Storage and Handling Effects on a CO₂-related Internal Browning Disorder of 'Braeburn' Apples

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Abstract. 'Braeburn' apple (*Malus ×domestica* Borkh.) fruit can be susceptible to the development of an internal disorder called "Braeburn" browning disorder" (BBD). Factors associated with development of this disorder were investigated. Susceptibility to injury was greater in fruit exposed to 2 or 5 kPa CO₂ than to 0 kPa CO₂ during storage. Susceptibility also increased with decreasing O₂ partial pressure in the range of 5 to 1 kPa in the storage atmosphere. However, fruit stored in 1 kPa O₂ remained firmer than those stored at higher partial pressures, regardless of CO₂ level. BBD appeared to develop during the first 2 weeks of storage, and delays in air at 0 °C prior to controlled-atmosphere (CA) storage decreased incidence and severity of the disorder. The incidence of BBD was also reduced when the time to establish CA conditions was prolonged. We recommend that 'Braeburn' apples be stored under CA conditions of ≤1.0 kPa CO₂ and 3.0 kPa O₂. Delayed application of CA for 2 weeks after fruit enter the coldstorage may also reduce development of BBD.

The storage quality of 'Braeburn' apples is limited by the development of a browning disorder within the fruit flesh. Isolated instances of the disorder were first observed commercially in 1978, but more recently, the disorder has caused erratic, but often major losses (up to 5%) in fruit from some growing regions (New Zealand Apple and Pear Marketing Board, unpublished data). The disorder appears to be more prevalent in southern or colder regions in the South Island of New Zealand (Central Otago and Canterbury), and in colder districts or at higher altitudes within regions of the North Island (Central Hawke's Bay and Wairarapa). 'Braeburn' apples can develop brownheart under controlled-atmosphere (CA) conditions (Padfield, 1975). However, because internal browning also has been observed in air-stored fruit, in fruit remaining on the tree late in the season (industry observations), and in fruit that have been fumigated with methyl bromide (Lay-Yee, 1993), the internal browning observed commercially may not be the same as that described earlier as brownheart. The disorder has also been observed in 'Braeburn' fruit grown in Washington State (Curry, 1996; Hansen, 1996). Since 1993, the disorder has been referred to as "Braeburn" browning disorder" (BBD) by the New Zealand industry.

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The symptoms of BBD include browning of the flesh and formation of lens-shaped cavities (Fig. 1) typical of CO₂ injury (Lidster et al., 1990; Meheriuk et al., 1994). In severe cases, the browning is visible on the surface of the fruit and may be associated with surface depressions. Anecdotal observations by storage operators in the New Zealand industry suggest that the type and severity of flesh browning and cavitation varies with harvest maturity and storage atmosphere. Typically, areas of medium- to dark-brown tissue are found throughout the cortex and core areas. These tend to be concentrated in the calyx end of the fruit and the midcortex initially, but are visible throughout the cortical tissues of severely affected fruit. They also tend to be associated with the shaded, nonblushed side of

the fruit, and, except for severe cases, a layer of flesh under the skin is usually unaffected. The affected areas are firm and are either dry or wet when cut, with the wet type of lesion being darker brown than the dry type. The cavities may be present within the brown tissue regions of either the core or cortical areas of the fruit. They are generally dry when cut, and presumably are formed when the brown tissues become dehydrated.

The incidence of BBD in New Zealand and the United States is having a major impact on confidence in the 'Braeburn' cultivar, and strategies to avoid occurrence of the disorder are needed by industry. Therefore, the objectives in this study were to investigate the influences of CO₂ and O₂ levels on quality and development of BBD in 'Braeburn' apples, and to determine how storage conditions may be manipulated to reduce the disorder. We investigated the effects of five factors: 1) CO₂ levels; 2) the O₂:CO₂ ratio; 3) exposure of fruit to air and CA storage for varying time intervals; 4) delays in air storage before application of CA, and 5) atmosphere drawdown rate.

