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Metabolic Response of 'Delicious' Apples to Carbon Dioxide in Anoxic and Low-oxygen Environments

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Abstract. **'Delicious' apples (*Malus domestica* Borkh.) were transferred from commercial controlled atmosphere (CA) storage after 7 months into a factorial series of CO_2 (0%, 3%, 6%, and 12%) and O_2 (0.0%, 0.5%, and 1.0%) concentration mixtures at 0.5°C for up to 14 weeks. Fruit tolerance to specific atmospheres that yielded anaerobic products was determined. Tissue ethanol levels ranged from about 2700 to 5800 µl·liter^{-1} in apples stored in 12:0 (CO_2:O_2) and 0:0 atmospheres, respectively, indicating CO_2 inhibition of ethanol accumulation in the absence of O_2. Less than 360 µl ethanol/liter was produced in the 0.5% and 1.0% O_2 treatments, No CO_2 inhibition of ethanol or acetaldehyde production occurred in the 0.5% and 1.0% O_2 treatments. Tissue acetaldehyde concentrations ranged from 6 to 14 µl·liter^{-1} in fruit held in 0.0% O_2 and 3 to 9 µl·liter^{-1} in fruit held in 0.5% O_2. No visible injury developed from the high CO_2 and low O_2 concentrations used in any of the storage treatments. After a week in air at 20°, following 0.0% O_2 storage, the fruit tissue ethanol content decreased while the acetaldehyde content increased.**

Current Washington State recommendations call for maintaining CO_2 levels below 2% in controlled atmosphere (CA) storage (13). Storage costs increase as CO_2 concentration is decreased due to the increased costs of removal. These low CO_2 guidelines arose in part from research that showed that elevated CO_2 can promote internal browning (7, 9, 17) and mealiness (8). However, elevated CO_2 has been shown to retard respiration, inhibit scald development (4, 14), and retain fruit firmness (14). High CO_2 treatments have been employed during the first weeks of storage to successfully retain firmness in 'Golden Delicious' (11). The potential for injury prevents its use on 'McIntosh' (2).

Plant storage organs have been shown to respond in diverse ways to anaerobic environments with and without CO_2. Anaerobic respiration in sweet potato (3) and asparagus (M.E. Patterson, unpublished data) is accelerated by an elevated CO_2 atmosphere while persimmon (15), carrot (R.M.M. Crawford, personal communication), and blueberry (16) are not affected. Anaerobic respiration in chickpea is inhibited by high CO_2 levels (6).

The possibility for deviations from precise control of desired gas mixtures in commercial CA storage is always present. We are interested in the effect of CO_2 on the anaerobic respiration and the low O_2 tolerance of 'Delicious' apples late in the CA storage season. We report on the accumulation of ethanol and acetaldehyde in CA-stored 'Delicious' apples during a 14-week exposure to anaerobic or low O_2 CAs containing varying levels of CO_2 at the end of the storage season. Knowledge of the susceptibility of 'Delicious' apples to critical ranges of O_2 and CO_2 concentration mixtures will provide valuable storage management information.

'Delicious' apples (*Malus domestica* Borkh.) were obtained from a commercial packinghouse in north central Washington on 8 May 1984 after being stored at 0°C in 1.6% O_2 with <1% CO_2 for 7 months. The fruit had been presized (180 g/fruit); therefore, specific identity as to rootstock and strain was unknown. The apples were subsequently treated and observed at the Washington State Univ. Postharvest Laboratory. One hundred fruit were placed in each of 12, 160-liter, CA chambers and held at 0.5°. Three O_2 levels (0.0%, 0.5%, and 1.0%), each maintained to a precision of ±0.1% was combined with four CO_2 levels (0%, 3%, 6%, and 12%), each to a precision of ±1%, yielding 12 environments in a factorial arrangement. To establish and maintain atmospheres, N_2, air, and CO_2 from cylinders were mixed and metered into the chambers at a continuous flow rate of 6 liter·hr^{-1}. Humidity was provided by a water moat making a gas-tight seal between chamber and chamber lids. Gas concentrations were verified daily with paramagnetic and infrared gas analyzers.

