

Quality of Pink Lady Apples in Relation to Maturity at Harvest, Prestorage Treatments, and Controlled Atmosphere during Storage

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Abstract. Apple [*Malus ×sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf. ‘Cripps Pink’] fruit were harvested yearly, at two or three maturity stages, from the same California orchard in 2002 through 2005. Fruit firmness, soluble solids, titratable acidity, background color, and percent blush were correlated with the starch pattern index at harvest. Fruit from each harvest were stored at 0.5 °C in air or in a controlled atmosphere (CA) with 1.5 or 2 KPa O₂ in combination with 1, 3, and 5 KPa CO₂. Subsets of fruit were treated with 1 μL·L⁻¹ 1-methylcyclopropane for 24 hours at 0 °C or 2200 μL·L⁻¹ diphenylamine (DPA) for 5 minutes. Ethylene production was measured for 30 days after harvest. Ethylene concentration in the storage atmosphere was also monitored during storage. Fruit quality was evaluated after storage plus 5 days of ripening at 20 °C. Fruit in a CA with 1 or 3 KPa CO₂ maintained firmness and green background color, and produced less ethylene during ripening at 20 °C than fruit stored in a CA with 5 KPa CO₂; however, quality of all CA-stored fruit was better than air-stored fruit. Flesh browning developed only in CA storage, appearing by 2 months and not increasing in incidence with further storage periods. 1-Methylcyclopropane conserved fruit quality in air as well as CA during 4 months of storage, and DPA-treated fruit were firmer after CA storage, but similar after air storage, compared with untreated fruit. Diphenylamine did not control a stem-end scald disorder, which increased with time in storage and affected more than 80% of the fruit after 6 months of air storage.

‘Cripps Pink’ apple was developed in Australia in the late 1960s from a cross between ‘Golden Delicious’ and ‘Lady Williams’. (‘Cripps Pink’ apples of an appropriate quality may be sold using the trademarked brand name Pink Lady.) It had an appealing appearance, good sensory quality, and performed as well as other late-harvested varieties during storage in Australia (Corrigan et al., 1997). It was introduced into central California in the 1990s, where there has been some difficulty growing a colorful apple because of the warm climate, and this has resulted in delays in harvest that can affect fruit quality. Nevertheless, Californian Pink Lady apples are marketed all over the United States, especially in the northeast, as well as in the United Kingdom.

Controlled-atmosphere (CA) storage extends the life and preserves the quality of Pink Lady apples (Cripps et al., 1993). However, CA can also cause physiological

disorders, such as internal browning in apples (Volz et al., 1998).

1-Methylcyclopropane is (1-MCP) an inhibitor of ethylene action that can reduce senescence processes in fruit (Watkins and Miller, 2006). Its benefits in fruit conservation are numerous, and some of them remain unknown. Watkins et al. (2000) showed that 1-MCP reduced superficial scald incidence on apple fruit, and there was an additive effect of 1-MCP in a CA. If a mechanism that causes quality loss is ethylene dependent, 1-MCP has the potential to reduce that loss of quality. Our goal was to determine the effect of harvest maturity of Pink Lady apples on the quality attributes after storage and to determine the most appropriate postharvest treatments and storage conditions to maintain quality during storage.

Material and Methods

Fruit material. Pink Lady apples were harvested on 21 Sept. and 20 Oct. 2002, 6 Oct. and 20 Oct. and 6 Nov. 2003, 21 Sept. and 5 Oct. and 22 Oct. 2004, and 28 Sept. and 27 Oct. 2005 in the early morning from five 40-tree plots in the same orchard near Stockton, Calif. About 50 apples were harvested per tree, and each tree was harvested once. To determine the progress of starch conversion to sugars over time, 30 apples were collected from orchard trees weekly starting the first week of September each year. Starch pattern index was measured at harvest [Center Technique Interprofessionnel des Fruits et Legumes (CTIFL), Pink Lady Association Europe, Paris, France], and indices were ≈3.0 to 4.0 for the first harvest, 6.0 to 6.5 for the second harvest, and 8.0 to 8.5 for the third harvest.

