Internal Browning Disorder and Fruit Quality in Modified Atmosphere

Packaged ‘Bartlett’ Pears during Storage and Transit

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ABSTRACT

Internal browning (IB) can be a serious problem with the use of modified atmosphere packaging (MAP) for ‘Bartlett’ pears (Pyrus communis L.) grown in the Pacific Northwest during storage and transit to distant markets. To investigate this disorder, ‘Bartlett’ pears harvested at commercial maturity were packed in a commercial MAP (MAPc), an experimental MAP (MAPe) and commercial perforated plastic bags (control) and stored in air at -1.1 °C. After 1 and 3 months of storage, samples of MAPc and control fruit were transferred to rooms at temperatures of 2, 4.5, 7.5, and 10 °C for 3 weeks to simulate transit temperatures and the time required to reach distant markets. MAPc maintained an average internal atmosphere of 12.3% O₂ + 5.6% CO₂ and significantly extended ‘Bartlett’ pear storage life with high eating quality and without IB and other disorders for up to 4 months at -1.1 °C. The internal gas atmosphere of MAPe equilibrated at 2.2% O₂ + 5.7% CO₂, which resulted in fruit with 25.5 and 62.3% IB after 3 and 4 months of storage, respectively. During simulated transit conditions of 2, 4.5, 7.5, and 10 °C, the CO₂ level in MAPc was maintained at 5.6-7.9%, while O₂ was reduced dramatically to 10.5, 5.0, 2.5, and 1.0%, respectively. IB developed at 7.5 and 10 °C but not at 2 and 4.5 °C, regardless of pre-transit storage duration (1 and 3 months) at -1.1 °C. The longer the storage duration and the higher transit temperature, the higher the incidence and severity of IB. The MAP-related IB disorder observed in this study included two types of symptoms: classic pithy brown core and wet brown flesh. The MAPc storage gas atmospheres maintained fruit firmness, color and higher eating quality after ripening, eliminated senescent scald and core breakdown, suppressed the loss of ascorbic acid (AsA) and titratable acidity, and slowed the accumulation of malondialdehyde (MDA) during storage at -1.1 °C for up to 4 months or 3 months + 3 weeks at simulated transit temperatures of 2 and 4.5 °C. In contrast, fruit held in MAP with low O₂ levels (1.0-2.5 %)
developed IB that appeared to be associated with a reduction in AsA, accumulated MDA and exhibited an increase in membrane leakage. MAP inhibited ripening at high CO₂ + high O₂ but lead to IB when the packaging material or elevated temperatures resulted in high CO₂ + low O₂ conditions. The incidence of IB closely correlated with lipid peroxidation and appeared to be related to fruit AsA concentration. The MAPc designed for pears appears to be suitable for ‘Bartlett’ fruit stored at -1.1 °C for up to 4 months or storage for 3 months and a transportation duration of up to 3 weeks at 0-4.5 °C during the early season and at 0-2 °C during the late packing season. These conditions yielded fruit of high eating quality and without IB or over-ripening upon arrival at distant markets.

*Keywords:* ‘Bartlett’ pear, modified atmosphere packaging, high CO₂/O₂ injury, ascorbic acid, internal browning disorder, eating quality
1. Introduction

Modified atmosphere packaging (MAP) and controlled atmosphere (CA) storage are used to supplement low temperature management to delay ripening, reduce physiological disorders, and suppress decay in many fresh fruit and vegetable products (Kader et al., 1989; Smith et al., 1987). The benefits of MAP are derived primarily from the altered gas atmosphere surrounding the commodity that is created by the respiration of the product and the polymeric film’s resistance to O₂ and CO₂ diffusion. MAP also maintains a high relative humidity which reduces water loss, greatly improving the preservation of product quality and the potential storage duration (Mir and Beaudry, 2004). The steady-state O₂ and CO₂ concentrations within the package are a result of the interaction of a number of factors (e.g., permeability characteristics of the package, respiratory behavior of the plant material, conditions of the surrounding storage environment) (Beaudry, 1999; Mir and Beaudry, 2004). In spite of the benefits, MAP can induce undesirable effects such as fermentation and off-flavors if the O₂ concentration decreases to a point that will not sustain aerobic respiration (Kays, 1997). Similarly, injury can occur if the CO₂ concentration exceeds tolerable levels (Kader et al., 1989; Beaudry, 1999, 2000). As a consequence, film gas permeability must match the requirement of the commodity and the storage temperature to ensure creating the gas atmosphere and relative humidity needed to maintain quality and extend postharvest life without creating undesirable conditions (e.g., anaerobic condition, CO₂ damage, condensation within the package) (Lange, 2000).

Export plays a key role in keeping tree fruit production profitable in the Pacific Northwest (PNW). About one third of the pear (Pyrus communis L.) production is exported to foreign markets (Warner, 2012) and the major quality issues at distant markets are over-ripening, especially yellowing, and physiological disorders (industry communication). The pear industry
has experimented with MAP to ensure arrival quality of ‘Bartlett’ pears shipped to distant markets (i.e., within 3 months after harvest). However, MAP-related internal browning (IB) has presented a problem for buyers and receivers (industry communication). In the PNW, the majority of ‘Bartlett’ pears are normally held for 1-2 months in traditional refrigerated air (RA) or 3-4 months in controlled atmosphere (CA) storage (Drake et al., 2004; Kupferman, 2003; Richardson and Kupferman, 1997), although in some years a portion of the crop can be stored for 3 and 5 months in RA and CA, respectively (industry communication). As a consequence, there is considerable interest in the possible use of MAP for maintaining quality similar to CA storage without the extensive investment in infrastructure and instrumentation. However, there is insufficient information on optimum MAP conditions for pears (e.g., most efficient O₂ and CO₂ ranges, low O₂ and/or high CO₂ injury thresholds, effect of ethylene accumulation during MAP storage on fruit quality, safe storage life).

