Physiology and Prediction of Fruit Tolerance to Low-oxygen Atmospheres

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Abstract. Fruits of 'Granny Smith' and 'Yellow Newtown' apples (*Malus domestica* Borkh), '20th Century' pear (*Pyrus serotina* L.), and 'Angeleno' plum (*Prunus domestica* L.) were kept in air and in 0.25% or 0.02% O₂ at 0, 5, or 10°C for 3, 7, 14, 25, or 35 days to study the effects of low-O₂ atmospheres on their postharvest physiology and quality attributes. Soluble solids content (SSC), pH, and external appearance were not significantly influenced, but resistance to CO₂ diffusion was increased by the low-O₂ treatments. Exposures to the low-O₂ atmospheres inhibited ripening, including reduction in ethylene production rate, retardation of skin color changes and flesh softening, and maintenance of titratable acidity. The most important detrimental effect of the low-O₂ treatments was development of an alcoholic off-flavor that had a logarithmic relation with ethanol content of the fruits. The ethanol content causing slight off-flavor (E_o) increased with SSC of the commodity at the ripe stage, and it could be estimated using the following formula: $(\log E_o)/SSC = 0.228$. Using SSC of ripe fruits and average ethanol accumulation rate per day (V_e) from each low-O₂ treatment, the tolerance limit (T) of fruits to low-O₂ atmospheres could be predicted as follows: $T_i = E_o / V_e = (10^{0.228SSC}) / V_e$.

Extensive studies have been done to investigate the effects of controlled atmospheres (CA) on postharvest physiology and quality attributes of apples (Bramlage, 1977; Chen et al., 1985, 1989; Couey and Olsen, 1977; Dewey and Bourne, 1982; Johnson and Ertan, 1983; Lau, 1983; Lidster et al., 1983; Little et al., 1982; Liu and Ci, 1986; Porritt and Meheriuk, 1977). Beneficial effects of low-O₂ and/or high-CO₂ atmospheres on extending the storage life of apples include reduction in rates of respiration and ethylene production, color changes, and softening; maintenance of vitamins, sugars, acids, and flavor; and inhibition of some physiological disorders and decay. However, prolonged storage of apples in stress levels of low-O₂ and/or high-CO₂ atmospheres may cause detrimental effects, such as accumulation of ethanol and acetaldehyde, development of off-flavor, failure to ripen after removal to air, and development of low-O₂ and/or high-CO₂ injury (Fidler and North, 1971; Little and Pegg, 1987; Nichols and Patterson, 1987; Patterson and Nichols, 1988).

Very little work has been done to study the effects of low O₂ and/or high CO₂ on Asian pears. Zagory et al. (1989) found no obvious benefits for CA in long-term storage of 'Early Gold' and 'Shinko' pears. Asian pears are also quite susceptible to low-O₂ and high-CO₂ injuries (such as discolored surface depressions or flesh browning) that were observed after 4 to 6 months of storage in 1% to 3% O₂ or in air enriched with 5% CO₂ (Richardson, 1985).

Low-O₂ and/or high-CO₂ atmospheres reduced respiration rate, softening, decay, and loss of soluble solids and extended the storage life of plums (Claypool and Allen, 1951; Couey, 1960, 1965; Maxie et al., 1958; Sive and Resnizky, 1979). Storage of peaches and nectarines in 1% O₂ + 5% CO₂ with intermittent warming reduced internal breakdown, extended storage life, and maintained levels of sugars, acids, and unsaturated fatty acids (Wang and Anderson, 1982). Treatments with low O₂ and/or high CO₂ were also reported to retard softening and color changes

and reduce respiration and ethylene production rates of peaches and nectarines (Anderson, 1982; Kerbel et al., 1989; Smilanick and Fouse, 1989; Wang and Anderson, 1982). Storage of cherries in CA conditions retarded color darkening and reduction in SSC and titratable acidity (Chen et al., 1981; Patterson, 1982). CA treatments were also reported to be beneficial to the storage of apricots (Wankier et al., 1970). However, when stone fruits were stored in stress-inducing O₂ and/or CO₂ concentrations for a period longer than tolerable, detrimental effects, such as abnormal ripening, flesh browning, and large increases in ethanol and acetaldehyde contents, occurred (Kader, 1986; Smilanick and Fouse, 1989).