Fruit were held overnight at 10 °C after harvest before being sorted to obtain

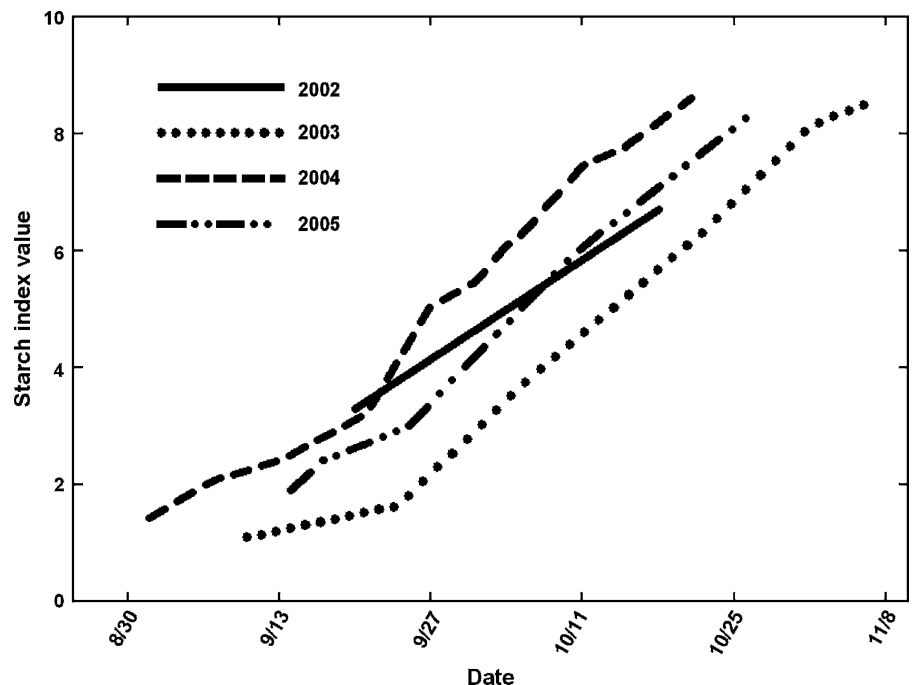


Fig. 1. Starch index of apples during maturation on the tree versus calendar date for 2002, 2003, and 2004. Starch index value from 1 to 10 (Center Technique Interprofessionnel des Fruits et Legumes).

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undamaged fruit of uniform size and background color, and were immediately cooled to 0.5 °C in air for 24 h before starting the CA treatments. Atmospheres were established in air-tight 120-L cylinders using an automatic control system adding N₂, CO₂, or air as needed to maintain the set point (TechniSystems, Chelan, Wash.). After a predetermined storage period plus 5 d at 20 °C, fruit were assessed for quality attributes.

Harvest evaluations. On the day of harvest, six fruit were selected randomly from each of five plots for a total of 30 fruit for evaluation of starch pattern index, soluble solids content (SSC), titratable acidity (TA), firmness, percent skin blush, and background color. Ethylene production was also measured during 25 d at 20 °C from a second group of 30 apples selected in an identical manner. The average fruit size was determined by weighing each box of fruit and dividing by the number of apples.

The background color was assessed with a CTIFL color scale (3 = green to 7 = yellow). The percentage of skin blush was visually estimated. Firmness was measured as resistance to penetration with an 11-mm probe using a Fruit Texture Analyzer (Güss, South Africa) on opposite sides of the fruit after removing a small area of peel. Juice was extracted from a composite sample of six wedges cut from stem to blossom end from six separate fruit from each plot for determination of SSC and TA. Percent SSC was determined with a digital refractometer (Abbe model 10450; American Optical, Buffalo, N.Y.), and TA (malic acid equivalents) was determined with an automatic titration system (TIM 850; Radiometer, Copenhagen, Denmark). For starch index determination, apples were cut in half at the equator, immersed for 2 min in 3% iodine-potassium iodide and rinsed with fresh water. The starch index was scored using a 10-point CTIFL scale.

Carbon dioxide and ethylene production were determined from six fruit from each of the five plots. The six fruit were placed together in a 3.78-L jar connected to a flow board that supplied a uniform flow of air at 120 mL·min⁻¹ for 24 to 30 d at 20 °C. During ripening, the outlet gases were monitored daily for CO₂ and ethylene by collecting samples in a syringe. Carbon dioxide concentrations were measured using an infrared gas analyzer (Horiba VIA 510; Horiba Instruments Inc., Irvine, Calif.), and ethylene was measured using a gas chromatograph with a flame ionization detector using N₂ as the carrier gas at 80 °C and 8% NaCl on alumina F-1 column (Carl Gas Chromatograph model 211; Chandler Engineering, Tulsa, Okla.).

Treatments. In 2002, fruit were stored in air, or 1.5, 3.0, or 20 KPa O₂ in a factorial with 1.0, 3.0, or 5.0 KPa CO₂ at 0.5 °C for 2 or 6 months. In 2003, fruit were stored in air or 2 KPa O₂ in a factorial with 1 or 3 KPa CO₂ at 0.5 °C for 0.5, 1, 2, or 4 months. A separate set of apples from the second harvest in 2003, were precooled at 0.5 °C and then placed into plastic bins in gas-tight 300-L stainless steel

tanks for 1-MCP treatment. A small electric fan was placed inside each tank to ensure even distribution of 1-MCP gas around the fruit. SmartFresh tablets (AgroFresh, Springhouse, Pa.) were used to generate 1 µL·L⁻¹ 1-MCP gas, and apples were treated for 24 h at 0.5 °C and then placed into air or CA storage at 0.5 °C for 4 months. A third set of fruit from the same harvest was treated with 2200 µL·L⁻¹ diphenylamine (DPA; Pace International, Seattle, Wash.) in water by immersion for 5 min, air-dried at 20 °C, cooled overnight at 0.5 °C, and then placed into air or CA storage at 0.5 °C for 4 months. In 2004, fruit were stored in air or 1.5 KPa O₂ in a factorial with 1, 3, or 5 KPa CO₂ at 0.5 °C for 2, 4, or 6 months. An additional set of fruit from the second harvest was treated with 2200 µL·L⁻¹

DPA as described previously and stored for 4 months. In 2005, fruit were stored in air or 1.5 KPa O₂ and 5.0 KPa CO₂ at 0.5 °C for 1.5, 2, or 4 months. An additional set of fruit from the first harvest was treated with 2200 µL·L⁻¹ DPA as described previously, and two subsamples were stored for 2 and 4 months, respectively.

Ethylene concentration in storage. Ten milliliters of gas from each 120-L CA chamber containing 105 or 125 fruit in 2003 and 2004, respectively, were collected every 10 d during storage to determine ethylene concentration as previously described.

