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# Cold storage conditions affect ‘Shepard’ avocado fruit ripening and quality

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**ABSTRACT**

There has been little research into long storage of ‘Shepard’ avocado. Simulated conditions for sea freight of Australian-grown fruit to key Asian markets were investigated. Thirteen storage treatments were applied to fruit of premium quality sourced from two commercial farms. Compared with the generally recommended 7°C, fruit stored at 5.5°C for up to 28 days were firmer at removal, slow to ripen, and had less severe flesh rots at the ripe stage. Compared with regular air storage (Air), controlled atmosphere (CA; 2% $O_2$ and 5% $CO_2$) decreased weight loss at removal, retarded ripening and, after 28 days of storage, reduced discrete patches on the skin of ripe fruit. However, CA storage also resulted in ripe fruit with more severe shrivel, stem end rot, and vascular browning. For 14 or 21 days at 5.5°C, Air resulted in the best overall quality of ‘Shepard’ fruit. CA was comparatively advantageous after a 28-day storage in improving external quality at ripe. There was considerable variation between both farms in storage effects on fruit ripening and quality. Given the high variability in fruit chilling responses apparently dependent on preharvest factors, further research is warranted to elucidate these results and better evaluate risks under commercial sea freight conditions.

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**KEYWORDS**
*Persia americana* Mill.; storage; controlled atmosphere; fruit quality; fruit ripening

## Introduction

Avocado (*Persia americana* Mill.) is an important horticultural crop worldwide. In Australia, ‘Shepard’ is the second main cultivar after ‘Hass’, with a market share of approximately 20% by volume (Anon, 2023). ‘Shepard’ is an early-maturity fruit and is a green-skin type with a thinner skin compared to ‘Hass’, which does not darken as the fruit ripens (Crane et al., 2013). The fruit is reported to be more resistant to diseases than other green-skin cultivars (Whiley, 1987) and to have an overall higher proportion of fruit with no flesh defects at retail display compared to ‘Hass’, as per surveys in Australia (Petty & Embry, 2011). The flesh does not discolour readily when cut, in addition to having excellent eating quality (Crane et al., 2013). It is the main cultivar in Australia between February to April when there is limited supply of ‘Hass’ fruit in the market and due to previous strong market promotion (Anon, 2023; Kernot, 1995).

In 2021–22, Australia exported approximately 10% of its fresh avocado production, mainly to South-East Asia (Anon, 2023). Global competitiveness coupled with higher air freight costs during and after the recent pandemic have resulted in horticulture export businesses in Australia increasingly exploring sea freight as a more viable alternative, including the avocado industry (Marques et al., 2023). However, produce needs to be robust to endure the extended in-transit conditions encountered by refrigerated sea containers and arrive with adequate quality and shelf life at the importing country.

Low temperature, often combined with controlled atmosphere (CA), are two major conditions commercially used to slow down biological processes, especially fruit respiration rate and ethylene production, and thus maintain avocado fruit quality for the long durations typically required under sea freight (Hofman et al., 2013). The recommended temperature for postharvest handling, including pre-cooling, transport, and short-term storage of ‘Shepard’ avocado in Australia is generally 7°C (Anon, 2018; Ledger et al., 2011). That guideline was likely due to preliminary information that the fruit was more chilling sensitive than ‘Hass’ (Crane et al., 2013), which was mostly based on a retail survey in Australia comparing near-ripe fruit held at 2°C or 7°C, but not at 5°C (Ledger & Barker, 1995a). In a later study, partially ripened ‘Shepard’ fruit was held for shorter periods of up to 15 days, with little difference in internal quality between 4 and 6°C (Marques et al., 2011). However, there has been no published research into long-term cold storage of hard green ‘Shepard’ avocado. Other green-skin cultivars such as ‘Fuerte’ and ‘Edranol’ are reported worldwide to be generally held at 5.0–5.5°C under 2–4% $O_2$ and 3–10% $CO_2$ for periods of 28–50 days with generally adequate quality at removal and/or

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at ripe (Ahmed et al., 2007; Bower et al., 1989; Eksteen & Truter, 1985; Olivares et al., 2020).