Twenty apples were removed from each chamber after 3.5, 7, and 14 weeks of storage. Ten apples were analyzed immediately upon removal from CA chambers and the remaining 10 were analyzed following 1 week at 20°C. Fruit were individually analyzed for firmness with a Topping mechanical penetrometer on three sectors, for refractive index (RI) using an Abbé benchtop refractometer, and for titratable acidity (TA) with a Metrohm autotitrator titrating 10 ml of juice to a pH 8.1 endpoint. For ethanol and acetaldehyde analysis, a 1-ml sample of juice was prepared by adding 100 µl of 1 N trichloroacetic acid (TCA) and 10 mg of

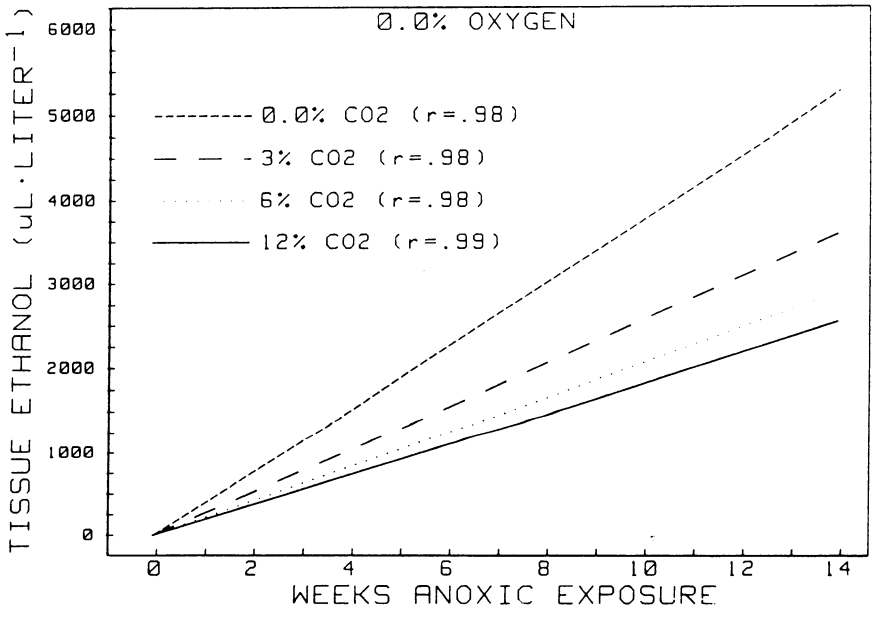

Fig. 1. Linear regression of tissue ethanol accumulation in 'Delicious' apples as a function of time in anoxic atmospheres containing 0%, 3%, 6%, or 12% CO_2 at 0.5°C. Fruit were exposed to mixtures for up to 14 weeks following 7 months in commercial storage room where O_2 concentration was 1.6%.

