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Residual Effects of Controlled Atmospheres on Postharvest Physiology and Quality of Strawberries

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Abstract. Low O₂ (2.0%, 1.0%, or 0.5%) or elevated CO₂ (10%, 15%, or 20%) concentrations and their combinations reduced respiration and ethylene production rates of 'Selva' strawberries (*Fragaria × ananassa* Duch.) stored at 2°C. After transfer from the various controlled atmospheres (CA) to air, respiration and ethylene production rates increased, but were still significantly lower than those of the fruits kept continuously in air. Atmospheres of 1.0% O₂ + 15% CO₂ and 0.5% O₂ + 20% CO₂ led to the accumulation of ethanol, which was still present at concentrations >100 µl-liter⁻¹ (ppm) in strawberry juice after their transfer to air for several days. Keeping strawberries in CA had residual effects on maintaining their flesh firmness and color, but had no significant effects on skin or juice color, titratable acidity, pH, soluble solids content, and ascorbic acid content. In general, as O₂ concentration was decreased or as CO₂ concentration was increased, the residual effects were more pronounced. The combinations of reduced O₂ and elevated CO₂ had more pronounced residual effects than either reduced O₂ or elevated CO₂ alone. Exposures to CA for 4 days had greater residual effects than exposure to CA for 1 to 3 days.

Controlled atmospheres with reduced O₂ and/or elevated CO₂ concentrations have been successfully used in extending the postharvest life and maintaining quality in strawberries. The effects of CA on postharvest physiology and quality attributes of strawberries include controlling postharvest decay (Couey et al., 1966; Couey and Wells, 1970; Sommer et al., 1973; El-Kazzaz et al., 1983; Harvey, 1982; Prasad and Stadelbacher, 1974; Shaw, 1969; Smith, 1957; Wells, 1970; Woodward and Topping, 1972), reducing rates of respiration (Siriphanich, 1980; Tomalin and Robinson, 1971; Woodward and Topping, 1972) and ethylene production (El-Kazzaz et al., 1983; Siriphanich, 1980), retarding softening (El-Kazzaz et al., 1983) and increasing accumulation of certain volatiles (Prasad and Stadelbacher, 1974; Shaw, 1969; Smith, 1957; Woodward and Topping, 1972).

Although the effects of CA storage on postharvest life of strawberries have been investigated, no work has been done to quantify the residual effects of CA on rate of strawberry deterioration after removal from CA to air. Such information can be used in managing the use of CA during transit and temporary storage of strawberries to maximize maintenance of their quality and reduction of postharvest losses.

The objectives of this research were to study the residual

effects of exposure to various O₂ or CO₂ levels and their combinations for various durations on strawberries following transfer to air.

Materials and Methods

Fruit source and preparation. During the period from July to Oct. 1987, 'Selva' strawberries were obtained on the day of harvest from Naturipe Cooperative in Watsonville, Calif. After transport (3 hr in an air-conditioned car) to the laboratory, the strawberries were sorted to remove damaged, unripe, and over-ripe fruits. Then, ≈35 selected fruits were put in a 2-liter glass jar as one replicate; three replicates were used per treatment.

Treatments and storage conditions. The jars were then placed at 2°C and ventilated with air or the desired gas mixtures at a continuous flow of about 25 ml·min⁻¹, using flow boards and capillary tubing for flow control.

The first three tests dealt with the effects of the following groups of CA combinations on strawberries: Test 1: air, 2.0%, 1.0%, or 0.5% O₂; Test 2: air, or air + 10%, 15%, or 20% CO₂; Test 3: air, 2.0% O₂ + 10% CO₂, 1.0% O₂ + 15% CO₂, or 0.5% O₂ + 20% CO₂. The air control strawberries were stored for 14 days, while all CA-treated fruits were stored in CA conditions for 7 days and then transferred to air for another 7 days. In the second group of three tests, the strawberries were stored in air for 8 days or in 0.5% O₂ (Test 4); 20% CO₂ (Test 5); or 0.5% O₂ + 20% CO₂ (Test 6) for 1, 2, 3, or 4 days; followed by holding in air for 7, 6, 5, or 4 days, respectively.

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Gas mixing and analysis. The desired CA combinations were obtained by mixing air, N₂, and/or CO₂ in various volume ratios. The compositions of all gas mixtures were verified each day by taking a 10-ml gas sample and analyzing it by a Carle gas chromatograph equipped with a thermal conductivity detector.

