High Storage Humidity Affects Fruit Quality Attributes and Incidence of Fruit Cracking in Cold-stored ‘Royal Gala’ Apples

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Abstract. ‘Royal Gala’ apples can be susceptible to the incidence of fruit cracking and senescent flesh breakdown during cold storage. Because the development of these physiological disorders in other cultivars can be influenced by humidity during storage, the objective of this study was to evaluate the effect of high storage humidity on fruit quality attributes and incidence of physiological disorders in cold-stored ‘Royal Gala’ apples. Fruit obtained from a commercial orchard were kept in cardboard boxes with or without a perforated polyethylene liner during and after cold storage. High storage humidity induced by the perforated polyethylene liner reduced fresh weight loss but enhanced the change of fruit circumference after cold storage. High storage humidity contributed the most reduction of cortex tightness (L*) and hue angle (h°) in stem-end cortex tissues during shelf life. Fruit stored with liners had reduced internal ethylene concentration (IEC) and outer cortex firmness after removal from storage compared with control fruit. Furthermore, high storage humidity prevented shriveling but provoked fruit cracking. The incidence and severity of flesh breakdown were further aggravated during shelf life, compared with cold storage, regardless of a liner application. Overall, maintaining high storage humidity by applying a perforated polyethylene liner can contribute to delaying fresh weight loss, reducing IEC, and preventing fruit shriveling but can enhance cortex tissue browning, loss of flesh firmness, and incidence of fruit cracking during cold storage and shelf life.

‘Gala’ apple strains are susceptible to stem-end fruit cracking at harvest (Fallahi et al., 2013) and during storage (Lee et al., 2013, 2016). The incidence of stem-end fruit cracking at harvest is influenced by several factors such as rootstock (Fallahi et al., 2013), irrigation (Opara et al., 2000), nutrient management (Opara et al., 1997b; Perring, 1984), and fruit maturity (Byers, 1998; Opara et al., 1997b). During and after cold storage, fruit size positively contributes to increases in fruit circumference, thereby inducing fruit cracking (Lee et al., 2013). Stem-end splitting during storage also increases with advanced fruit maturity at harvest in cold-stored ‘Honeycrisp’ apples (Wargo and Watkins, 2004). ‘Royal Gala’ cracking incidence and severity increase with increased storage temperatures (Lee et al., 2016). However, 1-methylcyclopropene (1-MCP) can reduce fruit cracking during and after cold storage (Lee et al., 2013). Reduced fruit cracking in 1-MCP treated fruit may be associated with reduced fresh weight loss and ripening during cold storage (Bai et al., 2005; Fan et al., 1999). The incidence of ‘Royal Gala’ flesh breakdown increases with increased fruit size but flesh breakdown severity is inconsistently associated with fruit size (Lee et al., 2013). Symptoms in untreated fruit can appear first as diffuse browning in the stem-end cortex tissue, and symptoms can progress through the equator and into the calyx-end cortex tissue (Lee et al., 2013). In contrast, symptoms in 1-MCP treated fruit often are only detected in the stem-end cortex tissue with a radial pattern rather than with diffuse pattern of browning (Lee et al., 2013, 2016). 1-MCP also contributes to reduced development of senescent flesh browning during controlled atmosphere (CA) storage (Argenta et al., 2006). Fuit cracking is also suppressed by 1-MCP treatment (Lee et al., 2013, 2016). The terminology of fruit cracking and splitting was well defined by Opara et al. (1997a).

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Materials and Methods

Plant material and fruit storage. ‘Royal Gala’ apples (Malus ×domestica Borkh.) harvested from mature trees in a commercial orchard near Vantage, WA were transported to a laboratory in Wenatchee, WA. Fruit without external blemishes that weighed more than 230 g were placed on pressed fiber trays (18 fruit/tray) and packed with or without a perforated polyethylene liner (polyliner) in cardboard boxes. Polyliner dimensions were 521 mm length, 330 mm width, and 762 mm depth with 5 mm hole diameter. Each liner had 16 holes, eight on each long side in two rows of four. Holes were 130 mm apart, and they were located starting 90 mm from the bag bottom and bag side. All fruit were stored in air at 0.5 °C in a cold room with ≈90% relative humidity (RH) for 6 months, followed by 20 °C in a room with 60 ± 10% RH for 7 d. The RH and temperature were recorded using a Veriteq Spectrum SP-2000-2OR humidity and temperature sensor (Vaisala Canada Inc., Richmond, B.C., Canada).