‘Bartlett’ pear fruit can be stored in MAP (1.6-9.0% O₂ + 2.9-6.5% CO₂) at 1 °C for 3 months with quality (firmness and color) equal to fruit stored in CA (Drake et al., 2004). However, Sugar (2001) reported that ‘Bartlett’ pears in MAP storage (1-2% O₂ + 5% CO₂) developed significant internal injury after 3-4 months at -0.5 °C. The IB disorder pithy brown core (PBC) has frequently been reported in pears (i.e., ‘d’Anjou’, ‘Bosc’, and ‘Bartlett’) with prolonged CA storage, especially when the CO₂ concentration exceeds 2% (Chen, 2004; Hansen and Mellenthin, 1962; Yoshida et al., 1986). The O₂ and CO₂ concentrations in MAP typically differ significantly from CA storage and the symptoms of MAP-related IB reported by the industry appear to differ from that of PBC caused by CA storage. While occurring in cold storage, the MAP-related IB may also develop during transportation due to altered atmospheres within MAP at elevated temperatures. The most efficient storage temperature for pears is -1.1 °C.
Porritt, 1964; Hansen and Mellenthin, 1979). Allen and Claypool (1948) reported that ‘Bartlett’ pears stored at 2.8 °C had higher respiration rates than those stored at 0 °C irrespective of the O₂ and CO₂ concentration in storage. Therefore, changes in temperature during shipment and distribution of MAP pears could also be a critical factor inducing IB.

Although MAP and CA can decrease oxidative stress through the retention of ascorbic acid (AsA) and other antioxidants in vegetables and fruit (Barth and Zhuang, 1996; Hodges and Forney, 2000; Yang, 1997; Zhuang et al., 1994), CA atmosphere (i.e., 2% O₂ + 5% CO₂) resulted in membrane breakdown due to lipid peroxidation and resulted in core browning of ‘Conference’ pears (Larrigaudiere et al., 2001a,b). IB of pears caused by CA is believed to be due more to oxidative damage than fermentation (Pinto et al., 2001; Franck et al., 2007).

The objectives of this research were to (1) evaluate MAP for preserving ‘Bartlett’ pear quality during storage and transit, (2) determine the effect of O₂ and CO₂ concentrations in MAP on MAP-related IB during cold storage; (3) identify temperatures and gas atmospheres that result in MAP-related IB during transit; and (4) determine the possible relationship of both the development of ripening and IB with AsA retention and membrane lipid peroxidation.

2. Materials and Methods

2.1. Fruit materials

Seventy-five ~20 kg boxes of commercially packed ‘Bartlett’ pears (90-100 fruit/box) were obtained from Duckwall-Pooley Packing Company (Hood River, Oregon) shortly after harvest. The initial flesh firmness (FF) was 85.9 N (N x 0.2246 = pounds), which met the recommended commercial harvest maturity. The fruit were washed and packed using standard industry procedures. The fruit pulp temperature was reduced to 4.5 °C prior to MAP and further
reduced to -1.1 °C using forced air cooling within 4-5 d after packing. The fruit were immediately transported to the Mid-Columbia Agricultural Research and Extension Center (MCAREC), Hood River, Oregon and stored at -1.1 °C until assessment. Two types of MAP bags were tested: commercially-available LifeSpan® L254 (MAPc) (Amcor, Victoria, Australia) and an experimental low density polyethylene (LDPE) bag (MAPe) (source withheld upon request of the manufacturer). Control fruit were packed in commercial perforated plastic bags. Box samples of MAPc (after 1 and 3 months) and control (after 1 month of storage) were randomly selected and transferred to 2, 4.5, 7.5, and 10 °C for 21 d to simulate possible temperature conditions during transit to distant markets.

2.2. Gas atmosphere determination

The concentrations of O₂ and CO₂ in MAPc, MAPe and perforated plastic bags were determined every other week during storage using an O₂ and CO₂ analyzer (Model 900151, Bridge Analyzers Inc., Alameda, California, USA). During simulated transit, O₂ and CO₂ concentrations were monitored every 2 d in MAPc and perforated plastic bags at the elevated temperatures (i.e., 2, 4.5, 7.5, and 10 °C) for 20 d. A silicon septum was glued to each MAP bag to prevent gas leakage at the sampling site.

2.3. Fruit quality evaluation

2.3.1 Cold storage study

After 3 and 4 months of storage at -1.1 °C, ten fruit were randomly selected from each box of MAPc, MAPe, and perforated plastic bags (control) for assessment of fruit skin color and flesh firmness (FF) after fruit pulp temperature was equilibrated to room temperature. Color was determined based on the CIE hue angle (h°) value using a white reference tile calibrated spectrophotometer (Model CR-2500d, Minolta, Tokyo, Japan). FF was determined by a texture
analyzer (Model GS-14, Guss Manufacturing Ltd., Strand, South Africa) with an 8-mm plunger that penetrated 9 mm in 0.9 s. Two measurements were obtained per fruit from opposite sides after 16 mm diameter peel discs were removed.

Fifty fruit in each box of MAPc and MAPe and controls were randomly selected and cut longitudinally and transversely to assess for IB, which was categorized as clear (C), very slight (VSL), slight (SL), moderate (MOD), and severe (SEV) (Fig. 1). Fruit with IB that were rated as SL, MOD and SEV were considered commercially unacceptable and combined to calculate the percent IB.

