Textural property and cell wall metabolism of ‘Golden Bosc’ and ‘d’Anjou’ pears as influenced by oxygen regimes after long-term controlled atmosphere storage

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1. Introduction

‘Golden Bosc’ and ‘d’Anjou’ are European pear cultivars (*Pyrus communis* L.) extensively grown in the Pacific Northwest of United States (U.S.). The recommended harvest maturity for ‘Golden Bosc’ and ‘d’Anjou’, as indicated by flesh firmness (FF), is 62–71 N and 58–67 N, respectively (Hansen and Mellenthin, 1979; Sugar, 2007). The two cultivars require ‘21 and ‘60 d of low temperature conditioning, respectively, to attain ripening competency. Well-managed pears develop a buttery-juicy texture, sweetness, and full flavor with low rate of postharvest disorders during marketing and retailing (Chen et al., 1983, 1986; Sugar and Basile, 2009; Sugar and Einhorn, 2011; Dong et al., 2018). ‘Golden Bosc’ and ‘d’Anjou’ pears can maintain desirable textural properties when stored at −1.1 °C for a maximum of ‘4 and ‘6 months, respectively (Sugar, 2007; Xie et al., 2014, 2017). However, fruit quality tends to deteriorate more rapidly in long storage (i.e. > 10 months). Specifically, consumers report an unpleasant taste, and coarse and dry (mealy) texture in pears stored beyond the optimum duration (Dong et al., 2018). Therefore, pear growers and packers face significant quality issues when attempting to extend the marketing window as fresh-market demand increase, while still offering fresh-eating quality.

Controlled atmosphere (CA) is the primary postharvest storage method in the Pacific Northwest regions. For about 17 years, the Pear Bureau Northwest has encouraged retailers and packers to build CA rooms (Kevin Moffitt, personal communication). This practice has been shown to extend fruit storage life when O2 concentrations are reduced to 1–3%, ethylene gas is removed, and pathogen germination/growth is inhibited (Bai et al., 2006; Kader, 2007; Lum et al., 2017; Zhi et al., 2019). The commercial standard CA regimes for ‘Golden Bosc’ and

Keywords:

- *Pyrus communis*
- Controlled atmosphere
- Melting texture
- Cell wall polyuronides
- Cell wall-modifying enzymes

ABSTRACT

Controlled atmosphere (CA) allows long-term storage of European pears (*Pyrus communis* L.) without chemical treatment to deliver a natural melting (buttery and juicy) texture for consumers. However, the relationship between textural properties and cell wall metabolism has not been comprehensively determined. In this study, ‘Golden Bosc’ and ‘d’Anjou’ pears were stored in 21 (air), 2, 1, or 0.5% O2 with < 0.5% CO2 for up to 8 and 10 months at −1.1 °C plus 7 d of ripening at 20 °C, respectively. Melting texture development in both cultivars showed high correlations with the level of water-soluble polyuronides (WSP) and activity of β-galactosidase (β-GAL). Also, activities of pectin methylesterase (PME) and α-arabinofuranosidase (α-ARF) were associated with softening in ‘Golden Bosc’ pears. Concentrations of CDTA-soluble polyuronides (CSP) and WSP + CSP + sodium carbonate-soluble polyuronides (SSP), as well as ethylene production showed positive correlations to melting texture in ‘d’Anjou’ pears. Reducing O2 concentrations from 21% to 2–1% and 1–0.5% in ‘Golden Bosc’ and ‘d’Anjou’ pears allowed pears to develop a desirable eating texture during ripening for up to 8 and 10 months, respectively, with a commercially acceptable levels of postharvest disorders. Use of 1–0.5% O2 resulted in a melting texture in ‘Golden Bosc’ pears, but fruit developed more core browning when ripe. These results indicate that O2 regimes regulate textural qualities by influencing WSP level and β-GAL activity.