Measurements of respiration and ethylene production rates. A 10-ml gas sample was taken from each jar. Carbon dioxide production rates of fruits kept under reduced O₂ atmospheres or air were based on analyses of concentration measured with a Horiba infrared CO₂ gas analyzer (Model SX-2), O₂ consumption rates of fruits stored in CO₂-enriched atmospheres on measurements made with an electrochemical S-3A oxygen analyzer, and ethylene production rates on those made with a Carle gas chromatograph equipped with a flame ionization detector.

Determination of volatile compounds. Frozen juice of strawberries was thawed and then centrifuged at 25,000 × g for 20 min. at 0°C. One milliliter supernatant was diluted with 10 ml of double-deionized water and filtered through a 0.45-μm microfilter in a 0°C room. The filtrate was used for determining the concentrations of ethanol, methanol, and acetaldehyde by a HP5890A gas chromatograph with a flame ionization detector, the oven at 85°C and the detector at 250°C.

Measurements of firmness and color. Flesh firmness was measured as penetration force with a U.C. Fruit Firmness Tester, using a 3.2-mm plunger tip. A round area 2 cm in diameter was peeled at both sides of a fruit and measurements were made on the peeled areas. Skin, flesh, and juice colors were measured by a Gardner XL-23 Tristimulus Colorimeter, using the 'a' value. Positive and negative 'a' values indicate red and green color, respectively.

Determination of titratable acidity, pH, and soluble solids content. An automatic titrator was used to determine pH and titratable acidity. Fresh juice (4 g) was diluted with 20 ml deionized water, its pH was measured, then it was titrated with 0.1 N NaOH. Titratable acidity was calculated as percent citric acid. Undiluted fresh juice was used to measure soluble solids content (SSC) by an Abbé Refractometer.

Determination of ascorbic acid. Frozen strawberries were thawed and 50 g of tissue was taken from eight fruit and sliced. The tissue was blended by a Polytron blender in 100 ml of 0.1% (w/v) oxalic acid solution. The homogenate was filtered through four layers of cheesecloth and centrifuged for 20 min at 25,000 × g at 0°C. One milliliter of supernatant was diluted with 9 ml of 0.1% oxalic acid solution and filtered through a 0.45-μm microfilter. The filtrate was used to determine ascorbic acid content. A Bio-Rad high-performance liquid chromatograph system with an ion exclusion HPX-87H column and a Bio-Rad UV Monitor (Model 1306) at 245 nm wavelength was used for the analysis at 20°C. Mobile phase was 0.01 N H₂SO₄ and flow rate was 0.8 ml·min⁻¹.

Statistical analysis was done using analysis of variance for each test and, when differences were significant, LSD test was used for mean separation.

Results and Discussion

Effects of exposure to various O₂ and CO₂ levels and their combinations on strawberries.

Respiration. Strawberries kept in 0.5 to 2% O₂ had lower respiration rates, based on CO₂ evolution, than those held in air (Fig. 1A). Upon transfer to air, respiration rates increased gradually but remained lower than those of the fruits continuously stored in air. The 0.5% O₂ atmosphere was more effective in