To investigate the feasibility and requirements for sea freight of 'Shepard' avocado grown in Australia to key Asian markets, it was important to confirm the optimum transport temperature and to what extent CA would be beneficial compared to regular air storage for the typical fruit in-transit duration of up to 21 days. We hypothesised that hard-green 'Shepard' fruit could respond well to long-term storage at 5.5°C, especially under CA conditions, as reported for other green-skin cultivars. We verified this by sourcing fruit from the two major growing locations in Australia and simulating typical export supply chain conditions in the laboratory. We tested the impact of temperature and storage conditions at various durations on fruit quality at removal from cold storage and after ripening to determine shelf life.

## Materials and methods

### Fruit sampling

Green mature (19.4–25.8% dry matter, mean of 22.0%) 'Shepard' fruit ($n = 912$) were sampled from two commercial orchards at the major growing regions in Australia, one near Atherton (AT; 17.27° S, 145.48° E) and the other near Childers (CH; 25.24° S, 152.28° E) in Queensland. Trees were 18 or 6 years old on 'Velvick' or 'Zutano' rootstocks for AT and CH farms, respectively. Fruit were grown, harvested, and packed under standard commercial practices, which included fruit being picked by hand with the use of mechanical work platforms (cherry pickers), placed in 450 kg plastic field bins, then transported to the packing facility adjacent to each farm (Anon, 2018). On the next day, fruit were put through a commercial automated packing line, which included being washed, brushed, sprayed with postharvest fungicide, dried, sorted, and manually packed into cardboard trays. Two major sizes for export fruit from Australia to Asia were sampled, i.e. large (count 18) and small (count 28), with fruit mass ranges of 305–330 g and 195–210 g, respectively. Each tray was lined with polyethylene inserts (~0.12 mm thick) providing moulded cups to hold either 18 or 28 fruit as a single layer and held ~ 5.5–5.9 kg. At AT farm, two batches of fruit were collected from two different field blocks on 31 January 2022, with fruit being held in a coldroom set at 7°C for 3 days before transport in a refrigerated commercial truck set at 7°C to Brisbane (~1,700 km away), then transferred by an air-conditioned car set at 21°C (~115 km away) to a research facility at Nambour, Queensland. In total, the storage treatments started 7 days after harvest. At CH farm, two batches of fruit were harvested from a field block on two consecutive harvest days, 1 and 2 March 2022. These fruit were handled and packed in similar conditions as described above for AT fruit, except that they were held in a coldroom set at 7°C for 1–2 days before being collected directly from the pack facility and transferred (~220 km away) by the same car under similar conditions to the same research facility as AT fruit. In total, the storage treatments commenced 2–3 days after harvest. The 4–5-day variation from harvest to the start of the storage treatments between AT and CH fruit was due to farms being located ~ 1,570 km apart, resulting in large variation in fruit handling and transport logistics to the seaport, aligned with commercial practices. Sampling fruit that represented real export supply chain conditions was critical to achieving the objectives of the experiment.

### Treatments

Thirteen storage conditions were applied as treatments: a control (non-stored), regular air (Air), or controlled atmosphere (CA; with 2% $O_2$ and 5% $CO_2$) storage applied at either 5.5 and 7.0°C for 14, 21, or 28 days. Each treatment was applied to 32 fruit per farm ($n = 416$), which were individually labelled and divided into four lots (replicates) of eight fruit each, with 16 fruit from each size from each of the two farm batches. Each farm batch of 16 fruit (8 fruit of each size) was randomly allocated to a cardboard tray lined with a polyethylene insert as a single layer. The trays were randomly placed into four aluminium chambers of about 0.5 $m^3$ each, two for Air and two for CA treatments, which were positioned into two coldrooms, one for each temperature. Each chamber held six trays (two farm batches x three removal times) per farm. The chambers were sealed, and their internal temperature, relative humidity, and atmospheric conditions fully controlled and monitored throughout the trial using an automatic gas control unit (EC12 Freshview Environmental Control System, Pacific Data Systems, Eight Miles Plains, QLD, Australia).