Materials and Methods

Fruit source. Export quality 'Braeburn' fruit were harvested from commercial orchards in the Hawke's Bay region of New Zealand during commercial harvest periods in the 1992, 1995, and 1996 seasons. Unless indicated otherwise, fruit from four orchards were used in each experiment. All experiments were carried out with size 100-count fruit, each weighing between 174 and 199 g.

Quality assessment. Maturity and quality characteristics (flesh firmness, soluble solids, starch index, internal ethylene concentration, background skin color, and titratable acidity) were assessed at harvest as described by Watkins et al. (1992). Flesh firmness and incidence of disorders were assessed after storage at both 1 and 7 d after transfer of fruit to 20 °C. Cut fruit from each treatment were also sampled by the assessors to detect any off-flavor development.

Storage disorders. In the initial trial, flesh

Fig. 1. CO₂-related injury of 'Braeburn' apples showing: (left) cavity formation and (right) browning of internal tissues.

browning and cavities were ranked separately on a scale of 0 to 5, where 0 = no disorder and 5 = severe disorder. An ENZA (New Zealand Apple and Pear Marketing Board) 1995 'Braeburn' browning disorder chart, which ranks the flesh browning and cavitation symptoms together over a range of 1 (slight) to 5 (severe), was used to describe the incidence of disorders in subsequent trials. In all cases, sound fruit were those with no symptoms of BBD or coreflush.

CA treatments. Fruit were exposed to CA conditions either in chambers, buckets, or a commercial CA facility. Each 360-L plastic chamber held four export tray cartons of 100 fruit and was ventilated with humidified atmosphere at a rate of 300 mL·min⁻¹. Each 20-L bucket held 40 to 50 fruit and was ventilated with humidified atmospheres at 200 mL·min⁻¹. The commercial CA store utilized a nitrogen-generating system (Floax SPI-101 membrane unit; Air Liquide, Paris), with purging used to control CO₂. The storage was fully loaded and operational when fruit were placed into it through the sampling hatch. Atmospheres in the chambers and buckets were established within 2 and 1 d, respectively, monitored daily for 1 week, and then twice weekly. In the latter trial, atmospheres in the buckets were applied gradually over the time periods indicated in the experiment on effect of atmosphere drawdown rate.

Effects of CO₂ levels and of delaying CA storage. Fruit (100 from each of four orchard blocks) were harvested either during the first (23 Mar. 1992) or last (13 Apr. 1992) week of the commercial season. Fruit from different growers were used for the early and late harvests. Fruit were stored in 2 kPa O₂ with either 0.2, 2, or 5 kPa CO₂ for 31 weeks. Late-harvested fruit were also held at a temperature of ≈20 °C for 5 d, or in air at 0 °C for 2 or 3 weeks prior to storage in 2 kPa O₂/2 kPa CO₂.

Effects of CO₂ and O₂ levels. Fruit were harvested from four orchard blocks during the last week of the commercial harvest (27 Apr. 1995). Two replicates of 100 fruit from each orchard were stored at 0 °C for 12 weeks in chambers under 1, 2, or 5 kPa O₂ with 2 kPa CO₂, 1 or 2 kPa O₂ with 0 kPa CO₂, or air. To control the level of CO₂ from fruit respiration, nominal 0 kPa CO₂ atmospheres were maintained at ≤0.2 kPa by including a sachet of 250 g hydrated lime per 20 kg fruit in the chambers.

Effect of timing of CA storage. Two replicates of 100 fruit from each of the same orchard blocks used above (1995) were subjected to each of the following storage regimes: continuous air storage; 1 week of CA (2 kPa O₂/2 kPa CO₂), then air storage; 1 week of air storage, then 1 week of CA storage, then air storage; 2 weeks of CA storage, then air storage; 2 weeks of air storage, then CA storage; continuous CA storage. This trial was carried out in a commercial CA facility, with fruit stored for a total period of 11 weeks at 0 °C.