The remaining 40 fruit in each box were held at 20 ± 1 °C for determinations of sensory quality, total soluble solids (TSS), total acidity (TA), senescent scald (SS), and senescent core breakdown (SCB) at day 5. Sensory quality (texture and flavor) of the ripe fruit was evaluated organoleptically by an experienced two-three member panel using a nine-point hedonic scale with 9 = buttery and juicy texture with full flavor and 1 = coarse or mealy and dry texture with off flavor (Chen et al., 1996; McBride, 1986). Scale anchor points and definitions were determined in an orientation session prior to the first evaluation. An average score of 5 or higher was defined as commercially acceptable. Each assessor tasted one small fruit sector sliced from each of five fruit. The procedures for sensory evaluation of horticultural crops (Heintz and Kader, 1983) were adopted by the panelists. For TSS and TA determinations, juice from 0.1 kg of flesh tissue of 5 fruit was obtained using a juice extractor (Acme Model 6001, ACME Juicer Mfg. Co., Sierra Madre, California, USA) for 1 min. TSS was determined using a hand held refractometer (Atago, Tokyo, Japan) and expressed as a percent. TA was determined by titrating 10 mL of juice to pH 8.1 using 0.1 N NaOH with a commercial titration system (Model T80/20, Schott-Gerate, Hofheim, Germany) and expressed as meq L⁻¹ of juice. SS and SCB were
evaluated for 30 fruit and recorded as the percentage of total fruit (Ju et al., 2001). SS was defined as a dark discoloration on the fruit skin and SCB as a brown and watery discoloration in the core area (Meheriuk et al., 1994).

2.3.2. Simulated transit study

Following the simulated transit conditions, 10 fruit per box of each treatment were randomly selected for FF and color determinations as described above. Ten randomly selected fruit from each box that had been held at 2 and 4.5 °C for 21 d were held at 20 ± 1 °C for 5 d and then evaluated for sensory quality, TSS and TA as described above. The remaining fruit that had been held at 2, 4.5, 7.5 and 10 °C (80/box) were evaluated for IB, SS, and SCB. Fruit at 7.5 and 10 °C were not evaluated for eating quality due to the presence of physiological disorders.

2.4. Sample preparation for ascorbic acid (AsA), membrane peroxidation, and tissue leakage determinations

AsA, malondialdehyde (MDA), and tissue leakage were determined in the fruit at harvest and at each evaluation period before ripening. In treatments with IB, fruit were divided into two categories: undamaged and damaged (i.e., with IB). Five fruit of each category were selected for evaluation from treatments with IB present. Five undamaged pears were also selected in treatments without IB for biochemical analysis. The fruit were peeled using a hand-held peeler and cut length-wise. Four plugs were taken using a #4 cork borer from the cortex tissue just above the core of each half fruit. The tissue plugs of five pears were cross-cut into 2 mm sections using a razor blade and homogenized at 2 °C to give 3 replications per treatment.

2.5. AsA determination

L-ascorbic acid (AsA) concentration was measured based on the method of Cheng and Ma (2004) and Logan et al. (1998). Briefly, 5 g of cold fruit tissue were ground in 10 mL ice-
cold 6% (v/v) HClO₄. The extract was centrifuged at 10,000 g for 10 min at 2 °C and the supernatant used immediately for the measurement. A portion of the extract was neutralized with 1.5 M Na₂CO₃. Thirty to one hundred μL (corresponding to the concentrations of AsA in the samples) of the neutralized sample was used to assay the AsA. The AsA concentration was determined spectrophotometrically (Model Ultrospec 3100 pro, Biochrom Ltd, Cambridge, England) at 265 nm in 100 mM potassium phosphate buffer (pH 5.6), before and after 15 min incubation with 5 units of Cucurbita AsA oxidase (Sigma-Aldrich, St. Louis, MO, USA). The AsA concentration was determined from the absorbance difference and compared to a standard curve with the results expressed as mg kg⁻¹ FW.

2.6. Measurement of tissue leakage

Tissue leakage was determined using the method described by Lu and Toivonen (2000) and Redman et al. (1986). Sliced plugs (5 g each) were rinsed twice in distilled water, then placed in 30 mL of distilled water and exposed to a -25 kPa vacuum for 2 min to facilitate rapid infusion of the water into the tissue and enhance the effusion of solutes from the tissue. The sample was then shaken for 30 min. One mL of the solution was centrifuged at 10,000 g for 5 min and the absorbance of the supernatant measured at 280 nm using a spectrophotometer (Ultrospec 3100 pro, Biochrom Ltd., Cambridge, England). The samples were then frozen at -20 °C, thawed and a second absorbance measurement of the medium taken. The ratio of the first and second absorbance measurements was defined as the relative leakage ratio (RLR). This method measures leakage of ultraviolet light-absorbing compounds from the tissue, which are primarily free amino acids. Leakage of these compounds is considered to reflect relative cellular membrane leakage within the tissue (Redmann et al., 1986)

2.7. Membrane peroxidation determinations
Malondialdehyde concentration was determined using the method of Dhindsa et al. (1981). Two grams of cold fruit tissue was ground in 5 mL 10% (w/v) trichloroacetic acid (TCA). After centrifugation at 10,000 g for 15 min, a 2 mL aliquot of the supernatant was mixed with 2 mL 10% TCA containing 0.6% (w/v) thiobarbituric acid (TBA). The mixture was heated to 100 °C for 20 min, quickly cooled and centrifuged at 10,000 g for 10 min. The supernatant was collected and the absorbance at 450, 532, and 600 nm determined using a spectrophotometer (Ultrospec 3100 pro, Biochrom Ltd, Cambridge, England). The MDA concentration was calculated according to the formula: 6.45 × (A532–A600) − 0.56 × A450 and the results expressed as μmol kg⁻¹ FW.

2.8. Statistical analyses

Experimental units were boxes and there were three replications per treatment at each evaluation period. The experimental design was completely randomized and the data were subjected to analysis of variance (ANOVA) using StatSoft® Statistica version 6. When appropriate, means were separated by Fisher’s Protected LSD test at p ≤ 0.05.

3. Results

3.1. Cold storage study

3.1.1. Gas analysis

The concentrations of O₂ and CO₂ in MAPc reached equilibrium at 12.3% O₂ + 5.6% CO₂ by the second week in storage at -1.1 °C which was maintained throughout the 4 months of storage (Fig. 2A). MAPe maintained a similar CO₂ concentration as that of MAPc, but with a substantially lower O₂ concentration (2.2% O₂ + 5.7% CO₂) (Fig. 2B). There was no
accumulation of CO₂ or reduction of O₂ in the perforated plastic bags compared to the storage environment (Fig. 2C).

