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% O_2 + 5.6% CO_2 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% O_2 + 5.7% CO_2, 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 CO_2 level in MAPc was maintained at 5.6-7.9%, while O_2 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 O_2 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 CO_2 + high O_2 but lead to IB when the packaging material or elevated temperatures resulted in high CO_2 + low O_2 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 CO_2/O_2 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 O_2 and CO_2 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 O_2 and CO_2 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 O_2 concentration decreases to a point that will not sustain aerobic respiration (Kays, 1997). Similarly, injury can occur if the CO_2 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, CO_2 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

93 has experimented with MAP to ensure arrival quality of 'Bartlett' pears shipped to distant
94 markets (i.e., within 3 months after harvest). However, MAP-related internal browning (IB) has
95 presented a problem for buyers and receivers (industry communication). In the PNW, the
96 majority of 'Bartlett' pears are normally held for 1-2 months in traditional refrigerated air (RA)
97 or 3-4 months in controlled atmosphere (CA) storage (Drake et al., 2004; Kupferman, 2003;
98 Richardson and Kupferman, 1997), although in some years a portion of the crop can be stored for
99 3 and 5 months in RA and CA, respectively (industry communication). As a consequence, there
100 is considerable interest in the possible use of MAP for maintaining quality similar to CA storage
101 without the extensive investment in infrastructure and instrumentation. However, there is
102 insufficient information on optimum MAP conditions for pears (e.g., most efficient O_2 and CO_2
103 ranges, low O_2 and/or high CO_2 injury thresholds, effect of ethylene accumulation during MAP
104 storage on fruit quality, safe storage life).
105 'Bartlett' pear fruit can be stored in MAP (1.6-9.0% O_2 + 2.9-6.5% CO_2) at 1 °C for 3
106 months with quality (firmness and color) equal to fruit stored in CA (Drake et al., 2004).
107 However, Sugar (2001) reported that 'Bartlett' pears in MAP storage (1-2% O_2 + 5% CO_2)
108 developed significant internal injury after 3-4 months at -0.5 °C. The IB disorder pithy brown
109 core (PBC) has frequently been reported in pears (i.e., 'd'Anjou', 'Bosc', and 'Bartlett') with
110 prolonged CA storage, especially when the CO_2 concentration exceeds 2% (Chen, 2004; Hansen
111 and Mellenthin, 1962; Yoshida et al., 1986). The O_2 and CO_2 concentrations in MAP typically
112 differ significantly from CA storage and the symptoms of MAP-related IB reported by the
113 industry appear to differ from that of PBC caused by CA storage. While occurring in cold
114 storage, the MAP-related IB may also develop during transportation due to altered atmospheres
115 within MAP at elevated temperatures. The most efficient storage temperature for pears is -1.1 °C

116 (Porritt, 1964; Hansen and Mellenthin, 1979). Allen and Claypool (1948) reported that 'Bartlett'
117 pears stored at 2.8 °C had higher respiration rates than those stored at 0 °C irrespective of the O_2
118 and CO_2 concentration in storage. Therefore, changes in temperature during shipment and
119 distribution of MAP pears could also be a critical factor inducing IB.
120 Although MAP and CA can decrease oxidative stress through the retention of ascorbic
121 acid (AsA) and other antioxidants in vegetables and fruit (Barth and Zhuang, 1996; Hodges and
122 Forney, 2000; Yang, 1997; Zhuang et al., 1994), CA atmosphere (i.e., 2% O_2 + 5% CO_2) resulted
123 in membrane breakdown due to lipid peroxidation and resulted in core browning of 'Conference'
124 pears (Larrigaudiere et al., 2001a,b). IB of pears caused by CA is believed to be due more to
125 oxidative damage than fermentation (Pinto et al., 2001; Franck et al., 2007).
126 The objectives of this research were to (1) evaluate MAP for preserving 'Bartlett' pear
127 quality during storage and transit, (2) determine the effect of O_2 and CO_2 concentrations in MAP
128 on MAP-related IB during cold storage; (3) identify temperatures and gas atmospheres that result
129 in MAP-related IB during transit; and (4) determine the possible relationship of both the
130 development of ripening and IB with AsA retention and membrane lipid peroxidation.
131
132 **2. Materials and Methods**
133 *2.1. Fruit materials*
134 Seventy-five ~20 kg boxes of commercially packed 'Bartlett' pears (90-100 fruit/box)
135 were obtained from Duckwall-Pooley Packing Company (Hood River, Oregon) shortly after
136 harvest. The initial flesh firmness (FF) was 85.9 N (N × 0.2246 = pounds), which met the
137 recommended commercial harvest maturity. The fruit were washed and packed using standard
138 industry procedures. The fruit pulp temperature was reduced to 4.5 °C prior to MAP and further

139 reduced to -1.1 °C using forced air cooling within 4-5 d after packing. The fruit were
140 immediately transported to the Mid-Columbia Agricultural Research and Extension Center
141 (MCAREC), Hood River, Oregon and stored at -1.1 °C until assessment. Two types of MAP
142 bags were tested: commercially-available LifeSpan® L254 (MAPc) (Amcor, Victoria, Australia)
143 and an experimental low density polyethylene (LDPE) bag (MAPe) (source withheld upon
144 request of the manufacturer). Control fruit were packed in commercial perforated plastic bags.
145 Box samples of MAPc (after 1 and 3 months) and control (after 1 month of storage) were
146 randomly selected and transferred to 2, 4.5, 7.5, and 10 °C for 21 d to simulate possible
147 temperature conditions during transit to distant markets.

148 *2.2. Gas atmosphere determination*

149 The concentrations of O_2 and CO_2 in MAPc, MAPe and perforated plastic bags were
150 determined every other week during storage using an O_2 and CO_2 analyzer (Model 900151,
151 Bridge Analyzers Inc., Alameda, California, USA). During simulated transit, O_2 and CO_2
152 concentrations were monitored every 2 d in MAPc and perforated plastic bags at the elevated
153 temperatures (i.e., 2, 4.5, 7.5, and 10 °C) for 20 d. A silicon septum was glued to each MAP bag
154 to prevent gas leakage at the sampling site.

155 *2.3. Fruit quality evaluation*

156 *2.3.1 Cold storage study*

157 After 3 and 4 months of storage at -1.1 °C, ten fruit were randomly selected from each
158 box of MAPc, MAPe, and perforated plastic bags (control) for assessment of fruit skin color and
159 flesh firmness (FF) after fruit pulp temperature was equilibrated to room temperature. Color was
160 determined based on the CIE hue angle ($h°$) value using a white reference tile calibrated
161 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

185 evaluated for 30 fruit and recorded as the percentage of total fruit (Ju et al., 2001). SS was
186 defined as a dark discoloration on the fruit skin and SCB as a brown and watery discoloration in
187 the core area (Meheriuk et al., 1994).