Quality evaluations. After storage plus 5 d at 20 °C, 60 fruit were selected to determine firmness and background color, as previously described, and were then used to assess

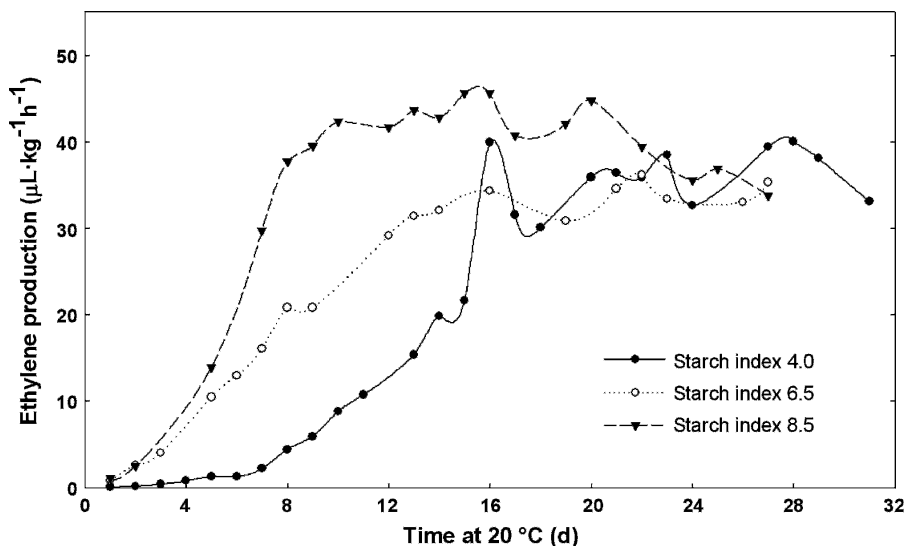


Fig. 2. Ethylene production at 20 °C in Pink Lady apples harvested at starch index 4.0, 6.5, or 8.5 for 30 d after harvest in 2003.

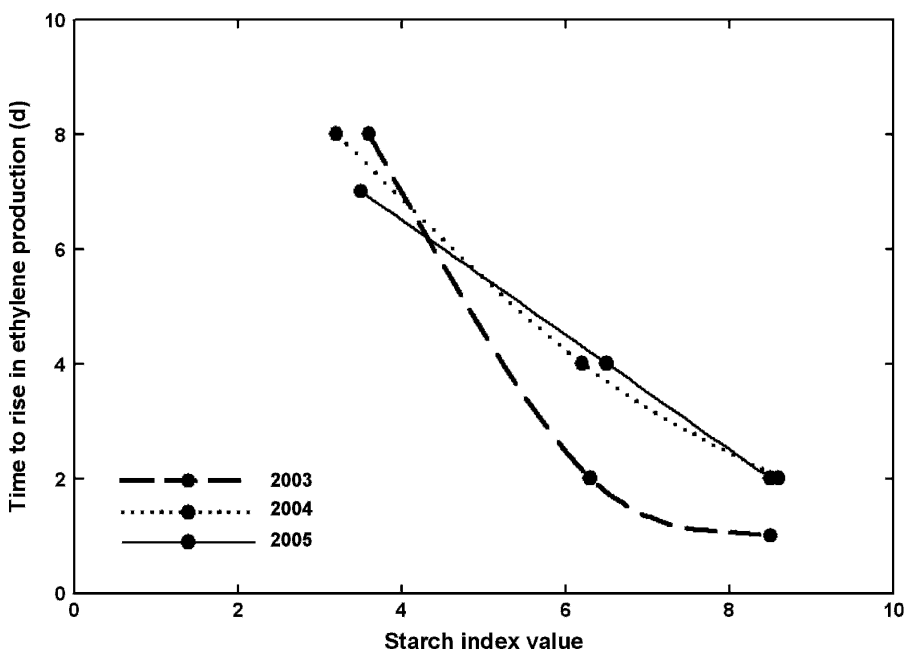


Fig. 3. Days at 20 °C before rise in ethylene production versus the starch index value at harvest in 2003, 2004, and 2005.

external disorders. Ethylene and CO₂ production were also measured, as previously described, in 2003 and 2004 during 15 d at 20 °C after storage from a separate set of 30 fruit. To calculate the total ethylene produced during 10 d, the daily ethylene production was summed for those 10 d.

The internal condition, particularly flesh browning, of the fruit was evaluated after storage using 80, 105, and 125 fruit per treatment in 2002, 2003, and 2004 respectively. Fruit were cut equatorially in three equally spaced locations, resulting in ≈2-cm thick slices, and the percentage of flesh area showing browning was visually estimated.

Results and Discussion

The starch index value on a given calendar date varied among seasons (Fig. 1).

However, except for 2002, the rate of degradation of starch per interval of time was similar. For 2003 to 2005, the differences among years may be a function of the day that starch conversion started, rather than the rate of conversion.

Pink Lady apples harvested in 2003 at a starch pattern index of 4 required at least 6 d at 20 °C before ethylene production began to increase (Fig. 2). A clear ethylene production peak was reached on day 16. When harvest was delayed until a starch index of ≥6.5 was reached, the fruit showed a nearly immediate increase in ethylene production on day 2. There was a similar relationship between ethylene production at harvest and starch pattern index in other years, indicating a close relationship for these two parameters (Fig. 3).