Effects of CO₂ and O₂ levels and delaying CA storage. Four orchard blocks of fruit were harvested during the last week of the commercial harvest season (22–23 Apr. 1996) and

held for 16 weeks of CA storage at 0.5 °C. One replicate of 100 fruit from each orchard was stored under each of the following storage regimes: air, 2 kPa, 2.5 kPa, or 3 kPa O₂, with either 0 or 1 kPa CO₂. In addition, CA was applied after either 1 or 14 d in air at 0.5 °C.

Effect of atmosphere drawdown rate. Fruit from the same orchards used above (1996) were placed into flow-through buckets (four buckets/treatment; 40 fruit/bucket) and subjected to varying stepwise reductions in controlled atmosphere during the initial storage period. Fruit were brought under an atmosphere of 1.5 kPa O₂/2.5 kPa CO₂ over 1, 2, 4, 8, or 16 d by daily adjustments to the composition of the atmosphere flowing through the buckets. Fruit were stored for 4 weeks at 0.5 °C.

Statistical analysis. Analysis of variance (ANOVA) was used to make statistical inferences from the randomized complete-block designs. Percentage data were arcsine transformed to obtain homogeneity of variance in the data. Mean separation was by LSD at the 5% level.

Results

Effects of CO₂ levels and of delaying CA storage. The incidence of flesh browning, cavities and coreflush in 'Braeburn' apples after 31 weeks of storage was higher with greater CO₂ partial pressure in the storage atmosphere (Table 1). The severity of each disorder followed the same statistical relationship as did incidence (data not shown). The incidence of disorders was orchard-dependent, with fruit from some orchards having <10% incidence while other lines had >90% (data not shown). However, trends were the same for each orchard. When fruit were held in air at 0 °C for 2 or 3 weeks prior to CA storage, the incidence of flesh browning and cavities decreased markedly. However, the incidence of coreflush, although slight in severity, increased, such that the proportion of sound fruit was largely unchanged. When fruit were held in air at 20 °C for 5 d prior to CA storage, the incidence of flesh browning was slightly reduced, but the incidence of cavities and coreflush was unchanged.

Late-harvested fruit had slightly more starch loss than early-harvested fruit (3.3 vs. 2.5 on

the 'Braeburn' starch chart; scale 0–6), but both groups had very similar internal ethylene concentrations, firmness, and soluble solids levels at harvest (data not shown). Separate comparative analyses of early-harvested and late-harvested fruit, without incorporation of delayed CA and/or low-temperature treatments, were performed. Although harvest dates differing by 3 weeks had similar BBD incidence (Table 1), the orchards used were not identical at both harvests, thus making any conclusion about effect of harvest date tentative. However, alcoholic off-flavors developed in late-harvested fruit immediately following removal from CA storage after 31 weeks at 0 °C, as detected by informal tasting of fruit, indicating that harvest date may be a factor influencing tolerance of fruit to CA conditions. These off-flavors had largely dissipated after 7 d at 20 °C, except for fruit that had been held for 5 d at 20 °C prior to CA storage.

Flesh firmness after 31 weeks of storage at 0 °C was generally above 80 N, and was not significantly affected by harvest date, storage atmosphere, or delay in CA storage (data not shown).

Effects of CO₂ and O₂ levels. Incidence of BBD in fruit stored in air for 12 weeks was only 2%. Its incidence (Table 2) and severity (data not shown) were greater in 2 kPa CO₂ than in 0 kPa CO₂, and were enhanced by decreasing O₂ partial pressure over the range of 1 to 5 kPa. Once again, incidence of BBD was highly variable among orchard sites, but fruit from all orchards responded in a similar manner to changes in storage atmosphere. There was a small interaction between orchard and storage atmosphere on BBD, with fruit from two orchards being more severely affected by lowering O₂ partial pressure than were those from the other two orchards (data not shown).

After storage for 12 weeks at 0 °C, fruit stored under CA were firmer than those stored in air, with fruit stored at low O₂ partial pressure retaining greater firmness than those stored at higher O₂ partial pressures, regardless of CO₂ partial pressure (Table 2).