To meet quarantine requirements for fresh fruits and vegetables, there has been an increased interest in recent years in studying the potential of short-term exposure to O₂ levels below 1% and/or CO₂ levels above 50% for postharvest insect disinfestation (Aharoni et al., 1979; Lidster et al., 1981, 1984; Soderstrom and Brandl, 1987). CA treatments can be used for quarantine procedures only when the fresh commodities can tolerate the low-O₂ and/or high-CO₂ conditions longer than the insects. Through studying the physiological processes that limit the tolerance of fruits to low-O₂ and/or high-CO₂ atmospheres and predicting the tolerance of fruits and insects to CA conditions, the optimum CA treatments that will effectively kill the insects of concern without detrimental effects on the fruits can be selected.

In this paper, we report on the physiological processes and factors that determine the tolerance of fresh fruits to low-O₂ atmospheres and present a mathematical model that predicts the tolerance of fruits to stress-inducing low-O₂ conditions.

Materials and Methods

Materials and treatments. Fruits of 'Granny Smith' and 'Yellow Newtown' apples (both waxed), '20th Century' pear (non-waxed), and 'Angeleno' plum (waxed) were obtained on the day of harvest from commercial shippers in San Joaquin, Monterey, Solano, and Fresno Counties of California, respectively, and transported in an air-conditioned car to our laboratory at the Univ. of California, Davis, where they were kept overnight at 0°C. The experiments were initiated the next morning. Fruits with defects were sorted out and discarded, and six good fruits

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were put into a 4- or 8-liter glass jar as one replicate, with three replicates used per treatment in a flow system. In one set of experiments, the fruit samples were kept in humidified air, 0.25% O₂, or 0.02% O₂ (balance was N₂) at 0, 5, or 10C for 3 days before they were used for the determinations of respiration and ethylene production rates, internal CO₂ concentration, and resistance to gas diffusion. In another set of experiments, the fruit samples were kept in the stated atmospheres at 0, 5, or 10C for 7, 14, 25, or 35 days followed by holding in air for 7 days to allow the ethanol content to decrease at 0C (for 'Granny Smith' apple, '20th Century' pear, and 'Angeleno' plum) or 5C (for 'Yellow Newtown' apple). Then the fruits were transferred to air at 20C for 2 to several days to allow them to ripen before final quality evaluations.

Gas measurements. The O₂ and CO₂ concentrations of all gas mixtures were measured by analysis of a 10-ml gas sample using an O₂ analyzer (Model S-3All, 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. The vacuum extraction method (Saltveit, 1982) was used to determine internal CO₂ concentration and resistance to gas diffusion. The vacuum apparatus consisted of a steel vessel 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. Preliminary experiments showed that the extracted internal gas concentrations did not significantly change over 1 to 5 min under the vacuum and, therefore, a 3-min vacuum time was used in the experiments. The vacuum was then released and the vessel opened. Samples were taken through the septum using a 1-ml syringe for the determination of internal CO₂ concentration. Resistance to CO₂ diffusion was calculated by the ratio: $[(CO_2)_{int} - (CO_2)_{ext}]/CO_2$ production rate (Trout et al., 1942). Ethylene production rate was estimated by detecting the ethylene concentration of the gas sample from each jar using a Carle gas chromatography (Model 211, Carle; EG & G Chandler Engineering, Tulsa, Okla.) with a flame ionization detector, the flow rate used, and the fresh weight of the sample.

Determinations of quality attributes. Three initial samples of six fruits each were evaluated for external appearance, skin color, flesh firmness, SSC, pH, titratable acidity, ethanol concentration, and flavor score. Similar measurements were done in the final quality evaluations. External appearance was scored using a subjective scale of 1 to 5, where 5 = excellent, 4 = good, 3 = fair, 2 = slight defects, 1 = severe defects. Skin color was measured with a Gardner XL-23 Tristimulus Colorimeter (Gardner Laboratory, Bethesda, Md.) using the color difference meter (CDM) "a" value in which a more negative number means increasing greenness, while a larger positive number indicates increasing redness. Flesh firmness was measured as penetration force with a Univ. of California fruit firmness tester, using an 11-mm plunger tip for apples and an 8-mm tip for pears and plums. Fruit juice was extracted from four flesh sections of each fruit with a hand-press juicer. SSC was measured with an Abbé refractometer (VWR Scientific, San Francisco), and pH and titratable acidity were measured using an automatic titrator with a PHM85 Precision pH meter (Radiometer, Copenhagen, Denmark), an AUB80 autoburette (Radiometer), a PRS12 Alpha printer (Radiometer), and a SAC80 sample changer (Radiometer).

