Tolerance of 'Valencia' Oranges to Controlled Atmospheres, as Determined by Physiological Responses and Quality Attributes

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Abstract. 'Valencia' oranges [*Citrus sinensis* (L.) Osbeck] tolerated up to 20 days of exposure to 0.5%, 0.25%, or 0.02% O₂ at 5 or 10C followed by holding in air at 5C for 7 days without any detrimental effects on external and internal appearance. Oranges stored in 0.5%, 0.25%, or 0.02% O₂ had lower respiration rates, but higher resistance to CO₂ diffusion and higher ethanol evolution rates than those stored in air at 10C. Similar, but less pronounced, effects of the low O₂ atmospheres were observed at 0 and 5C. Respiration rates, internal CO₂ concentrations, and ethanol evolution rates were generally higher at 10C than at 0C, while resistance to CO₂ diffusion was lower at the higher temperature. 'Valencia' oranges kept in 60% CO₂ at 5C for 5 to 14 days followed by holding in air at 5C for 7 days developed slight to severe injury that was characterized by skin browning and lowered external appearance scores. Juice color, soluble solids content, pH, titratable acidity, and ascorbic acid content were not significantly influenced by either the low O₂ or the high CO₂ treatments. However, these treatments increased ethanol and acetaldehyde contents, which correlated with the decrease in flavor score of the fruits. Ethanol content of the oranges transferred to air following low O₂ treatment correlated with CO₂ production rate of the fruits at the transfer temperature and was related to ethanol evolution and probably production rates after the transfer.

Long-term storage of oranges in controlled atmospheres (CA) is not recommended because CA has little or no advantage for extending the storage life of the fruits (Hatton and Cubbedge, 1977a). Low O₂ or high CO₂ atmospheres stimulated accumulation of ethanol, acetaldehyde, and several other volatile compounds in oranges (Davis et al., 1973; Norman and Craft, 1971; Pesis and Avissar, 1989) and increased activity of pyruvic decarboxylase (EC 4.1.1.1) and alcohol dehydrogenase (EC 1.1.1.1) (Davis et al., 1973). CA storage delayed skin degreening and extended storage life of limes and lemons (Hatton and Cubbedge, 1977a; Hatton and Reeder, 1969). Prestorage treatments with 10% to 40% CO₂ in air reduced chilling injury and stem-end rind breakdown of grapefruits (Hatton and Cubbedge, 1977b; Hatton et al., 1975).

Although oranges can not tolerate long-term storage in CA conditions, the fruits may tolerate a short period of exposure to low O₂ and/or high CO₂ atmospheres. In recent years, there has been an increased interest in studying the responses of fresh fruits and vegetables to short-term exposure to O₂ levels at or below 1% and CO₂ levels at or above 50% because these atmospheres may be effective as a quarantine treatment for post-harvest insect control (Aharoni et al., 1979; Benshote, 1987; Lidster et al., 1981, 1984; Soderstrom and Brandl, 1987). CA treatment can be used for quarantine procedure only when they can effectively kill the insects of concern without detrimental effects on quality attributes of the fresh commodities.

In this study, we investigated the effects of short-term exposures to O₂ levels at or below 0.5% and CO₂ level at 60% at 0, 5, and 10C on postharvest physiology and quality attributes of 'Valencia' oranges to determine their tolerance to these treatments, which may be used for postharvest insect control.

Materials and Methods

Materials and treatments. Fungicide-treated 'Valencia' oranges were obtained on the day of harvest from a commercial shipper in Fresno, Calif., and transported in an air-conditioned car to our laboratory at Davis, where they were kept at 5C. The experiments were initiated the next morning. Fruits with defects were sorted out and six acceptable fruits were put into a 4-liter glass jar as one replicate with three replicates used per treatment (with the exception that in sensory panel experiment, more jars were used so that enough fruits were available for the sensory evaluation). In one experiment, the fruit samples were kept in air and in 0.5%, 0.25%, or 0.02% O₂ (balance was N₂) at 0, 5, or 10C for 3 days before they were used for the determinations of respiration rate, internal O₂ and CO₂ concentrations, resistance to gas diffusion, and ethanol evolution rate. Flow rates were 15, 25, and 40 ml·min⁻¹·kg⁻¹ at 0, 5, or 10C, respectively. In a second experiment, the fruit samples were kept in the atmospheres noted at 5 or 10C for 5, 9, 15, or 20 days, followed by holding in air at 5C for 7 days before final quality evaluations. In a third experiment, the fruit samples were kept in 0.02% O₂ at 5C for 7 days and then in air at 0, 5, or 10C for another 7 days to examine the effects of transfer temperature on ethanol content, ethanol evolution rate, and CO₂ production rate. In a fourth experiment, the fruit samples were kept in air and in air enriched with 60% CO₂ at 5C for 5, 9, or 14 days, followed by holding in air at 5C for 7 days before final quality evaluations.

