Fermentative Volatile Production in Relation to Carbon Dioxide–induced Flesh Browning in ‘Fuji’ Apple

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Abstract. Apple (Malus ×domestica Borkh., cv. Fuji) fruit were harvested from two California orchards 190 and 210 days after full bloom and from an additional three orchards at 190 days after full bloom. Fruit were immediately exposed to 20 or 50 kPa CO2 in air at 20 °C. Area of flesh browning and tissue ethanol, acetaldehyde, and ethyl acetate concentrations for individual fruit were determined immediately before exposure and after 3 and 7 days (20 kPa) or 1 and 3 days (50 kPa) exposure to CO2. Area of flesh browning and concentrations of all compounds increased with increasing duration of exposure to high CO2, were greater in response to 50 kPa than to 20 kPa CO2, and were greater for fruit harvested later in the season. For individual orchards and for individual fruit within most orchards, greater flesh browning was associated with higher acetaldehyde concentrations after 7 days exposure to 20 kPa CO2, or 3 days exposure to 50 kPa CO2. Similarly, flesh browning was positively correlated with ethanol concentrations after 7 days at 20 kPa CO2, but was not related to tissue ethyl acetate concentrations at either CO2 partial pressure. However, higher production of ethanol, acetaldehyde, or ethyl acetate relative to flesh browning occurred during exposure to 50 kPa than to 20 kPa CO2. This suggests that the relationship between accumulation of these compounds and CO2-induced flesh browning in ‘Fuji’ is not simply causal.

Elevated CO2, partial pressure retards senescence and maintains fruit quality during storage of many fruit and vegetable commodities, and is therefore a valuable component of controlled-atmosphere (CA) and modified-atmosphere (MA) storage. For apple, the use of high CO2 in such storage systems is limited by the risk of damage to fruit, and recommended partial pressures in CA storage, for instance, usually do not exceed 5 kPa (Kupferman, 1997). ‘Fuji’ apples grown in California are particularly sensitive to CO2 gas used in CA storage (Volz et al., 1998). Flesh browning exacerbated by CO2 injury has been observed in some lots of fruit held under CA with CO2 partial pressures as low as 0.4 kPa (Grant et al., 1996).

In ‘Fuji’ (Volz et al., 1998), ‘Mcintosh’ (Bramlage et al., 1977), and ‘Golden Delicious’ (Meherutik, 1977), fruit susceptibilities to CO2 partial pressures used in CA (1–5 kPa), as well as to much higher partial pressures (>10 kPa) can vary according to harvest date, orchard, and season. Although the cause of this variability in fruit response is unknown for ‘Fuji’, it may be due to differing tissue sensitivities to CO2 (Volz et al., 1998).

Many commodities accumulate ethanol and acetaldehyde when exposed to high CO2, and this has often been associated with tissue browning and cell death (Kader, 1986; Lidster et al., 1990). High levels of CO2 divert carbon from the Krebs cycle to the fermentative pathway whereby pyruvate is converted to acetaldehyde and ethanol (Ke et al., 1993). Exposure of fruit of some apple cultivars to high CO2 (≥20 kPa) can result in rapid accumulation of acetaldehyde and ethanol within the tissue (Pesis et al., 1994; Thomas, 1925, 1929); however, relationships between these compounds and damage are unclear.

Similarly, high concentrations of ethanol and acetaldehyde can be induced and tissue damage can sometimes occur under hypoxic conditions, although there is considerable debate as to the cause(s) of damage and the roles of ethanol and acetaldehyde under anaerobiciosis (Perata and Alpi, 1993). For instance, ethanol and acetaldehyde have been induced in apple under very low O2 atmospheres with no accompanying damage (Blamiped and Jozwiak, 1993; Ke and Kader, 1992; Ke et al., 1990; Patterson and Nichols, 1988). Smagula and Bramlage (1977), in reviewing past literature, concluded that acetaldehyde accumulation was probably a result rather than a cause of tissue injury, although they acknowledged that deficiencies in analytical methods may have obscured results.