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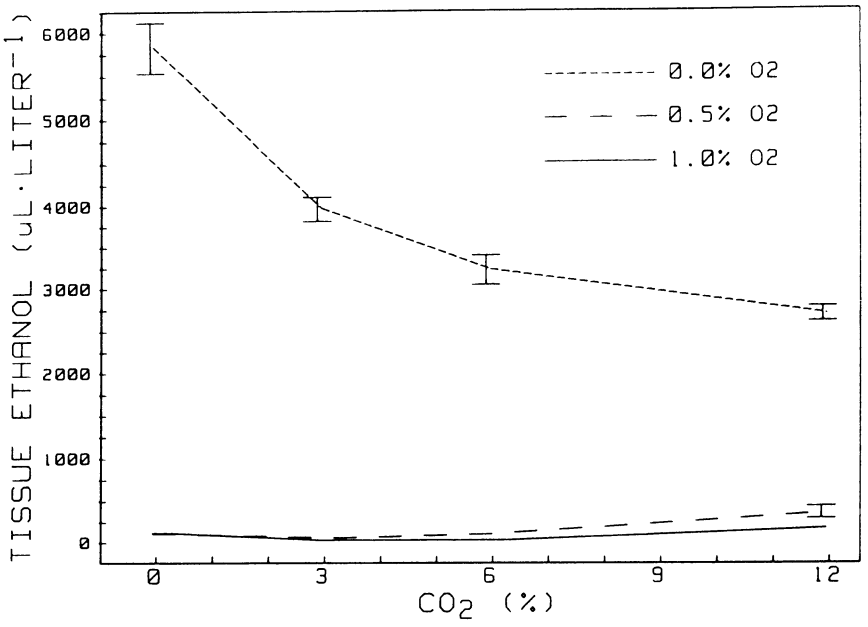

Fig. 2. Tissue ethanol accumulation in 'Delicious' apples held 14 weeks in various O_2 and CO_2 atmosphere combinations at 0.5°C following 7 months in a commercial storage room where O_2 concentration was 1.6%. Vertical bars represent SE.

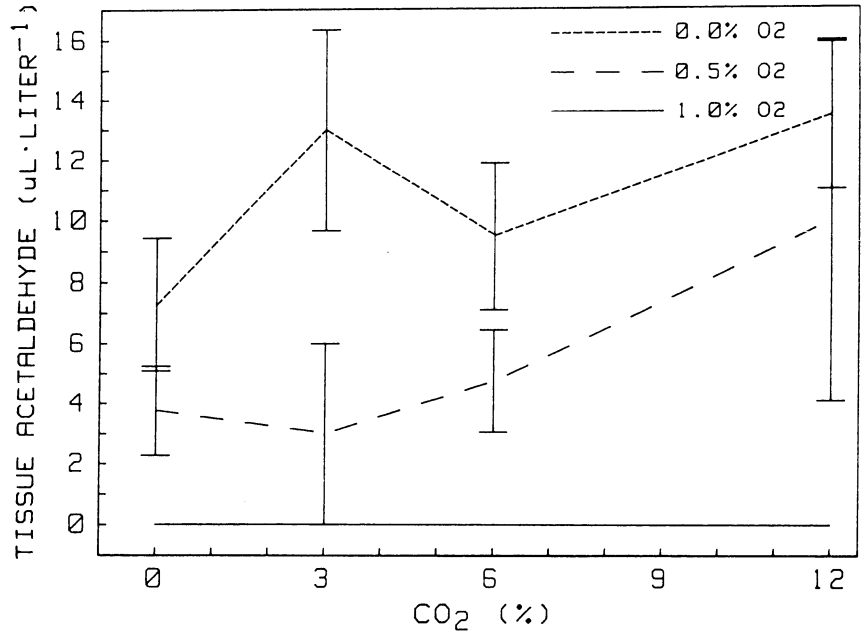

Fig. 3. Tissue acetaldehyde accumulation in 'Delicious' apples held 14 weeks in various O_2 and CO_2 atmosphere combinations at 0.5°C following 7 months in a commercial storage room where O_2 concentration was 1.6%. Vertical bars represent SE.

polyvinyl-polypyrrolidone (PVPP) in a micro-centrifuge tube. After the tubes were centrifuged, 1 ml of supernatant was injected into a gas chromatograph (model Hewlett Packard 5830A) equipped with an autosampler and a 3.2 mm × 6 m stainless steel Porapack T column. CO_2 and O_2 effects were analyzed by analysis of variance (ANOVA).

Ethanol and acetaldehyde measurements were performed individually on 10 fruit upon their removal from CA chambers. An additional measurement followed after the apples had been held 1 week in air at 20°C when a single composite sample comprising all 10 apples was analyzed.