Gas measurements. The required O₂ and CO₂ concentrations of all gas mixtures were verified by analysis of a 10-ml gas sample using an O₂ analyzer (Model S-3AII, Applied Electrochemistry, Sunnyvale, Calif.) in series with an infrared CO₂ analyzer (Model PIR-2000, Horiba Instruments, Irvine, Calif.). Respiration rate was estimated by detecting the CO₂ concentration from the headspace of the jar holding the fruits, the flow rate used, and the fresh weight of the sample.

A vacuum extraction method (Saltveit, 1982) was used to determine internal O₂ and CO₂ concentrations and resistance to gas diffusion. The vacuum apparatus consisted of a steel vessel

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and a heavy-duty funnel with a plastic gas sampling septum attached to the tip of the funnel. The funnel was placed inside the vessel, which was filled to the top with degassed water. Six fruits were placed under water in the inverted funnel. The vessel was closed and a vacuum of ≈ 2300 kPa applied. A preliminary experiment showed that the extracted internal gas concentrations of 'Valencia' oranges did not significantly change during 1 to 5 min under the vacuum condition and, therefore, 3-min vacuum time was used in the experiment. The vacuum was then released and the vessel opened. Samples were taken through the septum with a 1-ml syringe for the determination of internal O_2 and CO_2 concentrations. Resistance to CO_2 diffusion was calculated by the ratio: $[(CO_2)_{int} - (CO_2)_{ext}] / (CO_2 \text{ production rate})$.

Determinations of quality attributes. For the determination of quality attributes, three initial samples of six fruits each were evaluated for external and internal appearances, juice color, soluble solids content (SSC), pH and titratable acidity, ascorbic acid content (AA), and ethanol and acetaldehyde concentrations. Similar measurements were made in the final quality evaluations. External and internal appearance was scored using a subjective scale of 1 to 5, where 5 = excellent, 4 = good, 3 = fair, 2 = slight defects, 1 = severe defects. Juice was extracted with a hand-press juicer, using two flesh sections from each fruit. Juice color ("b" value) was measured with a color difference meter (Minolta Chroma Meter, Model CR-200), in which a high positive number indicates an intense yellow. Soluble solids content was measured with an Abbé refractometer and pH and titratable acidity were measured using an automatic titrator equipped with a PHM85 Precision pH meter, an AUB80 autoburette, a PRS12 Alpha printer, and a SAC80 sample changer.

For the determination of AA content, previously frozen orange juice (up to 30 days at -30°C) was thawed and a 2-ml sample was diluted with 18 ml of 0.1% oxalic acid solution. The sample solution was centrifuged for 20 min at $25,000 \times g$ at 0°C and ≈ 2 ml of supernatant was filtered through a $0.45\text{-}\mu\text{m}$ microfilter. The filtrate was used to determine AA content using a Bio-Rad (Richmond, Calif.) high performance liquid chromatography system with an ion exclusion HPX-87H column and a Bio-Rad UV monitor (Model 1306) at a 245-nm wavelength. Mobile phase was $0.01 \text{ N H}_2\text{SO}_4$ and flow rate was $0.8 \text{ ml}\cdot\text{min}^{-1}$.

The method for measurements of ethanol and acetaldehyde contents of orange juice was slightly modified from that of Davis and Chace (1969). Frozen orange juice was thawed and a 5-ml sample was put in a 10-ml screw-cap test tube. The test tube was closed with a plastic cap and incubated in a water bath at 60°C . After 60 min, a headspace sample was taken with a 1-ml glass syringe for determination of ethanol and acetaldehyde concentrations using a HP5890A gas chromatography (Hewlett Packard, Palo Alto, Calif.) with a flame ionization detector (at 250°C) and a glass column ($2 \text{ mm} \times 1.0 \text{ m}$) containing 5% Carbowax on 60/80 Carbowax as stationary phase (at 85°C).