Determination of ethanol content. Frozen fruit juice was thawed and a 2-ml sample was diluted with 18 ml deionized water. The diluted sample was centrifuged at $25,000\times g$ for 20 min at 0C, and then ≈ 2 ml supernatant was filtered through a 0.45- μ m microfilter in a 0C room. The filtrate was used for the determination of ethanol concentration using a HP5890A gas chromatography (Hewlett Packard, Palo Alto, Calif.) with a flame ionization detector (at 250C) and a glass column (2 mm $\times$ 1.8 m) containing 5% Carbowax on 60/80 Carpack (at 85C).

Estimation of low-O₂ injury. Low-O₂ injury was estimated by a pretransformed scale of 1 to 5 according to the percentage of brown area in the core and/or flesh of a longitudinal fruit section: 1 = no injury; 2 = slight injury, up to 15% browning; 3 = moderate injury, 16% to 50% browning; 4 = severe injury, 51% to 85% browning; 5 = extreme injury, 86% to 100% browning.

Estimation of flavor score. Flavor score was estimated by tasting using a subjective scale ranging from 1 to 7, where 7 = excellent, 6 = good, 5 = fair, 4 = slight off-flavor, 3 = moderate off-flavor, 2 = severe off-flavor, 1 = extreme off-flavor. Three fruits were tested in each treatment, with one section tasted in each fruit. The evaluation was done in the Postharvest Laboratory, Pomology Dept. The judge rinsed ad lib with drinking water between tastings to clear his mouth of residual samples and to avoid an influence by prior fruits. Previous studies with strawberries and oranges used an untrained taste panel of ≈ 100 people and a trained taste panel of four people to conduct sensory evaluations (Ke and Kader, 1990; Ke et al., 1991). D.K. participated in such studies and gained substantial experience in sensory evaluation; due to the limited resources available, only he estimated flavor in this research. This approach reduced the variation in the ability to detect off-flavor, but potentially limited the reproducibility of the results.

Statistical analysis. Data were treated for multiple comparisons by analysis of variance with least significant difference (LSD) between means determined at $P = 0.05$. A computer curve fit program (Cricket Graph, Macintosh SE, Apple Computer, Cupertino, Calif.) was used to analyze the regression and correlation between ethanol content and flavor score.

Results

Storage of 'Granny Smith' apples in 0.25% or 0.02% O₂ at 0 or 10C generally slightly reduced respiration and ethylene production rates but increased internal CO₂ concentration and resistance to CO₂ diffusion (Table 1). Respiration and ethylene production rates and internal CO₂ concentration were higher at 10C than those at 0C, but resistance to CO₂ diffusion was lower at the higher temperature. The low-O₂ treatments did not significantly influence skin color, flesh firmness, and external appearance of 'Granny Smith' apples during the experimental period but slightly retarded the reduction in titratable acidity. As acidity decreased, pH slightly increased in all treatments (Table 1). No visible injury or decay was observed in any of the treatments during the experiment. The low-O₂ treatments did not influence SSC but greatly increased ethanol content (EC) and reduced the flavor score of 'Granny Smith' apples (Fig. 1). The low-O₂ effects were more pronounced at 10C than those at 0C. The effects of 0.02% O₂ were greater than those of 0.25% O₂. Flavor scores of 4 or below indicate that alcoholic off-flavor could be detected in low-O₂-treated fruits.

Exposure of 'Yellow Newtown' apples to 0.25% or 0.02% O₂ at 5 or 10C increased CO₂ production rate, internal CO₂ concentration, and resistance to CO₂ diffusion (Table 2). Res-

Table 1. Effects of O₂ level and temperature on respiration and ethylene production rates, internal CO₂ concentration, resistance to CO₂ diffusion (r_{CO_2}), and quality attributes of 'Granny Smith' apples. Skin color, flesh firmness, external appearance, titratable acidity, and pH were measured after specified days under treatment followed by holding in air at 0C for 7 days and then at 20C for 7 days.