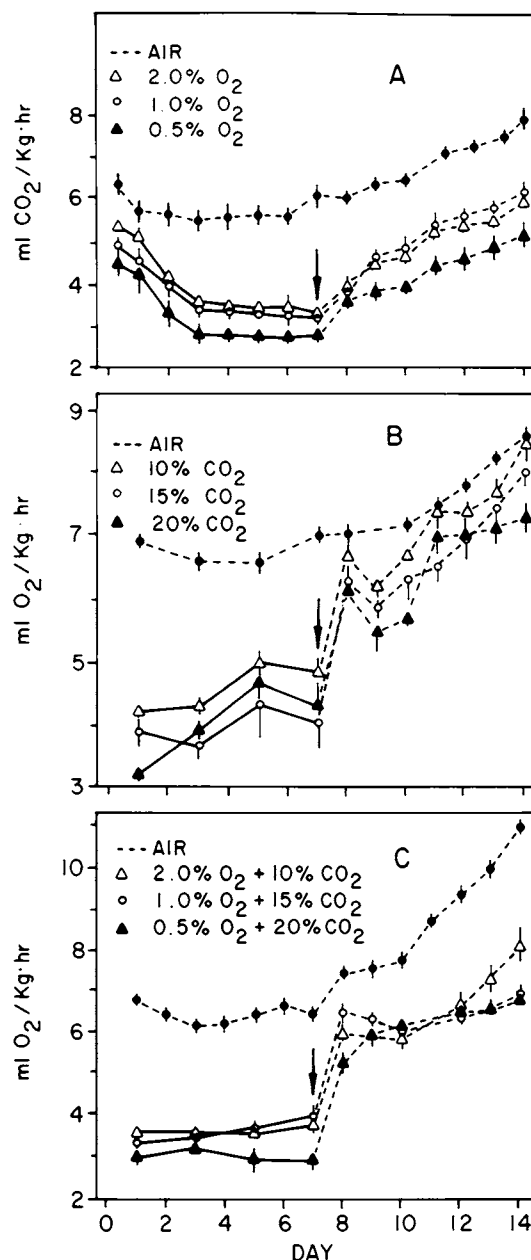


Fig. 1. Effects of exposure at 2°C to various levels of O₂ (A), CO₂ (B), or their combinations (C) for 7 days followed by 7 days in air at 2°C on respiration rate of 'Selva' strawberries. Bars on data points represent SDs; arrows indicate date of transfer to air.

reducing respiration rates, and its residual effect was greater than those with 1.0% and 2.0% O₂. There was no significant difference between 1.0% and 2.0% O₂ in reducing respiration rates.

The respiration rates of the strawberries held in CO₂-enriched atmospheres, as determined by O₂ uptake, were lower than those of the fruits kept in air (Fig. 1B). Generally, 20% and 15% CO₂ were more effective than 10% CO₂ in lowering respiration rate. When the fruits were transferred to air, their respiration rates increased, but were still lower than those of the strawberries continuously stored in air.

CA combinations of reduced O₂ and elevated CO₂ lowered respiration rates, based on O₂ consumption, of strawberries (Fig. 1C); 0.5% O₂ + 20% CO₂ was the most effective. There was no significant difference in respiration rates between strawber-

ries kept in 2.0% O₂ + 10% CO₂ and those stored under 1.0% O₂ + 15% CO₂. When the fruits were transferred to air, respiration rates increased, but were still lower than those of the strawberries continuously exposed to air.

As measured after 7 days storage in CA, reduced O₂ was ≈10% more effective than elevated CO₂ in lowering respiration rates, and the combinations of reduced O₂ and elevated CO₂ were no more effective than reduced O₂ alone (38% to 55% vs. 45% to 54%, respectively). After storage in air for another 7 days (day 14), the reduction of respiration rates of the strawberries previously stored in reduced-O₂ atmospheres was greater than that in the fruits previously treated with elevated CO₂ (21% to 35% vs. 2% to 15%, respectively). The residual effects of the combinations were comparable to those of reduced O₂ alone, except the 1.0% O₂ + 15% CO₂, which had a greater effect than that of either 1.0% O₂ or 15% CO₂ alone (36% vs. 21% vs. 7% reduction, respectively).

Ethylene production. Ethylene production rates of strawberries stored in 2.0%, 1.0%, or 0.5% O₂ were greatly reduced as compared to the air control (Fig. 2A); 0.5% O₂ was more effective than 1.0% and 2.0% O₂. When transferred to air, C₂H₄ production rates increased, but remained lower than C₂H₄ production by fruits kept continuously in air for 14 days.

When strawberries were stored in air + 10%, 15%, or 20% CO₂, C₂H₄ production rates were reduced in proportion to the increase in CO₂ concentration (Fig. 2B). Upon transfer of the strawberries from elevated CO₂ atmospheres to air, C₂H₄ production rates gradually increased, but remained lower than those of the control fruits; 15% and 20% CO₂ had more pronounced residual effect than 10% CO₂.

The strawberries stored in CA combinations of reduced O₂ and elevated CO₂ had lower C₂H₄ production rates than the fruits stored in air (Fig. 2C). Siriphanich (1980) and El-Kazzaz et al. (1983) reported similar effects of CA on reducing C₂H₄ production by strawberries. There was no significant difference among these CA treatments. After the CA-treated fruits were transferred to air, C₂H₄ production rates increased, but were still lower than those of fruits kept continuously in air. The residual effects of reducing C₂H₄ production rates were especially noteworthy for the 0.5% O₂ + 20% CO₂ and 1.0% O₂ + 15% CO₂ treatments.