### Fruit quality assessments

On arrival at the laboratory, fruit were individually weighed and assessed for firmness using the Bareiss hardness tester (model HPE II Fff, Arden, NC, USA) fitted with a 5 mm diameter spherical indenter. This digital durometer determines non-destructively (i.e. no peeling of the skin is required) the yielding of the fruit surface at a given contact force. It then displays a resulting spring loading as Shore Fff degree, which decreases at the fruit gets softer. Fruit were then allocated to the treatments described above. Non-stored fruit at arrival and all other fruit after removal from cold storage were held at 22°C for ripening and ongoing assessments as described below. A slight higher ripening temperature than the 20°C recommended for less mature fruit in Australia (Ledger et al., 2011) was adopted to reflect more closely typical commercial ripening conditions encountered by major Australian importing countries.

At removal from cold storage, fruit were individually assessed for firmness, weight loss, and severity of discrete patches on the skin, typically associated with chilling damage (Hofman et al., 2013). During ripening, fruit firmness was checked every 2–3 days until close to ripe. To determine the ripe stage, a Shore Fff degree close to 38 was targeted based on assessments previously done by our research team on a separate batch of 106 ‘Shepard’ fruit across a broad range of firmness stages. This target value equated to a firmness of ~ 3–5 N as measured by the Shimadzu firmness tester (model EZ, Kyoto, Japan), i.e. the force required to push a 12 mm spherical plunger 2 mm into the fruit. When reaching the ripe stage, each fruit was externally assessed for severity of discrete patches (DP) on the skin and fruit shrivel. Fruit were then cut in half, the flesh scooped and individually assessed for severity of flesh body rots, stem end rots, diffuse discolouration, and vascular browning. The severity of DP on the skin and all the above flesh defects was visually determined as the percentage of either skin surface area or flesh volume affected as per rating systems and photographs shown in the ‘International Avocado Quality Manual’ (White et al., 2009). Shrivel was recorded in those fruit with a severity of 3 on a 0 (no shrivel) to 3 (severe shrivel, fruit appear puckered) rating scale shown in the same manual. The proportion (%) of ripe fruit with severe DP or shrivel was determined by the number of fruit with over 10% of skin surface area affected or a rating of 3, respectively, relative to the total number of fruit per replicate. Accordingly, the proportion (%) of unacceptable fruit at ripe was determined by the number of fruit with over 10% of flesh volume affected by all flesh defects combined relative to the total number of fruit per replicate. This is based on retail quality surveys conducted in Australia showing that future purchase intent for fresh avocados by consumers is negatively impacted when that limit is exceeded (Gamble et al., 2010).

An additional 10 fruit from each size class within each batch, total of 40 fruit per farm ($n = 80$), were sampled for flesh dry matter, which was determined for each fruit using the coring method as described by Anon (2018). Samples were processed one day after arrival in the laboratory and dried in a dehydrating oven set at 65°C to constant weight (~2 days).

### Experimental analysis

Statistical analyses were done with Genstat® 22nd Edition (VSN International Ltd., UK). Results were analysed separately for each of the two farms due to the large differences in growing conditions, harvest time at each area (aligned with commercial maturity), and logistics of fruit handling before the start of the treatments as detailed above. The ‘General Analysis of Variance’ model with a split–split plot design was used to analyse the multiple treatment factors and their interactions. Storage atmosphere x temperature x duration x fruit size was adopted as the treatment structure, with fruit batches as blocks, storage atmosphere and temperature as whole plots, storage duration as sub-plots, and fruit size as sub-sub-plots. Non-stored fruit was excluded from the analysis, with means for each quality parameter used as references only in the results and discussion. Residual plots were assessed to confirm that the model assumptions were met. Data on the proportion of acceptable fruit and the proportion of fruit with severe discrete patches or shrivel at ripe were analysed using the ‘Generalised Linear Models’ procedure for a ‘Modelling of binomial proportions’ with a logit transformation (link). Back-transformed means as percentage are presented. Whenever the treatment effect was significant ($p \leq 0.05$), pairwise comparisons were made using ‘Fishers protected Least Significant Difference’ (LSD) test. Different letters for separating treatment means are presented in graphs and tables.