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2.2. Evaluation of fruit quality

CO2 at cultivar were loaded into each of 18 gas-tight cabinets, then sealed and cold storage room at (any visible signs of damage or fungal infection and were uniform in size per cabinet). Concentrations of O2 and CO2 were monitored every 2 d were maintained at < 0.5% by adding su modifying enzymes (Chen and Borgic, 1985; Arpaia et al., 1987; Chen and Varga, 1997; Bai et al., 2009). Reduced O2 concentrations and pithy brown core of sensitive cultivars (Mellenthin et al., 1980; Siddiqui et al., 2004; Ortiz et al., 2011a). However, the relationship between textural properties and cell wall metabolism during ripening of 'Golden Bosc' or 'd'Anjou' pears as influenced by different O2 regimes has not yet been elucidated.

An accurate description of the development of a melting or mealy texture and cell wall metabolism in CA stored pears would be lead to a better understanding of the softening processes after long-term storage. Therefore, we examined the changes of FF, texture quality, pectic polyuronides, cell wall-modifying enzymes, ethylene production, and related physiological disorders in 'Golden Bosc' and 'd'Anjou' pears during ripening after storage at −1.1 °C with 21 (air), 2, 1, or 0.5% O2 and < 0.5% CO2 for 8 and 10 months, respectively.

2. Materials and methods

2.1. Fruit materials and experimental design

'Golden Bosc' and 'd'Anjou' pears were hand-harvested at FF of 62.2 and 65.1 N, respectively, 1 d before commercial harvest from mature trees from the orchard of the Mid-Columbia Agriculture Research and Extension Center in Hood River, OR, USA (45.71 °N, 121.51 °W, elevation 150 m and average annual rainfall ~800 mm). Fruit were free of any visible signs of damage or fungal infection and were uniform in size and color. The fruit were transferred into forty-eight 18-kg plastic boxes (70 fruit per box) with standard polyethylene bags (12 holes with 3 mm diameter were made nearby bottom of the bags) and placed in a cold storage room at −1.1 °C. After 2 d of storage, four boxes of each cultivar were loaded into each of 18 gas-tight cabinets, then sealed and flushed with pure nitrogen (N2) generated from a membrane gas generator (CPA-5, Permea, St. Louis, MO, USA). CO2 concentrations were maintained at < 0.5% by adding sufficient hydrated lime (0.5 kg per cabinet). Concentrations of O2 and CO2 were monitored every 2 d using an O2/CO2 analyzer (Storex, Gravendeel, Netherlands). After sealing the cabinets, three replicates of fruit were established at 2, 1, or 0.5% O2 and 90–94% relative humidity in each cabinet within 6 d. Air stored fruit were stored in a cold storage room at 21% O2 and −1.1 °C. 'Golden Bosc' and 'd'Anjou' pears were sampled at 4, 6, or 8 months and 6, 8, or 10 months, respectively. A sample of 165 fruit per treatment at each time was transferred to 20 °C. CA conditions were re-established within 3 h by flushing cabinets with N2. Thirty fruit were evaluated for FF on day 1, 90 fruit were evaluated for sensory quality and physiologial disorders after 7 d, and 45 fruit were used for evaluation of FF, pectic polyuronides, cell wall-modifying enzymes, ethylene production and respiration rates on day 7. Flesh tissue from each treatment were quick-frozen and ground in liquid N2, then stored at −80 °C.

2.2. Evaluation of fruit flesh firmness, ripening capacity, and sensory quality

Flesh firmness (FF) of 10 fruit per replicate was measured at 20 °C on days 1 and 7 after removal from cold storage. FF was measured on opposite sides of the equator of each fruit after removing 2-mm thick peel discs using a texture analyzer (model GS-14, Güüs Manufacturing Ltd., Strand, South Africa) equipped with a 8-mm probe and a penetration speed of 10 mm s−1. The maximum force was recorded and expressed in newtons (N). Ripening capacity was identified as fruit FF < 24 N after 7 d at 20 °C and determined as competency of fruit softening.

Sensory quality (melting/mealy texture score) of fruit on day 7 was evaluated on a 5-point hedonic scale (Dong et al., 2018) by ten experienced panelists. Fruit with highly, moderately, or slightly melting texture were rated as 5, 4, or 3, respectively, and those rated as moderately or very firm (i.e., underripe pears) or moderately or very mealy texture (i.e., overripe pears) were rated as 2, or 1, respectively. Scale anchor points and definitions were determined in an orientation session before the first evaluation. Each panelist tasted one small fruit slice from each treatment of the five fruit.