Arelationship between flesh browning and production of ethanol and acetaldehyde in ‘Fuji’ as induced by high CO2 partial pressures has not been demonstrated. Recently, we found that individual ‘Fuji’ fruit that had browned, in an atmosphere of 2 kPa CO2 and 2 kPa O2 at 0 °C after 17 weeks, had higher ethanol and acetaldehyde production than those not browned, but stored in the same atmosphere (unpublished data). Our objective in the following study was to determine and compare relationships between flesh browning and ethano,n acetaldehyde, and ethyl acetate production for ‘Fuji’ during exposure to CO2. Because of the sporadic occurrence of damage in ‘Fuji’ fruit exposed to the low CO2 atmospheres typically used in CA (Grant et al., 1995; Volz et al., 1998), this study employed high CO2 partial pressures at ambient temperatures to ensure rapid development of injury.

Materials and Methods

Fruit from 5 two-tree plots were harvested 190 (1–5 Oct.) and 210 d after full bloom (DAFB) from each of two ‘Fuji’ orchards located in the San Joaquin Valley, Calif. Fruit were also harvested from 5 two-tree plots at 190 DAFB from an additional three ‘Fuji’ orchards.

The day following harvest, fruit were sorted and 10 fruit per orchard (two fruit per plot) were placed into each of eight 19-L glass jars. The jars were connected to a humidified flow-through gas system (300 mL-min−1) in which the CO2 partial pressure was 50 kPa (balance = 10.3 kPa O2 + 38.6 kPa N2 + 2.4 kPa H2O) for four jars, or 20 kPa (balance = 16.6 kPa O2 + 62.3 kPa N2 + 2.4 kPa H2O) for the remaining four jars, all at 20 °C. In addition, from the two orchards that were harvested twice, five fruit per harvest date were sampled immediately before exposure to high CO2. Fruit were exposed to 50 kPa CO2 for 1 and 3 d, and to 20 kPa CO2 for 3 and 7 d. Two jars were removed for each atmosphere at the completion of each exposure period. Each fruit was then sliced transversely into five sections and assessed for the proportion of flesh area that showed browning. In addition, five fruit were selected at random from each jar, and a 10-mm-thick equatorial slice was cut from the equator of each fruit. Also, from each of the two orchards harvested twice, five fruit were sliced immediately before exposure to high CO2, and another five after storage at 20 °C for 7 d. Each slice, cortical plugs were taken and bulked together (5–8 g), weighed, placed in a 12-mL screw-cap test tube, and immediately stored at −20 °C.

For ethanol, acetaldehyde, and ethyl acetate determinations, each test tube was warmed for 1 h in a water bath at 65 °C (Beaulieu et al., 1997). A 1-mL headspace sample was taken from the tube and injected into a gas chromatograph (model GC-8A; Shimadzu, Columbia, Md.) fitted with a flame ionization detector (250 °C) and a glass column (2 mm × 1.8 m) containing 5% Carbowax on 60/80 Carpack (Supelco, Bellefonte, Pa.), at 85 °C. Tissue concentration was calculated using a standard curve, generated by injecting a 1-mL headspace sample into the gas chromatograph from warmed 5-mL standard solutions of known concentrations in 12-mL test tubes. All values are reported on a fresh-weight basis.
For the two orchards harvested at 190 and 210 DAFB, data were analyzed as a split-split plot design, with orchard analyzed as the main effect, harvest date as the split, treatment as a further split, and using the individual plots as replicates. Treatments were defined as the initial (before CO₂ exposure) sample plus the combination of each CO₂ partial pressure × exposure time. Variation due to orchard site at 190 DAFB was analyzed separately and, in this case, each CO₂ partial pressure exposure time was analyzed independently. All tissue concentration data were log transformed to stabilize the variance before each analysis of variance (ANOVA).

Results and Discussion

Harvest date. Orchard had a significant effect on area of flesh browning and acetaldehyde concentration (P = 0.04 and 0.03, respectively) (see below), but not on ethanol and ethyl acetate concentrations. There were no significant interactions between harvest date and treatment on flesh browning or tissue concentrations of ethanol, acetaldehyde, and ethyl acetate, so data were pooled across the two orchards at 190 and 210 DAFB.

Flesh browning increased with increasing time of exposure to high CO₂ partial pressures and was greater for the later harvest and the longer exposure times (Fig. 1A). After 3 d, more browning was observed in fruit in the 50 kPa than in the 20 kPa CO₂ treatment, but browning was greatest after 7 d at 20 kPa CO₂. These results are consistent with those reported earlier for ‘Fujis’ apples grown in California (Volz et al., 1998). Browning was not observed in fruit stored in air.