## Results and discussion

### Fruit quality at removal from storage and ripening time

At removal, there was a significant ($p \leq 0.05$) interaction between storage atmosphere, temperature, and duration for fruit firmness determined by the Bareiss hardness tester in samples from both farms, and an added interaction with fruit size in fruit from AT farm (Table 1). Generally, ‘Shepard’ fruit were firmer on removal when stored at 5.5°C than at 7.0°C after 21 and 28 days storage regardless of the storage atmosphere. In contrast, there was little difference in firmness between both temperatures in fruit at removal after 14 days. Compared to air storage, fruit from both farms held under CA were generally firmer at removal after 28 days storage, and after 21 days at 7.0°C for fruit from CH farm, with no differences between Air and CA after 14 days for either farm. For samples from AT farm, large fruit were firmer after 21 days under CA at 5.5°C or after 28 days at 7.0°C, with no differences after 14 days storage in any atmosphere or temperature. Fruit size had no significant effect on samples from CH farm, so results shown in Table 1 are averaged across both sizes. The removal from storage equates to the critical point at which the fruit would arrive at the importing country in a commercial setting, and firmness is the key criteria to determine the ripeness stage of the avocado fruit (Hofman et al., 2013). Comparatively, non-stored fruit on arrival at the lab had initial Shore Fff degrees of 82.4 and 84.7 for samples from AT and CH farms, respectively. There were no signs of discrete patches on the skin at removal.

**Table 1.** 'Shepard' avocado fruit firmness (Shore Fff degree) at removal from storage as affected by the interaction between storage atmosphere, i.e. regular air (Air) or controlled atmosphere (CA), temperature, duration, and fruit size.

| | | | Fruit firmness (Shore Fff degree) at removal from storage* | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Air | | | CA (2% $O_2$ and 5% $CO_2$) | | |
| | | | Storage duration (days) | | | Storage duration (days) | | |
| Farm | Storage temperature | Fruit size# | 14 | 21 | 28 | 14 | 21 | 28 |
| AT | 5.5°C | Large | $79.5^{i}$ | $77.8^{ghi}$ | $75.3^{cefg}$ | $80.0^{i}$ | $77.7^{ghi}$ | $79.0^{hi}$ |
| | | Small | $79.5^{i}$ | $75.7^{efgh}$ | $72.1^{bcd}$ | $79.7^{i}$ | $74.1^{bcdef}$ | $78.7^{ghi}$ |
| | 7.0°C | Large | $79.0^{hi}$ | $72.2^{bcde}$ | $64.1^{a}$ | $78.4^{ghi}$ | $71.7^{bc}$ | $79.0^{hi}$ |
| | | Small | $78.0^{ghi}$ | $70.6^{b}$ | $64.5^{a}$ | $76.5^{fghi}$ | $73.4^{bcde}$ | $66.9^{a}$ |
| CH§ | 5.5°C | | $82.0^{cdefg}$ | $82.4^{cefghi}$ | $81.4^{bcd}$ | $81.6^{cde}$ | $83.4^{fghi}$ | $83.2^{fghi}$ |
| | 7.0°C | | $81.1^{c}$ | $79.7^{b}$ | $72.9^{a}$ | $81.7^{cdef}$ | $83.3^{egi}$ | $82.2^{cdefgh}$ |

*Means (n = 192, 128, 192, and 48 for atmosphere, duration, fruit size, and the interaction, respectively) for each farm and effect with different letters indicate significant differences ($p \leq 0.05$) by the LSD test.

#Large fruit size: count 18 (305–330 g); small fruit size: count 28 (195–210 g).

§Means were averaged across the two fruit sizes.