2.3. Extraction and measurement of cell wall polyuronides

Flesh powder (15 g) from 10 fruit per replicate were homogenized in 30 mL of 80% ethanol using a hand-held homogenizer (D1000, Benchmark Scientific Inc., Sayreville, NJ, USA), then boiled for 30 min, as describe by Murnayama et al. (2002). After centrifugation at 8000 g for 15 min at 20 °C, the residue was treated with 30 mL of 100% ethanol and again centrifuged. Next, the residue was suspended in 100% acetone, centrifuged, and repeated. An alcohol insoluble residue (AIR) fraction was obtained by air drying at 20 °C for 48 h. AIR (50 mg) were fractionated with distilled water and continuously shaken for 1 h, then centrifuged. This procedure was repeated and supernatants were collected as water-soluble polyuronides (WSP). The residue was then treated with 50 mM sodium acetate (pH 6.5) containing 50 mM cyclohexanetrans-1, 2-diaminotetra (CDTA) and shaken for 1 h. This procedure was repeated and the resultant supernatants were collected as CDTA-soluble polyuronides (CSP). Finally, the residue was suspended in 50 mM sodium carbonate containing 10 mM sodium borohydride, and subjected to shaking and centrifugation. The supernatants were collected as sodium carbonate-soluble polyuronides (SSP).

Uronic acids were determined as described by Blumenkranz and Asboe-Hansen (1973). Each extracted polyuronides solution (200 µL) was added to H2SO4 solution with 75 mM sodium borate. After boiling, 0.15% (w/v) m-phenylphenol was added to the mixture and loaded into a 96-well plate. Absorbance was measured at 550 nm using a plate reader (ELx800, Bio-Tek Instruments Co., Winooski, VT, USA). A standard calibration curve was constructed using galacturonic acid and the data were expressed as mg kg−1 on a fresh weight basis.

2.4. Activities of cell wall-modifying enzymes

Polygalacturonase (PG) was measured according to the method described by Gross (1982). Power samples (500 mg) from 10 fruit per replicate were homogenized in 0.3 M NaCl and centrifuged at 10,000g for 15 min at 4 °C. The supernatant (50 µL) was added to a solution of 0.1% (w/v) polygalacturonic acid and 50 mM acetic-acid-sodium acetate buffer (pH 4.5), then incubated at 37 °C for 2 h. After adding 0.1 M borate buffer (pH 9.0) and 1% (w/v) 2-cyanoacetamide, the mixture was boiled for 10 min. One unit of PG activity was defined as 1 mg of galacturonic acid released per min at 276 nm absorbance using a UV/visible spectrophotometer (Ultrospec 3100 pro, Biochrom Ltd, Cambridge, UK). Data were expressed on a fresh weight basis.

Pectin methylsterase (PME) was measured according to the method described by Basak and Ramaswamy (1996). Power samples (4 g) from 10 fruit per replicate were homogenized in 0.3 M NaCl and centrifuged. The supernatant (50 µL) was added to distilled water and continuously shaken for 1 h. After centrifugation at 10,000 g for 15 min at 4 °C, the supernatant (50 µL) was added to a solution of 0.1% (w/v) polygalacturonic acid and 50 mM acetic-acid-sodium acetate buffer (pH 4.5), then incubated at 37 °C for 2 h. After adding 0.1 M borate buffer (pH 9.0) and 1% (w/v) 2-cyanoacetamide, the mixture was boiled for 10 min. One unit of PG activity was defined as 1 mg of galacturonic acid released per min at 276 nm absorbance using a UV/visible spectrophotometer (Ultrospec 3100 pro, Biochrom Ltd, Cambridge, UK). Data were expressed on a fresh weight basis.
ester hydrolyzed released per min on a fresh weight basis. Pectate lyase (PL) was measured according to the method described by Chourasia et al. (2006). Power samples (1.5 g) from 10 fruit per replicate were homogenized in 50 mM Tris-HCl (pH 8.5) containing 0.6 mM CaCl2, 5 mM EDTA and 0.05% (v/v) Triton X-100 and centrifuged. The supernatant (0.2 mL) was added to 50 mM Tris-HCl (pH 8.5) containing 0.6 mM CaCl2 and 0.24% (w/v) polygalacturonic acid, then incubated at 37 °C for 30 min and boiled for 10 min. One unit of PL activity was defined as 1 mM of 4, 5-unsaturated product at 232 nm on a fresh weight basis. The molar extinction coefficient for the 4, 5-unsaturated product at 232 nm is 4600 M⁻¹ cm⁻¹.