As measured after 7 days of storage in CA, reduced O₂ was more effective in lowering C₂H₄ production rates than elevated CO₂, and the combinations of both were more effective than either alone (63% to 70% vs. 42% to 58% vs. 67% to 75% reduction, respectively). Similar synergistic effects were noted after the fruits had been transferred to air for 7 days (day 14); the percent reductions were 16% to 40% vs. 24% to 48% vs. 43% to 73%, respectively.

Accumulation of volatile compounds. Strawberries stored with 1.0% or 0.5% O₂ for 7 days contained more ethanol than the air control fruits (Table 1). However, after the fruits had been held in air for another 7 days, the differences in ethanol content among these three treatments were very small. Low O₂ atmospheres had no effect on methanol or acetaldehyde concentrations, which ranged from 20 to 60 and from 80 to 100 μl·liter⁻¹ juice, respectively (data not shown).

The fruits treated with 15% or 20% CO₂ had higher ethanol concentration than air control fruits (Table 1). After the high-CO₂-treated fruits had been held in air for 7 days, ethanol concentration declined in all treatments except the air control. In both tests, control strawberries continued to accumulate ethanol, but the concentration range after 14 days at 2C was still between

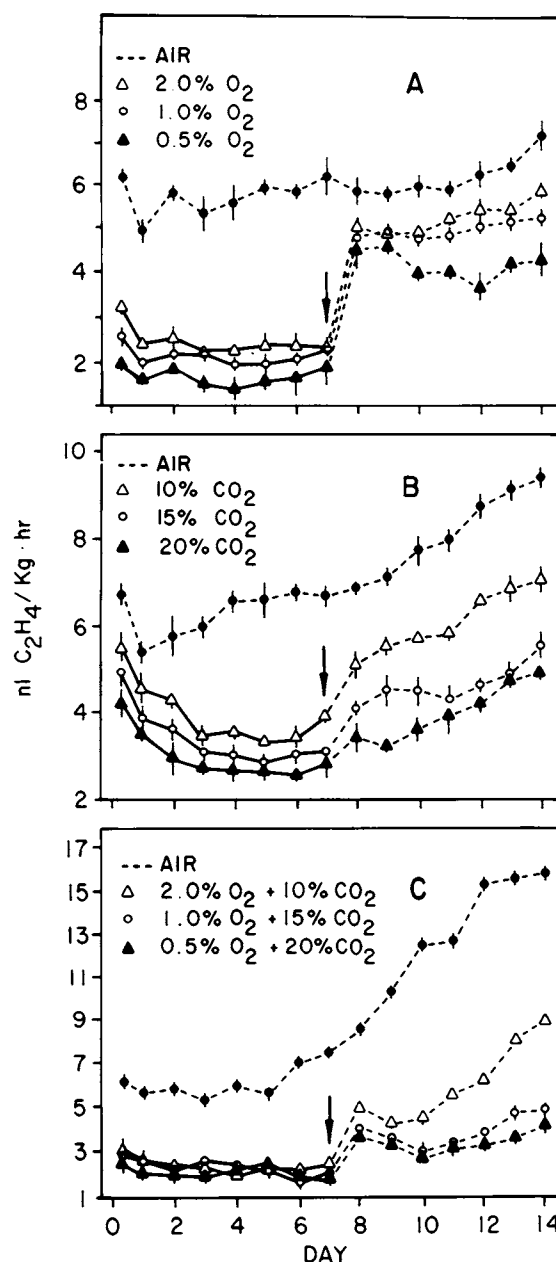


Fig. 2. Effects of exposure at 2C to various levels of O₂ (A), CO₂ (B), or their combinations (C) for 7 days followed by 7 days in air at 2C on ethylene production rate of 'Selva' strawberries. Bars on data points represent SDs; arrows indicate date of transfer to air.

30 and 60 μl·liter⁻¹ juice, which is not likely to have a negative effect on flavor.