Observation	Days under treatment	0C			10C			LSD at 5%
		Air	0.25% O ₂	0.02% O ₂	Air	0.25% O ₂	0.02% O ₂	
CO ₂ production rate (ml·h ⁻¹ ·kg ⁻¹)	3	1.6	1.2	1.4	4.8	4.4	4.2	0.3
C ₂ H ₄ production rate (μl·h ⁻¹ ·kg ⁻¹)	3	0.1	ND ²	ND	0.4	ND	ND	0.1
Internal CO ₂ (%)	3	2.0	2.0	2.4	3.8	5.1	4.8	0.8
r_{CO_2} [% (ml·h ⁻¹ ·kg ⁻¹) ⁻¹]	3	1.2	1.6	1.7	0.8	1.1	1.1	0.3
Skin color (CDM ^y "a" value)	7	-14	-14	-14	-14	-14	-14	
	35	-14	-13	-13	-12	-13	-14	1
Flesh firmness (N)	7	69	73	80	72	73	76	
	35	76	72	68	65	71	70	6
External appearance ^x	7	5.0	5.0	4.9	4.9	4.8	4.9	
	35	4.5	4.8	4.4	5.0	5.0	4.8	0.5
Titratable acidity (%)	7	0.57	0.57	0.65	0.52	0.56	0.58	
	35	0.46	0.55	0.51	0.41	0.54	0.52	0.03
pH	7	3.5	3.5	3.5	3.5	3.5	3.5	
	35	3.6	3.6	3.6	3.6	3.6	3.6	0.1

²ND = Not detected.

^yCDM = color difference meter; a more negative "a" value indicates a greener appearance.

^xEstimated using a scale of 1 to 5, where 5 = excellent, 4 = good, 3 = fair, 2 = slight defects, and 1 = severe defects.

Fig. 1. Effects of O₂ level and temperature on the soluble solids content (SSC), ethanol content (EC), and flavor score of 'Granny Smith' apples kept in air, 0.25% O₂, or 0.02% O₂ at 0 or 10C for 7, 14, or 25 days followed by holding in air at 0C for 7 days and then at 20C for 7 days. Flavor score was estimated using a scale of 1 to 7, where 7 = excellent, 6 = good, 5 = fair, 4 = slight off-flavor, 3 = moderate off-flavor, 1 = extreme off-flavor.

piration rate and internal CO₂ concentration were higher in fruits at 10C than in those at 5C, while resistance to CO₂ diffusion was lower at 10C. No ethylene production was detected in 'Yellow Newtown' apples from any treatment in this experiment. The low-O₂ treatments slightly retarded skin yellowing, flesh

softening, and decreases in titratable acidity of the fruits, but external appearance and pH were not significantly influenced (Table 2). No visible injury or decay was observed during the experiment. Treatments with 0.25% or 0.02% O₂ did not significantly affect SSC but greatly increased the ethanol content and reduced the flavor score of 'Yellow Newtown' apples (Fig. 2). The effects at 10C were greater than those at 5C.

Storage of '20th Century' pears in 0.25% or 0.02% O₂ at 0 or 5C generally decreased respiration rate but decreased internal CO₂ concentration consistently only at 0C and increased resistance to CO₂ diffusion (Table 3). No ethylene production was detected in '20th Century' pears from any treatment. Skin color, flesh firmness, and external appearance of the fruits were not significantly influenced by the low-O₂ treatments. Exposure of '20th Century' pears to 0.25% or 0.02% O₂ at 0 or 5C for 7 days did not cause any visible injury; but longer exposures (14 to 35 days) resulted in core browning. This result indicates that '20th Century' pears were sensitive to low-O₂ injury. The low-O₂ atmospheres did not have consistent effects on titratable acidity and pH of the fruits (Table 3). No decay was observed during the experiment. The low-O₂ treatments at 0 or 5C did not significantly influence SSC but greatly increased ethanol content and decreased the flavor score of '20th Century' pears (Fig. 3). Generally, the effects of low-O₂ treatments were more pronounced at 5C than at 0C; and the effects of 0.02% O₂ were greater than those of 0.25% O₂.

Exposure to 0.25% or 0.02% O₂ at 5 or 10C reduced respiration and ethylene production rates but increased resistance to CO₂ diffusion of 'Angeleno' plums (Table 4); internal CO₂ concentration was not significantly influenced by the low-O₂ treatments. Respiration and ethylene production rates and internal CO₂ concentration were higher at 10C than at 5C, while resistance to CO₂ diffusion was lower at the higher temperature. As the plums ripened, skin color changed from red to dark red (lower "a" values) and flesh firmness decreased. The low-O₂

Table 2. Effects of O₂ level and temperature on respiration rate, internal CO₂ concentration, resistance to CO₂ diffusion (r_{CO_2}), and quality attributes of 'Yellow Newtown' apples. Skin color, flesh firmness, external appearance, titratable acidity, and pH were measured after specified days under treatment followed by holding in air at 5C for 7 days and then at 20C for 14 days.