β-galactosidase (β-GAL) and α-arabinofuranosidase (α-ARF) were measured according to the methods described by Brummell et al. (2004) and Sozzi et al. (2002). Power samples (1.5 g) from 10 fruit per replicate were homogenized in 0.3 M NaCl, then centrifuged. The supernatant (0.4 mL) was added to 0.04% (w/v) p-nitrophenyl-β-D-galactoside or p-nitrophenyl-α-D-arabinofuranoside. After incubation at 37 °C for 30 min and boiled for 10 min. One unit of β-GAL and α-ARF activity were defined as 1 mg of p-nitrophenol released per min at 400 nm on a fresh weight basis.

2.5. Ethylene production and respiration rates

Ethylene production (EPR) and respiration rates (RR) were measured from each treatment of five fruit per replicate on day 7 at 20 °C. Fruit were sealed in a 3.8-L jar at 20 °C for 1 h. One mL of gas sample was withdrawn and injected into a gas chromatograph (GC-8A, Shimadzu, Tokyo, Japan) equipped with a flame ionization detector. The injection and detector port temperature were 90 °C and 140 °C, respectively. The carrier gas was nitrogen at a flow rate of 0.8 mL s⁻¹. Ethylene production rate was expressed as ng kg⁻¹ s⁻¹. Headspace CO2 was measured with a CO2 analyzer (900161, Bridge Analyzers Inc., Alameda, CA, USA) and expressed as CO2 μg kg⁻¹ s⁻¹.

2.6. Evaluation of physiological disorders

Core browning of ‘Golden Bosc’ pears and superficial scald of ‘d’Anjou’ pears was evaluated from each treatment of 30 fruit per replicate on day 7 at 20 °C. ‘Golden Bosc’ pears were cut longitudinally and transversely to assess core browning. Any browning in the core tissue was considered core browning and as expressed as percentage of incidence. Superficial scald incidence was expressed as the percentage of fruit affected with approximately 0.6 cm² or more peel area on the entire surface of pear.

2.7. Statistical Analysis

Experiments were performed using a completely randomized design. One-way analysis of variance (ANOVA) was carried out to determine the significance of differences at P < 0.05 according to Fisher’s protected LSD test at P < 0.05, n = 3.
Fishers protected least significant difference (LSD) test using IBM SPSS Statistics (IBM Co., Armonk, NY, USA). A principal component analysis (PCA) was performed using Origin 2017 software (OriginLab, Northampton, MA, USA). PCA was carried out among treatments of each cultivar and represented the associations among FF on day 7 at 20 °C, sensory score of textural quality (TQ), cell wall polyuronides (WSP, CSP, SSP, and WSP + CSP + SSP), cell wall-modifying enzymes (PG, PME, PL, β-GAL, and α-ARF), and EPR during storage. Principle components (PC) with variance under 10% or eigenvalues under 1.0 were dropped from the data (Supplement 1 and 2).

3. Results

3.1. Flesh firmness, ripening capacity, and textural quality

During 4–8 months of storage at −1.1 °C, air stored ‘Golden Bosc’ pears softened, while FF gradually increased from 25 to 30 N after fruit were held at 20 °C for 7 d, indicating a loss in ripening capacity (> 24 N) (Fig. 1A). Sensory scores of textural quality decreased from 3.7 to 2.1; these fruit developed a mealy texture (Fig. 1C). Decreasing O2 concentrations from 21% to 2, 1, or 0.5%, fruit maintained relatively higher FF than air stored fruit after 1 d subjected to 20 °C and developed ripening capacity (< 24 N) with melting texture during ripening after 4–6 months of storage. Beyond 6 months, softening in 2% O2 stored fruit was impaired; the FF increased from 18 to 25 N with a moderately mealy textural quality (sensory score at 2.9). In contrast, one and 0.5% O2 stored fruit maintained ripening capacity with the melting texture until 8 months. For ’dAnjou’, the FF of air stored fruit decreased faster than CA stored fruit on day 1 at 20 °C after removal from storage, but fruit developed ripening capacity after storage for 6 to 8 months. Beyond 8 months, FF on day 7 at 20 °C increased from 21 to 27 N with an intermediate softening and mealy texture (Fig. 1B and D). Fruit stored at 1 or 2% O2 maintained ripening capacity with buttery and juicy properties throughout the entire storage period. The FF of 0.5% O2 stored fruit decreased from 39 to 28 N by day 7, but the fruit did not ripen.