Sensory panel evaluation of flavor. Four judges (two males, two females, ages 25 to 30) were selected to be trained and to conduct a sensory panel evaluation of the flavor of the oranges kept in various atmospheric compositions at 5 or 10°C for various durations. The judges rated the flavor on a subjective scale of 1 to 9, where 9 = excellent, 8 = very good, 7 = good, 6 = fair, 5 = minimally acceptable, 4 = slight off-flavor, 3 = moderate off-flavor, 2 = severe off-flavor, 1 = extreme off-flavor. The fruits were washed with water and held in a 8-liter glass jar and each jar was coded with a three-digit random num-

ber. The judges tasted, in random order, a section from each fruit with three fruits in each treatment, without knowing their identities. The tasting was performed in the Postharvest Laboratory of the Pomology Dept., Univ. of California, Davis. Judges rinsed ad-lib with tap water between tastings to avoid influence by the flavor of prior sample.

Estimation of CO_2 injury. CO_2 injury was estimated by a pretransformed scale of 1 to 5 according to the percentage of brown skin area: 1 = no injury; 2 = slight injury, up to 15% brown skin area; 3 = moderate injury, 16% to 50% brown skin area; 4 = severe injury, 51% to 85% brown skin area; 5 = extreme injury, 86% to 100% brown skin area.

Statistical analysis. Data were treated for multiple comparisons by analysis of variance with LSD between means determined at $P = 0.05$. Linear regression and correlation was also done in some analysis.

Results and Discussion

Keeping 'Valencia' oranges in 0.5%, 0.25%, or 0.02% O_2 at 10°C for 3 days decreased respiration rates (Table 1), but the effect of low O_2 was less pronounced when the fruits were kept at 5°C and was absent at 0°C . Fruits kept in 0.02% O_2 at 10°C had a higher CO_2 production rate than those kept in 0.25% O_2 , probably due to anaerobic respiration (Boersig et al., 1988). Fruits kept in low- O_2 atmospheres generally had higher internal CO_2 concentrations, resistance to CO_2 diffusion, and ethanol evolution rates than those kept in air. Carbon dioxide production rates, internal CO_2 concentrations, and ethanol evolution rates were generally higher at 10°C than those at 0°C , while resistance to CO_2 diffusion was generally lower at the higher temperature. Although the external CO_2 concentration was kept below 0.25% during the experiment, the internal CO_2 concentration was ≈ 20 times higher than the external levels. Therefore, a large CO_2 concentration gradient existed between the outside and inside atmospheres. A preliminary experiment showed that ethylene production rate of 'Valencia' oranges was almost undetectable; thus, no further work was done to study the effect of low O_2 on ethylene production in these fruits.

The oranges kept in air or in 0.5%, 0.25%, or 0.02% O_2 at 5 or 10°C for 5, 9, 15, or 20 days followed by transfer to air at 5°C for 7 days all had similar external and internal appearance scores (4.4 to 4.9 and 4.8 to 5.0, respectively). No external or internal injury was observed and decay was negligible during the entire experiment.

Low- O_2 treatments did not significantly influence juice color (range of "b", 14-16), SSC (11%), pH (range 3.5-3.6) titratable acidity (range 0.9% to 1.2%), or AA content (range 78 to 86 mg/100 ml) of 'Valencia' oranges. The oranges treated with low O_2 had similar or slightly lower flavor scores than those of the fruits kept in air after 5 to 20 days of treatment (Fig. 1 A and B), but all the flavor scores were still acceptable (with scores of near or above 5 compared to the initial score of 7). All four judges detected moderate to severe off-flavor in oranges that were kept in 0.027002 at 5°C for 26 days before transfer to air for tasting (with an average flavor score of 2.4). Therefore, 26 days was beyond the limit for storing 'Valencia' oranges in 0.029002 at 5°C . Large variations were observed among the flavor scores estimated by different judges, due in part to the subjective nature of the flavor scale used and to differences in individual preferences.

All low- O_2 treatments increased ethanol and acetaldehyde contents of 'Valencia' oranges (Fig. 1 C-F), indicating that anaerobic respiration occurred in all the low- O_2 -treated fruits.