Fruit quality assessment. Fruit fresh weight and circumference were measured with an analytical balance and a tape measure, respectively. Measurements were conducted at harvest and after storage on the same fruit. Peel color variables on an unblushed area of the fruit equator region were measured with a chromameter (Minolta CR-200; Minolta Co., Osaka, Japan). Flesh color was assessed at the stem-end (1.5 cm from the stem-end toward the equator, cut horizontally), equator (at the fruit equator, cut horizontally), and the calyx-end (1.5 cm from the calyx-end toward the equator, cut horizontally), with six readings per region using the same chromameter. Color measurements were expressed as lightness (L*, 0–100), chroma (C*), and hue angle (h*, 0–360) (McGuire, 1992).

Internal ethylene concentration (IEC) was measured by withdrawing a 0.5-mL gas sample from the core cavity using a syringe and analyzing the sample using a Hewlett-Packard 5890A gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a flame ionization detector and 46-cm (length) × 0.32-cm (diameter) glass column packed with Porapack Q (Supelco Co., Bellefonte, PA). Flow rates for N2 carrier, H2, and air were 0.5, 0.17, and 3.3 mL·s−1, respectively. Oven, injector, and detector temperatures were 60, 100, and 200 °C, respectively.

The CO2 production rate was determined using three replicates of four fruit placed into 3.79-L glass jars sealed with Teflon lids with two gas ports. Jars were purged with air at 1.7 mL·s−1 for 1 h; then, 3 mL of head space gas collected from the lid outlet port was used for the CO2 analysis with a Hewlett Packard 5890 gas chromatograph (Agilent, Palo Alto, CA) equipped with a 0.5-m, 3.2-mm i.d., stainless steel column packed with Porapak Q (Supelco), a methanizer (John Booker & Co., Austin, TX), and a flame ionization detector. The N2 carrier, H2, and air flow rates were 0.5, 0.5, and 5 mL·s−1, respectively. Oven and injector temperatures were 35 and 300 °C, respectively. The methanizer temperature was 290 °C and was controlled by an instrumentation temperature controller (Valco Instruments Inc., Houston, TX) with an H2 flow of 0.5 mL·s−1.

Flesh firmness, tissue tensile strength (TTS), and crispness were assessed using a penetrometer (Mohr Digi-Test; Mohr & Associates, Richland, WA) equipped with a cylindrical plunger 11 mm in diameter. Measurements were performed on two pared surfaces on opposite sides of the fruit equator region. Maximum cortex firmness of the outer 8 mm (outer cortex, M1) and 8 mm from the core line boundary (inner cortex, M2), TTS at 8 mm, and crispness were assessed (Mattheis, 2017). TTS indicated the relaxation rate of the fruit with 4.54 kg of creep force for 0.5 s at the boundary between regions from the outer 8 mm and

Table 1. Fresh weight loss (Δfresh wt), fruit circumference change (Δfruit circumference), and peel color changes in terms of lightness (L*), chroma (C*), and hue angle (h*) of ‘Royal Gala’ apple fruit stored in air at 0.5 °C for 6 mo., followed by 7 d at 20 °C.

| Storage duration | ΔFresh wt (g/fruit) | ΔCircumference' (mm/fruit) | Peel lightness (L*) | Peel chroma (C*) | Peel hue angle (h*) |
|------------------|---------------------|--------------------------|--------------------|----------------|------------------|
|                  | Control Liner       | Control Liner            | Control Liner      | Control Liner  | Control Liner   |
| 0 mo.            | 18.9              | 6.4                      | 6.9                 | 0.8            | 55.8             | 60.9             | 53.9             | 45.3             | 53.9             |
| 6 mo. + 0 d      | 22.5              | 8.6                      | 4.8                 | 2.9            | 57.5             | 59.3             | 36.5             | 37.3             | 50.8             | 51.9             |
| 6 mo. + 7 d      | 22.5              | 8.6                      | 4.8                 | 2.9            | 57.5             | 59.3             | 36.5             | 37.3             | 50.8             | 51.9             |