Soluble solids content and TA at harvest were similar among seasons at equivalent

starch levels; there was a high correlation between these two attributes and starch index (Fig. 4). In Australia, Pink Lady is a cultivar that at commercial maturity remains very tart, with a TA of 0.7% to 0.9%, and very sweet, with 12.5% to 13.5% SSC (Cripps et al., 1993). In the central valley of California, harvest is often delayed to obtain increased blush, and the TA can decrease to ≈0.6% while the sugars are high at 14% SSC. Other fruit characteristics at harvest, such as blush, background color, and firmness correlated to starch index at harvest. Other factors, such as crop load, biennial bearing, mineral content, and weather, which vary seasonally, would have more influence on variability in fruit growth and fruit size. Red color development depends on temperature and light exposure. Low temperatures, such as those encountered on cold nights, enhance anthocyanin synthesis; high temperatures inhibit

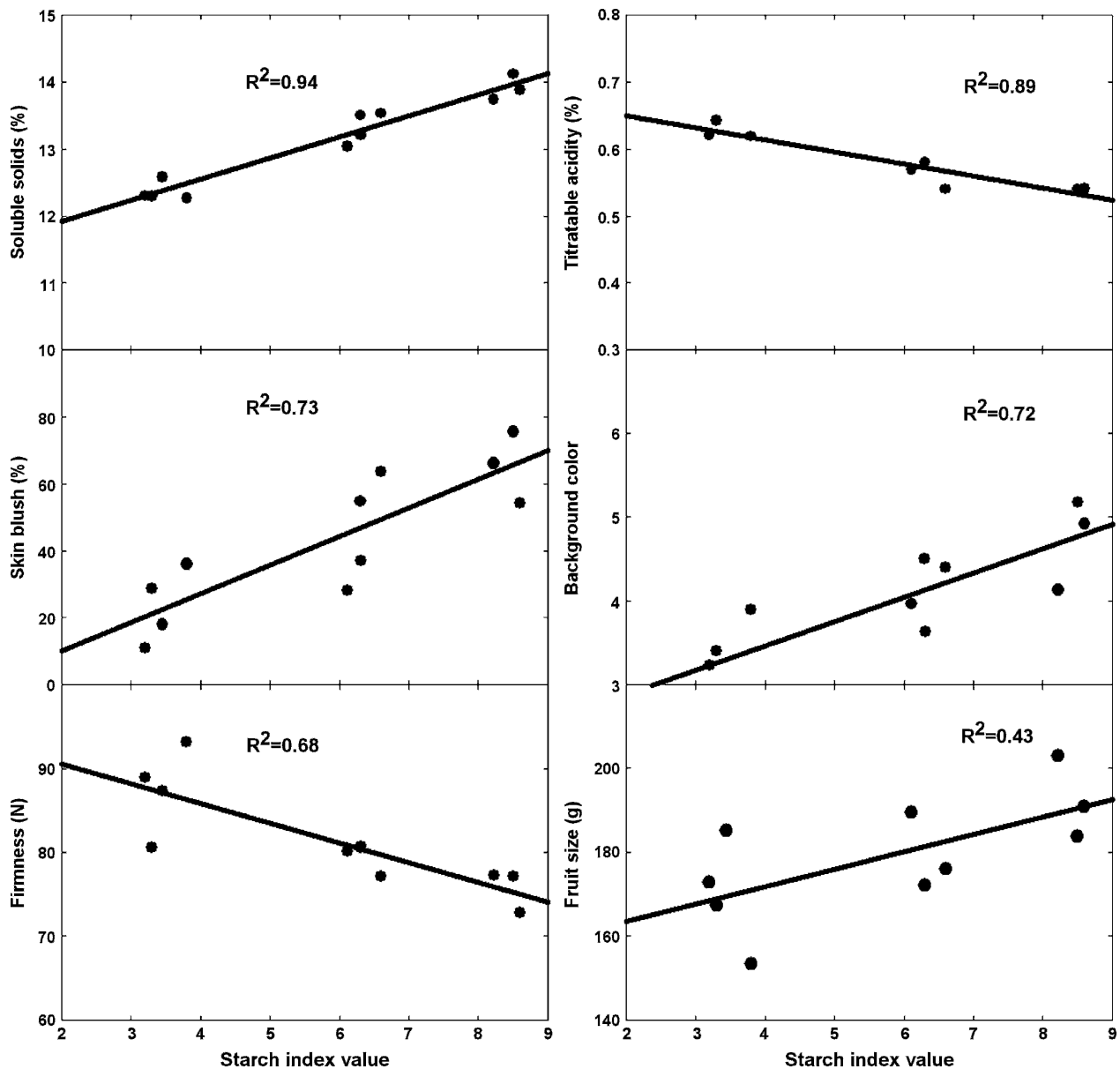


Fig. 4. Mean apple fruit soluble solids (in grams per 100 mL), skin blush (as a percent), background color (on a scale of 3–7 points), firmness (Newtons), and fruit size (in grams) for each harvest and each year for 2002, 2003, 2004, and 2005; and titratable acidity (as a percent) for 2002, 2003, and 2004 versus the starch index (scale, 1–9 points) at harvest.

anthocyanin accumulation in apple fruit (Reay, 1999). Pink Lady harvest dates in central California are strongly influenced by the percentage of fruit surface with blush and are not based as strongly on starch conversion. Therefore, in some years, apple quality can be compromised, because delaying harvest can result in detrimental losses during storage. However, a delay improves fruit size and sweetness, and provides an excellent fruit for rapid marketing.