188 *2.3.2. Simulated transit study*

189 Following the simulated transit conditions, 10 fruit per box of each treatment were
190 randomly selected for FF and color determinations as described above. Ten randomly selected
191 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
192 then evaluated for sensory quality, TSS and TA as described above. The remaining fruit that had
193 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
194 10 °C were not evaluated for eating quality due to the presence of physiological disorders.

195 *2.4. Sample preparation for ascorbic acid (AsA), membrane peroxidation, and tissue leakage*
196 *determinations*

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

205 *2.5. AsA determination*

206 L-ascorbic acid (AsA) concentration was measured based on the method of Cheng and
207 Ma (2004) and Logan et al. (1998). Briefly, 5 g of cold fruit tissue were ground in 10 mL ice-

208 cold 6% (v/v) $HClO_4$. The extract was centrifuged at 10,000 g for 10 min at 2 °C and the
209 supernatant used immediately for the measurement. A portion of the extract was neutralized with
210 1.5 M Na_2CO_3. Thirty to one hundred µL (corresponding to the concentrations of AsA in the
211 samples) of the neutralized sample was used to assay the AsA. The AsA concentration was
212 determined spectrophotometrically (Model Ultrospec 3100 pro, Biochrom Ltd, Cambridge,
213 England) at 265 nm in 100 mM potassium phosphate buffer (pH 5.6), before and after 15 min
214 incubation with 5 units of *Cucurbita* AsA oxidase (Sigma-Aldrich, St. Louis, MO, USA). The
215 AsA concentration was determined from the absorbance difference and compared to a standard
216 curve with the results expressed as mg kg^{-1} FW.

217 *2.6. Measurement of tissue leakage*

218 Tissue leakage was determined using the method described by Lu and Toivonen (2000)
219 and Redman et al. (1986). Sliced plugs (5 g each) were rinsed twice in distilled water, then
220 placed in 30 mL of distilled water and exposed to a -25 kPa vacuum for 2 min to facilitate rapid
221 infusion of the water into the tissue and enhance the effusion of solutes from the tissue. The
222 sample was then shaken for 30 min. One mL of the solution was centrifuged at 10,000 g for 5
223 min and the absorbance of the supernatant measured at 280 nm using a spectrophotometer
224 (Ultrospec 3100 pro, Biochrom Ltd., Cambridge, England). The samples were then frozen at -20
225 °C, thawed and a second absorbance measurement of the medium taken. The ratio of the first and
226 second absorbance measurements was defined as the relative leakage ratio (RLR). This method
227 measures leakage of ultraviolet light-absorbing compounds from the tissue, which are primarily
228 free amino acids. Leakage of these compounds is considered to reflect relative cellular
229 membrane leakage within the tissue (Redmann et al., 1986)

230 *2.7. Membrane peroxidation determinations*

231 Malondialdehyde concentration was determined using the method of Dhindsa et al.
232 (1981). Two grams of cold fruit tissue was ground in 5 mL 10% (w/v) trichloroacetic acid (TCA).
233 After centrifugation at 10,000 g for 15 min, a 2 mL aliquot of the supernatant was mixed with 2
234 mL 10% TCA containing 0.6% (w/v) thiobarbituric acid (TBA). The mixture was heated to 100
235 °C for 20 min, quickly cooled and centrifuged at 10,000 g for 10 min. The supernatant was
236 collected and the absorbance at 450, 532, and 600 nm determined using a spectrophotometer
237 (Ultrospec 3100 pro, Biochrom Ltd, Cambridge, England). The MDA concentration was
238 calculated according to the formula: $6.45 \times (A_{532}–A_{600}) – 0.56 \times A_{450}$ and the results expressed as
239 μmol kg^{-1} FW.

240 *2.8. Statistical analyses*

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

245

246 **3. Results**

247 *3.1. Cold storage study*

248 *3.1.1. Gas analysis*

249 The concentrations of O_2 and CO_2 in MAPc reached equilibrium at 12.3% O_2 + 5.6%
250 CO_2 by the second week in storage at -1.1 °C which was maintained throughout the 4 months of
251 storage (Fig. 2A). MAPe maintained a similar CO_2 concentration as that of MAPc, but with a
252 substantially lower O_2 concentration (2.2% O_2 + 5.7% CO_2) (Fig. 2B). There was no

253 accumulation of CO_2 or reduction of O_2 in the perforated plastic bags compared to the storage
254 environment (Fig. 2C).
255 *3.1.2. FF, color, and IB during cold storage*
256 Control fruit held in commercially perforated plastic bags had significantly ($p \leq 0.05$)
257 reduced FF and color ($h°$) after 3 and 4 months of storage. The control fruit started to display
258 visible yellowing after 3 months of storage. MAP slowed the rate of ripening significantly ($p \leq$
259 0.05) during storage as indicated by both FF and color ($h°$) (Fig. 3A,B). Fruit in MAPc
260 maintained 80.1 N FF and were green in color after 4 months at -1.1 °C.
261 Fruit in MAPc and perforated plastic bags had no IB disorder after 4 months in storage,
262 while fruit in MAPe developed 25.5 and 62.3% IB after 3 and 4 months of storage, respectively
263 (Fig. 3C). The primary difference between MAPc and MAPe bags was in the O_2 concentration
264 (12.3 versus 2.2% O_2, respectively).
265 Two symptoms of IB were identified: the classic pithy brown core (PBC) (Fig. 1B) and
266 wet brown flesh (WBF) (Fig. 1A). Most of the IB (91%) during storage was PBC, the symptoms
267 of which are a brownish colored core and many sponge-like small pores. The injured core tissue
268 was quite dry and typically restricted to the core although it occasionally spread to outside the
269 core where it was often accompanied by dried lesions and cavities. In contrast, WBF affected the
270 flesh tissue (cortex) turning it a dark brown color with the injured tissue having a water-soaked
271 appearance. The flesh tissue at the stem end of fruit with IB was intact and uninjured.
272 *3.1.3. Fruit quality and disorders after ripening*
273 After 3 and 4 months in storage at -1.1 °C, MAPc, MAPe and control fruit were ripened
274 at 20 ± 1 °C for 5 d to determine TA, TSS, and sensory quality. Due to significant IB, quality
275 data of MAPe fruit after ripening is not presented. While TA declined gradually in both MAPc

276 and control fruit during storage, MAPc fruit displayed a significantly ($p \leq 0.05$) slower decrease
277 in the rate of decline (Fig. 4A). In both MAPc and control the fruit increased in TSS a small but
278 significant ($p \leq 0.05$) amount after 3 and 4 months of storage compared to their initial values.
279 There was no difference in TSS content between MAPc and control fruit during storage (Fig. 4B).
280 Panelists rated the flavor and texture of MAPc and control fruit as comparable after 3 months of
281 storage, while after 4 months MAPc fruit had a higher eating quality than control fruit (both
282 flavor and texture) (Fig. 4C,D).