**Table 2.** 'Shepard' avocado fruit weight loss (%) at removal from storage as affected by storage atmosphere, i.e. regular air (Air) or controlled atmosphere (CA; 2% $O_2$ and 5% $CO_2$), storage duration, and fruit size.

| | Fruit weight loss (%) at removal from storage* | | | | | | |
|---|---|---|---|---|---|---|---|
| | Storage atmosphere# | | Storage duration (days)§ | | | Fruit size± | |
| Farm | Air | CA | 14 | 21 | 28 | Large | Small |
| AT | $2.8^{b}$ | $2.1^{a}$ | $1.7^{a}$ | $2.6^{b}$ | $3.1^{c}$ | $2.3^{a}$ | $2.6^{b}$ |
| CH | $2.5^{b}$ | $1.8^{a}$ | $1.5^{a}$ | $2.2^{b}$ | $2.7^{c}$ | 2.1 | 2.2 |

*Means for each farm for either atmosphere (n = 192), duration (n = 128), or fruit size (n = 192) with different letters indicate significant differences ($p \leq 0.05$) by the LSD test. The absence of letters indicates the effect was not significant. Means were averaged across two storage temperatures (5.5 and 7.0°C).

#Means were averaged across the three storage durations and two fruit sizes.

§Means were averaged across the two storage atmospheres and two fruit sizes.

±Means were averaged across the two storage atmospheres and three storage durations.

Fruit weight loss at removal from storage increased with longer durations and in air-stored compared to CA-stored fruit from both farms (Table 2). Small fruit from AT farm lost more weight than large fruit, whereas that response was not significant in fruit from CH farm. The effect of storage temperature on fruit weight loss was not significant in fruit from either farm.

The effect of storage conditions on ripening time varied depending on farm. For fruit from AT farm, there was a significant interaction between storage atmosphere, temperature, and fruit size (Figure 1). Fruit generally ripened faster when stored under Air compared to CA. Large fruit tended to soften more rapidly than small sized fruit. There was little difference between temperatures except for small Air-stored fruit at 7.0°C ripening faster than at 5.5°C. Means in Figure 1 are averaged across the three storage durations due to no significance of that treatment factor. For fruit from CH farm, there were significant single treatment effects for storage atmosphere (5.2 and 6.4 days for Air and CA, respectively), temperature (6.1 and 5.5 days for 5.5°C and 7.0°C, respectively), and duration (6.6, 5.7, and 5.1 days, for 14, 21, and 28 days of storage, respectively), whereas the effects of fruit size or interactions were not significant. Comparatively, non-stored fruit took 7.8 and 9.2 days to ripen at 22°C for samples from AT and CH farms, respectively. These results show a considerably shorter ripening time at 22°C after storage, especially for Air-stored fruit held at 7.0°C for longer periods. It confirms an earlier study in Australia showing a relatively short ripening time for 'Shepard' fruit after treatment with ethylene for 2 days and ripening at 20°C (Ledger & Barker, 1995b). This highlights the importance of maintaining the cold chain after arrival and the need for an efficient distribution to ensure sufficient

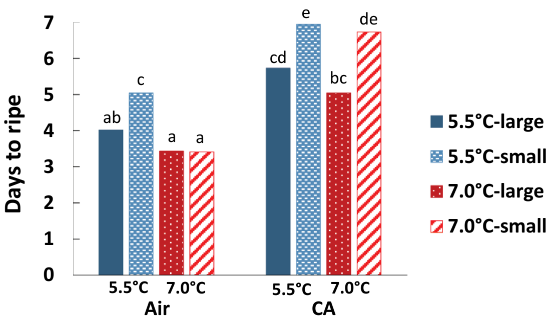

**Figure 1.** Ripening time of 'Shepard' avocado fruit as affected by the interaction between storage atmosphere, storage temperature, and fruit size. Fruit were sampled from the AT farm, held under either regular air (Air) or controlled atmosphere (CA; 2% $O_2$ and 5% $CO_2$) for 14, 21, or 28 days at either 5.5 or 7.0°C, before ripening at 22°C. Bars (*n* = 128) with different letters indicate significant differences (*p* ≤0.05) by the LSD test.