The CO_2 and O_2 atmosphere treatments did not significantly influence firmness, RI, or TA (data not presented).

Ethanol accumulation in fruit stored in 0.0% O_2 within a given CO_2 concentration was linearly related to duration of exposure (Fig. 1). However, increasing the CO_2 concentration markedly reduced the rate of ethanol accumulation and the quantity accumulated. At the end of the 14-week sampling period, ethanol levels had reached nearly 5400 $\mu l \cdot liter^{-1}$ in the 0:0 (CO_2:O_2) and nearly 2700 $\mu l \cdot liter^{-1}$ in the 12:0 treatments (Figs. 1 and 2). CO_2 clearly inhibits anaerobic production of ethanol in 'Delicious' apples in the absence of O_2. In the presence of O_2 (0.5% nd 1.0%), no CO_2 inhibition of ethanol production was seen. Although little ethanol accumulated in the tissue in 0.5% and 1.0% O_2, 12:0.5 treatment yielded the highest tissue ethanol level (354 $\mu l \cdot liter^{-1}$) among these low O_2 treatments. High CO_2 concentrations did not induce any internal browning, mealiness, or surface injury during the experiment.

Acetaldehyde concentrations ranged from 6 to 14 $\mu l \cdot liter^{-1}$ and 4 to 9 $\mu l \cdot liter^{-1}$ in the 0.0% and 0.5% O_2 treatments, respectively, and were not related to CO_2 concentration in any consistant pattern (Fig. 3). No acetaldehyde production was detected in 1.0% O_2. Concentration of O_2 yielded a strong linear relationship with acetaldehyde production (P = 0.0001) (orthogonal polynomial contrasts not shown).

During a week in air at 20°C, following 14 weeks in experimental atmospheres, ethanol levels decreased. A greater percentage of ethanol was lost from fruit from the 12:0 and 6:0 treatments than from fruit from the 3:0 and 0:0 treatments. Although fruit from 12:0 and 6:0 accumulated less ethanol in storage, they lost >45% of their accumulated ethanol in air at 20°. Fruit from the 0:0 treatment, which produced the most alcohol (5837 $\mu l \cdot liter^{-1}$), lost only 28% of it during a week in air. If ethanol were lost by gas-exchange diffusion, those fruit containing the most ethanol should have lost the most during a week in air. It is possible that levels of ethanol were reached that inhibited metabolism of ethanol and/or acetaldehyde when the 0:0 atmosphere was replaced with air.

Following a week in air, no acetaldehyde was detected in fruit from the 0.5% and 1.0% O_2 storage chambers. Acetaldehyde concentrations increased to as high as 29 $\mu l \cdot liter^{-1}$ during a week in air following holding in 0:0. An increase in acetaldehyde concentration can be explained as a function of reversible alcohol dehydrogenase (ADH) activity during ethanol oxidation. Ethanol oxidation appeared to produce acetaldehyde faster than diffusion and acetaldehyde oxidation could eliminate it.

Phytotoxic levels of ethanol, although subject to some controversy, have been suggested to be about 2800 $\mu l \cdot liter^{-1}$ (60 mM) in pea seedlings (1) and >4600 $\mu l \cdot liter^{-1}$ (100 mM) in a variety of tissues (10). Ethanol toxicity is reported to be reduced in flow through storage systems such as that used in this study (6). Acetaldehyde is much more toxic than ethanol, with as little as 30 μM reported to cause damage (5).

We saw no internal or external anaerobic injury symptoms after 14 weeks of complete anoxia, nor after fruit subsequently were exposed to air for a week. In previous experiments with fruit treated immediately after harvest, injury symptoms appeared in all fruit subjected to 14 weeks of anoxia followed by a week in air (12). Although almost twice as much ethanol was produced in this experiment using apples previously stored 7 months in a 1.6% O_2 commercial CA storage, the

lack of visible injury confirms that these injury symptoms were not related to specific tissue ethanol level.