3.2. Pectic polyuronides

WSP in air and 2% O2 stored ‘Golden Bosc’ pears decreased from 56 to 33 mg kg−1 and from 58 to 39 mg kg−1, respectively, over 8 months of storage plus 7 d at 20 °C (Fig. 2A). Storage in 0.5 or 1% O2 maintained higher WSP over the entire storage period, and differences between these two treatments were not detected. CSP in air stored fruit decreased from 43 to 34 mg kg−1 after 8 months (Fig. 2C). By contrast, CSP increased in low O2 concentrations. SSP was unaffected by treatment (Fig. 2E). For ’dAnjou’, the air and 2% O2 stored fruit maintained relatively higher SSP and WSP between 6–8 months storage, but afterwards decreased until 10 months (Fig. 2B and D). Levels of WSP and CSP in 0.5 or 1% O2 stored fruit markedly increased throughout storage. Compared with the air stored fruit, CA stored fruit had higher SSP, which remained relatively constant (Fig. 2F).

3.3. Cell wall-modifying enzymes

For ‘Golden Bosc’, PG activity increased during storage and no differences were observed among treatments (Fig. 3A). Air stored fruit had lower activities of PME, β-GAL, and α-ARF after 4 months; these activities decreased with each additional 2 months of storage (Fig. 3C, G, and I). Low O2 concentrations inhibited the decrease of the activities of the enzymes, especially in 0.5% O2 treatment, which remained relatively constant and displayed higher activity throughout the entire storage period. Similar to PG, PL activity increased in all fruit; air stored fruit had the highest PL activity after 8 months (Fig. 3E). For ’dAnjou’, the air stored fruit had increasing in PG activity during storage, while activities of PME, PL, β-GAL, and α-ARF decreased (Fig. 3B, D, F, H, and J). Low O2 treatments maintained relatively stable activities of PG and α-ARF throughout the 8-month storage period, and no difference was observed among treatments. Decreasing O2 concentrations from 2% to 0.5% resulted in higher activity of PME, but activities of PL and β-GAL were inhibited after 6 months. However, an increase in the activities of PL and β-GAL was observed in 0.5 and 1% O2 stored fruit.

3.4. Ethylene synthesis and respiration rate

For ‘Golden Bosc’, EPR and RR of air stored fruit decreased from 22 to 19 ng kg−1 s−1 and from 30 to 20 CO2 μg kg−1 s−1, respectively, during 4–8 months storage plus 7 d at 20 °C (Fig. 4A and C). Compared with the air stored fruit, low O2 resulted in lower EPR and RR. After 8 months of storage, the EPR of the 2% O2 stored fruit decreased, although the RR continued to increase. For ’dAnjou’, the EPR in air stored fruit decreased from 5 to 1 ng kg−1 s−1, while the RR increased from 11 to 13 CO2 μg kg−1 s−1 (Fig. 4B and D). Low O2 treatments had lower EPR and RR at six months of storage, but they increased thereafter. The EPR of 2% O2 stored fruit increased, reaching a maximum of 7 ng kg−1 s−1 after 8 months of storage, before decreasing to 4 ng kg−1 s−1. The EPR of 0.5 and 1% O2 stored fruit continued to increase after 10 months of storage, reaching 3 and 5 ng kg−1 s−1, respectively.