3.1.2. FF, color, and IB during cold storage

Control fruit held in commercially perforated plastic bags had significantly \( (p \leq 0.05) \) reduced FF and color \( (h^\circ) \) after 3 and 4 months of storage. The control fruit started to display visible yellowing after 3 months of storage. MAP slowed the rate of ripening significantly \( (p \leq 0.05) \) during storage as indicated by both FF and color \( (h^\circ) \) (Fig. 3A,B). Fruit in MAPc maintained 80.1 N FF and were green in color after 4 months at -1.1 °C.

Fruit in MAPc and perforated plastic bags had no IB disorder after 4 months in storage, while fruit in MAPe developed 25.5 and 62.3% IB after 3 and 4 months of storage, respectively (Fig. 3C). The primary difference between MAPc and MAPe bags was in the O₂ concentration (12.3 versus 2.2% O₂, respectively).

Two symptoms of IB were identified: the classic pithy brown core (PBC) (Fig. 1B) and wet brown flesh (WBF) (Fig. 1A). Most of the IB (91%) during storage was PBC, the symptoms of which are a brownish colored core and many sponge-like small pores. The injured core tissue was quite dry and typically restricted to the core although it occasionally spread to outside the core where it was often accompanied by dried lesions and cavities. In contrast, WBF affected the flesh tissue (cortex) turning it a dark brown color with the injured tissue having a water-soaked appearance. The flesh tissue at the stem end of fruit with IB was intact and uninjured.

3.1.3. Fruit quality and disorders after ripening

After 3 and 4 months in storage at -1.1 °C, MAPc, MAPe and control fruit were ripened at 20 ± 1 °C for 5 d to determine TA, TSS, and sensory quality. Due to significant IB, quality data of MAPe fruit after ripening is not presented. While TA declined gradually in both MAPc
and control fruit during storage, MAPc fruit displayed a significantly ($p \leq 0.05$) slower decrease in the rate of decline (Fig. 4A). In both MAPc and control the fruit increased in TSS a small but significant ($p \leq 0.05$) amount after 3 and 4 months of storage compared to their initial values. There was no difference in TSS content between MAPc and control fruit during storage (Fig. 4B). Panelists rated the flavor and texture of MAPc and control fruit as comparable after 3 months of storage, while after 4 months MAPc fruit had a higher eating quality than control fruit (both flavor and texture) (Fig. 4C,D).

Control fruit developed 3.3 and 38.3% SCB after 3 and 4 months of cold storage plus 5 d ripening, respectively (Fig. 4F). There was no SS after 3 months, but about half of the fruit with SCB displayed mild SS (i.e., 19.5%) after 4 months in storage (Fig. 4E).

3.1.4. AsA, cell membrane peroxidation, and tissue leakage

The pears contained AsA of 51 mg kg$^{-1}$ FW of fruit flesh at harvest and the peel content was significantly higher (68 mg kg$^{-1}$ FW). The AsA content of the flesh decreased significantly during storage (Fig. 5A). The concentration in the control fruit had declined to 28 mg kg$^{-1}$ FW after 3 months and 15 mg kg$^{-1}$ FW by 4 months of storage. MAPc significantly ($p \leq 0.05$) inhibited the decline in AsA concentration (41 and 30 mg kg$^{-1}$ FW after 3 and 4 months of storage, respectively). After 4 months of storage, fruit without IB in MAPe had significantly ($p \leq 0.05$) lower AsA than MAPc fruit and equal to control fruit. Fruit with IB in MAPe had an extremely low AsA content (5 and 4 mg kg$^{-1}$ FW after 3 and 4 months of storage, respectively) (Fig. 5A).

MDA, a secondary end product of polyunsaturated fatty acid oxidation, is widely used as an indicator of membrane lipid peroxidation and cell integrity when assessing senescence and environmental stresses (Hodges et al., 2004; Zhang et al., 2011). MDA concentration in control
fruit increased during storage (Fig. 5B). The change in MDA concentration in MAP fruit displayed a similar trend, although the increase in MAPc fruit was significantly ($p \leq 0.05$) less by the end of 3 and 4 months in storage. The MDA concentration in fruit without IB in MAPe was the same as in MAPc fruit after 3 months in storage. By 4 months, fruit in MAPe had a higher MDA concentration than MAPc fruit but the same concentration ($p \leq 0.05$) as control fruit. IB damaged fruit had a significantly higher MDA content than undamaged fruit held in MAPe after 3 and 4 months in storage (increases of 180 and 160%, respectively).

The relative leakage ratio (RLR) is an indicator of tissue and membrane integrity in fruit and other plant materials (Lu and Toivonen, 2000, Redmann et al., 1986). The increased RLR observed with storage time (Fig. 5C) is likely due to the increasing senescence of the fruit held in perforated plastic bags. Fruit in MAPc displayed lower RLR values than control fruit during storage. The RLR of fruit without damage in MAPe was not different than in MAPc fruit after 3 months in storage, but was higher ($p \leq 0.05$) after 4 months of storage. Fruit with IB had significantly higher RLR values ($p \leq 0.05$) than undamaged fruit in MAPe after 3 and 4 months of storage.

3.2. Simulated transit study

3.2.1. Gas analysis

The $O_2$ and $CO_2$ concentrations in MAPc were 12.5 and 5.6%, respectively, at the time when boxes were sampled after 1 month of storage at -1.1 °C. The $O_2$ concentration decreased dramatically while $CO_2$ increased mildly with elevated temperatures reflecting the differential permeability of the packaging materials for the two gases. At the holding temperatures of 2 and 4.5 °C, the $O_2$ concentration decreased to 10.5 and 5.0%, respectively, while the $CO_2$ concentration increased to 5.8 and 6.2%, respectively, within 48 h and maintained those levels.
through a period of 20 d. At 7.5 and 10 °C, the O₂ concentration was dramatically reduced to 2.5 and 1.0%, respectively, while CO₂ increased to 7.0 and 7.9%, respectively (Fig. 6). Storage duration (1 and 3 months) prior to subjecting the fruit to simulated transit treatments did not have a significant effect on O₂ and CO₂ concentrations within the MAPc at each of the elevated holding temperatures (Fig. 6). This was possibly due to a suppressed respiration rate of the fruit in the high CO₂/low O₂ environment during storage. ‘Bartlett’ pears in MAPc maintained a low, stable respiration rate at -1.1 °C throughout 4 months in storage (data not shown).