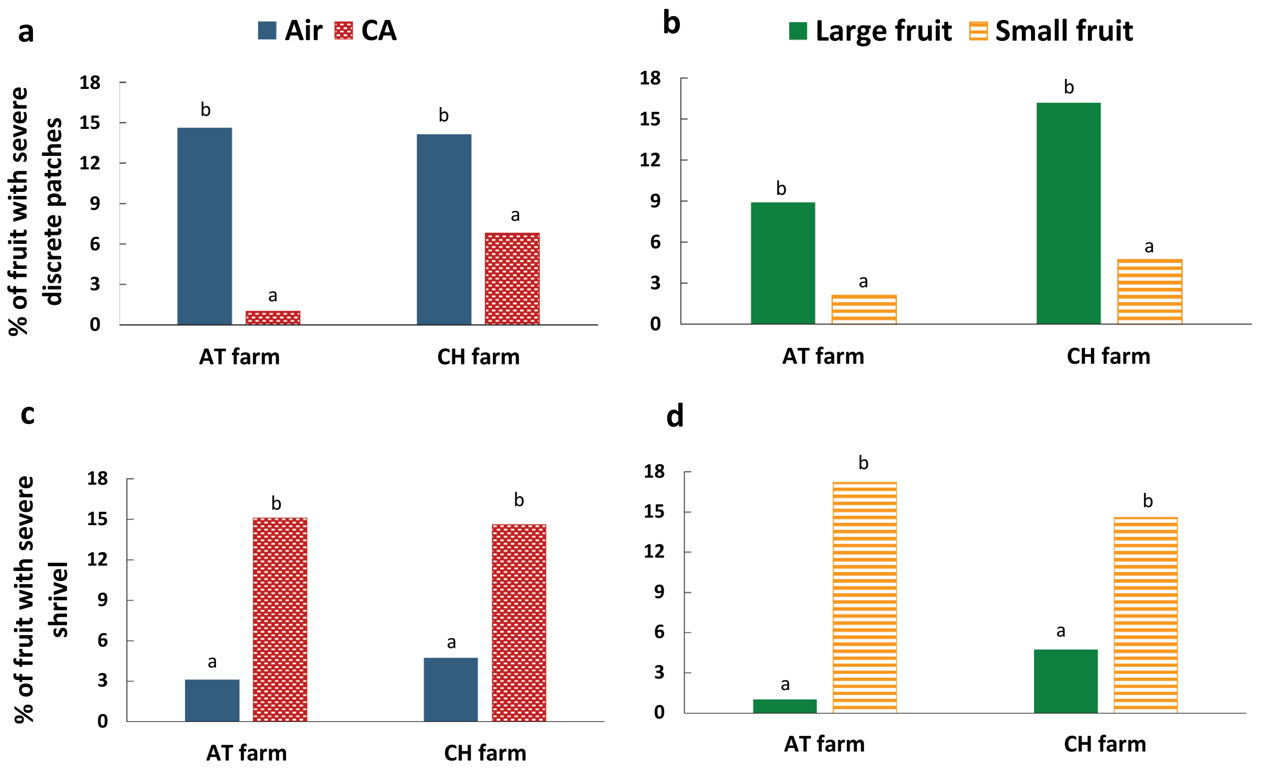

**Figure 2.** The proportion (%) of 'Shepard' avocado ripe fruit with severe discrete patches (>10% of skin area affected; a and b) or with severe shrivel (rating 3 on a 0 to 3 scale; c and d), as affected by storage atmosphere (a and c) or fruit size (b and d). Fruit were sampled from two farms, held under either regular air (Air) or controlled atmosphere (CA; 2% $O_2$ and 5% $CO_2$) for 14, 21, or 28 days at either 5.5 or 7.0°C, before ripening at 22°C. Bars ($n = 192$) for each farm with different letters indicate significant differences ($p \leq 0.05$) by the LSD test.

product shelf life at retail. Similar results for fruit quality at removal were reported with green-skin cultivars 'Fuerte' and 'Edranol' stored at 5.0°C for 30 or 50 days, with fruit held under CA (4% $O_2$ and 6% $CO_2$) being generally firmer and having reduced weight loss at removal, and taking longer to ripen, compared with fruit stored in regular air (Olivares et al., 2020).