Effect of timing of CA storage. Timing of imposition of CA regimes greatly affected the development of BBD. Fruit stored continuously in air, or in CA after 2 weeks in air, were

Table 1. Percentage of sound fruit and fruit with internal browning, cavities, and coreflush in early- (23 Mar.) and late-harvested (13 Apr. 1992) 'Braeburn' apples stored in varying controlled atmospheres immediately or after delays at 0 or 20 °C. Fruit were stored for 31 weeks at 0 °C, and quality assessed after an additional week at 20 °C. Means are the average of four orchard replicates.

Harvest	Treatment (kPa O ₂ : kPa CO ₂)	Sound (%)	Disorder incidence (%)		
			Browning	Cavities	Coreflush
Early	2:0	81 a ²	1 c	5 cd	5 b
	2:2	58 ab	3 bc	21 ab	6 b
	2:5	17 c	18 ab	33 a	14 ab
Late	2:0	84 a	0 c	6 bcd	6 b
	2:2	65 ab	4 bc	20 ab	4 b
	2:5	20 c	29 a	14 abc	17 ab
Late	2:2; 5-d delay at 20 °C	56 b	0 c	29 a	6 b
	2:2; 2-week delay at 0 °C	71 ab	1 c	3 cd	27 a
	2:2; 3-week delay at 0 °C	70 ab	0 c	1 d	29 a

²Mean separation within columns by LSD at *P* ≤ 0.05. Data were arcsin transformed for statistical analysis.

Table 2. Incidence (%) of “‘Braeburn’ browning disorder” (BBD) and flesh firmness (N) of ‘Braeburn’ fruit stored under air or various controlled-atmosphere regimes for 12 weeks at 0 °C. Each mean is the average of fruit from four orchards, harvested 27 Apr. 1995.

Treatment (kPa O ₂ : kPa CO ₂)	BBD (%)	Flesh firmness (N)
Air	1 f ^a	75 c
1:2	88 a	83 a
2:2	67 b	81 b
5:2	25 d	80 b
1:0 ^b	43 c	83 a
2:0	17 e	81 b

^aMean separation within columns by LSD at $P \leq 0.05$. Percent BBD data were arcsin transformed for statistical analysis.

^bNominal 0 kPa CO₂ treatments were ≤ 0.2 kPa CO₂ due to fruit respiration.

mostly sound, while fruit stored in CA for only 1 week, immediately after harvest or after 1 week in air, had 5% and 1% incidence of BBD, respectively (Table 3). Fruit stored in CA for the first 2 weeks of storage had 10% BBD. However, when fruit were stored in CA for the entire storage period, the incidence of BBD was significantly lower for three of the four orchards than was found with shorter exposures (data not shown). This may indicate that continuous CA has a moderating effect on injury development after an initial period of high susceptibility. A further feature of this experiment is that while fruit used were from the same source as fruit used in the previous experiment (Table 2), the incidence of BBD was dramatically lower. The reasons for the difference are not yet understood, but may relate to the different storage conditions in a flow-through CA system compared with a commercial CA storage facility.

Fruit that had been CA-stored for 2 weeks or longer were generally 5 to 8 N firmer than fruit that had been kept continuously in air (Table 3). No significant effects of orchard or treatment $\times$ orchard interactions were detected.

Effects of CO₂ and O₂ levels and delays to CA storage. The major consideration with storage of ‘Braeburn’ apples is to balance firmness retention with the minimization of BBD incidence. To develop a protocol to successfully store ‘Braeburn’ under CA conditions, a trial was designed to compare the

Table 3. Incidence (%) of “‘Braeburn’ browning disorder” (BBD) and flesh firmness (N) of ‘Braeburn’ fruit after storage for 11 weeks at 0 °C under varying storage regimes combining air and controlled-atmosphere (CA) storage at 2 kPa O₂ : 2 kPa CO₂. Each mean is the average of fruit from four orchards.