The combinations of reduced O₂ and elevated CO₂ levels dramatically increased ethanol accumulation, especially for 0.5% O₂ + 20% CO₂ and 1.0% O₂ + 15% CO₂ (Table 1). These treatments also had increased acetaldehyde concentration by ≈50% and 20% on days 7 and 14, respectively, but had no effect on methanol content (data not shown). After the fruits had been transferred to air for 7 days (day 14), ethanol content of strawberries previously exposed to 1% O₂ + 15% or 0.5% O₂ + 20% CO₂ declined, but still was much higher than that of strawberries previously kept in 2% O₂ + 10% CO₂ or in air. Although only small residual effects on ethanol concentrations were observed with either reduced O₂ or elevated CO₂ alone, the combinations of 1% O₂ + 15% CO₂ and 0.5% O₂ + 20%

Table 1. Effects of exposure at 2C with various O₂ or CO₂ levels, or their combinations for 7 days followed by 7 days in air at 2C on ethanol content, flesh color, and firmness of 'Selva' strawberries.

Treatment (7 days) before holding in air for 7 days	Ethanol concn ² (μl·liter ⁻¹)		Firmness on day 14 (N)	Color on day 14 (CDM ³ 'a' value)
	Day 7	Day 14		
Air	10.0	55.0	2.5	54.6
2.0% O ₂	13.2	28.6	2.5	57.5
1.0% O ₂	35.2	66.0	2.6	54.8
0.5% O ₂	63.8	72.6	2.8	53.4
LSD at 5%	15.0	21.4	0.1	2.8
Air	18.9	42.8	2.5	55.4
Air + 10% CO ₂	19.8	17.6	2.8	52.9
Air + 15% CO ₂	40.5	19.8	2.7	52.8
Air + 20% CO ₂	97.3	39.6	3.0	53.1
LSD at 5%	15.9	16.1	0.2	1.9
Air	28.6	30.8	2.6	53.7
2.0% O ₂ + 10% CO ₂	54.3	55.0	2.7	49.9
1.0% O ₂ + 15% CO ₂	270.6	143.0	3.3	48.0
0.5% O ₂ + 20% CO ₂	292.6	178.2	3.2	47.0
LSD at 5%	20.2	22.8	0.1	3.5

²Initial concentration was 9.2 ± 1.2 μl·liter⁻¹.

³CDM = Color Difference Meter.

CO₂ resulted in a pronounced residual effect. On day 14, ethanol concentrations in strawberries exposed to these treatments were three to four times greater than the control fruits. The higher concentrations of ethanol and acetaldehyde (100 to 200 μl·liter⁻¹) may cause off-flavors in these strawberries.

Quality attributes. Some residual effects of CA treatments were observed with flesh firmness and flesh color (Table 1). As measured on day 14, the fruits previously kept in 0.5% O₂ were slightly firmer than those continuously exposed to air or 2.0% or 1.0% O₂. The fruits previously treated with elevated CO₂ were all firmer than those continuously exposed to air; 20% CO₂ was the most effective. The combinations of reduced O₂ and elevated CO₂ also resulted in better firmness retention, which may delay incidence of decay (El-Kazzaz et al., 1983).

Reduced O₂ (0.5% and 1%) did not influence and the 2% O₂ slightly influenced the development of redness in the flesh of strawberries (Table 1). Elevated CO₂ slightly retarded color development, but no differences were noted among the three CO₂ concentrations tested. The combinations of reduced O₂ and elevated CO₂ retarded color development (relative to their control) to a slightly greater extent than elevated CO₂ alone (relative to their control).

None of the CA combinations tested had a significant effect on skin or juice color, titratable acidity, pH, soluble solids content, and ascorbic acid content of strawberries (data not shown).

Effects of exposure to 0.5% O₂, 20% CO₂, or 0.5% O₂ + 20% CO₂ for various durations

Since 0.5% O₂, 20% CO₂, and 0.5% O₂ + 20% CO₂ were generally the most effective treatments among the various CA combinations tested, these three treatments were selected to study the residual effects of exposure to CA for various durations.

Respiration. Strawberries kept in 0.5% O₂ had lower respiration rates than those stored in air (Fig. 3A). When transferred to air following storage in 0.5% O₂ for 1 to 4 days, respiration rates increased, but were still lower than those of the fruits

continuously exposed to air, indicating the increasing residual effect of 0.5% O₂, which increased (from 7% to 21% reduction on day 8) with exposure time.