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

286 *3.1.4. AsA, cell membrane peroxidation, and tissue leakage*

287 The pears contained AsA of 51 mg kg^{-1} FW of fruit flesh at harvest and the peel content
288 was significantly higher (68 mg kg^{-1} FW). The AsA content of the flesh decreased significantly
289 during storage (Fig. 5A). The concentration in the control fruit had declined to 28 mg kg^{-1} FW
290 after 3 months and 15 mg kg^{-1} FW by 4 months of storage. MAPc significantly ($p \leq 0.05$)
291 inhibited the decline in AsA concentration (41 and 30 mg kg^{-1} FW after 3 and 4 months of
292 storage, respectively). After 4 months of storage, fruit without IB in MAPe had significantly ($p \leq$
293 0.05) lower AsA than MAPc fruit and equal to control fruit. Fruit with IB in MAPe had an
294 extremely low AsA content (5 and 4 mg kg^{-1} FW after 3 and 4 months of storage, respectively)
295 (Fig. 5A).

296 MDA, a secondary end product of polyunsaturated fatty acid oxidation, is widely used as
297 an indicator of membrane lipid peroxidation and cell integrity when assessing senescence and
298 environmental stresses (Hodges et al., 2004; Zhang et al., 2011). MDA concentration in control

299 fruit increased during storage (Fig. 5B). The change in MDA concentration in MAP fruit
300 displayed a similar trend, although the increase in MAPc fruit was significantly ($p \leq 0.05$) less by
301 the end of 3 and 4 months in storage. The MDA concentration in fruit without IB in MAPe was
302 the same as in MAPc fruit after 3 months in storage. By 4 months, fruit in MAPe had a higher
303 MDA concentration than MAPc fruit but the same concentration ($p \leq 0.05$) as control fruit. IB
304 damaged fruit had a significantly higher MDA content than undamaged fruit held in MAPe after
305 3 and 4 months in storage (increases of 180 and 160%, respectively).

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

314 *3.2. Simulated transit study*

315 *3.2.1. Gas analysis*

316 The O_2 and CO_2 concentrations in MAPc were 12.5 and 5.6%, respectively, at the time
317 when boxes were sampled after 1 month of storage at -1.1 °C. The O_2 concentration decreased
318 dramatically while CO_2 increased mildly with elevated temperatures reflecting the differential
319 permeability of the packaging materials for the two gases. At the holding temperatures of 2 and
320 4.5 °C, the O_2 concentration decreased to 10.5 and 5.0%, respectively, while the CO_2
321 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 O_2 concentration was dramatically reduced to 2.5 and 1.0%, respectively, while CO_2 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 O_2 and CO_2 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 CO_2/low O_2 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 the 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

345 firmness level at which the fruit would be at risk for mechanical damage, i.e., 59.6 N and 40.5 N
346 respectively, for fruit that had been stored 1 month at -1.1 °C and 38.3 N and 28.9 N after 3
347 months of storage, respectively (Fig. 7A).

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

353 *3.2.3. Fruit quality and disorders after ripening*

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

364 *3.2.4. AsA, cell membrane peroxidation, and tissue leakage during simulated transit conditions*
365 *after 1 month of storage*

366 After 1 month of storage at -1.1 °C, MAPc fruit had higher AsA concentrations after 3
367 weeks at simulated transit temperatures of 2 and 4.5 °C, but they were lower at 7.5 and 10 °C

368 compared to control fruit. The AsA levels were 45, 46, 20, and 11 mg kg^{-1} FW for fruit held in
369 MAPc and 41, 34, 33, and 23 mg kg^{-1} FW for control fruit at 2, 4.5, 7.5, and 10 °C, respectively.
370 Fruit with IB had extremely low AsA concentrations of 6 and 7 mg kg^{-1} FW for MAP fruit after
371 3 weeks at 7.5 and 10 °C, respectively (Fig. 9A).

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

378

379 **4. Discussion**

380 *4.1. Effect of MAP on fruit quality and IB development during cold storage*

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

387 High CO_2 (i.e., 5.6%) appears to play a major role in maintaining fruit quality based on
388 the fact that the reduction in O_2 concentration from 12.3 (MAPc) to 2.2% (MAPe) at 5-6% CO_2
389 did not improve storage life or quality. The reduction in O_2 concentration from 21 to 4-5% did
390 not significantly reduce the respiration or ethylene synthesis rates in either unripe or ripened

391 'Bartlett' pears (unpublished data). Kerbel et al. (1988) found that 'Bartlett' pears held in
392 elevated CO_2 concentrations had reduced respiratory and ethylene synthesis rates, and
393 maintained firmer, greener fruit compared to those stored in air. The physiological effect of CO_2
394 on respiration has been related to an inhibition of the Krebs cycle (Ke et al., 1994) and glycolytic
395 pathway (Kerbel et al., 1988, 1990). High CO_2 is also thought to inhibit the ethylene biosynthetic
396 pathway in pears (Yashida et al., 1986) at the conversion of 1-aminocyclopropane-1-carboxylic
397 acid to ethylene, a reaction catalyzed by 1-aminocyclopropane-1-carboxylic acid oxidase (de
398 Wild et al., 1999, 2003). High CO_2 is also a competitive inhibitor of ethylene action (Burg and
399 Burg, 1967). De Wild et al. (1999, 2003) suggested that CO_2 might antagonize the ethylene
400 receptor in pear fruit. Elevated CO_2 can also affect other metabolic pathways involved in
401 secondary metabolism, i.e., pigments, phenolics, volatiles (Beaudry, 1999; Kader, 1989;
402 Watkins, 2000).
403 Modified aerobic atmospheres are believed to extend the storage life of fruit and
404 vegetables in part through reduced oxidative stress, while a number of postharvest procedures
405 can lead to increased oxidative stress and/or injury (Hodges et al., 2004; Toivonen, 2004). In
406 broccoli, MAP (11.2% O_2 + 7.5% CO_2) retarded senescence and decreased oxidative stress
407 through the retention of AsA and other antioxidants (Zhuang et al., 1994; Barth and Zhuang,
408 1996). Yang (1997) reported that low O_2 (<3%) reduced AsA loss, membrane leakage and the
409 accumulation of MDA in 'Niitaka' pears, and 3% CO_2 + 1.2% O_2 was more effective in
410 maintaining fruit firmness than 1.1% CO_2 + 1.2% O_2. Storage of 'Pink Lady' apples at 1.5% O_2
411 + 3-5% CO_2, in contrast with air storage, maintained significantly higher AsA concentrations in
412 fruit without FB (flesh browning) after 4 months of storage (de Castro et al., 2008). In the
413 present study, 'Bartlett' pears stored in MAPc at 12.3% O_2 + 5.6% CO_2 displayed a reduced loss

414 of AsA, a reduced accumulation of MDA, a reduced increase of membrane leakage, and
415 extended storage life.