3.5. PCA of softening-related parameters, cell wall polyuronides, cell wall-modifying enzymes, and EPR influenced by O2 regimes during storage

For ‘Golden Bosc’, three principal components accounted for 92.60% of total variation of the data with 63.86% from PC1, 18.10% from PC2, and 10.64% from PC3 (Supplement 1). The TQ, WSP, PME, β-GAL, and α-ARF were highly positively correlated with PC1, whereas FF on day 7 at 20 °C was negatively correlated, suggesting that PC1 might be associated with melting texture development (Supplement 2). With increasing period of storage, scores of the air stored fruit rapidly moved away from TQ, while low O2 stored fruit showed a slow movement, especially in 0.5% O2 treatment (Fig. 5A). For ’dAnjou’, two principal components accounted for 80.82% of total variation of the data with 52.88% from PC1 and 27.94% from PC2. The TQ, WSP, CSP, WP + CSP + SSP, β-GAL, and EPR were highly positively correlated with PC1, whereas FF on day 7 at 20 °C and PG was negatively correlated. Over the 6 to 10 months storage period, scores of air and 2% O2 stored fruit moved away from TQ, while scores of 0.5 and 1% O2 stored fruit moved towards TQ (Fig. 5B).

3.6. Postharvest disorders

The air and 2% O2 stored ‘Golden Bosc’ pears were free of core browning throughout the 8-month storage period (Fig. 6A). Core browning was first found in 0.5 and 1% O2 stored fruit at 6 months, increasing to 36 and 21%, respectively, by month 8. For ’dAnjou’, the air stored fruit displayed 100% of scald damage during 6–10 months of storage (Fig. 6B). The 2% O2 stored fruit developed 68% scald at 6 months and increased to 90% after 10 months. Lower O2 concentrations of 0.5 and 1% inhibited the development of scald, 0.5% O2 stored fruit remaining free of superficial scald throughout the entire storage period.

4. Discussion

4.1. Impact of O2 regimes on melting texture development and ethylene synthesis

After exposure to temperature of less than 1 °C for a length of time, conditioned pears can soften and reach a desirable eating quality characterized by a buttery and juicy properties with full flavor at ambient temperature (Sugar and Basile, 2009). However, it is difficult to manage storage beyond a maximum time and develop the melting texture. European pears are prone to losses of ripening capacity, which
results in a coarse and dry (mealy) texture with off flavor and a high rate of disorders; these conditions diminish repeated purchase by consumers (Chen et al., 1983; Ma and Chen, 2003; Gallardo et al., 2011; Dong et al., 2018). In this study, ‘Golden Bosc’ and ‘d’Anjou’ pears stored in air developed a melting texture after storage at −1.1 °C for 4 and 8 months, respectively. When stored for ≥8 months, both cultivars lost ripening capacity with fruit texture remaining a firm, coarse, and dry (mealy), as was previously observed in ‘Comice’ and ‘d’Anjou’ pears that were incapable of softening during ripening after 8 months of regular-air storage (Dong et al., 2018). The cause of inferior texture was initially thought to be due to a rapid decline in the rate of ethylene production, which prevented development of a melting pattern of softening after excessive long-term storage (Hiwasa et al., 2003; Obenland et al., 2008). Our results support the observation that a decreased EPR was accompanied by the development of a mealy texture in both cultivars of air stored fruit. Therefore, to maintain ripening capacity after long-term storage, an effective means to manage ethylene biosynthesis during fruit storage must be identified.

In Washington and Oregon, CA is the primary storage method and provides tremendous benefits in lowering the rates of ethylene production and respiration, reducing ethylene action, and delaying ripening and senescence processes (Chen and Borgic, 1985; Chen and
Fig. 3. Activity of polygalacturonase (PG), pectin methylesterase (PME), pectate lyase (PL), \( \beta \)-galactosidase (\( \beta \)-GAL), and \( \alpha \)-arabinofuranosidase (\( \alpha \)-ARF) in 'Golden Bosc' (A, C, E, and G) and 'd'Anjou' (B, D, F, and H) pears during storage in 21% (air), 2%, 1%, and 0.5% O2 (with < 0.5% CO2) at \(-1.1^\circ C\) plus 7 d at 20°C. Values are presented as the means ± SD, \( n = 3 \).
Low-O₂ atmospheres inhibit the production of ethylene by stored pears. Low ethylene concentrations in CA environment thus stimulates fruit softening (Villalobos-Acuña and Mitcham, 2008; Ma and Chen, 2003; Zhi et al., 2019). In this study, 1 and 2% O₂ stored ‘d’Anjou’ fruit were capable of

Fig. 4. Ethylene production (EPR) and respiration rates (RR) in ‘Golden Bosc’ (A and C) and ‘d’Anjou’ (B and D) pears during storage in 21% (air), 2%, 1%, and 0.5% O₂ (with < 0.5% CO₂) at -1.1 °C plus 7 d at 20 °C. Values are presented as the means ± SD, n = 3.