High CO_2 (up to 12%) treatments combined with low O_2 (0.5% and 1.0%) during the last 3 months of the storage season did not cause any physiological disorders or loss in quality or condition. Thus, this and a previous study (12), combine to suggest that the period in the storage season when fruit are exposed to accidental anaerobisis may influence their susceptibility to visible low O_2 injury. The substantial tolerance of 'Delicious' apples late in the CA storage season suggest that considerable deviations from target atmospheres, other than total anoxia, are possible without injury late in the CA storage season.

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Alleviation of Chilling Injury in Papayas

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Abstract. **Susceptibility to chilling injury in 'Kapoho' papayas (*Carica papaya* L.) was reduced by ripening the fruit before storage at 5°C. The relationship between the decrease in chilling injury (CI) and postharvest ripening time at 24° was found to follow first order kinetics, hence, the half-lives for reducing the symptoms of CI of scald and hard core are 14.8 and 33.5 hr, respectively.**

Papayas (*Carica papaya* L.) develop chilling injury (CI) when stored at temperatures below 10° to 12°C. CI in papayas is characterized by uneven ripening, blotchy coloration, development of skin scald, hard core, and increases in electrolyte leakage, ethylene production, and susceptibility to fungal decay (2–4, 7, 8). In 1983, a series of experiments at this laboratory showed that preconditioning at elevated temperatures for alleviation of CI necessitated shorter preconditioning storage times. This led to the belief that ripening could be a factor in alleviating CI in papayas. This was consistent with the work of Chen and Paull (1986) who reported that papayas >60% yellow (one-half ripe) could be stored at a chilling temperature of 2° for 17 days without developing CI.

Chilling sensitivity of avocados and honeydew melons are related to stage of ripeness (9, 10, 16). There is evidence that this might be the case for papayas (1, 12, 15). Hence, I report in this paper the sensitivity of papayas to CI at various stages of postharvest ripeness.

'Kapoho' papayas were harvested at the "color break" stage of ripeness (i.e., fruit showing a tinge of yellow, with Hunter "b" values of 13.5–17.0) from a commercial field on the Island of Hawaii. The papayas were exposed to a recommended hot water treatment (42°C for 40 min followed by 49° for 20 min) for quarantine purposes (6) and to (2-[4-thiazolyl]benzimidazole (thiabendazole) (4 g·liter^{-1}) to reduce fungal decay (5). The treated fruit sample was subdivided into 8 lots of 17 fruits each. Each lot was placed into a 19-liter respiration chamber. One lot (control) was stored at 24° and the remaining seven lots were placed sequentially into 5° storage after 11, 24, 48, 72, 96, 120, and 144 hr of postharvest holding at ambient temperature. The 11-hr time lag was the preparation time required and also served to simulate the time lag occurring under commercial practice. Before cold storage, the surface of fruit in each lot was measured for color with a Hunter colorimeter (LS 5100). The respiration chambers were sealed for 4 hr once every 24 hr to allow detectable levels of ethylene to accumulate. Head space samples (5 ml) were taken with a syringe and analyzed for ethylene with a gas chromatograph equipped with a flame ionization detector. The carrier gas (helium) flow rate was 31 ml·min^{-1}. The 1.2 × 2.3 mm i.d. stainless steel column was packed with Porapak QS 100/120 mesh. The column was operated isothermally at 60° with the detector temperature at 250° and the injector at 150°. After 7 days of storage at 5°C the fruit were allowed to ripen at 24° for 2 to 3 days and then were evaluated for the presence of CI symptoms (scalding and hardcore). Ethylene evolution was monitored 11 hr postharvest and every 24 hr thereafter. Replicate samples were taken monthly, beginning in Sept. 1984 and, with the exception of four occasions, continued to June 1986 for a total of 18 sam-

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