416 While the atmosphere created by MAPc was safe for PNW 'Bartlett' pears with regard to
417 IB development, an atmosphere with the same concentration of CO_2 but a reduced O_2 (i.e., 2.2%
418 O_2 + 5.7% CO_2) created by MAPe induced significant IB development during cold storage. In a
419 similar MAP atmosphere, 'Bartlett' fruit grown in southern Oregon developed 23 and 38% IB
420 after 3 and 4 months in storage at -0.5 °C (Sugar, 2001). In California, 1.5-2% O_2 + 1-5% CO_2
421 are recommended CA conditions for long-term storage of early- and mid-season 'Bartlett' pears
422 (Mitcham et al., 2012). Kader (2007) indicated that 'Bartlett' pears grown in regions of the US
423 other than California do not tolerate CO_2 levels above 1% regardless of the harvest date. The
424 PNW pear industry utilizes 2% O_2 + <1% CO_2 for 'Bartlett' pears in CA storage (Chen, 2004;
425 Richardson and Kupferman, 1997). Results of this study demonstrate that the tolerance of PNW
426 'Bartlett' pears to high CO_2 is influenced by the O_2 concentration, with low O_2 enhancing CO_2-
427 induced injury. Two types of MAP-related IB for 'Bartlett' pears have been reported by the
428 industry (PBC and WBF) and each was found in this study. In CA storage, PBC in 'd'Anjou',
429 'Bosc', and 'Bartlett' pears is caused by high CO_2 and aggravated by low O_2 (Chen, 2004; Chen
430 et al., 1986; Hansen and Mellenthin, 1962; Yoshida et al., 1986). In contrast, Ong (1987)
431 reported that 1.5% O_2 depressed flesh browning induced by 20% CO_2 in 'Bartlett' pears grown in
432 California. Yang (1997) reported that high O_2 levels increased a disorder in Asian pears known
433 as "skin blackening". The disorder did not develop in CA combinations of CO_2 (1.2 and 3%) and
434 O_2 (1.2 and 3%). However, 20% O_2 + 3% CO_2 caused "skin blackening" of 'Niitaka' pears.
435 While the reasons for the contradictory results are not currently understood, growing location
436 and cultivar may be critical factors affecting the response of pears to high CO_2/O_2 injury.

437 *4.2. IB development and quality of MAP fruit during transit*

438 The increase in respiratory rate between 0 and 10 °C is 2 to 3 times the rate of gas
439 permeability of low density polyethylene MAP films (Mir and Beaudry, 2004). While pears
440 stored in MAP are generally held at -1.1 °C (Porritt, 1964; Hansen and Mellenthin, 1979), this
441 study has demonstrated that increased temperatures during shipment and distribution can be a
442 critical factor in the induction of IB due to the dramatic alteration in the gas atmosphere within
443 the MAP. At the simulated transit temperatures tested, the atmosphere in MAPc was maintained
444 at 5.0-10.5% O_2 + 5.8-6.2% CO_2 at 2 and 4.5 °C and the fruit in these environments were free
445 from IB during the 3 week simulated transit period. However, CO_2 accumulated to 7.0 and 7.9%
446 and O_2 was reduced to 2.5 and 1.0% at 7.5 and 10 °C, respectively. Control fruit packed in
447 commercially perforated plastic bags did not display an accumulation of CO_2 nor reduced O_2 at
448 any of the simulated transit conditions and did not develop IB. Fruit held in MAPc maintained a
449 significantly higher fruit firmness and better color than the control fruit at each of the simulated
450 transit temperatures after both 1 and 3 months in storage. This indicated that MAPc can maintain
451 'Bartlett' pear quality at slightly elevated temperatures (e.g., 2-4.4 °C) during transit. However,
452 higher post-storage temperatures (e.g., 7.5 and 10 °C) caused reduced O_2 (i.e., <2.5%) and
453 elevated CO_2 concentrations in MAPc that resulted in significant increase in the incidence of IB.

454 'Bartlett' pears are extremely susceptible to friction discoloration (FD) due to mechanical
455 failure in response to friction (scuffing) and bruising throughout the supply chain (harvest,
456 packinghouse operations, transit, wholesale and retail marketing). According to Thompson
457 (2007), 'Bartlett' pears with a FF of 66.7 N would likely suffer vibration and/or impact bruising
458 damage during transportation. MAPc packed 'Bartlett' fruit maintained FF higher than 66.7 N at
459 2 °C for 21 d regardless of storage durations up to 3 months at -1.1 °C. While MAP packed fruit

460 maintained FF of 71.2 N at 4.5 °C for 21d in the early storage season (1 month storage), this was
461 reduced to 60.1 N after 3 months of storage. Therefore, MAPc packed 'Bartlett' pears can be
462 shipped at 0-4.5 °C in the early storage season, but the post-storage temperature should be 0-2 °C
463 during the later season to avoid softening. At 7.5 °C and 10 °C for 21 d, fruit FF was reduced to
464 lower than 66.7 N regardless of storage duration (Fig. 7). The simulated transit temperatures did
465 not significantly affect fruit green color significantly under the MAP conditions.

466 *4.3. Mechanisms of MAP-related IB*

467 The mechanism of IB in pears is not sufficiently understood. Fruit tissue browning
468 involves the enzymatic oxidation of phenolic compounds by polyphenoloxidase (PPO) to o-
469 quinones, which form brown colored polymers (Mathew and Parpia, 1971; Mayer, 1987).
470 Although susceptibility to tissue browning among pear cultivars and production factors have
471 been related to the concentration of certain phenolic compounds (Hamauzu and Hanakawa,
472 2003; Franck, 2007), neither PPO activity nor the concentrations of polyphenol compounds
473 appear to be limiting factors in the process of browning development in pears (Larrigaudiere et
474 al., 1998; Veltman et al., 1999b). Since PPO and its substrate phenolic compounds are located in
475 different cellular compartments (cytoplasm/plastids and vacuole, respectively) (Nicolas et al.,
476 1994; Dixon and Paiva, 1995), enzymatic browning only occurs after cellular
477 decompartmentalization caused by membrane disintegration. Cell membrane damage is thought
478 to occur due to an imbalance between oxidative and reductive processes, when degradative
479 processes exceed those of maintenance (Shewfelt and del Rosario, 2000).

480 Several lines of evidence indicate that excessive CO_2 plus low O_2 increases oxidative and
481 reduces reductive processes in pear fruit. Elevated CO_2, especially with low O_2, results in an
482 accumulation of alcohol and acetaldehyde in 'Bartlett' pears (Ke et al, 1990, 1994) and active

483 oxygen species (AOS) such as O_2^- and H_2O_2 in 'Conference' pears (Larrigaudiere et al., 2001a).
484 These compounds are either toxic to fruit tissues or cause lipid peroxidation and protein
485 denaturation (Halliwell and Gutteridge, 1989) and as a consequence cause membrane
486 disintegration and cellular decompartmentalization (Veltman et al., 1999a). Plants have several
487 enzymatic (e.g., superoxide dismutase, catalase, peroxidase) and non-enzymatic antioxidant
488 defense mechanisms that counteract membrane oxidative damage caused by AOS (Larrigaudiere
489 et al., 2001b). Among the antioxidant systems, AsA appears to play a critical role in the
490 occurrence of browning disorder of 'Conference' pears (Eccher Zerbini et al., 2002; Franck et
491 al., 2003a,b). It has been suggested that AsA protects against browning and that browning in
492 pears is initiated when AsA drops below a threshold value (Eccher Zerbini et al., 2002; Veltman
493 et al., 1999a). This study demonstrated that MAP-related IB in 'Bartlett' fruit appears to occur
494 through the loss of membrane integrity as indicated by increased MDA concentration and RLR
495 values in fruit with IB damage. AsA content dropped dramatically in IB-damaged fruit stored at
496 high CO_2 and low O_2. Fruit without IB-damaged when stored at high CO_2 + low O_2 also
497 displayed lower AsA concentrations compared to those stored in a safe atmosphere (e.g., high
498 CO_2 + high O_2). What predisposes some fruit in the same atmosphere (i.e., 5.7% CO_2 + 2.2%
499 O_2), to maintain a higher concentration of AsA and resistance to IB than other fruit from the
500 same box warrants further study.