Fig. 5. Principal component analysis of FF on day 7 at 20 °C, sensory scores of textural quality (TQ), cell wall polyuronides (WSP, CSP, SSP, and WSP + CSP + SSP), cell wall-modifying enzymes (PG, PME, PL, β-GAL, and α-ARF), and EPR in ‘Golden Bosc’ (A) and ‘d’Anjou’ (B) pears during storage in 21% (air), 2%, 1%, and 0.5% O₂ (with < 0.5% CO₂) at -1.1 °C. Scores of 21 (square), 2 (circle), 1 (triangle), and 0.5 (reverse triangle) % O₂ stored fruit after 4 (red), 6 (yellow), and 8 (green) months of storage for ‘Golden Bosc’ pears and after 6 (red), 8 (yellow), and 10 (green) months of storage for ‘d’Anjou’ pears are in biplot (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).
ripening normally with desirable eating quality over the entire storage period. Conversely, development of a melting texture was not noted in 0.5% O2 stored pears, indicating that this concentration contributed greatly to decreasing internal or external ethylene of the fruit. As a result, in the minimum ethylene ripening requirement had not been met after removal from CA and, ultimately, these fruit failed to develop a buttery/melting texture. However, the 0.5% O2 stored ‘Golden Bosc’ pears developed a normal fruit-softening pattern over the 8 months storage. Chen et al. (1982) reported that ‘Bosc’ pears had higher EPR than ‘d’Anjou’ pears during any period of cold storage, suggesting that the overall pathway of ethylene biosynthesis in ‘Bosc’ were more sensitive to chilling. In this study, even though the lower O2 concentration reduced the sensitivity of ethylene response in ‘Golden Bosc’, a longer chilling period stimulated ethylene production inside the fruit and drove the ripening process.

4.2. Impact of O2 regimes on cell wall polyuronides and related modifying enzymes

Consumers enjoy European pears because of their buttery and juicy textural properties with full flavor when ripe. Development of this melting texture is primarily due to an increase in WSP and the activities of enzymes associated with cell wall degradation (Chen et al., 1985; Hiwasa et al., 2004; Murayama et al., 2002, 2006; Dong et al., 2018). WSP shows a strong hygroscopic binding capacity to absorb and retain water, and as a result, allows pears to develop the buttery and juicy mouth feel preferred by consumers (Ma and Chen, 2003). In this study, high concentrations of WSP were measured in ‘Golden Bosc’ and ‘d’Anjou’ pears stored in air after 4 and 6 months of storage plus 7 d at 20 °C, respectively. However, after long-term storage, a decline in WSP paralleled the observed decrease in FF on day 7 at 20 °C and sensory scores of textural quality. This finding was in line with our previous study, which demonstrated higher level of WSP in fruit contributed to the development of melting texture (Dong et al., 2018). However, it is unknown why ‘Golden Bosc’ pears did not show trends of WSP increase similar to those of ‘d’Anjou’ pears after they were subjected to different O2 treatments. For example, lowering O2 concentrations to 0.5 or 1% maintained WSP of ‘Golden Bosc’ pears at high concentrations over the entire storage period. At those O2 concentrations, WSP development in ‘d’Anjou’ pears was suppressed through month 6, before increasing by month 10. These results suggested that the lower O2 concentration reduced the solubility of pectin polyuronides in ‘d’Anjou’ pears, but not in ‘Golden Bosc’, and therefore the ‘d’Anjou’ fruit remained firmer earlier during the ripening test. It may be that a longer chilling period or other regulatory mechanisms such as the activation of cell wall-modifying enzymes induced a stronger cell wall degradation response after long-term storage. In apple fruit, higher yields of CSP and SSP are related to higher flesh firmness (Ortiz et al., 2011b). In this study, CSP showed a strong relation to melting texture development in ‘d’Anjou’ pears, but not in ‘Golden Bosc’. Moreover, the change in SSP was not in accordance with the FF loss on day 7 at 20 °C in either cultivar. Whether the changes in CSP or SSP affected pear softening requires further exploration and clarification.