3.2.2. FF, color, and IB

During the 21 d simulated transit period, 7.5 °C was the minimum temperature that resulted in IB, regardless of the prior storage duration (1 or 3 months). While there was no IB after 21 d when held at 2 and 4.5 °C, IB did occur at the higher simulated transit temperatures (10 and 7.5 °C) and was greater the longer storage period (Fig. 7C). The IB index displayed the same trend as the incidence of IB (data not shown). At the higher simulated transit temperatures, after 21 d MAPc pears had developed 3.3% (7.5 °C), 6.3% (10 °C), 10.7% (7.5 °C) and 38.3% (10 °C) IB when the fruit was stored 1 versus 3 months. Most of the IB (85%) that developed at elevated temperatures was WBF (Fig. 1A). Control fruit held in perforated plastic bags for 1 month at -1.1 °C did not develop IB at any of the simulated transit temperatures.

Fruit held in MAPc maintained a FF of 79.2 N and 71.2 N, higher than the critical value (66.7 N) for susceptibility to impact and vibration damage (Thompson, 2007), when held at 2 °C during the 21 d simulated transit period for fruit that had been stored at 1 and 3 months, respectively. MAP fruit maintained a FF of 71.2 N at 4.5 °C during the 21 d simulated transit period after having been stored for 1 month, while after 3 months in storage FF was reduced to 60.1 N. At simulated transit temperatures of 7.5 and 10 °C for 21 d, fruit FF was reduced to a
firmness level at which the fruit would be at risk for mechanical damage, i.e., 59.6 N and 40.5 N respectively, for fruit that had been stored 1 month at -1.1 °C and 38.3 N and 28.9 N after 3 months of storage, respectively (Fig. 7A).

Elevated temperatures during simulated transit did not significantly ($p < 0.05$) affect the color ($h^o$ value) of fruit in MAPc from either storage duration. At each temperature, fruit color was significantly ($p < 0.05$) reduced with increasing storage duration (1 and 3 months) (Fig. 7B). Fruit held in MAPc for 1 month maintained a significantly higher FF and color than control fruit at each of the simulated transit temperatures.

3.2.3. Fruit quality and disorders after ripening

After 1 month storage at -1.1 °C followed by 21 d at 2 and 4.5 ºC, MAPc and control fruit were ripened at 20 ± 1 ºC for 5 d to determine TA, TSS, and sensory quality. No differences were found in TA, TSS, and flavor and texture sensory quality between MAPc and control fruit when held at a simulated transit temperature of 2 ºC, however, MAPc fruit had a higher TA and flavor and texture sensory scores than control fruit when held at 4.5 ºC. After 3 months of storage and 21 d at transit temperatures of 2 and 4.5 ºC, MAPc fruit had reduced TA and texture sensory scores compared to fruit stored for 1 month, although in each instance the flavor and texture scores (8.3 and 8.3 at 2 ºC and 7.8 and 7.5 at 4.5 ºC, respectively) were quite good (Fig. 8). SCB and SS were not found in the simulated transit treatments for control fruit after 1 month or for MAPc fruit that had been previously stored for 1 or 3 months at -1.1 ºC.

3.2.4. AsA, cell membrane peroxidation, and tissue leakage during simulated transit conditions after 1 month of storage

After 1 month of storage at -1.1 ºC, MAPc fruit had higher AsA concentrations after 3 weeks at simulated transit temperatures of 2 and 4.5 ºC, but they were lower at 7.5 and 10 ºC.
compared to control fruit. The AsA levels were 45, 46, 20, and 11 mg kg\(^{-1}\) FW for fruit held in MAPc and 41, 34, 33, and 23 mg kg\(^{-1}\) FW for control fruit at 2, 4.5, 7.5, and 10 °C, respectively. Fruit with IB had extremely low AsA concentrations of 6 and 7 mg kg\(^{-1}\) FW for MAP fruit after 3 weeks at 7.5 and 10 °C, respectively (Fig. 9A).

There were no significant differences \((p \leq 0.05)\) in MDA concentration and RLR values between MAP and control fruit after 3 weeks at simulated transit temperatures of 2 and 4.5 °C for fruit that had been stored for 1 month at -1.1 °C. MAP fruit without IB displayed an increase in MDA concentration and RLR values at 7.5 and 10 °C, compared to control fruit. Fruit with IB had significantly \((p \leq 0.05)\) higher MDA concentrations and RLR values compared to undamaged fruit (Fig. 9B,C).

4. Discussion

4.1. Effect of MAP on fruit quality and IB development during cold storage

This study demonstrated that MAP is an excellent addition to cold temperature management for extending the storage and shipping life of ‘Bartlett’ pears produced in the PNW. The atmosphere generated by MAPc (12.3% \(O_2\) + 5.6% \(CO_2\)) extended the storage life, minimized the loss of fruit firmness and color, and provided fruit that were free of IB, SCB, and SS for up to 4 months at -1.1 °C. Fruit held in MAPc for either 3 or 4 months had higher eating quality after ripening than control fruit.