The fruits stored in 20% CO₂ had lower respiration rates than strawberries kept in air (Fig. 3B). Respiration rates of fruits that had been stored in 20% CO₂ for 1 to 4 days before transfer to air increased during the first day and then leveled off, but were still lower than those of fruits continuously stored in air. The residual effect of 4 days of exposure to 20% CO₂ was greater than those of shorter exposures (35% vs. 24% to 29% reduction on day 8).

Strawberries kept in 0.5% O₂ + 20% CO₂ had lower respiration rates than strawberries stored in air (Fig. 3C). Upon transfer to air, respiration rates rapidly increased during the first day and then leveled off, but remained lower than those of the fruits continuously stored in air. As measured on day 8, the residual effects of air + 20% CO₂ and 0.5% O₂ + 20% CO₂ on respiration rates were comparable and generally greater than the effects of the 0.5% O₂ treatment (24% to 35% vs. 19% to 24% vs. 7% to 21% reduction, respectively).

Ethylene production. The fruits stored in 0.5% O₂ had much lower C₂H₄ production rates than those of the strawberries stored in air (Fig. 4A). Upon transfer of the 0.5% O₂-treated fruits to air, C₂H₄ production rates rapidly increased, but were still lower by 2% to 14% on day 8 than those of the fruits continuously kept in air.

Storage in air + 20% CO₂ reduced C₂H₄ production rates (Fig. 4B). When transferred to air, C₂H₄ production rates by the strawberries increased rapidly, but were still lower than those of the air control. The residual effect of 4 days of exposure to 20% CO₂ was much more pronounced than that of shorter exposure times (43% vs. 10% or lower reduction on day 8).

Treatment with 0.5% O₂ + 20% CO₂ significantly reduced C₂H₄ production rates of strawberries (Fig. 4C). Upon transfer to air, C₂H₄ production rates rapidly increased, but remained lower by 15% to 30% than those of the control fruits. The residual effect of 0.5% O₂ + 20% CO₂ increased with exposure time from 1 to 4 days (15%, 21%, 27%, and 30% reduction on

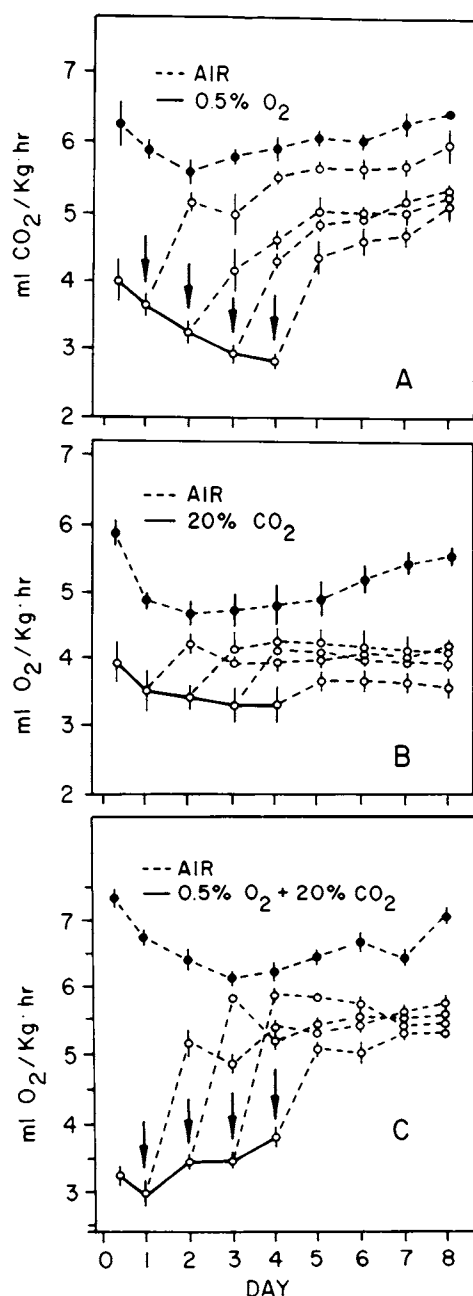


Fig. 3. Effects of exposure at 2°C to 0.5% O₂ (A), 20% CO₂ (B), or 0.5% O₂ + 20% CO₂ (C) for 1, 2, 3, or 4 days and subsequent holding in air for 7, 6, 5, or 4 days at 2°C on respiration rate of 'Selva' strawberries. Bars on data points represent SDs; arrows indicate times of transfer to air.

day 8, respectively). Treatment with 0.5% O₂ + 20% CO₂ for 1 to 3 days induced a larger residual effect than exposure to either 0.5% O₂ or 20% CO₂ alone (15% to 27% vs. 1% to 3% vs. 0% to 10% reduction on day 8, respectively), but 4 days of exposure did not show similar synergistic effects (14% vs. 43% vs. 30% reduction, respectively).