*ΔFresh weight and circumference difference = harvest value – poststorage (or after 7-d shelf life) value (n = 18).
*Values are means (n = 18).
*Values of color parameters are means (n = 18), with three readings per replicate.
*NS, **, ****Nonsignificant or significant at P < 0.01 or 0.0001, respectively.
8 mm from the core line boundary. TTS and crispness values were recorded as Co and Cn, respectively, as Mohr Digi-test data. Starch pattern index was estimated by cutting each fruit horizontally through the equator and then staining the cut surface using a potassium-iodine (1.5% KI, 0.6% I) solution. Starch pattern index from 1 (100% starch) to 6 (0% starch) was determined using the scale described by Brookfield et al. (1997).

Fruit index of absorbance difference (IAD) was determined using a DA meter (35300 DA meter; T.R. Turono srl, Forli, Italy). IAD on the fruit blush and shade sides was used to calculate the overall mean for each fruit (Costamagna et al., 2013). Soluble solids concentration (SSC) and titratable acidity (TA) in freshly juice with 0.1 M KOH to pH 8.1.

Incidence and severity of peel shriveling and cracking were recorded and then fruit were horizontally sliced into five or six sections. Incidence and severity of flesh breakdown were assessed. Disorder incidence was expressed as percent fruit affected (n = 18). The severity of external and internal physiological disorders was subjectively scored from 0 to 6 (0 = 0%, 1 = 1% to 10%, 2 = 11% to 25%, 3 = 26% to 50%, 4 = 51% to 75%, and 5 = 76% to 100% of the area of the peel or the slice with the largest area with cracking and breakdown) (Lee et al., 2013).

Experimental Design and Statistical Analysis

Statistical analyses. The experiment was conducted with the presence or absence of a perforated polyethylene liner during cold storage and shelf life. Fruit quality attributes and incidence and severity of physiological disorders were analyzed according to a completely randomized experimental design. To assess fruit quality attributes and physiological disorders, 18 fruit per treatment were used, with three replicates of six fruit each. All statistical analyses were performed with SAS version 9.3 (SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) using the general linear model (GLM) procedure was used to determine the main and interaction effects, and Duncan’s multiple range test was used to determine the mean separation. Pearson correlation coefficient analysis (PROC CORR) was performed to identify relationships between response variables, fruit quality, and storage disorders.

Results

Cold room RH was not monitored during storage. During shelf life, the RH fluctuated between 46 and 72% in the 20 °C room in which fruit were kept (Fig. 1). RH inside the polyliner during this period rapidly increased and was more than 90% for most of the shelf life.

Fruit fresh weight loss and decreased circumference during cold storage and shelf life were highest for controls (Table 1). Liner fruit circumference actually increased during shelf life. Peel L* was highest for liner fruit at removal from cold storage but similar for controls and liner fruit after shelf life. Peel C* and h° increased during cold storage and shelf life regardless of liner use. Liner x storage duration was not significant for any of these variables.

Table 1. Internal ethylene concentration (IEC), CO₂ production, and IAD of ‘Royal Gala’ apple fruit stored in air at 0.5 °C for 6 mo. followed by 7 d at 20 °C.

| Storage duration | IEC (μL·L⁻¹) | CO₂ production (μmol CO₂·kg⁻¹·h⁻¹) | IAD (ΔA₆₇₀ – ΔA₇₂₀) |
|------------------|-------------|-----------------------------------|---------------------|
|                  | Control     | Liner | Control | Liner | Control | Liner |
| 0 mo.            | 1.0°C       |       |         |       |         |       |
| 6 mo. + 0 d      | 11.6 bc     | 15.3 b | 303.3   | 317.9 | 0.06    | 0.06  |
| 6 mo. + 7 d      | 162.8 a     | 17.7 b | 507.4   | 490.8 | 0.04    | 0.05  |

Source: IEC, CO₂ production, IAD (ΔA₆₇₀ – ΔA₇₂₀).