501 The precise mechanism by which high CO_2/O_2 modulates AsA metabolism is not known.
502 While the AsA biosynthetic pathway in plants has yet to be fully elucidated, two distinct
503 pathways have been proposed (Noctor and Foyer, 1998). The decrease in AsA in pears caused by
504 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 CO_2 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 CO_2/O_2 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 CO_2 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 CO_2 injury (Hansen and Mellenthin, 1962). Additional information on pre-harvest factors
518 affecting the susceptibility of 'Bartlett' pears to high CO_2/O_2 injury during storage in MAP is
519 needed.

520

521 **5. Conclusions**

522 The goal of MAP for fresh produce is to create an equilibrium package atmosphere with
523 O_2 low enough and CO_2 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 O_2 and CO_2 for commodities is
527 critical for the successful commercial application of MAP. The optimum CA conditions for long-

528 term storage of 'Bartlett' pear grown in the PNW are believed to be approximately 1-2% O_2 and
529 0-0.5% CO_2 (Chen, 2004; Richardson and Kupferman, 1997). The current study has
530 demonstrated that the susceptibility of 'Bartlett' pears to CO_2 injury is highly dependent upon the
531 O_2 concentration and storage duration. Optimal CO_2 and O_2 concentrations differed between
532 MAP and recommended CA storage conditions. MAP developed an atmosphere of ~5.5% CO_2
533 with a relatively high O_2 concentration and appears to be excellent addition to low temperature
534 management for extending the storage life and potential transportation distances for 'Bartlett'
535 pears. Good temperature management is the key to avoiding CO_2 injury during transit. The
536 MAPc for pears created an appropriate O_2 and CO_2 atmosphere within the bags for maximizing
537 keeping quality without CO_2 injury and over-ripening at transit temperature of 0-4.5 °C in early
538 season and 0-2 °C in late season pears. When properly used, MAP has the potential to
539 substantially extend the 'Bartlett' marketing season, expand long-distance exports into new
540 markets, and increase the total volume of PNW fruit that can be sold each year.
541
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544
545 **References**
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710 Fig. 1. Grading scales for two types of MAP-related internal browing disorders: wet brown flesh

711 (WBF) (A) and pithy brown core (PBC) (B).

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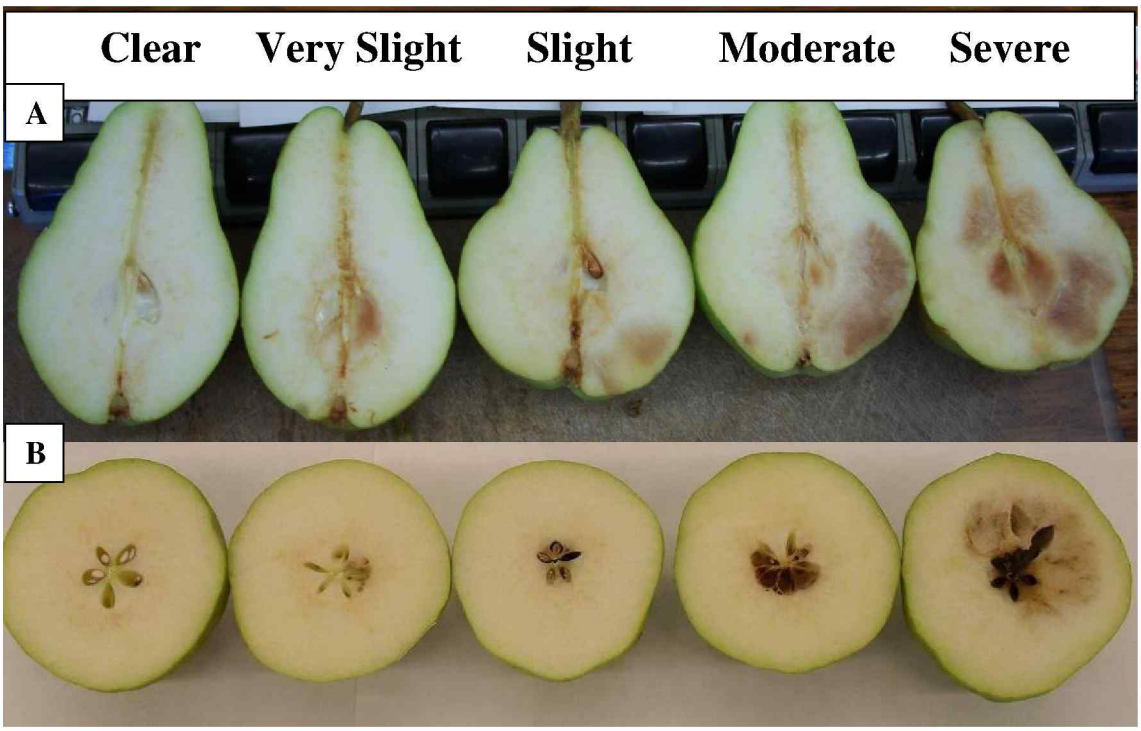

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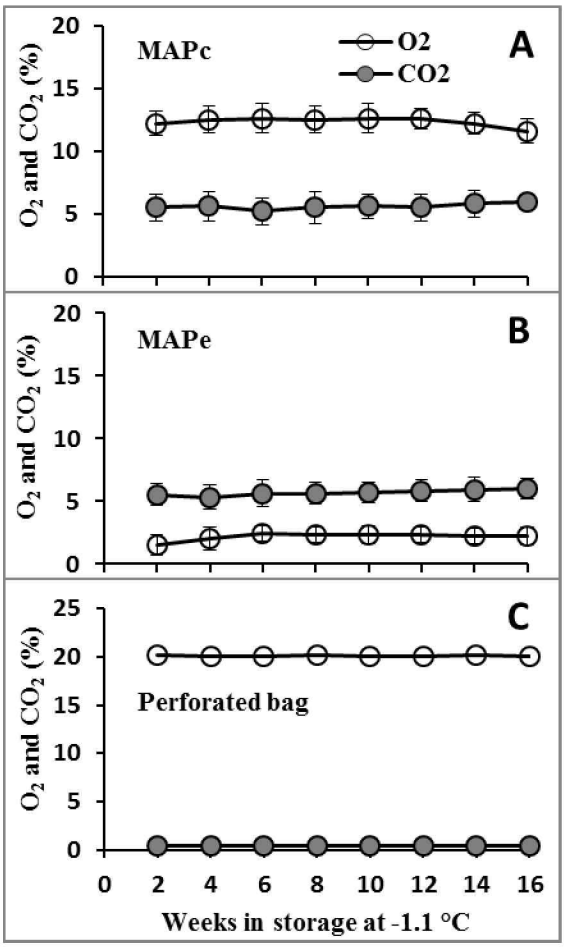
Fig. 2. O_2 and CO_2 concentrations in a commercial modified atmosphere packaging (MAPc) (A), an experimental modified atmosphere packaging (MAPe) (B), and commercial perforated plastic bags (C) containing 'Bartlett' pears stored at -1.1 °C. Vertical bars represent standard deviations (5% level).