Accumulation of volatile compounds. The fruits stored in 0.5% O₂ or in 20% CO₂ had higher ethanol concentrations than the fruits stored in air, but, in all treatments, ethanol concentrations remained <30 µl·liter⁻¹ (Table 2). The ethanol concentrations increased with exposure time. On day 8, no differences were observed in ethanol concentration among the 0.5% O₂ treatments. Ethanol content on day 8 was greater in the air control fruits than in the 20% CO₂-treated fruits.

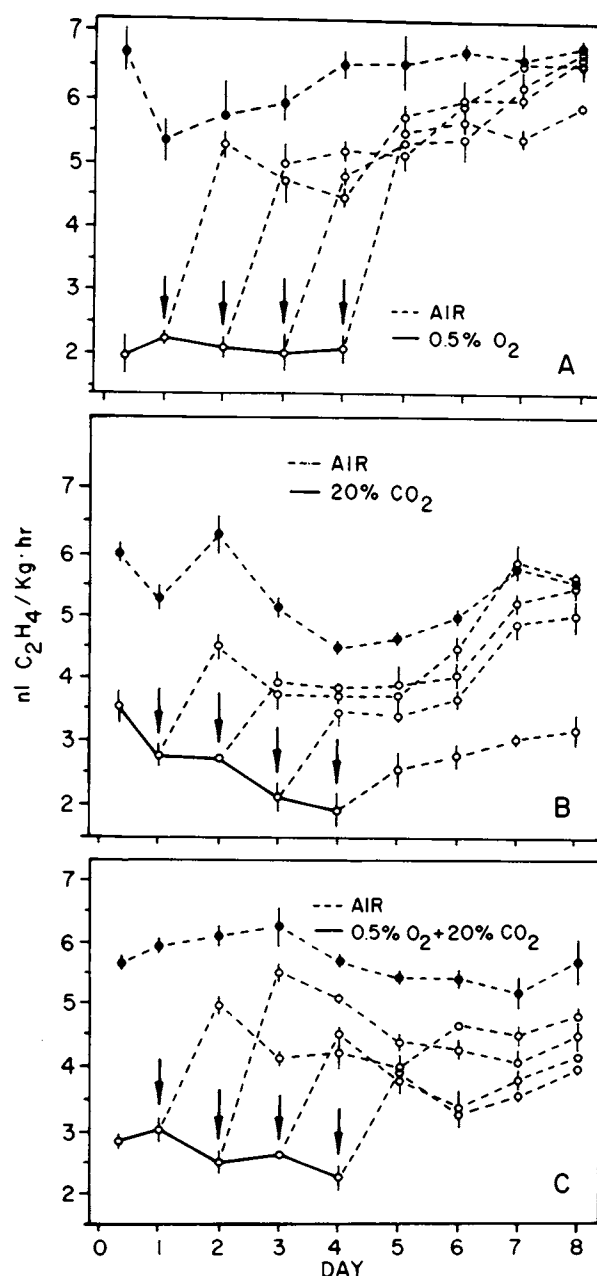


Fig. 4. Effects of exposure at 2°C to 0.5% O₂ (A), 20% CO₂ (B), or 0.5% O₂ + 20% CO₂ (C) for 1, 2, 3, or 4 days and subsequent holding in air for 7, 6, 5, or 4 days at 2°C on ethylene production rate of 'Selva' strawberries. Bars on data points represent SDs; arrows indicate times of transfer to air.

Exposure to 0.5% O₂ + 20% CO₂ dramatically increased ethanol accumulation; the magnitude of this effect increased with exposure time. Ethanol concentration in the fruits treated with 0.5% O₂ + 20% CO₂ was much higher than that in the strawberries treated with either 0.5% O₂ or 20% CO₂ alone (Table 2). However, after transfer to air, ethanol concentration declined and differences among treatments on day 8 were small. This pattern was also true for methanol and acetaldehyde concentrations (range from 20 to 30 and 100 to 120 µl·liter⁻¹, respectively). Such acetaldehyde concentrations are not likely to have any effects on flavor quality of strawberries (Shaw, 1969; Prasad and Stadelbacher, 1974).