Table 2. Cortex tissue color variables (L*, C*, h°) of stem-end, equator, and calyx-end internal tissues of ‘Royal Gala’ apple fruit stored in air at 0.5 °C for 6 mo. followed by 7 d at 20 °C.

| Storage duration | Stem-end |          |          | Equator |          |          | Calyx-end |          |          |
|------------------|----------|----------|----------|---------|----------|----------|-----------|----------|----------|
|                  | Control  | Liner    | Control | Liner | Control | Liner | Control   | Liner    |          |
| 0 mo.            | 82.1*    | -        | 83.6     | -      | 82.6     | -      |          |          |          |
| 6 mo. + 0 d      | 80.6     | 80.9     | 82.3     | 82.9   | 80.4     | 80.6   |          |          |          |
| 6 mo. + 7 d      | 77.7     | 76.1     | 80.5     | 80.9   | 79.5     | 78.6   |          |          |          |

Source: Lightness (L*), Chroma (C*), and Hue angle (h°).

Table 3. Internal ethylene concentration (IEC), CO₂ production, and IAD of ‘Royal Gala’ apple fruit stored in air at 0.5 °C for 6 mo. followed by 7 d at 20 °C.

Table 4. Outer cortex firmness (M1), inner cortex firmness (M2), tissue tensile strength (TTS), and crispness of ‘Royal Gala’ apple fruit stored in air at 0.5 °C for 6 mo. followed by 7 d at 20 °C.

| Storage duration | Firmness M1 (N) | Firmness M2 (N) | TTS (cm) | Crispness |
|------------------|-----------------|-----------------|----------|-----------|
|                  | Control | Liner | Control | Liner | Control | Liner |
| 0 mo.            | 79.3*    |       | 105.5   | -      | 0.000*   | 182.9 |
| 6 mo. + 0 d      | 61.4     | 51.7   | 52.6    | 57.7   | 0.097    | 121.5 |
| 6 mo. + 7 d      | 43.8     | 33.9   | 42.4    | 42.8   | 0.235    | 114.4 |

Source: Firmness M1, Firmness M2, Strength, Crispness.
storage and shelf life, regardless of tissue or liner use. Cortex $L^*$ decreased most at the stem end in liner fruit. Cortex $C^*$ values increased during cold storage and shelf life. Flesh $h^*$ decreased during cold storage and shelf life and with liner use. The reduction in flesh $h^*$ was higher at the stem-end, compared with calyx-end tissue.

IEC was higher relative to harvest after storage and shelf life, but fruit stored in liners had lower values, compared with controls (Table 3). CO$_2$ production increased during shelf life but was not influenced by liner. $I_{AD}$ was not influenced by shelf life or liner.

Outer cortex firmness (M1) decreased during cold storage and shelf life, with the lowest values found for liner fruit (Table 4). Inner cortex firmness (M2) also decreased during cold storage and shelf life, but liner fruit had a higher M2 compared with controls at the time of removal from cold storage. TTS decreased (increased values) during cold storage and shelf life but were unaffected by liner. Fruit crispness decreased during cold storage and shelf life but liner storage decreased crispness loss.

Incidence and severity of fruit shriveling occurred only on controls (Table 5). Cracking only occurred on liner fruit and incidence and severity increased during shelf life. Incidence and severity of flesh breakdown increased during shelf life with no influence of liner.

Correlations between variables for fruit quality attributes and physiological disorders were in general low. Nonetheless, there were 87 positive and 79 negative correlations for control fruit, while liner fruit had 105 positive and 111 negative correlations (Fig. 2). IEC was highly correlated with peel and flesh color, fruit fresh weight loss, and incidence and severity of senescent breakdown in control fruit but not in liner fruit. Fruit fresh weight loss was positively correlated with fresh weight and circumference at harvest, IEC, fruit circumference change, and incidence and severity of shriveling in control fruit but more diversely correlated with peel and cortex tissue color variables, along with the incidence and severity of storage disorders in liner fruit. In liner fruit, peel $L^*$ was negatively correlated with fresh weight loss, $I_{AD}$, and the incidence and severity of senescent breakdown. Peel $h^*$ was positively correlated with IEC and peel $L^*$ but negatively with $I_{AD}$ in control fruit. In contrast, peel $h^*$ was positively correlated with peel $L^*$, stem-end cortex tissue $L^*$, calyx-end cortex tissue $L^*$, and stem-end cortex tissue $h^*$ but negatively with $I_{AD}$ and the incidence and severity of senescent breakdown in liner fruit.