High CO\(_2\) (i.e., 5.6%) appears to play a major role in maintaining fruit quality based on the fact that the reduction in \(O_2\) concentration from 12.3 (MAPc) to 2.2% (MAPe) at 5-6% CO\(_2\) did not improve storage life or quality. The reduction in \(O_2\) concentration from 21 to 4-5% did not significantly reduce the respiration or ethylene synthesis rates in either unripe or ripened
‘Bartlett’ pears (unpublished data). Kerbel et al. (1988) found that ‘Bartlett’ pears held in elevated CO₂ concentrations had reduced respiratory and ethylene synthesis rates, and maintained firmer, greener fruit compared to those stored in air. The physiological effect of CO₂ on respiration has been related to an inhibition of the Krebs cycle (Ke et al., 1994) and glycolytic pathway (Kerbel et al., 1988, 1990). High CO₂ is also thought to inhibit the ethylene biosynthetic pathway in pears (Yashida et al., 1986) at the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene, a reaction catalyzed by 1-aminocyclopropane-1-carboxylic acid oxidase (de Wild et al., 1999, 2003). High CO₂ is also a competitive inhibitor of ethylene action (Burg and Burg, 1967). De Wild et al. (1999, 2003) suggested that CO₂ might antagonize the ethylene receptor in pear fruit. Elevated CO₂ can also affect other metabolic pathways involved in secondary metabolism, i.e., pigments, phenolics, volatiles (Beaudry, 1999; Kader, 1989; Watkins, 2000).

Modified aerobic atmospheres are believed to extend the storage life of fruit and vegetables in part through reduced oxidative stress, while a number of postharvest procedures can lead to increased oxidative stress and/or injury (Hodges et al., 2004; Toivonen, 2004). In broccoli, MAP (11.2% O₂ + 7.5% CO₂) retarded senescence and decreased oxidative stress through the retention of AsA and other antioxidants (Zhuang et al., 1994; Barth and Zhuang, 1996). Yang (1997) reported that low O₂ (<3%) reduced AsA loss, membrane leakage and the accumulation of MDA in ‘Niitaka’ pears, and 3% CO₂ + 1.2% O₂ was more effective in maintaining fruit firmness than 1.1% CO₂ + 1.2% O₂. Storage of ‘Pink Lady’ apples at 1.5% O₂ + 3-5% CO₂, in contrast with air storage, maintained significantly higher AsA concentrations in fruit without FB (flesh browning) after 4 months of storage (de Castro et al., 2008). In the present study, ‘Bartlett’ pears stored in MAPc at 12.3% O₂ + 5.6% CO₂ displayed a reduced loss
of AsA, a reduced accumulation of MDA, a reduced increase of membrane leakage, and extended storage life.

While the atmosphere created by MAPc was safe for PNW ‘Bartlett’ pears with regard to IB development, an atmosphere with the same concentration of CO2 but a reduced O2 (i.e., 2.2% O2 + 5.7% CO2) created by MAPe induced significant IB development during cold storage. In a similar MAP atmosphere, ‘Bartlett’ fruit grown in southern Oregon developed 23 and 38% IB after 3 and 4 months in storage at -0.5 °C (Sugar, 2001). In California, 1.5-2% O2 + 1-5% CO2 are recommended CA conditions for long-term storage of early- and mid-season ‘Bartlett’ pears (Mitcham et al., 2012). Kader (2007) indicated that ‘Bartlett’ pears grown in regions of the US other than California do not tolerate CO2 levels above 1% regardless of the harvest date. The PNW pear industry utilizes 2% O2 + <1% CO2 for ‘Bartlett’ pears in CA storage (Chen, 2004; Richardson and Kupferman, 1997). Results of this study demonstrate that the tolerance of PNW ‘Bartlett’ pears to high CO2 is influenced by the O2 concentration, with low O2 enhancing CO2-induced injury. Two types of MAP-related IB for ‘Bartlett’ pears have been reported by the industry (PBC and WBF) and each was found in this study. In CA storage, PBC in ‘d’Anjou’, ‘Bosc’, and ‘Bartlett’ pears is caused by high CO2 and aggravated by low O2 (Chen, 2004; Chen et al., 1986; Hansen and Mellenthin, 1962; Yoshida et al., 1986). In contrast, Ong (1987) reported that 1.5% O2 depressed flesh browning induced by 20% CO2 in ‘Bartlett’ pears grown in California. Yang (1997) reported that high O2 levels increased a disorder in Asian pears known as “skin blackening”. The disorder did not develop in CA combinations of CO2 (1.2 and 3%) and O2 (1.2 and 3%). However, 20% O2 + 3% CO2 caused “skin blackening” of ‘Niitaka’ pears. While the reasons for the contradictory results are not currently understood, growing location and cultivar may be critical factors affecting the response of pears to high CO2/O2 injury.
4.2. IB development and quality of MAP fruit during transit

The increase in respiratory rate between 0 and 10 °C is 2 to 3 times the rate of gas permeability of low density polyethylene MAP films (Mir and Beaudry, 2004). While pears stored in MAP are generally held at -1.1 °C (Porritt, 1964; Hansen and Mellenthin, 1979), this study has demonstrated that increased temperatures during shipment and distribution can be a critical factor in the induction of IB due to the dramatic alteration in the gas atmosphere within the MAP. At the simulated transit temperatures tested, the atmosphere in MAPc was maintained at 5.0-10.5% O₂ + 5.8-6.2% CO₂ at 2 and 4.5 °C and the fruit in these environments were free from IB during the 3 week simulated transit period. However, CO₂ accumulated to 7.0 and 7.9% and O₂ was reduced to 2.5 and 1.0% at 7.5 and 10 °C, respectively. Control fruit packed in commercially perforated plastic bags did not display an accumulation of CO₂ nor reduced O₂ at any of the simulated transit conditions and did not develop IB. Fruit held in MAPc maintained a significantly higher fruit firmness and better color than the control fruit at each of the simulated transit temperatures after both 1 and 3 months in storage. This indicated that MAPc can maintain ‘Bartlett’ pear quality at slightly elevated temperatures (e.g., 2-4.4 °C) during transit. However, higher post-storage temperatures (e.g., 7.5 and 10 °C) caused reduced O₂ (i.e., <2.5%) and elevated CO₂ concentrations in MAPc that resulted in significant increase in the incidence of IB. ‘Bartlett’ pears are extremely susceptible to friction discoloration (FD) due to mechanical failure in response to friction (scuffing) and bruising throughout the supply chain (harvest, packinghouse operations, transit, wholesale and retail marketing). According to Thompson (2007), ‘Bartlett’ pears with a FF of 66.7 N would likely suffer vibration and/or impact bruising damage during transportation. MAPc packed ‘Bartlett’ fruit maintained FF higher than 66.7 N at 2 °C for 21 d regardless of storage durations up to 3 months at -1.1 °C. While MAP packed fruit
maintained FF of 71.2 N at 4.5 °C for 21d in the early storage season (1 month storage), this was reduced to 60.1 N after 3 months of storage. Therefore, MAPc packed ‘Bartlett’ pears can be shipped at 0-4.5 °C in the early storage season, but the post-storage temperature should be 0-2 °C during the later season to avoid softening. At 7.5 °C and 10 °C for 21 d, fruit FF was reduced to lower than 66.7 N regardless of storage duration (Fig. 7). The simulated transit temperatures did not significantly affect fruit green color significantly under the MAP conditions.