Treatment during week			BBD (%)	Flesh firmness (N)
1	2	3–11		
Air	Air	Air	0.6 a ^a	74 d
CA ^a	Air	Air	4.5 b	77 c
Air	CA	Air	1.0 a	77 c
CA	CA	Air	10.1 d	79 b
Air	Air	CA	2.6 ab	79 b
CA	CA	CA	6.8 bc	82 a

^aMean separation within columns by LSD at $P \leq 0.05$. Percent BBD data were arcsin transformed for statistical analysis.

effects of a 2-week delay in air prior to CA application vs. immediate CA application, in combination with varying atmospheres. An atmosphere of 1 kPa CO₂ with 2–3 kPa O₂ resulted in higher levels of BBD than those incurred when CO₂ was scrubbed with hydrated lime (Table 4). Delaying application of CA for 2 weeks reduced BBD to levels near those of fruit stored continuously in air. As in earlier trials, BBD incidence increased with increasing CO₂ and decreasing O₂ partial pressures.

Fruit held under CA were firmer than those stored in air (Table 4). Firmness after 1 week at 20 °C ranged from 70 to 80 N for fruit stored under CA, and from 63 to 72 N for air-stored fruit (data not shown). Firmness of CA-stored fruit was not affected detrimentally by delay, CO₂ or O₂ partial pressure, or time at 20 °C after storage. Some minor orchard variations in firmness in response to CA treatments and delays were detected (data not shown).

Effect of atmosphere drawdown rate. Incidence of BBD decreased from 88% to 22% as the time taken to reach a CA of 1.5 kPa O₂ : 2.5 kPa CO₂ increased from 1 to 16 d (Table 5). Similarly, the average BBD score decreased from 3.2 to 0.5, indicating that the affected fruit in the longer drawdown treatment only exhibited slight disorder symptoms (data not shown). Regressions for the percentage of total BBD and BBD score vs. time taken to reach a final CA of 1.5 kPa O₂ : 2.5 kPa CO₂ were: $Y = 90.9 - 4.6X$, $r^2 = 0.87$, and $Y = 2.9$

$- 0.17X$, $r^2 = 0.78$, respectively. All regressions were statistically significant at $P < 0.01$.

Discussion

A high incidence of an internal browning disorder in ‘Braeburn’ apples stored in either air or CA has been observed as production of the cultivar has increased. Brownheart was observed in CA-stored or methyl bromide-treated ‘Braeburn’ apples by Padfield (1975) and Lay-Yee (1993). However, because the disorder appeared to occur occasionally on trees prior to harvest, and under air storage, it could have been a response to low temperatures, CO₂, or a combination of both factors. In prior studies, we have found no evidence that the disorder is a form of low-temperature breakdown (data not shown), and the experiments reported here conclusively indicate that BBD is a CO₂-related injury.

Two aspects of BBD induction support this conclusion. First, incidence of BBD is associated with high CO₂ partial pressures in the storage environment, and is aggravated by decreasing O₂ partial pressures when CO₂ partial pressures are kept constant. Second, susceptibility to injury appears greatest immediately after harvest and decreases markedly when fruit are kept in air for 2 weeks prior to CA storage. These responses are typical of those found for both external and internal CO₂-related disorders in a number of apple cultivars (Burmeister and Dilley, 1995; Johnson et al., 1998; Watkins et al., 1997; Wilkinson and Fidler, 1973).