The ethylene concentration in the storage chambers exhibited an unexpected pattern (Fig. 5A). We expected that high CO₂ concentrations in storage would suppress ethylene production and ethylene action as in 'Golden Delicious' (Gorny and Kader, 1997) and 'Granny Smith' (Chaves and Tomás, 1984) apples, both studies using 20 KPa CO₂. However, we found the highest ethylene concentration [$\approx 2 \mu\text{L}\cdot\text{L}^{-1}\cdot\text{kg}^{-1}$ fresh weight (FW)] in 5 KPa CO₂, followed by 3 KPa CO₂ and then 1 KPa CO₂. High CO₂ storage in Pink Lady may cause fruit stress and result in higher ethylene production. When fruit treated with DPA were stored in a CA, higher concentrations of ethylene accumulated in the chamber compared with untreated fruit in the same atmosphere, and the difference was larger in 3 KPa CO₂ than in 1 KPa CO₂ (Fig. 5B). Diphenylamine is an antioxidant used to inhibit superficial scald in apples (Lau, 1990). Diphenylamine has been shown to prevent development of CO₂-induced internal and external browning in some apple varieties (Fernandez-Trujillo et al., 2001; Watkins et al., 1997), including Pink Lady. However, its action did not inhibit the increase in ethylene production in Pink Lady apples stored in elevated CO₂ and appeared to increase ethylene production. Even though DPA inhibits flesh browning, the fact that the ethylene is still high in storage might mean that it does not prevent the underlying stress incited by high CO₂ in storage, just the last oxidative step of the browning.

After reaching a maximum at 130 d of storage, ethylene concentration in the storage atmosphere decreased to less than $1 \mu\text{L}\cdot\text{L}^{-1}\cdot\text{kg}^{-1}$ FW during the next 30 d (data not shown), indicating a ethylene production peak during storage in a CA at 0.5 °C. When fruit stored in a CA had been treated with 1-MCP at harvest, very low concentrations of ethylene were detected in both 1 and 3 KPa CO₂ (Fig. 5B).

Fruit softened during air storage, and flesh firmness was statistically lower after only 2 months for fruit harvested at early and intermediate maturities (Fig. 6). It decreased significantly further for the fruit harvested at the early-maturity stage (Fig. 6A), but not for the intermediate-maturity stage (Fig. 6B). Fruit stored in air was softer than fruit stored in a CA with 1.5 KPa O₂ after 2 or 6 months, regardless of the CO₂ concentration (Fig. 6). Flesh firmness was better maintained in storage with lower concentrations of O₂ and there was also a trend toward faster fruit softening with higher concentrations of CO₂,

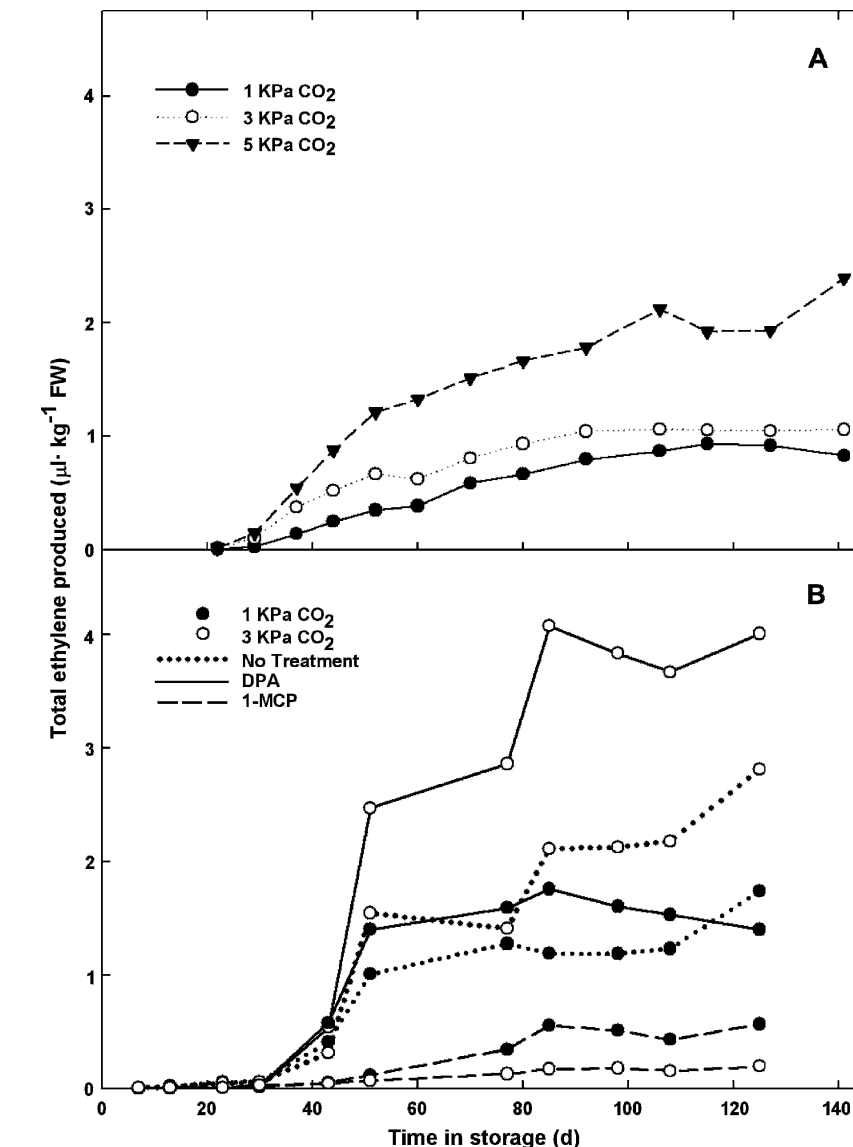


Fig. 5. Ethylene concentration (in microliters per liter per kilograms fresh weight) in controlled atmosphere chambers during storage of Pink Lady at 0.5 °C. (A) Apples harvested in 2004 at a starch index of 3.0 and stored in 1.5 KPa O₂ in combination with 1, 3, or 5 KPa CO₂. (B) Apples harvested in 2003 at a starch index of 6.5 and stored in 2 KPa O₂ in combination with 1 or 3 KPa CO₂. Diphenylamine 2200 $\mu\text{L}\cdot\text{L}^{-1}$ at harvest; 1-methylcyclopropane 1 $\mu\text{L}\cdot\text{L}^{-1}$ at harvest.