Observation	Days under treatment	5C			10C			LSD at 5%
		Air	0.25% O ₂	0.02% O ₂	Air	0.25% O ₂	0.02% O ₂	
CO ₂ production rate (ml·h ⁻¹ ·kg ⁻¹)	3	3.1	3.7	3.6	5.2	6.2	6.2	0.2
Internal CO ₂ (%)	3	5.3	7.6	8.0	6.1	9.6	11.4	1.0
r_{CO_2} [%·(ml·h ⁻¹ ·kg ⁻¹) ⁻¹]	3	1.7	2.0	2.2	1.1	1.5	1.8	0.2
Skin color (CDM ^a "a" value)	7	-14	-13	-13	-13	-13	-13	
	35	-11	-12	-13	-9	-12	-11	1
Flesh firmness (N)	7	99	102	100	104	99	103	
	35	73	81	79	70	92	89	9
External appearance ^b	7	4.6	4.2	4.1	4.2	4.6	4.5	
	35	4.2	4.4	4.3	4.3	4.6	4.0	0.8
Titratable acidity (%)	7	0.56	0.52	0.54	0.54	0.52	0.55	
	35	0.39	0.42	0.42	0.36	0.42	0.38	0.03
pH	7	3.6	3.6	3.6	3.6	3.6	3.6	
	35	3.7	3.7	3.7	3.8	3.7	3.8	0.1

^aCDM = color difference meter; a more negative "a" value indicates a greener appearance.

^bEstimated using a scale of 1 to 5, where 5 = excellent, 4 = good, 3 = fair, 2 = slight defects, and 1 = severe defects.

Fig. 2. Effects of O₂ level and temperature on soluble solids content (SSC), ethanol content (EC), and flavor score of 'Yellow Newtown' apples kept in air, 0.25% O₂, or 0.02% O₂ at 5 or 10C for 7, 14, or 25 days followed by holding in air at 5C for 7 days and then at 20C for 14 days. Flavor score was estimated using a scale of 1 to 7 (see legend of Fig. 1 for details).

treatments retarded skin color changes at both 5 and 10C, but the retardation effect on flesh softening was noted only at 10C (Table 4). External appearance, titratable acidity, and pH of the plums were not consistently influenced by the low-O₂ treatments. No visual low-O₂ injury was observed and decay was negligible during the experiment. The low-O₂ treatments did not significantly influence SSC but dramatically increased ethanol content and decreased flavor score of the plums (Fig. 4). The effects were more pronounced at 10C or in 0.02% O₂ than at 5C or in 0.25% O₂.

Discussion

The major beneficial effects of short-term exposure to 0.25% or 0.02% O₂ were the inhibition or delay of ripening processes, including reduction in respiration and/or ethylene production rates; retardation of skin color changes and flesh softening; and maintenance of acidity in the fruits. External appearance, pH, and SSC were not significantly influenced by the low-O₂ treatments. For all the fruits tested thus far, including apples, Asian pears, 'Bartlett' pears (Ke et al., 1990), nectarines (Kerbel et al., 1989), peaches, plums, strawberries (Ke et al., 1991), and 'Valencia' oranges (Ke and Kader, 1990), '20th Century' pear is the only one in which visible low-O₂ injury was observed during short-term exposure to low O₂ levels. However, alcoholic off-flavor was detected in the low-O₂-treated fruits of all the commodities after various durations during the experiments. Therefore, the occurrence of an alcoholic off-flavor is the most important detrimental effect with short-term low-O₂ treatments. A low-O₂-treated fruit may look good but taste bad. Thus, the tolerance of fresh fruits to short-term low-O₂ atmospheres is largely limited by the occurrence of off-flavor.

After the flavor score decreased to 4 (slight off-flavor) or a lower value, the flavor was not acceptable. Therefore, the number of days to reach a flavor score of 4 was used as the tolerance limit of fruits to low-O₂ atmospheres from the experiments (T_f). The T_f values for all treatments of the four commodities were obtained from Figs. 1-4 by linear extrapolation and are presented in Table 5. For each commodity, T_f was higher at the higher O₂ concentration and lower at the higher temperature.