735 Fig. 3. Fruit flesh firmness (FF) (A), color ($h°$) (B), and internal browning (IB) (C) of 'Bartlett'
736 pears in a commercial modified atmosphere packaging (MAPc), an experimental modified
737 atmosphere packaging (MAPe), and commercial perforated plastic bags during storage at -1.1 °C.
738 Vertical bars represent standard deviations (5% level). Different letters indicate significant
739 differences ($p \leq 0.05$) according to Fisher's protected LSD test.

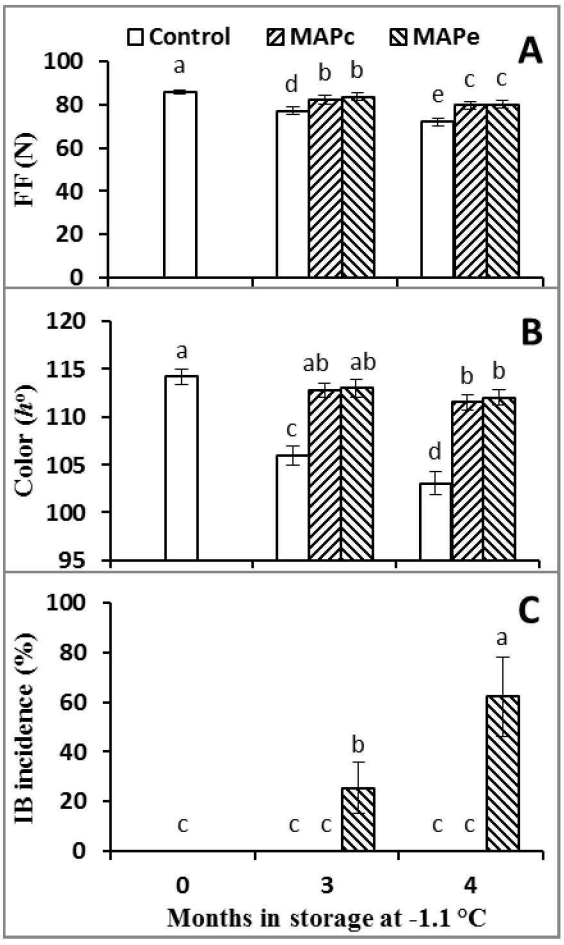


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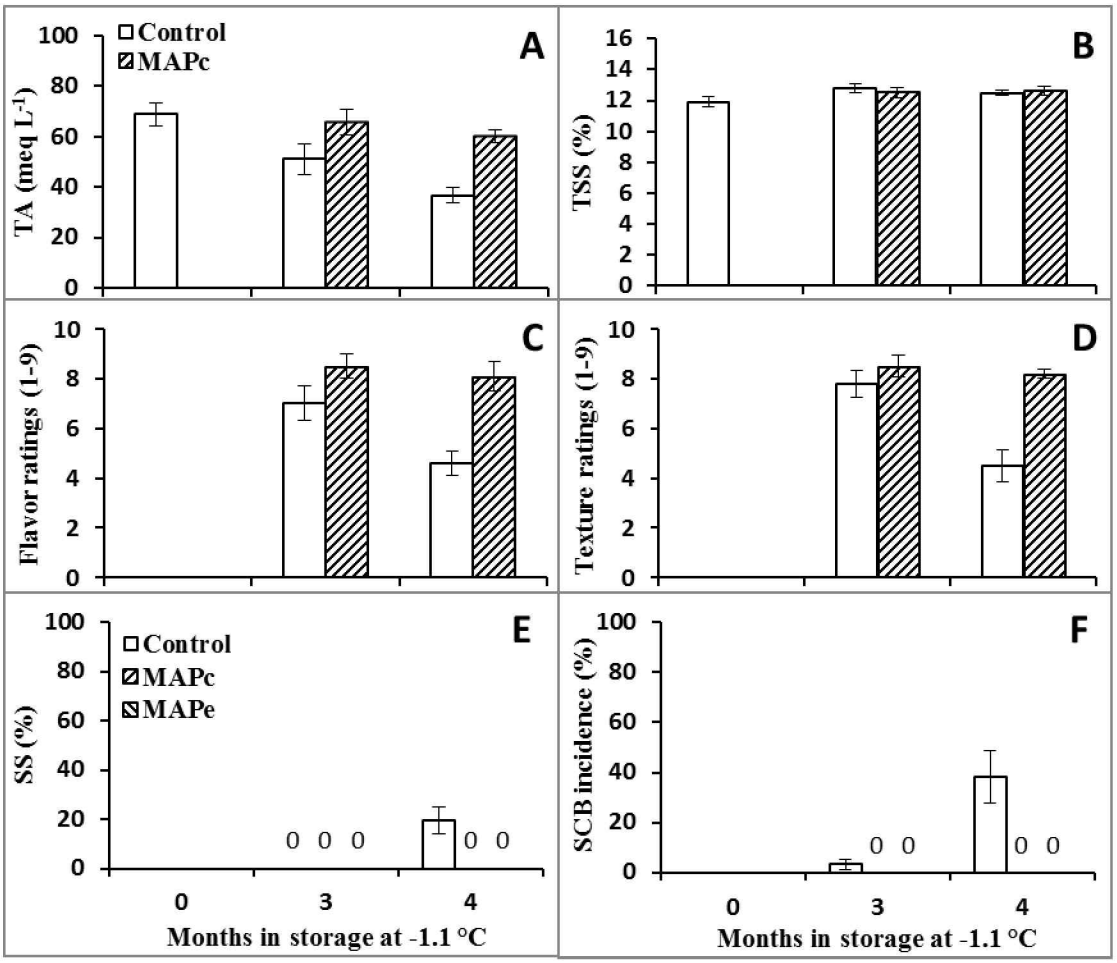
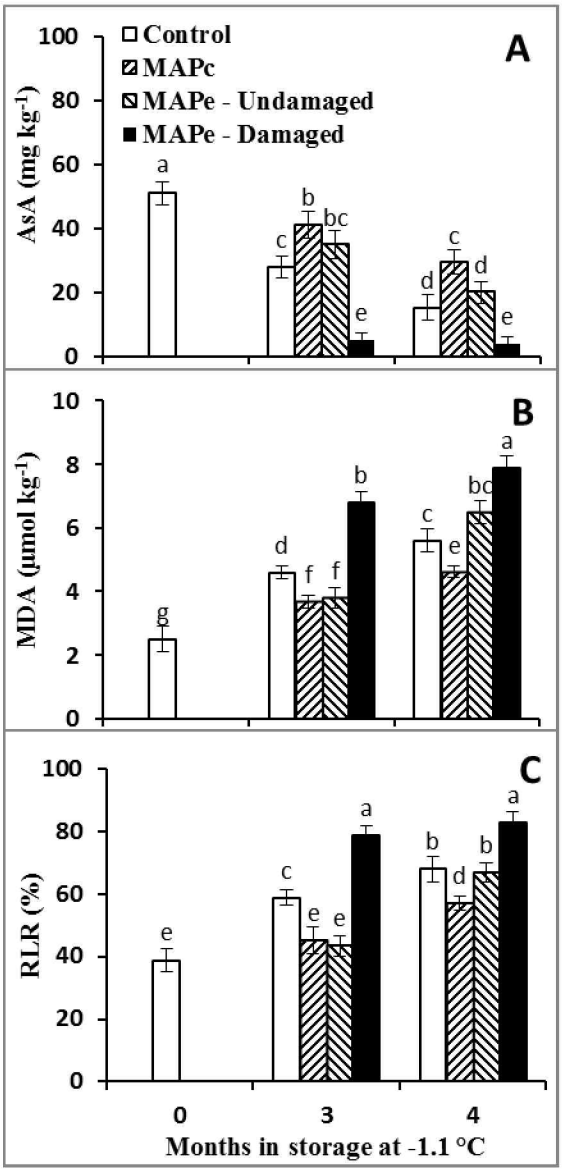
Fig. 4. Titratable acidity (TA) (A), total soluble solids (TSS) (B), sensory quality (C&D), senescent scald (SS) (E), and senescent core breakdown (SCB) (F) of ripened (5 d at 20 ± 1 °C) 'Bartlett' pears stored in a commercial modified atmosphere packaging (MAPc) and commercial perforated plastic bags after 3 and 4 months at -1.1 °C. Vertical bars represent standard deviations (5% level).