Ethanol (Fig. 1B) and acetaldehyde (Fig. 1C) concentrations increased within 1 to 3 d following exposure to high CO₂, and continued to increase with increasing exposure time. Production of these compounds was greater after 3 d at 50 kPa than at 20 kPa CO₂. For 50 kPa CO₂, ethanol and acetaldehyde concentrations had increased 100- and 10-fold, respectively, similar to the increases reported for ‘Newtown Wonder’ apples exposed to similar gas partial pressures and exposure times at 15 °C (Thomas, 1925). Storage in air at 20 °C for 7 d did not affect tissue concentrations of ethanol, acetaldehyde, or ethyl acetate (data not shown). CO₂ may stimulate ethanol and acetaldehyde production through its positive effects on activation and/or transcription of the fermentation enzymes, pyruvate dehydrogenase (EC 1.1.1.1), and alcohol dehydrogenase (EC 1.1.1.1), as well as through accumulation of pyruvate (Ke et al., 1993).

Later harvested fruit accumulated more ethanol and acetaldehyde than earlier harvested fruit at similar CO₂ partial pressures and exposure times (Fig. 1B and C). A similar harvest date effect was found with peach and pear fruit when exposed to very low O₂ partial pressures (Ke et al., 1993). Fruit resistance to diffusion of CO₂ and O₂ can be influenced by both cultivar and harvest date (Gran and Beaudry, 1993; Park et al., 1993; Rajapakse et al., 1990), thereby affecting internal gas composition within the fruit and the tissue’s response to external atmospheres. However, there is little influence of harvest date on skin resistance to CO₂ in ‘Fujis’ (Volz et al., 1998). Greater production of ethanol and acetaldehyde at a more advanced harvest date in ‘Fujis’ may reflect increased sensitivity of the tissue to high CO₂.

Ethyl acetate concentrations, while <1% of those of ethanol and acetaldehyde before exposure, increased by 70 times its initial level on exposure to CO₂. Alcohols are converted to esters by alcohol O-acetyltransferase (EC 2.3.1.84), and high concentrations of ethanol, the immediate precursor of ethyl acetate, may stimulate ethyl acetate production (Ke et al., 1993). While not all apple cultivars show a concurrent increase of ethyl acetate with increasing ethanol concentrations, this has also been observed in ‘Fujis’ parent, ‘Delicious’, and half-sibling, ‘Empire’ (Blanpied and Jozwiak, 1993).

Orchard variability. Tissue response to increasing exposure time for the two CO₂ atmospheres for fruit from all five orchards harvested at 190 DAFB was similar to those described above (Fig. 1). Differences were evident among orchards in the extent of browning and production of ethanol, acetaldehyde, and ethyl acetate, although these were not always consistent between the two CO₂ partial pressures. For instance, one orchard had greater area of flesh browning and higher ethanol and acetaldehyde production than another orchard under 50 kPa CO₂, but this relationship was reversed under 20 kPa CO₂.

Relationships between tissue concentrations of ethanol, acetaldehyde, and ethyl acetate and flesh browning were developed using data from all orchards and harvest dates. When orchard averages at each harvest date were considered (n = 7), acetaldehyde concentrations increased with increasing area of flesh browning after 7 d exposure to 20 kPa CO₂ or 3 d exposure to 50 kPa CO₂ (r = 0.88, P = 0.01 and r = 0.82, P = 0.03, respectively). For

Fig. 1. Effects of time of exposure to 20 and 50 kPa CO₂ on area of flesh browning (A) and tissue concentrations of ethanol (B), acetaldehyde (C), and ethyl acetate (D) in ‘Fujis’ apples harvested at 190 and 210 DAFB. Data pooled over two orchards and, for tissue concentrations, log transformed before ANOVA. Bar = LSD (P = 0.05).
individual fruit within any one orchard (n = 10), concentrations of acetaldehyde also increased with increasing area of flesh browning (r = 0.56, P < 0.05) after 7 d at 20 kPa CO₂ for all sites except one at 190 DAFB, and in four sites after 3 d at 50 kPa CO₂. Ethanol concentrations were also correlated with flesh browning for individual orchards (r = 0.79, P = 0.04), and for individual fruit within an orchard at six sites (r = 0.66, P < 0.05) after 7 d at 20 kPa CO₂, but not after 3 d at 50 kPa CO₂. Ethyl acetate concentrations were not correlated with flesh browning across orchards or for individual fruit at any one site for either CO₂ atmosphere (P > 0.09). Values and ranges of values in flesh browning among orchards were considerably less for the shorter than for the longer exposure times for each CO₂ partial pressure [e.g., 2% to 11% (3 d) vs. 28% to 62% (7 d) for 20 kPa]. That flesh browning and tissue concentrations of each compound were not correlated (P > 0.23) for the shorter CO₂ exposure times is therefore not surprising.