The accumulation of ethanol was accompanied by a decrease in the flavor score of the four commodities tested in this study (Figs. 1-4). A computer curve fit program (including linear, polynomial, logarithmic, and exponential regressions) was used to analyze the correlation between ethanol content and flavor score; the best curve fit was obtained by the logarithmic regression (Fig. 5). The correlation coefficients (*r*) were 0.93 to 0.94 for the four commodities. When the ethanol content data were transformed to a Log scale, straight lines were obtained from the correlation analysis. Therefore, ethanol content and flavor score, as related to ethanol content, have a logarithmic relation,

Table 3. Effects of O₂ level and temperature on respiration rate, internal CO₂ concentration, resistance to CO₂ diffusion (r_{CO_2}), and quality attributes of '20th Century' pears. Skin color, flesh firmness, external appearance, titratable acidity, and pH were measured after specified days under treatment followed by holding in air at 0C for 7 days and then at 20C for 3 days.

Observation	Days under treatment	0C			5C			LSD at 5%
		Air	0.25% O ₂	0.02% O ₂	Air	0.25% O ₂	0.02% O ₂	
CO ₂ production rate (ml·h ⁻¹ ·kg ⁻¹)	3	1.8	0.8	1.0	2.7	1.6	1.6	0.2
Internal CO ₂ (%)	3	0.35	0.23	0.26	0.48	0.39	0.46	0.12
r_{CO_2} [%·(ml·h ⁻¹ ·kg ⁻¹) ⁻¹]	3	0.14	0.23	0.21	0.14	0.21	0.24	0.05
Skin color (CDM ^z "a" value)	7	-2.1	-2.3	-2.6	-2.4	-1.2	-1.8	2.2
	35	-0.7	-2.4	-1.0	-0.2	-1.9	-2.6	
Flesh firmness (N)	7	32	37	37	34	32	34	4
	35	32	30	31	31	32	31	
Internal injury severity ^y	7	1.0	1.2	1.0	1.0	1.2	1.0	0.7
	14	1.0	1.8	1.9	1.2	1.5	2.0	
	35	1.0	3.1	3.2	1.0	1.8	2.3	
External appearance ^x	7	5.0	5.0	5.0	5.0	5.0	5.0	0.6
	35	4.8	4.9	4.3	4.9	4.8	4.9	
Titratable acidity (%)	7	0.22	0.25	0.25	0.24	0.21	0.21	0.03
	35	0.16	0.14	0.14	0.16	0.18	0.17	
pH	7	4.4	4.3	4.4	4.3	4.4	4.4	0.1
	35	4.6	4.6	4.7	4.5	4.5	4.6	

^zCDM = color difference meter; a more negative "a" value indicates a greener appearance.

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

^xEstimated using a scale of 1 to 5, where 5 = excellent, 4 = good, 3 = fair, 2 = slight defects, and 1 = severe defects.

Fig. 3. Effects of O₂ level and temperature on soluble solids content (SSC), ethanol content (EC), and flavor score of '20th Century' pears kept in air, 0.25% O₂, or 0.02% O₂ at 0 or 5C for 7, 14, 25, or 35 days followed by holding in air at 0C for 7 days and then at 20C for 3 days. Flavor score was estimated using a scale of 1 to 7 (see legend of Fig. 1 for details).

While it is well-known that the accumulation of ethanol may cause off-flavor in fruits and vegetables, the ethanol concentrations that cause alcoholic off-flavors varied greatly among commodities. For example, an ethanol content >200 μl·liter⁻¹ caused

a slight off-flavor (a flavor score of 4) in '20th Century' pears (Fig. 3), but it took >1000 and 2000 μl ethanol/liter to cause a slight off-flavor in 'Yellow Newtown' apples (Fig. 2) and 'Angeleno' plums (Fig. 4), respectively.

The variation in the threshold ethanol concentration causing off-flavor among commodities may be due to the interaction between ethanol and other compounds (such as sugars and organic acids). Using the data from Figs. 1-4 to analyze the relationship between SSC and the ethanol content causing slight off-flavor (E_o) in apples, 'Asian' pears, and plums, we found higher SSC to generally require higher ethanol concentrations to cause slight off-flavor (Fig. 6A). Since the relationship between ethanol content and flavor score is logarithmic (Fig. 5), the E_o data were transformed to a Log scale. The ratio of (Log E_o)/SSC is very constant (with a standard deviation of 0.019) for all low-O₂ treatments of the four commodities (Fig. 6B):

$$(\text{Log } E_o)/\text{SSC} = 0.228 \quad [1]$$

Thus, SSC plays a very important role in determining the ethanol level that causes off-flavor. SSC probably provides a background flavor for the interaction with ethanol; the higher the SSC, the higher the ethanol content required to cause off-flavor in the fruits. It is still not clear if this effect is due to a specific component of SSC, since SSC represents all the compounds from the fruits that are soluble in water.