**Discussion**

This study revealed that high humidity resulting from packing ‘Royal Gala’ apples in a perforated polyethylene liner can prevent shriveling but enhance cracking during and after cold storage. Apples stored in high humidity have reduced transpiration (Prange et al., 2001), reduced fruit fresh weight during (Lidster, 1990) and after cold storage (Tu et al., 2000). Modified atmosphere (MA) bags reduce fresh weight loss in sweet cherry fruit during cold storage (Padilla-Zakour et al., 2004) but enhance cracking development (Padilla-Zakour et al., 2004). Cracking development may be related to the number of microcracks on the fruit outer epidermal surface that increase in high humidity (Knoche and Peschel, 2006). Accordingly, it is assumed that higher storage humidity during apple cold storage due to a perforated polyethylene liner should affect physiological and physical properties of apple peel and cortex tissues during cold storage and shelf life in terms of fresh weight loss and cracking incidence, respectively. On the other hand, high storage humidity might contribute to reduced peel epicuticular wax thereby provoking fruit cracking during cold storage, as shown by the microscopic cracking of sweet cherry fruit cuticle (Knoche and Peschel, 2006). Furthermore, fruit ripening and senescence would be another factor to be considered for the development of fruit cracking during storage and shelf life because fruit cracking incidence increases with increased storage duration (Lee et al., 2013). However, fruit ripening and softening during cold storage and shelf life are strongly associated with the activation of cell wall solubilization, which is linked to the upregulation of numerous cell wall degradation enzymes (Harb et al., 2012; Roth et al., 2005). Therefore, cell wall loosening in long-term cold stored apples might be involved in provoking the development of fruit cracking under high storage humidity.

‘Royal Gala’ apples are susceptible to flesh breakdown during cold storage (Lee et al., 2013; Lee et al., 2016). In this study, higher storage humidity did not influence flesh breakdown during or after cold storage. However, the incidence and severity of flesh breakdown increased during shelf life, regardless of a liner application. Incidence of apple fruit internal disorders has been linked to storage humidity and temperature (Weber et al., 2012). Fruit ripening and senescence progress during long-term cold storage and subsequent warm temperature shelf life illustrated here in part by increased IEC and respiration. Although IEC was lower at the end of the shelf life period in liner fruit, compared with controls, flesh breakdown was not impacted by liner use. This result may indicate that metabolic events leading to flesh breakdown had occurred before fruit removal from cold storage; therefore, a higher incidence after the shelf life was not related to the poststorage IEC.

Physiological responses of fruit quality attributes to storage humidity were highly divergent, depending on fruit physiological characteristics. In this study, high storage humidity by using a liner application resulted in reduced fresh weight loss, delayed decline in peel $L^*$ and flesh crispness, and suppressed increase in IEC after storage. However, the liner resulted in enhanced fruit circumference change and firmness loss. High storage humidity contributes to delaying fresh weight loss (Tu et al., 2000). The alteration of fruit firmness was greater in high humidity in several ‘Gala’ apple strains (Weber et al., 2012). Furthermore, storage humidity may not be involved in CO$_2$ production and peel $C^*$ responses, but shelf life affected those. Peel $h^*$ and $I_{AD}$ were unaffected by liner use nor by shelf life. The results indicate that the divergence of fruit quality attributes to higher storage humidity would be influenced by fruit ripening and senescence during shelf life.