In ‘Newtown Wonder’, acetaldehyde was also associated with CO₂-induced flesh browning (Thomas, 1925) but at much higher concentrations on a fresh-weight basis (>2.2 mmol·kg⁻¹) than those concentrations found in our study (<2.2 mmol·kg⁻¹). Acetaldehyde concentrations that are toxic vary considerably among different plant species (Perata and Alpi, 1993), but variation in toxicity for different cultivars within a species is unknown.

Despite these significant relationships, accumulation of ethanol and/or acetaldehyde in cortical tissue, as induced by CO₂, may not be the sole causal agent in inducing browning, and other factors could be involved. Scatter graphs showing the association between flesh browning and acetaldehyde (Fig. 2), ethanol, or ethyl acetate concentrations (data not shown) for individual orchards indicate that production of each compound, relative to flesh browning area, was greater at 50 kPa than at 20 kPa CO₂. For individual fruit, relationships between compounds and browning were also dependent upon CO₂ partial pressure. For instance, at 210 DAFB, acetaldehyde and ethanol concentrations (0.32 and 3.0 mmol·kg⁻¹, respectively) were greater in fruit exposed to 50 kPa CO₂ for 3 d that did not brown, than in those showing 20% internal browning after exposure to 20 kPa for the same period. There may be some temporal differences in the CO₂- induction of the fermentative pathway and that of visible browning symptoms for ‘Fuji’. Similarly, acetaldehyde and ethanol production in pear relative to browning was greater under 50 kPa CO₂ than under 20 kPa CO₂ after 6 d exposure at 15 °C (Ke et al., 1990). Exposure of ‘Fuji’ apple fruit to exogenous acetaldehyde or ethanol may prove useful in understanding the possible relationship between these compounds and flesh damage.

In apple and pear, CO₂ inhibits the activity of succinic dehydrogenase (EC 1.3.99.1), which converts succinic acid to fumaric acid (Frenkel and Patterson, 1973; Knee, 1973). High succinic acid concentrations are associated with CO₂-induced injury in apple (Hulme, 1956). Initial measurements conducted in our laboratory have also shown succinic acid concentrations to be higher in browning than non-browned ‘Fuji’ fruit stored under high CO₂ atmospheres (unpublished data). However, at similar levels of flesh browning, concentrations were much greater under 50 kPa CO₂ than under 20 kPa CO₂. Exposure to high CO₂ may also reduce ATP : ADP ratios and thus the energy supply needed for maintenance of cellular processes (Lange and Kader, 1997), leading to loss of membrane function and compartmentalization (Cherwin et al., 1996).

Whatever the mechanism by which CO₂ induces flesh browning in ‘Fuji’ apples, it is probably the same in fruit exposed to very high (220 kPa) CO₂ atmospheres as in those exposed to lower concentrations at lower temperatures for longer periods, such as that used in CA. Visible symptoms of flesh browning are identical, and preharvest conditions affect flesh browning responses to CO₂ exposure under both CA conditions and under high CO₂ atmospheres at ambient temperatures (Volz et al., 1998). However, flesh browning in response to CO₂ occurs much more rapidly under high CO₂ (1–3 d) than in CA (14–56 d; unpublished data).

In conclusion, the area of flesh browning increased and fermentative production of ethanol, acetaldehyde, and ethyl acetate were stimulated by exposure of ‘Fuji’ fruit to high CO₂ atmospheres. While some positive correlations were found between flesh browning and tissue acetaldehyde and ethanol concentrations, the relationship between concentrations and area of browning differed for the two CO₂ atmospheres. The causal mechanism of CO₂ injury and the potential involvement of acetaldehyde and ethanol in ‘Fuji’ apple remain to be elucidated.

**Literature Cited**