From Eq. [1], the ethanol content causing slight off-flavor could be estimated from SSC of ripe fruits for all the commodities tested as follows:

$$E_o = 10^{0.228 \text{ SSC}} \quad [2]$$

From the estimated ethanol content causing slight off-flavor in Eq. [2] and the calculated average ethanol accumulation rate per day (V_e) of fruits under a low-O₂ treatment, the tolerance

Table 4. Effects of O₂ level and temperature on respiration and ethylene production rates, internal CO₂ concentration, resistance to CO₂ diffusion (*r*_{CO₂}), and quality attributes of 'Angeleno' plum. Skin color, flesh firmness, external appearance, titratable acidity, and pH were measured after specified days under treatment followed by holding in air at 0C for 7 days and then at 20C for 2 days.

Observation	Days under treatment	5C			10C			LSD at 5%
		Air	0.25% O ₂	0.02% O ₂	Air	0.25% O ₂	0.02% O ₂	
CO ₂ production rate (ml·h ⁻¹ ·kg ⁻¹)	3	4.0	2.2	3.2	7.5	3.7	4.5	0.5
C ₂ H ₄ production rate (μl·h ⁻¹ ·kg ⁻¹)	3	6.0	0.6	0.8	10.0	2.2	1.6	0.8
Internal CO ₂ (%)	3	1.5	1.4	1.5	1.9	1.6	1.9	0.3
<i>r</i> _{CO₂} [%·(ml·h ⁻¹ ·kg ⁻¹) ⁻¹]	3	0.34	0.63	0.46	0.24	0.40	0.41	0.08
Skin color (CDM ^a "a" value)	25	10	14	15	5	12	13	4
	35	6	14	12	3	14	14	
Flesh firmness (N)	25	33	34	35	5	37	32	11
	35	31	28	33	7	28	32	
External appearance ^y	25	4.3	4.0	4.8	4.9	4.8	4.8	0.9
	35	4.5	5.0	4.8	4.2	4.6	4.5	
Titratable acidity (%)	25	0.41	0.40	0.43	0.32	0.36	0.34	0.03
	35	0.37	0.35	0.40	0.30	0.34	0.32	
pH	25	4.1	4.0	4.0	4.4	4.1	4.1	0.1
	35	4.1	4.2	4.2	4.0	4.1	4.3	

^aCDM = color difference meter; a darker red resulted in a lower "a" value.

^yEstimated using a scale of 1 to 5, where 5 = excellent, 4 = good, 3 = fair, 2 = slight defects, and 1 = severe defects.

Fig. 4. Effects of O₂ level and temperature on soluble solids content (SSC), ethanol content (EC), and flavor score of 'Angeleno' plums kept in air, 0.25% O₂, or 0.02% O₂ at 5 or 10C for 7, 14, 25, or 35 days followed by holding in air at 0C for 7 days and then at 20C for 2 days. Flavor score was estimated using a scale of 1 to 7 (see legend of Fig. 1 for details).

limit (*T*₁) of fruits to low-O₂ atmospheres can be predicted by:

$$T_1 = E_0 / V_E = (10^{0.228 \text{ SSC}}) / V_E, \quad [3]$$

where *T*₁ = tolerance limit predicted, i.e., the number of days to cause slight off-flavor; *E*₀ = the ethanol content (μl·liter⁻¹) of the fruit that causes slight off-flavor; *V*_E = the average

ethanol accumulation rate (μl·liter⁻¹·day⁻¹) of the fruit under the low-O₂ treatment; and SSC = soluble solids content (%) of the fruit when ripe.

According to Eq. [3], two primary factors determine the tolerance of fruits to low-O₂ atmospheres: SSC of ripe fruits and average ethanol accumulation rate of the fruits under the low-O₂ treatment. Other factors may affect fruit tolerance to low O₂ through influencing these two primary factors. SSC of harvested ripe fruits is not greatly influenced by the O₂ level or temperature used during these short storage treatments (Figs. 1-4). Cultural conditions before harvest and fruit maturity at harvest may influence SSC of the fruit when ripe. However, ethanol accumulation rate can be greatly influenced by several factors including the following:

1) Respiration rate of the fruits under a low-O₂ treatment: *V*_E generally increases as respiration rate increases since, under the low O₂ levels (0.25% to 0.02%), respiration is largely anaerobic, which produces much ethanol. Respiration rate is commodity-specific and may be influenced by the O₂ level and temperature used.