757 Fig. 5. Ascorbic acid (AsA) (A), malondialdehyde (MDA) (B), and relative leakage ratio (RLR)
758 (C) of 'Bartlett' pears stored in a commercial modified atmosphere packaging (MAPc), an
759 experimental modified atmosphere packaging (MAPe) (with and without internal browning
760 discoloration), and commercial perforated plastic bags at -1.1 °C. Vertical bars represent
761 standard deviations (5% level). Different letters indicate significant differences ($p \leq 0.05$)
762 according to Fisher's protected LSD test.



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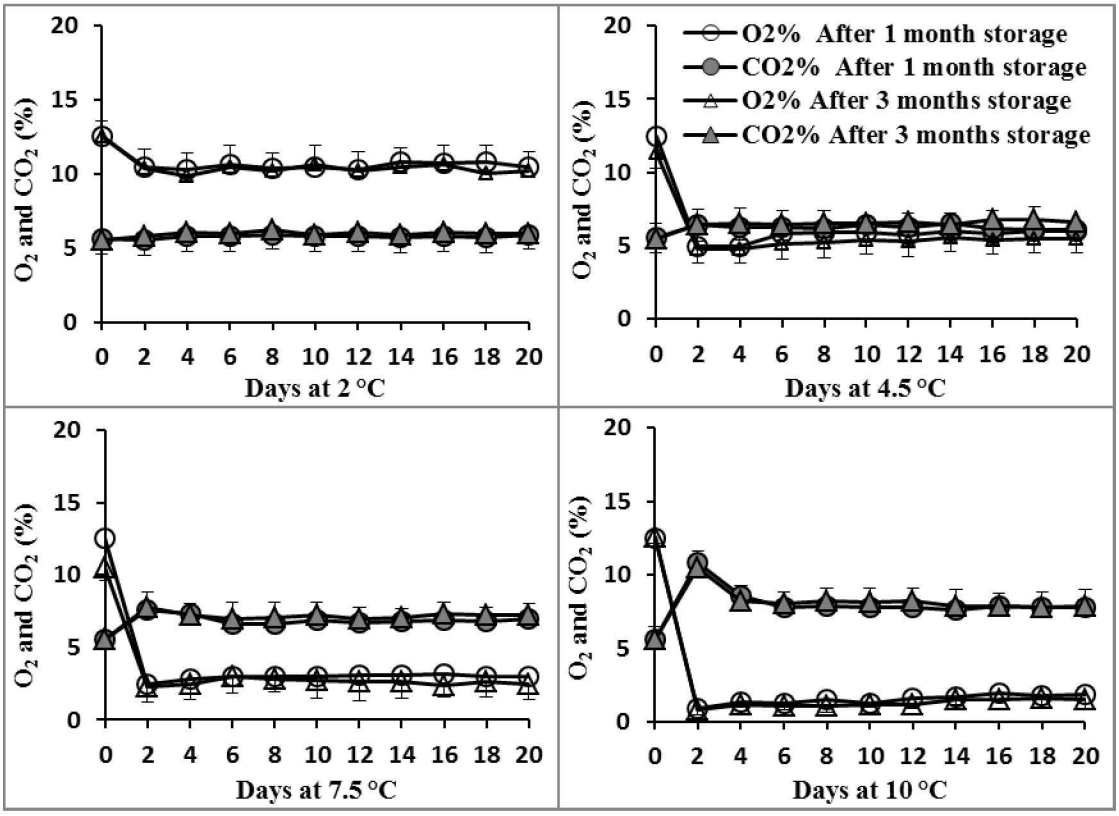
765 Fig. 6. O_2 and CO_2 concentrations in a commercial modified atmosphere packaging (MAPc)
766 containing 'Bartlett' pears at simulated transit temperatures for 3 weeks after 1 and 3 months of
767 storage at -1.1 °C. Vertical bars represent standard deviations (5% level).