perhaps as a result of elevated ethylene in storage or stress from the CO₂ atmosphere (Fig. 6). The firmness of fruit treated with DPA was similar to untreated fruit in air or CA storage after 4 months (Fig. 7). Fruit treated with 1-MCP did not soften noticeably during storage, independent of the storage atmospheres. Storage for more than 4 months may be necessary to see any loss in firmness of fruit treated with 1-MCP, as Watkins et al. (2000) showed for 'Empire', 'Delicious', and 'Law Rome' apples.

Background color changed gradually from green to yellow for fruit not treated with DPA or 1-MCP during storage, especially in air (Fig. 8). Storage in 3 KPa CO₂ delayed skin yellowing slightly better than 1 KPa CO₂, especially after 4 months. The total ethylene produced during 10 d at 20 °C after storage increased with time in air storage (Fig. 9). When the fruit was stored

in a CA, the total ethylene produced was similar among fruit stored for various times in storage and among CO₂ concentrations.

A percentage of apples stored in CA developed flesh browning. Flesh browning incidence tended to increase as the concentration of CO₂ in storage increased, but was variable (Table 1). Fruit stored in air never presented flesh browning at any evaluation time. The incidence of flesh browning did not change with additional time in storage (data not shown), and varied from year to year. Flesh browning was always higher with higher CO₂ concentration in CA storage. Similar symptoms have been described and related to CO₂ levels in storage in other apple cultivars, such as internal flesh browning in 'Fuji' apples (Volz et al., 1998) and 'Braeburn' browning disorder (Elgar et al., 1998).

Within 2 months of air storage, fruit exhibited stem-cavity browning or stem-end

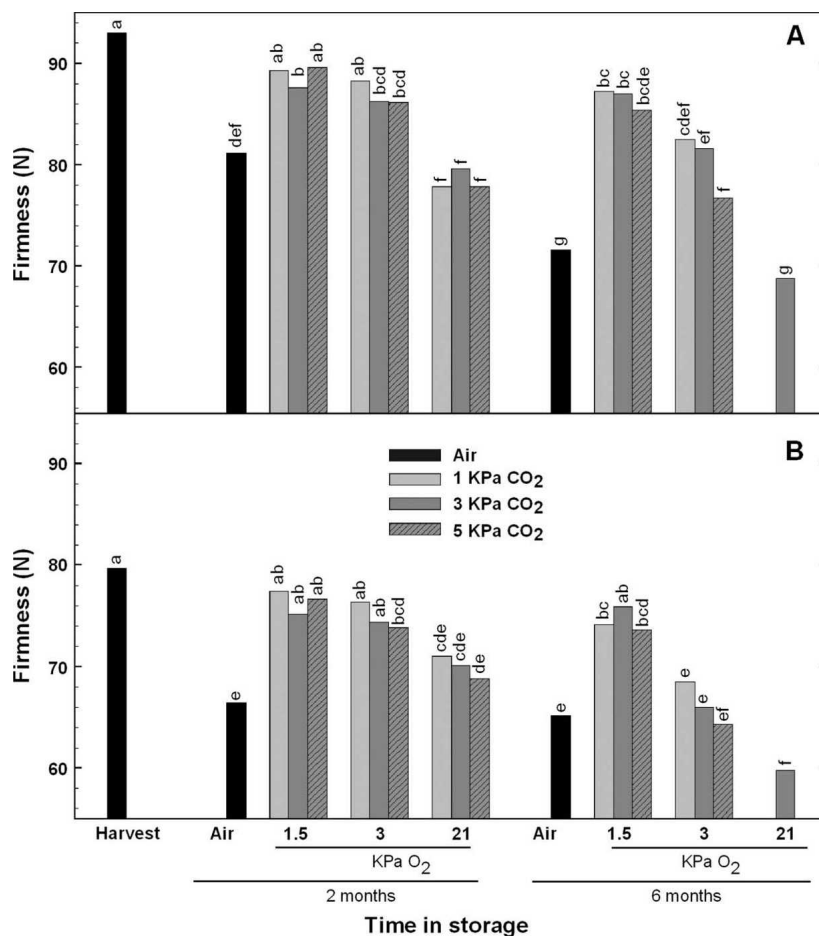


Fig. 6. Firmness (Newtons) of apples at harvest and after 2 or 6 months of air or controlled atmosphere storage (1, 3, or 5 KPa CO₂ in combination with 1.5, 3, or 20 KPa O₂) at 0 °C in 2002. Starch index at harvest was 3.5 (A) and 6.5 (B). Any two means not followed by the same letter are significantly different by Tukey's test at $P \leq 0.05$.