Table 1. Effect of O₂ level at several temperatures on respiration rate, internal CO₂ concentration, resistance to CO₂ diffusion (r_{CO₂}), and ethanol evolution rate of 'Valencia' oranges determined after 3 days under the conditions cited.

Temp (°C)	O ₂ concn (%)	CO ₂ production rate (ml·hr ⁻¹ ·kg ⁻¹)	Internal CO ₂ (%)	r _{CO₂} (% ml ⁻¹ ·hr ⁻¹ ·kg ⁻¹)	Ethanol evolution (μl·hr ⁻¹ ·kg ⁻¹)
0	21 (air)	2.0	3.3	1.5	0.1
	0.5	1.8	3.4	1.8	0.2
	0.25	1.8	3.7	2.0	0.2
	0.02	2.0	3.8	1.8	0.3
	LSD ^z	0.4	0.8	0.7	0.2
5	21 (air)	2.7	3.2	1.1	0.2
	0.5	2.4	4.2	1.7	0.5
	0.25	2.4	4.3	1.7	0.5
	0.02	2.4	3.7	1.5	0.7
	LSD ^z	0.3	0.5	0.2	0.3
10	21 (air)	5.4	3.7	0.6	0.8
	0.5	4.3	4.6	1.0	1.5
	0.25	4.0	4.3	1.1	1.5
	0.02	4.6	5.6	1.2	2.4
	LSD ^z	0.2	1.5	0.4	0.4

^zLSD at *P* = 0.05.

Fig. 1. Effects of temperature and O₂ level on flavor score (A and B), ethanol content (C and D), and acetaldehyde content (E and F) of 'Valencia' oranges kept in air and in 0.5%, 0.25%, or 0.02% O₂ at 5 or 10°C for 5, 9, 15, or 20 days followed by holding in air at 5°C for 7 days. The flavor scores were estimated by a sensory panel of four people using a subjective scale where 9 = excellent, 8 = very good, 7 = good, 6 = fair, 5 = minimally acceptable, 4 = slight off-flavor, 3 = moderate off-flavor, 2 = severe off-flavor, 1 = extreme off-flavor. The vertical bars represent LSD values at *P* = 0.05.

Ethanol and acetaldehyde accumulation was also observed in low-O₂-treated oranges by several other researchers (Davis et al., 1973; Norman and Craft, 1971; Pesis and Avissar, 1989). Generally, the fruits kept at 10°C had higher ethanol and acetaldehyde contents than those of the fruits kept at 5°C. The absolute ethanol contents of 'Valencia' oranges kept in low O₂ atmospheres were 50 to 100 times higher than the corresponding acetaldehyde contents. Fidler (1968) found that applied acetaldehyde could be converted to ethanol by plant tissue.

A correlation between ethanol or acetaldehyde content and flavor score was observed (Fig. 2 A and B). These correlations were statistically highly significant (*P* = 0.01), even though the *r* values were not near 1. Although higher ethanol and acetaldehyde contents generally resulted in lower flavor scores, the relation between flavor score and ethanol or acetaldehyde content is not straight-forward. Ethanol and acetaldehyde contents seemed to be important in determining flavor, but other factors, such as SSC and titratable acidity and their interactions with volatile compounds, may also have influenced the flavor of the oranges. While relatively high levels of ethanol and acetaldehyde may cause off-flavor (Ke et al., 1990; Smagula and Bramlage, 1977), relatively low concentrations of these compounds can enhance the sensory quality of fresh fruits (Morris et al., 1979; Paz et al., 1981). Also, the threshold levels of ethanol

Fig. 2. Correlation between flavor scores and ethanol (A) or acetaldehyde (B) contents. The flavor scores were estimated by a sensory panel of four people using a subjective scale of 1 to 9 (see Fig. 1 legend for details).

and acetaldehyde that cause off-flavor may vary considerably among different types of fruits. For example, ethanol content above $100 \mu\text{l}\cdot\text{liter}^{-1}$ may induce off-flavor in low- O_2 -treated strawberries (Ke et al., 1990); with 'Valencia' oranges, however, the ethanol level was higher than $100 \mu\text{l}\cdot\text{liter}^{-1}$, even in the fruits kept in air, and ethanol content up to $1000 \mu\text{l}\cdot\text{liter}^{-1}$ did not result in substantial decrease in the flavor score (Fig. 2A). Low- O_2 -treated 'Valencia' oranges accumulated very high ethanol concentrations (1000 to $4000 \mu\text{l}\cdot\text{liter}^{-1}$), confirming that oranges can not tolerate long-term low- O_2 storage. However, the results from this study indicate that 'Valencia' oranges may tolerate exposure to 0.25% or 0.5% O_2 for up to 20 days and still have acceptable flavor.