4.3. Mechanisms of MAP-related IB

The mechanism of IB in pears is not sufficiently understood. Fruit tissue browning involves the enzymatic oxidation of phenolic compounds by polyphenoloxidase (PPO) to o-quinones, which form brown colored polymers (Mathew and Parpia, 1971; Mayer, 1987). Although susceptibility to tissue browning among pear cultivars and production factors have been related to the concentration of certain phenolic compounds (Hamauzu and Hanakawa, 2003; Franck, 2007), neither PPO activity nor the concentrations of polyphenol compounds appear to be limiting factors in the process of browning development in pears (Larrigaudiere et al., 1998; Veltman et al., 1999b). Since PPO and its substrate phenolic compounds are located in different cellular compartments (cytoplasm/plastids and vacuole, respectively) (Nicolas et al., 1994; Dixon and Paiva, 1995), enzymatic browning only occurs after cellular decompartmentalization caused by membrane disintegration. Cell membrane damage is thought to occur due to an imbalance between oxidative and reductive processes, when degradative processes exceed those of maintenance (Shewfelt and del Rosario, 2000).

Several lines of evidence indicate that excessive CO₂ plus low O₂ increases oxidative and reduces reductive processes in pear fruit. Elevated CO₂, especially with low O₂, results in an accumulation of alcohol and acetaldehyde in ‘Bartlett’ pears (Ke et al, 1990, 1994) and active
oxygen species (AOS) such as $O_2^-$ and $H_2O_2$ in ‘Conference’ pears (Larrigaudiere et al., 2001a). These compounds are either toxic to fruit tissues or cause lipid peroxidation and protein denaturation (Halliwell and Gutteridge, 1989) and as a consequence cause membrane disintegration and cellular decompartmentalization (Veltman et al., 1999a). Plants have several enzymatic (e.g., superoxide dismutase, catalase, peroxidase) and non-enzymatic antioxidant defense mechanisms that counteract membrane oxidative damage caused by AOS (Larrigaudiere et al., 2001b). Among the antioxidant systems, AsA appears to play a critical role in the occurrence of browning disorder of ‘Conference’ pears (Eccher Zerbini et al., 2002; Franck et al., 2003a,b). It has been suggested that AsA protects against browning and that browning in pears is initiated when AsA drops below a threshold value (Eccher Zerbini et al., 2002; Veltman et al., 1999a). This study demonstrated that MAP-related IB in ‘Bartlett’ fruit appears to occur through the loss of membrane integrity as indicated by increased MDA concentration and RLR values in fruit with IB damage. AsA content dropped dramatically in IB-damaged fruit stored at high CO$_2$ and low O$_2$. Fruit without IB-damaged when stored at high CO$_2$ + low O$_2$ also displayed lower AsA concentrations compared to those stored in a safe atmosphere (e.g., high CO$_2$ + high O$_2$). What predisposes some fruit in the same atmosphere (i.e., 5.7% CO$_2$ + 2.2% O$_2$), to maintain a higher concentration of AsA and resistance to IB than other fruit from the same box warrants further study.

The precise mechanism by which high CO$_2$/O$_2$ modulates AsA metabolism is not known. While the AsA biosynthetic pathway in plants has yet to be fully elucidated, two distinct pathways have been proposed (Noctor and Foyer, 1998). The decrease in AsA in pears caused by high CO$_2$/O$_2$ may result from an inhibition of its biosynthesis. High CO$_2$/O$_2$ may additionally or
505 alternatively stimulate the oxidation of AsA, possibly via ascorbate peroxidase (Mehlhom,
506 1990).

507 The ‘Bartlett’ pears used in this study were harvested at the commercial maturity
508 standard. Susceptibility of pear fruit to CO2 injury has been reported to be closely associated
509 with factors that tend to advance maturity or senescence of fruit grown in Oregon (Hansen and
510 Mellenthin, 1962) and in California (Claypool, 1973). This study demonstrates that ‘Bartlett’
511 pears stored in MAP for 3 months are more susceptible to high CO2/O2 injury caused by elevated
512 post-storage temperatures than fruit stored for 1 month. Climatic conditions, such as a cool
513 production season, predispose the fruits to a greater susceptibility to CO2 injury. Hansen and
514 Mellenthin (1962) reported that pears from Hood River tended to be more susceptible than the
515 same cultivar from the Medford area in Oregon, where prevailing temperatures during the
516 growing season tend to be higher. Fruit from trees with low vigor are also more susceptible to
517 CO2 injury (Hansen and Mellenthin, 1962). Additional information on pre-harvest factors
518 affecting the susceptibility of ‘Bartlett’ pears to high CO2/O2 injury during storage in MAP is
519 needed.