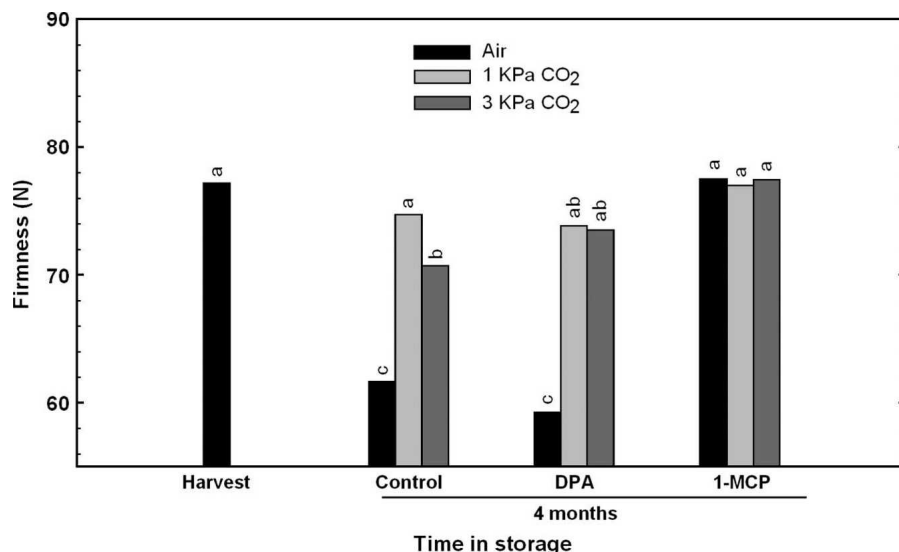


Fig. 7. Firmness (Newtons) of apples at harvest and after 4 months of air or controlled atmosphere storage (2 KPa O₂ in combination with 1 or 3 KPa CO₂) at 0 °C plus 5 d at 20 °C in 2003. Starch index at harvest was 6.5. 1-Methylcyclopropane (1-MCP; 1 $\mu\text{L}\cdot\text{L}^{-1}$) and diphenylamine (DPA; 2200 $\mu\text{L}\cdot\text{L}^{-1}$) were applied at harvest, before storage. Any two means not followed by the same letter are significantly different by Tukey's test at $P \leq 0.05$.

scald. The disorder was observed in all 4 years, but was studied in detail only in 2004 (Table 2). After 4 months of air storage, the incidence was often more than 50%. The

injury was primarily on the shoulder, radiating from the stem-end cavity, which remained relatively free of the disorder, as described by Bramlage (1988). As expected

Table 1. Incidence of flesh browning in 2002 and 2004 after 2 months of storage in air or indicated controlled atmosphere at 0.5 °C.

KPa O ₂ , KPa CO ₂	Incidence of flesh browning (%) ^z	
	2002	2004 ^y
Air control	0	0
1.5, 1	4	2
1.5, 3	14	10
1.5, 5	23	16

^zStarch index at harvest, ≈ 6.0 .

^y2003 had less than 2% of flesh browning in 2 KPa O₂ with 1 and 3 KPa CO₂ for the same starch index at harvest.

Table 2. Incidence of stem-cavity browning of apples after air storage at 0.5 °C in 2004.

Harvest	Incidence (%)		
	2 mo.	4 mo.	6 mo.
Harvest 1	13 a ^z	72 b	83 c
Harvest 2	7 a	48 a	82 c
Harvest 2 (DPA) ^y	— ^x	47 a	— ^x
Harvest 3	12 a	67 b	88 c

^zAny two means not followed by the same letter are significantly different by Tukey's test at $P \leq 0.05$.

^yTreated at harvest with 2200 $\mu\text{L}\cdot\text{L}^{-1}$ diphenylamine (DPA).

^xEvaluation not performed.

from previous reports (Lau and Lane, 1998; Lougheed et al., 1978), fruit stored in a CA had little stem-cavity browning. However, DPA treatment did not reduce this disorder for Pink Lady apple, as reported for 'McIntosh' apples (Lougheed et al., 1978). Stem-cavity browning may develop in overmature fruit as a result of ethylene action, which would explain how a CA and 1-MCP can prevent the development of this external defect. The disorder increased significantly with time in storage, but there were no differences among harvest dates, coinciding with some previous reports with 'McIntosh' (Elfving et al., 1990); however, the harvests in the study by Elfving et al. (1990) were only 1 week apart. Others have reported differences among maturity levels in 'McIntosh' and 'Sunrise', with more stem-cavity browning in early-harvested fruit than in late-harvested fruit (Lau and Lane, 1998; Lougheed et al., 1978).

Pink Lady quality, as assessed by commercial color criteria at harvest, is variable from year to year. The influence of weather determines the pace of red blush development and, consequently, the maturity of the fruit at commercial harvest. The desire for full red color by the market can be a limiting factor for the quality of the fruit, because fruit become overmature when left on the tree for too long.

Four seasons of evaluations in California have indicated that, at typical commercial harvest maturity, Pink Lady has an average of 14% SSC, 0.6% TA, and 75 N firmness. These values are similar to those reported as highly accepted by consumers who tasted Pink Lady apples, with the exception of the lower acidity values (Corrigan et al., 1997).