Our data indicate that BBD incidence can be significantly reduced by storing fruit under CA conditions of <1.0 kPa CO₂ and 3.0 kPa O₂. Also, while prompt application and rapid establishment of CA following cooling are commonly recommended to maximize the benefits of CA storage (Fica et al., 1985; Little and Pegg, 1987), a delay in CO₂ application can further reduce incidence of BBD. In our trials, delays did not result in significant loss of fruit texture. However, in one case, a delay before application of CA enhanced incidence of coreflush (Table 1), and ‘Braeburn’ fruit are susceptible to loss of firmness during storage (Harker and Hallett, 1992). Therefore, this method requires investigation using fruit of a range of maturities and harvest dates be-

Table 4. Incidence (%) of “‘Braeburn’ browning disorder” (BBD) and flesh firmness (N) of ‘Braeburn’ fruit stored for 16 weeks at 0.5 °C under various controlled atmospheres, applied either 1 d after packing or after a 14-d delay at 0.5 °C in air. Firmness was assessed after 1 d at 20 °C. BBD incidence and flesh firmness of air-stored fruit were 1% and 70 N, respectively. Each mean is the average of fruit from four orchards harvested 22–23 Apr. 1996.

Storage atmosphere		BBD (%)		Flesh firmness (N)	
O ₂ (kPa)	CO ₂ (kPa)	1-d delay	14-d delay	1-d delay	14-d delay
2.0	0	5 d ^a	4 de	78 ^a	75
2.5	0	6 d	1 fg	76	72
3.0	0	3 def	1 efg	75	73
2.0	1	37 a	1 efg	80	76
2.5	1	21 b	6 d	76	74
3.0	1	12 c	1 efg	78	74

^aMean separation among all treatments by LSD at $P \leq 0.05$. Percent BBD data were arcsin transformed for statistical analysis.

^bLSD at $P \leq 0.05$ for all firmness data = 4.5.

Table 5. Effect of the rate of atmosphere drawdown to 1.5 kPa O₂ : 2.5 kPa CO₂ on the incidence (%) of “‘Braeburn’ browning disorder” (BBD) in fruit from central Hawke’s Bay. Fruit were stored for 4 weeks at 0.5 °C. Each mean is the average of fruit from four orchards harvested 22–23 Apr. 1996.

Days to reach target atmosphere	BBD (%)
1	89 a ^a
2	88 a
4	68 b
8	46 c
16	22 d
Air (control)	0 e

^aMean separation within columns by LSD at $P \leq 0.05$. Percent BBD data were arcsin transformed for statistical analysis.

fore it can be commercially recommended.

The biochemical mechanisms of CO₂ injury, and the processes by which fruit become less susceptible to injury when CO₂ applications are delayed, are not well understood. Johnson et al. (1998) hypothesized that these physiological changes were possibly related to transiently high CO₂ concentration in the fruit during the 'respiratory burst' early in storage (Fidler, 1949; Kidd and West, 1937). Fruit subjected to CA during this time are more likely to have internal atmospheres with injurious O₂ and CO₂ concentrations. Johnson et al. (1998) found that high skin resistance to gas diffusion was associated with increased internal injury. 'Braeburn' had significantly higher skin resistances than other apple cultivars tested by Rajapakse et al. (1989, 1990) and this factor may contribute to its susceptibility to internal CO₂ injury. However, susceptibility of tissue to CO₂ injury also appears to decrease during the first few weeks of storage, suggesting that changes in metabolic responses of cells to CO₂ occur during this time. Inhibition of succinate dehydrogenase may be involved in accumulation of succinic acid in fruit held in high CO₂ atmospheres (Hulme, 1956). Inhibition of both external and internal CO₂ injuries by diphenylamine application (Burmeister and Dilley, 1995; Johnson et al., 1998; Watkins et al., 1997) suggests possible involvement of free radical damage to proteins and macromolecules in injury development. Acclimation of fruit in air may be due to changes in antioxidant concentrations and activities of free radical scavenging enzymes, such as superoxide dismutase and catalase.

Our studies have shown that, as with other CO₂-related disorders (Johnson et al., 1998; Watkins et al., 1997; Wilkinson and Fidler, 1973), the greatest source of variation in BBD development is the orchard sampled. While our study demonstrates that postharvest fac-

tors influence its expression during storage, preharvest factors, such as region, orchard, cropload and tree position, temperature during growth, fruit nutrient status, and water status, are all possible factors that predispose fruit to BBD.

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