4.7	4.4	5.1	4.4	2.5
		CA + 10% CO	4.7	5.3	5.1	5.3	1.9
		LSD 0.05	0.56	NS	NS	0.67	NS
G-3	21	Air (control)	3.6	4.0	4.6	3.8	3.1
		Air + C_2H_4 (20 μl/L)	3.4	3.8	5.0	3.7	3.5
		Air + 15% CO_2	4.5	4.4	4.2	3.4	3.6
		Air + 10% CO	4.1	3.9	4.7	3.9	2.7
		CA (2.3% O_2 + 5% CO_2	4.3	3.9	4.6	3.8	2.6
		CA + C_2H_4 (20 μl/L)	3.6	4.1	4.9	3.6	2.9
		CA + 10% CO	4.3	4.4	4.6	4.1	2.8
		LSD 0.05	0.5	NS	NS	NS	0.6

[a] Evaluation scores, mean of three replicates per treatment. Scores for each characteristic ranged between 0 = lowest and 10 = highest. NS = not significant.

DISCUSSION

Atmosphere modification by the addition of CO_2 has been commercially used in the transportation of strawberries in recent years for the control of *Botrytis cinerea* (gray mold). A problem with elevated CO_2 atmospheres has been the loss of flavor (10,20,25) or the development of objectionable or alcoholic flavors in fruits if the concentration is too high for too long (25). Accumulation of acetaldehyde, acetone, ethanol, ethyl acetate, and methyl acetate has been implicated with the objectionable change in taste (20). Off-flavor development as a result of elevated CO_2 appears to be dependent upon the cultivar, CO_2 concentration, and duration and temperature of storage.

The suitability of atmospheres containing CO for the suppression of disease during transit has not been thoroughly evaluated. An important suppression can be achieved with CO concentrations of 10 or 11%, providing the O_2 is less than about 4% (9,13). The use of CO has the advantage of not increasing in concentration during transit as does CO_2 and thus far no problem with the development of objectionable flavors has been reported. The significant decrease in the rate of respiration observed when CO was added to air (Fig. 1) is a very good indication that the storage life of the fruit has been increased as a consequence of the reduced consumption of energy reserves. On the other hand, the CO has been shown in this study to stimulate C_2H_4 evolution in the strawberry; similar results were previously shown in sweet cherries and are believed to occur in kiwifruits (22). Such C_2H_4 levels were not shown to have detrimental effects on quality or postharvest life of strawberries (21).

Our results do not support the point of view that C_2H_4 has either a fungistatic effect or a retarding effect on disease development (16,24). We found that the presence of C_2H_4 might stimulate fungal growth directly or might act indirectly by enhancing senescence as a result of increasing the rate of respiration or by other means. We also do not agree that removal of C_2H_4 from the air surrounding

TABLE 4. Effect of different atmospheres on firmness of G-3 and G-4 strawberry fruits held at 3.3 C for 7 and 21 days

Days in storage	Treatment	University of California firmness tester reading[a] (g): G-3	G-4
0	Initial	172	202
7	Air	96 c	138 b
	Air + C_2H_4 (20 μl/L)	117 abc	138 b
	Air + 15% CO_2	136 a	195 a
	Air + 10% CO	133 a	147 b
	CA (2.3% O_2 + 5% CO_2)	122 ab	159 b
	CA + C_2H_4 (20 μl/L)	108 bc	135 b
	CA + 10% CO	132 a	152 b
21	Air	63 d	88 cd
	Air + C_2H_4 (20 μl/L)	29 e	64 d
	Air + 15% CO_2	163 a	204 a
	Air + 10% CO	109 c	102 c
	CA (2.3% O_2 + 5% CO_2)	152 a	122 b
	CA + C_2H_4 (20 μl/L)	68 d	97 bc
	CA + 10% CO	141 ab	110 bc

[a] Within both columns, means with a common letter are not significantly different according to Duncan's new multiple range test ($P = 0.05$).

harvested strawberry fruits has no quality advantage (21). Our study, showing dramatic increases in disease development in the presence of 20 μl of C_2H_4 per liter suggests that the benefits of removal should be reevaluated with diseased berries present.

Storage and transit temperatures of near 0 C are desirable for postharvest handling of strawberries. Unfortunately, ideal temperatures can rarely be maintained in present transit vehicles. Use of CA + 10% CO, as a supplement to temperatures higher than the ideal, would be beneficial in extending the maximum transit time to about three weeks. This may permit the use of marine transport for exporting strawberries to various countries.

The explosive nature of CO above about 12.5% in air effectively limits the concentration for commercial use. Although errors in establishing the initial concentration might create a hazard, it appears impossible for the CO level to increase in transit. The poisonous nature of CO requires the installation of devices to warn of the presence of CO in case leakage should occur.

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