520 5. Conclusions

522 The goal of MAP for fresh produce is to create an equilibrium package atmosphere with
523 O2 low enough and CO2 high enough to be beneficial to the produce and not injurious. This is
524 accomplished through the proper balance of several variables that affect the package atmosphere,
525 (e.g., gas permeability of the film, respiration rate of the commodity, commodity weight, film
526 thickness, film surface area). Identifying safe concentrations of O2 and CO2 for commodities is
527 critical for the successful commercial application of MAP. The optimum CA conditions for long-
term storage of ‘Bartlett’ pear grown in the PNW are believed to be approximately 1-2% O₂ and
0-0.5% CO₂ (Chen, 2004; Richardson and Kupferman, 1997). The current study has
demonstrated that the susceptibility of ‘Bartlett’ pears to CO₂ injury is highly dependent upon the
O₂ concentration and storage duration. Optimal CO₂ and O₂ concentrations differed between
MAP and recommended CA storage conditions. MAP developed an atmosphere of ~5.5% CO₂
with a relatively high O₂ concentration and appears to be excellent addition to low temperature
management for extending the storage life and potential transportation distances for ‘Bartlett’
pears. Good temperature management is the key to avoiding CO₂ injury during transit. The
MAPc for pears created an appropriate O₂ and CO₂ atmosphere within the bags for maximizing
keeping quality without CO₂ injury and over-ripening at transit temperature of 0-4.5 °C in early
season and 0-2 °C in late season pears. When properly used, MAP has the potential to
substantially extend the ‘Bartlett’ marketing season, expand long-distance exports into new
markets, and increase the total volume of PNW fruit that can be sold each year.

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References

Allen, F.W., Claypool, L.L., 1948. Modified atmospheres in relation to the storage life of Bartlett

Barth, M.M., Zhuang, H., 1996. Packaging design affects antioxidant vitamin retention and

Beaudry, R.M., 1999. Effect of O₂ and CO₂ partial pressure on selected phenomena affecting

Beaudry, R.M., 2000. Responses of horticultural commodities to low oxygen: limits to the

Plant Physiol. 42:144-152.

Storage of Fruits, Vegetables, and Florist and Nursery Crops. USDA, ARS, Agriculture

Chen, P.M., Borgic, D.M., Sugar, D., Mellenthin, W.M., 1986. Influence of fruit maturity and
growing district on brown-core disorder in ‘Bosc’ pears. J. Amer. Soc. Hort. Sci. 21:1172-
1173.


Cheng, L., Ma, F., 2004. Diurnal operation of the xanthophyll cycle and the antioxidant system


with a CO2-induced fresh browning disorder of Pink Lady apples. Postharvest Biol. Technol.

ethylene production and respiration of pear fruit by different mechanisms. J. Exp. Bot.


Fig. 1. Grading scales for two types of MAP-related internal browning disorders: wet brown flesh (WBF) (A) and pithy brown core (PBC) (B).
Fig. 2. O\textsubscript{2} and CO\textsubscript{2} concentrations in a commercial modified atmosphere packaging (MAPc) (A), an experimental modified atmosphere packaging (MAPe) (B), and commercial perforated plastic bags (C) containing ‘Bartlett’ pears stored at -1.1 °C. Vertical bars represent standard deviations (5% level).
Fig. 3. Fruit flesh firmness (FF) (A), color (h°) (B), and internal browning (IB) (C) of ‘Bartlett’ pears in a commercial modified atmosphere packaging (MAPc), an experimental modified atmosphere packaging (MAPe), and commercial perforated plastic bags during storage at -1.1 °C. Vertical bars represent standard deviations (5% level). Different letters indicate significant differences (p ≤ 0.05) according to Fisher’s protected LSD test.
Fig. 4. Titratable acidity (TA) (A), total soluble solids (TSS) (B), sensory quality (C&D), senescent scald (SS) (E), and senescent core breakdown (SCB) (F) of ripened (5 d at 20 ± 1 °C) ‘Bartlett’ pears stored in a commercial modified atmosphere packaging (MAPc) and commercial perforated plastic bags after 3 and 4 months at -1.1 °C. Vertical bars represent standard deviations (5% level).
Ascorbic acid (AsA) (A), malondialdehyde (MDA) (B), and relative leakage ratio (RLR) (C) of ‘Bartlett’ pears stored in a commercial modified atmosphere packaging (MAPc), an experimental modified atmosphere packaging (MAPe) (with and without internal browning discoloration), and commercial perforated plastic bags at -1.1 °C. Vertical bars represent standard deviations (5% level). Different letters indicate significant differences ($p < 0.05$) according to Fisher’s protected LSD test.
Fig. 6. O₂ and CO₂ concentrations in a commercial modified atmosphere packaging (MAPc) containing ‘Bartlett’ pears at simulated transit temperatures for 3 weeks after 1 and 3 months of storage at -1.1 °C. Vertical bars represent standard deviations (5% level).
Fig. 7. Fruit flesh firmness (FF) (A), color ($h^\circ$) (B), and internal browning (IB) (C) of ‘Bartlett’ pears stored in a commercial modified atmosphere packaging (MAPc) and commercial perforated plastic bags at simulated transit temperatures at 2, 4.5, 7.5, and 10 °C for 3 weeks after 1 and 3 months of storage at -1.1 °C. Vertical bars represent standard deviations (5% level).
Fig. 8. Titratable acidity (TA) (A), total soluble solid (TSS) (B), and sensory quality (C&D) of ripened (5 d at 20 ± 1 °C) ‘Bartlett’ pears stored in a commercial modified atmosphere packaging (MAPc) and commercial perforated plastic bags at simulated transit temperatures of 2 and 4.5 °C for 3 weeks after 1 and 3 months of storage at -1.1 °C. Vertical bars represent standard deviations (5% level).
Fig. 9. Ascorbic acid (AsA) (A), malondialdehyde (MDA) (B), and relative leakage ratio RLR (C) of ‘Bartlett’ pears stored in a commercial modified atmosphere packaging (MAPc) and commercial perforated plastic bags at simulated transit temperatures of 2, 4.5, 7.5, and 10 °C for 3 weeks after 1 month of storage at -1.1 °C. Vertical bars represent standard deviations (5% level).