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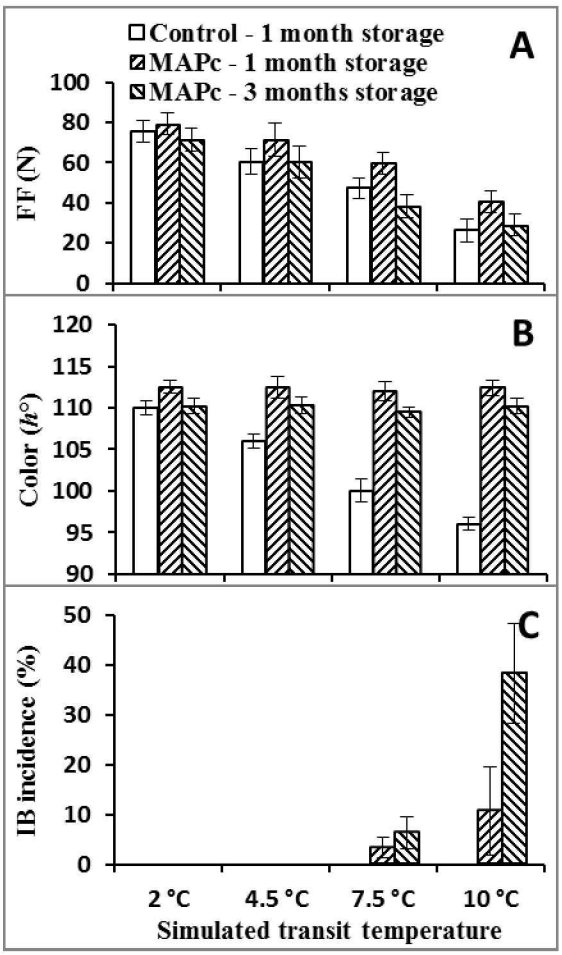
777 Fig. 7. Fruit flesh firmness (FF) (A), color ($h°$) (B), and internal browning (IB) (C) of 'Bartlett'
778 pears stored in a commercial modified atmosphere packaging (MAPc) and commercial
779 perforated plastic bags at simulated transit temperatures at 2, 4.5, 7.5, and 10 °C for 3 weeks
780 after 1 and 3 months of storage at -1.1 °C. Vertical bars represent standard deviations (5% level).



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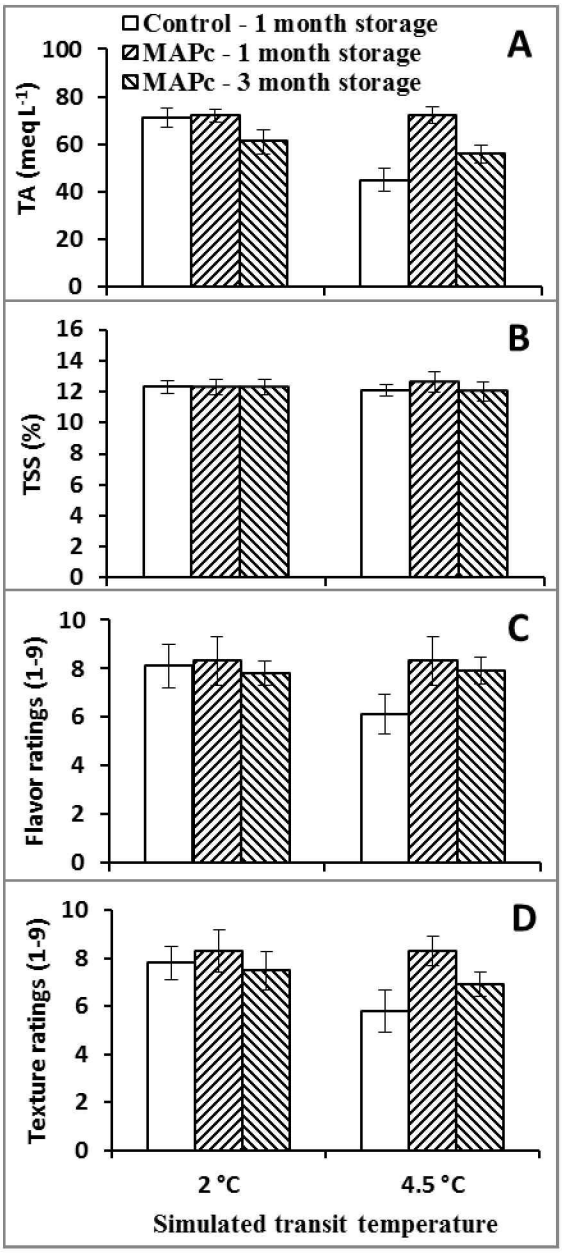
787 Fig. 8. Titratable acidity (TA) (A), total soluble solid (TSS) (B), and sensory quality (C&D) of
788 ripened (5 d at 20 ± 1 °C) 'Bartlett' pears stored in a commercial modified atmosphere packaging
789 (MAPc) and commercial perforated plastic bags at simulated transit temperatures of 2 and 4.5 °C
790 for 3 weeks after 1 and 3 months of storage at -1.1 °C. Vertical bars represent standard
791 deviations (5% level).



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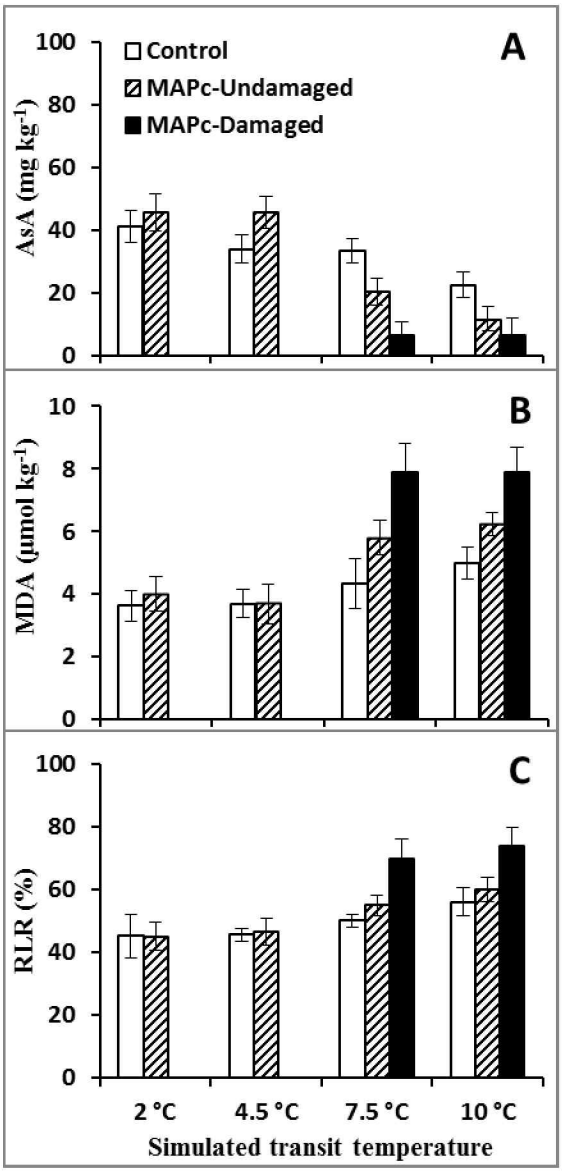
794 Fig. 9. Ascorbic acid (AsA) (A), malondialdehyde (MDA) (B), and relative leakage ratio RLR (C)
795 of 'Bartlett' pears stored in a commercial modified atmosphere packaging (MAPc) and
796 commercial perforated plastic bags at simulated transit temperatures of 2, 4.5, 7.5, and 10 °C for
797 3 weeks after 1 month of storage at -1.1 °C. Vertical bars represent standard deviations (5%
798 level).



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