Quality attributes. The strawberries stored in 0.5% O₂, 20% CO₂, or 0.5% O₂ + 20% CO₂ for 1 to 4 days before storage

Table 2. Residual effects of exposure at 2C to 0.5% O₂, 20% CO₂, or 0.5% O₂ + 20% CO₂ for 1, 2, 3, or 4 days and subsequent holding in air at 2C for 7, 6, 5, or 4 days on ethanol content, flesh color, and firmness of 'Selva' strawberries as measured on day 8.

Treatment	Ethanol concn ² (μl·liter ⁻¹)		Firmness (N)	Color (CDM ³ 'a' value)
	At transfer from CA	Day 8		
8 d in air	---	16.5	2.7	55.2
1 d (0.5% O ₂) + 7 d (air)	7.8	13.2	2.9	52.8
2 d (0.5% O ₂) + 6 d (air)	15.4	13.2	2.8	54.1
3 d (0.5% O ₂) + 5 d (air)	20.9	19.1	3.1	52.1
4 d (0.5% O ₂) + 4 d (air)	26.4	15.4	3.2	51.5
LSD at 5%	6.9	9.2	0.1	2.7
8 d in air	---	11.1	3.2	53.3
1 d (air + 20% CO ₂) + 7 d (air)	2.0	1.2	3.5	53.4
2 d (air + 20% CO ₂) + 6 d (air)	6.6	1.5	3.5	54.6
3 d (air + 20% CO ₂) + 5 d (air)	8.8	4.4	3.8	53.8
4 d (air + 20% CO ₂) + 4 d (air)	24.2	8.8	4.0	50.7
LSD at 5%	2.4	4.8	0.2	2.6
8 d in air	---	19.8	2.6	57.9
1 d (0.5% O ₂ + 20% CO ₂) + 7 d (air)	35.2	28.6	3.5	55.3
2 d (0.5% O ₂ + 20% CO ₂) + 6 d (air)	72.6	26.4	3.4	56.2
3 d (0.5% O ₂ + 20% CO ₂) + 5 d (air)	145.2	24.2	3.6	54.6
4 d (0.5% O ₂ + 20% CO ₂) + 4 d (air)	202.4	37.4	3.5	52.7
LSD at 5%	17.9	5.4	0.2	3.9

²Initial concentration was 2.3 ± 1.4 μl·liter⁻¹ and increased to 3.4 ± 2.8 , 4.9 ± 2.2 , 5.7 ± 1.4 , and 6.6 ± 2.3 μl·liter⁻¹ after 1, 2, 3, and 4 days, respectively.

³CDM = Color Difference Meter.

in air for 4 to 7 days were firmer than the fruits continuously stored in air (Table 2). Exposure to 0.5% O₂ or 20% CO₂ for 3 or 4 days had greater residual effect on firmness retention than exposure for 1 or 2 days. The residual effect of 0.5% O₂ + 20% CO₂ on flesh firmness was more pronounced than that of either 0.5% O₂ or 20% CO₂ alone relative to the control in each case.

Exposure to 0.5% O₂, air + 20% CO₂, or 0.5% O₂ + 20% CO₂ for 4 days retarded reddening in strawberry flesh relative to the controls (Table 2). None of the CA treatments tested had any significant effects on skin or juice color, titratable acidity, pH, soluble solids content, and ascorbic acid content of strawberries (data not shown).

Results from this research demonstrate the residual effects of CA on postharvest physiology and quality of strawberries. These residual effects of CA may include the reduction of respiration and C₂H₄ production rates, accumulation of ethanol and possibly other volatiles, maintenance of flesh firmness, and retardation of flesh color development. Generally speaking, as the concentration of O₂ is decreased, the concentration of CO₂ is increased, and the duration of exposure to CA conditions is increased and the residual effects become more prominent. Some combinations of reduced O₂ and elevated CO₂ have synergistic residual effects. Exposure of strawberries to controlled or modified atmospheres containing 15% to 20% CO₂ and 1% to 2% O₂ for at least 3 days will help maintain their quality during transport and temporary storage and will slow deterioration during subsequent handling in air.

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