One possible way to reduce or remove the undesirable effects of anaerobic respiration is to transfer the fruits to air after low- O_2 treatment so that ethanol and acetaldehyde concentrations may be reduced to below the threshold levels that cause off-flavor by evaporation and/or metabolism. The temperature to which the fruit is transferred influenced ethanol content, ethanol evolution rate, and respiration rate of the fruits (Fig. 3). When 'Valencia' oranges were kept in 0.02% O_2 at 5C for 7 days and then held in air at 0 or 5C for another 7 days, ethanol content declined. However, if transferred to 10C, ethanol content increased (Fig. 3A).

During the 7 days when the fruits were kept in 0.02% O_2 at 5C, ethanol content increased by $\approx 1000 \mu\text{l}/\text{kg}$ of fruit; only $\approx 110 \mu\text{l}$ of ethanol had evolved per kilogram of fruit during the same period (Fig. 3 A and B). This relationship indicates that only $\approx 10\%$ of the synthesized ethanol had diffused out.

Fig. 3. Effects of transfer temperature on ethanol content (A), ethanol evolution rate (B), and respiration rate (C) of 'Valencia' oranges after 7 days in 0.02% O_2 at 5C followed by transfer (indicated by arrows) to air at 0, 5, or 10C and holding there for 7 days. The vertical bars represent LSD values at $P = 0.05$.

Ethanol is highly soluble in water and the orange skin appeared to be quite resistant to ethanol diffusion even under vacuum; both factors may have contributed to the lower ethanol evolution rates. For example, after 3-rein vacuum extraction, the extracted CO_2 concentrations (3.2% to 5.6%) were ≈ 20 times higher than the external headspace concentrations (0.15% to 0.24%), while the extracted ethanol concentrations (0.1 to $0.3 \mu\text{l}\cdot\text{liter}^{-1}$) were the same as or lower than the external headspace concentrations (0.1 to $1.0 \mu\text{l}\cdot\text{liter}^{-1}$). Therefore, the resistance of orange skin to ethanol diffusion was much higher than its resistance to CO_2 diffusion.

During the 7 days after the oranges were transferred to air, ethanol content decreased by ≈ 500 and $300 \mu\text{l}/\text{kg}$ of fruit at 0 and 5C, respectively, while it increased by $\approx 500 \mu\text{l}/\text{kg}$ of fruit at 10C (Fig. 3A); the total amounts of ethanol evolved were 90, 140, and $270 \mu\text{l}/\text{kg}$ of fruit at 0, 5, and 10C, respectively (Fig. 3B). The decreases in ethanol contents of the fruits transferred to air at 0 or 5C may have been partly due to ethanol evolution and metabolic conversion. However, if ethanol content is at the level of thousands of microliters per liter when the fruits are kept in low O_2 , it would take a long time for the content to decrease to hundreds of microliters per liter after the fruits are transferred to air due to the low ethanol evolution rate.

Although transferring oranges to air at 10C resulted in an increase in ethanol evolution rate (Fig. 3B), the ethanol content increased (Fig. 3A). This result suggests that the residual effect of 0.0290 O_2 on anaerobic respiration kept ethanol production going even after the fruits were transferred to air. Because transferring the fruits to 10C greatly increased the respiration rate (Fig. 3C) and probably maintained a relatively high anaerobic respiration rate, the change likely was accompanied by a higher ethanol production rate that exceeded the ethanol evolution rate and resulted in the accumulation of ethanol in the fruits.