2) O₂ level used: ethanol accumulates at a higher rate at 0.02% than at 0.25% O₂ since anaerobic respiration rate is higher at the lower concentration.

3) Temperature used: as temperature increases, O₂ consumption rate and respiration rate increase, which cause an increase in *V*_E.

4) Resistance to gas diffusion: this is largely commodity-specific, but it maybe influenced by postharvest treatment, such as waxing, which causes a great increase in resistance to gas diffusion. Under the same O₂ level and temperature, a commodity with a higher resistance to gas diffusion will limit the efficiency of O₂ use and the diffusion of ethanol out of the fruit, causing a higher *V*_E.

Equation [3] was used as a model to predict the tolerance of fruits to low-O₂ atmospheres. Using the average SSC of the ripe fruits, *E*₀ was estimated by Eq. [2]. *V*_E values for all treatments

Table 5. Comparison of tolerance limit from experiments (T_{1e}) and predicted tolerance limit (T_1) of 'Granny Smith' and 'Yellow Newtown' apples, '20th Century' pears, and 'Angeleno' plums to 0.25% or 0.02% O_2 at several temperatures. T_1 is predicted by the model $T_1 = E_o/V_E$ or $T_1 = (10^{0.228 \text{ SSC}})/V_E$, where E_o is the predicted ethanol content causing slight off-flavor by using the average soluble solids content (SSC) of the commodity at the ripe stage and V_E is the average ethanol accumulation rate of the fruits in the low- O_2 treatment.

Commodity	Temp (°C)	O_2 (%)	SSC (%)	E_o ($\mu\text{l}\cdot\text{liter}^{-1}$)	V_E ($\mu\text{l}\cdot\text{liter}^{-1}\cdot\text{day}^{-1}$)	T_{1e} (days)	T_1 (days)	$T_{1e} - T_1$ (days)
'Granny Smith' apple	0	0.25	11.9	517	19	23.4	27.2	-3.8
	0	0.02	11.9	517	29	11.9	17.8	-5.9
	10	0.25	11.9	517	74	7.0	7.0	0.0
	10	0.02	11.9	517	94	5.0	5.5	-0.5
'Yellow Newtown' apple	5	0.25	13.4	1136	147	11.3	7.7	3.6
	5	0.02	13.4	1136	173	9.9	6.6	3.3
	10	0.25	13.4	1136	256	8.2	4.4	3.8
	10	0.02	13.4	1136	272	7.0	4.2	2.8
'20th Century' pear	0	0.25	10.7	275	8	35.0	34.4	0.6
	0	0.02	10.7	275	24	16.2	11.7	4.5
	5	0.25	10.7	275	22	13.1	12.4	0.7
	5	0.02	10.7	275	40	11.0	6.8	4.2
'Angeleno' plum	5	0.25	15.3	3079	68	40.6	45.3	-4.7
	5	0.02	15.3	3079	96	31.6	32.1	-0.5
	10	0.25	15.3	3079	164	14.0	18.8	-4.8
	10	0.02	15.3	3079	259	9.4	11.9	-2.5

Fig. 5. Correlation between ethanol content (EC) and flavor score of low- O_2 -treated fruits of 'Granny Smith' (A and B) and 'Yellow Newtown' apples (C and D), '20th Century' pears (E and F), and 'Angeleno' plums (G and H). Flavor score was estimated using a scale of 1 to 7 (see legend of Fig. 1 for details).

of the four commodities during the first 1 to 2 weeks were calculated from Figs. 1-4. The tolerance limit (T_{1e}) obtained from Figs. 1-4 of the experiments and the tolerance limit predicted by Eq. [3] (T_1) are quite close for most treatments (Table 5); the average deviation equals 2.9 days. Our later study on 'Bing' cherries (unpublished data) also indicated that Eq. [3]

Fig. 6. Relationship between soluble solids content (SSC) and ethanol content causing slight off-flavor (E_o) in low- O_2 -treated 'Granny Smith' and 'Yellow Newtown' apples, '20th Century' pears, and 'Angeleno' plums.

could reasonably predict fruit tolerance to low- O_2 atmospheres with a deviation of 1 to 2 days between the predicted and observed tolerance limits. For the practical application of the model to postharvest insect disinfestation, fruit tolerance to low- O_2 atmospheres appears to be predictable; with additional information from entomologists, optimum combinations of O_2 level and temperature could be selected so that the insects of concern could be killed without detrimental effects on the commodity.

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