Softening of pears is not only attributed to the solubilization and depolymerization of pectic polyuronides, but also associated with activity of cell wall-modifying enzymes (Brummell and Harpster, 2001; Ruiz-May and Rose, 2013). It was noted that the TQ, representative of melting texture development, in ‘Golden Bosc’ pears was strongly associated with the level of WSP and activities of PME, β-GAL, and α-ARF, and in turn inversely related to the FF on day 7 at 20 °C. For ‘d’Anjou’, TQ was strongly associated with the levels of WSP, CSP and WSP + CSP + SSP, β-GAL activity, and EPR, while inversely related to FF on day 7 at 20 °C and PG activity. Previous studies indicated that PME was a critical cell wall-modifying enzyme affecting development of melting texture in ‘Comice’ and ‘d’Anjou’ pears (Dong et al., 2018). In CA stored apple fruit, although PME contributed to cell wall degradation, no relative PME gene family was identified to regulate softening. Instead, β-GAL activity and the expression of related-gene (Mdβ-GAL2) were closely related to ripening (Gwanpua et al., 2016). Our study further demonstrated that the activity of β-GAL played a central role in determining that melting texture development was managed by O2 concentrations. In ‘Golden Bosc’ and ‘d’Anjou’ pears, the lower O2 concentrations decreased the activity of β-GAL at month 4 and 6, respectively. However, prolonged storage might recover the β-GAL activity, achieving the buttery and juicy textural properties. In addition, the strong correlation between β-GAL and textural properties in both cultivars, α-ARF contributed to melting texture development of ‘Golden Bosc’, which might be associated with the cultivar-specific response. This possibility will be considered and investigated in a future study.

4.3. Impact of O2 regimes on postharvest disorders

Irrespective of the textural properties of pears, retail chains have no tolerance for any physiological disorders in fruit offered for sale. In this study, the onset of core browning of 0.5 and 1% O2 stored ‘Golden Bosc’ pears occurred after 6 months, and reached severe levels by 8 months. By contrast, the air and 2% O2 stored fruit were free of disorders, indicating that ‘Golden Bosc’ pears were more sensitive to lower O2.
concentrations (< 2%), was confirmed in previous work in our laboratory (Chen et al., 1986). However, 0.5 and 1% O2 concentrations were more efficient in controlling superficial scald in ‘d’Anjou’ pears. Thus, to deliver higher-quality pears to consumers after long-term CA storage, O2 concentrations dropped to less than 1%, pears still maintained ripening activities of PME, and its manipulation in transgenic plants. Plant Mol. Biol. 47, 311–340. https://doi.org/10.1023/A:1016056104304.


Villalobos-Acuiña, M., Mitcham, E.J., 2008. Ripening of European pears: the chilling di-
postharvbio.2008.03.003.

Wang, Y., 2016. Storage temperature, controlled atmosphere, and 1-methylcyclopropene
effects on α-farnesene, conjugated trienols, and peroxidation in relation with su-
perficial scald, pithy brown core, and fruit quality of ‘d’Anjou’ pears during long-term

Xie, X., Song, J., Wang, Y., Sugar, D., 2014. Ethylene synthesis, ripening capacity, and
superficial scald inhibition in 1-MCP treated ‘d’Anjou’ pears are affected by storage
postharvbio.2014.06.002.

Xie, X., Fang, C., Wang, Y., 2017. Inhibition of ethylene biosynthesis and perception by 1-
methylcyclopropene and its consequences on chlorophyll catabolism and storage
21273/JASHS04017-16.

Zhi, H., Dong, Y., Wang, Y., 2019. Effects of controlled atmosphere, edible coating, and 1-
methylcyclopropene on improving storage quality of ‘Bartlett’ pears after long-term
2018.1450098.