Cortex tissue color variables ($L^*$, $C^*$, and $h^*$) were unaffected by high storage humidity during long-term cold storage, but they were significantly influenced during shelf life. These changes may result from accelerated fruit ripening and senescence during shelf life, compared with that occurring during long-term cold storage. The correlation

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**Table 5. Incidence and severity of shriveling, cracking, and flesh breakdown in ‘Royal Gala’ apple fruit stored in air at 0.5 °C for 6 mo. followed by 7 d at 20 °C.**

| Storage duration | Shriveling | Cracking | Flesh breakdown |
|------------------|------------|----------|----------------|
|                  | Incidence (%) | Severity (0–5) | Incidence (%) | Severity (0–5) | Incidence (%) | Severity (0–5) |
| 6 mo. + 0 d       | Control Liner | Control Liner | Control Liner | Control Liner | Control Liner | Control Liner |
| 6 mo. + 7 d       | 88.9 0.0 1.9 0.0 | 0.0 b 5.6 b 0.0 b 0.1 b | 44.4 16.7 0.5 0.3 |

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*The incidence of physiological disorders was expressed as the percent of fruit affected (n = 18).

*Severity rate of physiological disorders was subjectively evaluated by estimating the percentage of each incidence: 0 = 0%, 1 = 1% to 10%, 2 = 11% to 25%, 3 = 26% to 50%, 4 = 51% to 75%, and 5 = 76% to 100%.

*Means of each category followed by the same letters do not differ significantly. Duncan’s multiple range test at $P \leq 0.05$.

**NS, *,** **Non-significant or significant at $P < 0.05$, 0.01, or 0.0001, respectively.**
analysis linked cortex color variables with the incidence and severity of flesh breakdown, indicating that high storage humidity influences fruit ripening and senescence during shelf life. High storage humidity also differentially affected cortex tissue color variables, especially $h^\circ$, where the change in color was highest in stem-end cortex tissues. As reported previously (Lee et al., 2013), stem-end cortex tissues are where ‘Gala’ flesh breakdown symptoms first appear; however, due to the long cold storage period in this study, symptoms were well developed from stem- to calyx end. In long-term CA stored ‘Empire’ apples, $L^*$ values were much darker in stem-end regions than in calyx-end regions in which the color response coincided with internal browning incidence (Lee et al., 2012). Development of browning in cortex tissues has been linked to peroxidase activity (Ma et al., 2015). As peroxidase activity is associated with fruit ripening (Ingham et al., 1998) as well as internal browning (Richard-Forget and Gauillard, 1997), stem-end cortex tissues may be more susceptible to flesh breakdown, compared with calyx-end cortex tissues as ripening in ‘Gala’ tends to progress from the stem to calyx ends (Opara et al., 1997a).

In general, early harvest apple cultivars such as Gala could have much shorter storability and marketability at lower storage temperature than late harvest apple cultivars such as Fuji or Delicious (Lee et al., 2013; Watkins et al., 2000; Yoo et al., 2016). Nevertheless, new storage technologies such as 1-MCP have been introduced to extend fruit storability and marketability, particularly in combination with CA storage (Watkins et al., 2000). 1-MCP technology is routinely applied for retaining fruit quality during cold storage and CA storage (Mattheis, 2008). As done in this study,
polyethylene liner application or MA packaging (MAP) is an effective technology to maintain fruit quality and freshness along with conventional cold storage (Kim et al., 2018; O’Loughlin, 1975). These single post-harvest management technologies would not be sufficient enough to guarantee fruit marketability and consumer acceptability. Use of the combined technologies increases the likelihood of high fruit quality after storage compared with application of each technology alone.

In conclusion, high storage humidity achieved through the use of perforated polyethylene box liners influenced incidence of fruit shriveling and cracking of ‘Royal Gala’ apples during and after cold storage, respectively. In contrast, flesh breakdown was unaffected by humidity. The loss of fresh weight was reduced by higher storage humidity as it was shriveling during storage. High storage humidity resulted in increased fruit circumference and cracking during storage and shelf life. The decrease in flesh firmness in liner fruit may be associated with enhanced fruit ripening under high humidity, although flesh breakdown incidence was not influenced by humidity. In total, high humidity during ‘Royal Gala’ storage differentially impacts fruit quality and disorder development during and after removal from cold storage.

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