The correlation coefficients between transfer temperature and CO_2 production rate or ethanol content and that between CO_2 production rate and ethanol content are highly significant ($r = 0.992$ to 0.994 and $P < 0.01$). The higher ethanol content in the fruits transferred to 10C may have been caused by the higher respiration rate of the fruits, assuming the ratio of residual anaerobic respiration to total respiration was the same for the fruits transferred to the three temperatures. Therefore, it is desirable to transfer fruits to a low, but nonchilling, temperature after low O_2 treatment so that ethanol and acetaldehyde contents may be reduced to below the threshold levels that cause off-flavor. Chilling temperatures ($\leq 3\text{C}$) should be avoided because they may result in increased production and accumulation of ethanol and acetaldehyde, as has been reported for lemons (Eaks, 1980).

The 'Valencia' oranges kept in air and in air + 60% CO_2 at 5C for 9 days followed by holding in air at 5C for 7 days had similar juice color ("b" value 15–16), SSC (10% to 11%), pH (3.8), titratable acidity (0.8–0.9), and AA content (78–85 mg/100 ml). However, the high CO_2 level caused slight to severe external injury to the fruits after 5 to 14 days of treatment followed by holding in air at 5C for 7 days (Table 2). The CO_2 injury in 'Valencia' oranges was characterized by skin browning and occasional fruit softening, but no visual symptoms of internal injury were observed. After 9 to 14 days, more than half of the fruits were injured (Table 2). Associated with the CO_2 injury, external appearance of the fruits was greatly impaired, while internal appearance was not influenced.

The air + 60% CO_2 treatment enhanced ethanol and acetaldehyde contents (Table 2). After 5 days of CO_2 treatment followed by holding in air for 7 days, most fruits still had a good

Table 2. Appearance, injury scores, and ethanol and acetaldehyde contents of 'Valencia' oranges exposed to air or air + 60% CO₂ for 5, 9, or 14 days followed by holding in air for 7 days at 5C.

Days under treatment	External appearance ^z		Internal appearance ^z		External injury severity ^y		Injured fruit (%)		Ethanol (μl·liter ⁻¹)		Acetaldehyde (μl·liter ⁻¹)	
	Air	60% CO ₂	Air	60% CO ₂	Air	60% CO ₂	Air	60% CO ₂	Air	60% CO ₂	Air	60% CO ₂
0	4.9	4.9	4.6	4.6	1.0	1.0	0	0	575	575	14	14
5	4.6	4.3	4.9	4.9	1.0	1.2	0	11	792	2330	16	52
9	4.7	3.3	4.8	4.9	1.0	1.7	0	50	787	3550	16	83
14	4.4	2.3	4.4	4.9	1.0	2.8	0	72	793	4640	18	141
LSD, <i>P</i> = 0.05	0.9		0.3		0.6		9		586		15	

^zEstimated on a scale where 5 = excellent, 4 = good, 3 = fair, 2 = slight defects, 1 = severe defects.

^yEstimated by a pretransformed scale of 1 to 5 according to the percentage of brown skin area: 1 = no injury; 2 = slight injury, up to 15% brown skin area; 3 = moderate injury, 16% to 50% brown skin area; 4 = severe injury, 51% to 85% brown skin area; 5 = extreme injury, 86% to 100% brown skin area.

flavor, but some had a slight off-flavor. However, after 9 to 14 days of CO₂ treatment, all oranges had moderate to very severe off-flavor. Thus, we conclude that 'Valencia' oranges can not be stored in 60% CO₂ at 5C for 5 days or longer without detrimental effects on their quality attributes.

Results from this study indicate that 'Valencia' oranges can be treated with 0.5%, 0.25%, or 0.02% O₂ at 5 or 10C for up to 20 days without detrimental effects on their appearance or nutritional value, as expressed by SSC and AA content. The flavor of these low O₂-treated oranges was acceptable and close to that of air control fruits. Once entomologists have determined which of these treatments can effectively control specific insects at the temperatures tested, such treatments might be approved by quarantine authorities for commercial use. Since a CO₂ level > 50% is required for effective insect control (Soderstrom and Brandl, 1987), and our results indicated that even 5 days in 60% CO₂ at 5C could injure 'Valencia' oranges, such a treatment can not be used for insect control in the oranges.

Flavor of low-O₂-treated 'Valencia' oranges is related to the accumulation of the products of anaerobic respiration, i.e., ethanol and acetaldehyde. To reduce or remove the undesirable effects of anaerobic respiration, it would be desirable to transfer the fruits to air at a low, but nonchilling, temperature following low-O₂ treatment so that ethanol and acetaldehyde contents may be decreased to below threshold levels that cause off-flavor.

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