When Pink Lady apples are to be stored for up to 4 months, the optimum time to harvest is

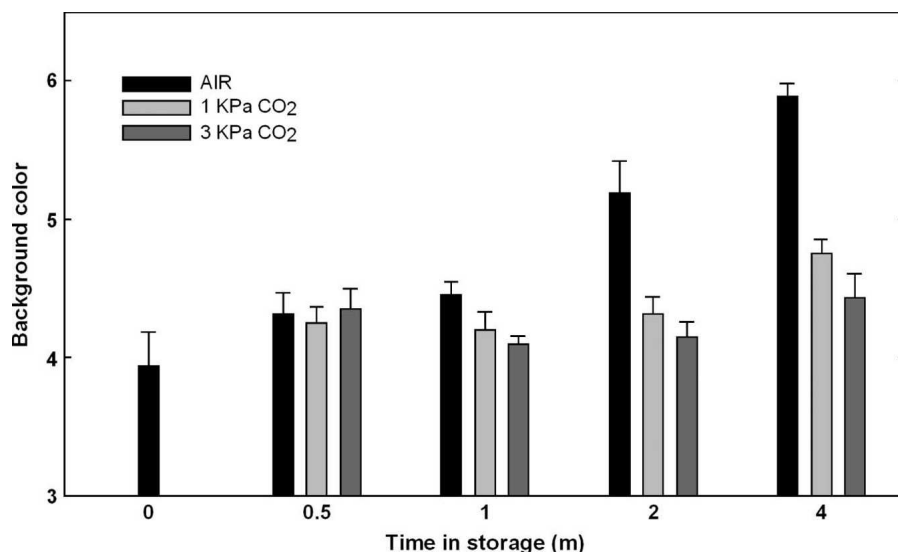


Fig. 8. Background color was measured after air or controlled atmosphere storage (2 KPa O₂ in combination with 1 or 3 KPa CO₂) at 0 °C for 0, 0.5, 2, or 4 months plus 5 d at 20 °C in 2003. Subjective color scale, 3 = green to 7 = yellow. Starch index score at harvest was 4.0.

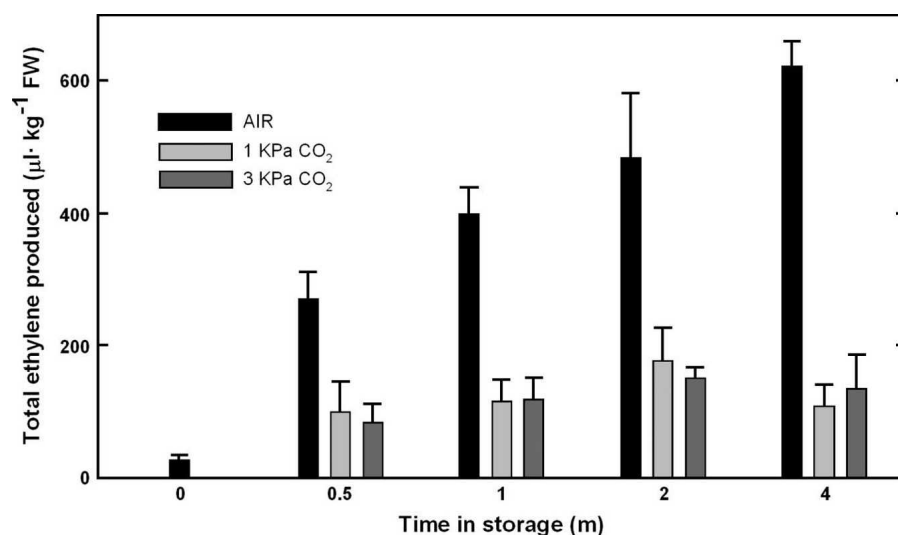


Fig. 9. Total ethylene produced during 10 d at 20 °C after air or controlled atmosphere storage (2 KPa O₂ in combination with 1 or 3 KPa CO₂) at 0 °C for 0, 0.5, 1, 2, or 4 months after harvest in 2003. Starch index score at harvest was 4.0. FW, fresh weight.

at early to intermediate maturity stages (starch index, 3.5–6.5). Fruit harvested late, at a starch index of ≈8.0, showed increased greasiness, even when stored in a CA (data not shown) and also lost ≈11% of their harvest firmness (Table 3), having an average penetration force of 64 N after 4 months.

In cooler climates, Pink Lady can attain brilliant pink color at the optimum maturity stage. However, central valley California growers may have to compromise the color of the apples or use color-enhancing treatments if they want to store the fruit for up to 4 months, or delay the harvest to increase red color and market the fruit within a shorter time frame.

Treatment with 1-MCP or storage in a CA with low CO₂ concentrations retarded Pink Lady ripening and senescence, and slowed detrimental changes after harvest. Brackmann et al. (2005), recommended an atmosphere

Table 3. Percent loss of firmness from initial values at harvest during 2 months or 4 months in air or controlled atmosphere (CA; 1.5–2 KPa O₂ with 1, 3 and 5 KPa CO₂) storage at 0.5 °C for three harvest dates in 2003 and 2004.

	Percent firmness (Newtons) loss					
	Harvest 1 ^a		Harvest 2 ^b		Harvest 3 ^c	
	2 mo.	4 mo.	2 mo.	4 mo.	2 mo.	4 mo.
Air	15 ^{**}	27	14	26	6	17
CA	5	7	2	5	2	11

^aStarch index, 3.0 to 4.0.

^bStarch index, 6.0 to 6.5.

^cStarch index, 8.0 to 8.5.

^{**}Data were pooled across atmospheres and years.

of 1.5 KPa O₂ with either 1 KPa or 2 KPa CO₂ at 0.5 °C for Pink Lady apple; however, our results show that CO₂ concentrations more than 1 KPa can cause CO₂-related disorders such as internal browning in California-grown

Pink Lady. High concentrations of CO₂ seem to stress the fruit, leading to higher ethylene production and resulting in softer fruit. To prevent quality loss and a high incidence of CO₂-induced flesh browning, CAs for Pink Lady apples should not exceed 1 KPa CO₂.

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