### *External quality of ripe fruit*

Compared to air-storage, CA-storage resulted in fewer ripe fruit from both farms with severe DP (>10% of the skin area affected), whereas that effect was reversed for severe shrivel (Figure 2). In ripe fruit from AT farm, severe DP occurred mostly after storage for 28 days (15.6% of fruit affected compared to nil and 0.8% after 21 and 14 days, respectively), whereas severe shrivel increased gradually with increased duration (4.7, 9.4 and 13.3% of affected fruit for storage after 14, 21, and 28 days, respectively; data not shown). In contrast, differences among removal times for severe DP or shrivel were not significant in fruit from CH farm. As the differences between both storage temperatures were not significant in fruit from either farm, means presented in Figure 1 are averaged across both temperatures and three storage times. Accordingly, the interactions between storage atmosphere, temperature, and duration were not significant in fruit from either farm (data not shown).

The fact that CA storage markedly reduced skin darkening in ripe fruit stored for 28 days suggests that CA may be a safer commercial option to preserve external quality if 'Shepard' fruit is exported to destinations requiring a sea journey longer than 3 weeks. Comparatively, non-stored ripe fruit from both farms had nearly no discrete patches on the skin (data not shown). Similar results of marked reduction in the severity of discrete patches (described as external damage or chilling injury in the references) in CA-stored compared to Air-stored fruit at ripe are reported for other green-skin cultivars such as 'Fuerte' and 'Edranol' fruit held at 5.0—5.5°C for periods of 30—50 days (Eksteen & Truter, 1985; Olivares et al., 2020). The reduced proportion of Air-stored ripe fruit with severe shrivel compared to CA-stored fruit may be associated with reduced moisture loss due to the shorter ripening time of Air-stored fruit as shown above. Severe shrivel was typically observed only in fruit in which weight loss at ripe exceeded around 8%. The effect of fruit size on severe shrivel could be associated with the larger surface area of small fruit relative to fruit volume compared to large fruit, in addition to small fruit taking longer to ripen in some cases, as shown in Figure 1.

Table 3. The average severity of flesh defects (% of flesh volume affected) in ripe 'Shepard' avocado fruit as affected by the storage atmosphere, i.e. regular air (Air) or controlled atmosphere (CA; 2% $O_2$ and 5% $CO_2$), temperature (5.5 or 7.0°C), duration (14, 21, or 28 days), and fruit size.

| | AT farm | | | CH farm | | | |
|---|---|---|---|---|---|---|---|
| Flesh defect | Significant effect* | | Severity (%) | Significant effect* | | | Severity (%) |
| | | | | Interaction between atmosphere, temperature, and fruit size)& | | Air | CA |
| Stem end rot | Atmosphere# | Air | $0.3^{a}$ | | 5.5°C/Large | $0.4^{a}$ | $1.9^{ab}$ |
| | | CA | $1.2^{b}$ | | 5.5°C/Small | $0.9^{a}$ | $0.7^{a}$ |
| | | | | | 7.0°C/Large | $2.8^{b}$ | $1.6^{ab}$ |
| | | | | | 7.0°C/Small | $0.2^{a}$ | $1.3^{ab}$ |
| Body rots | Fruit size§ | Large | 0.14 | Fruit size§ | | Large | $1.2^{b}$ |
| | | Small | 0.01 | | | Small | $0.6^{a}$ |
| Vascular browning | Fruit size§ | Large | $0.8^{a}$ | Atmosphere# | | Air | $0.5^{a}$ |
| | | Small | $2.0^{b}$ | | | CA | $1.3^{b}$ |
| All flesh defects combined | Fruit size§ | | | Duration (days)± | | 14 | $4.5^{b}$ |
| | | Large | $1.5^{a}$ | | | 21 | $2.1^{a}$ |
| | | Small | $2.2^{b}$ | | | 28 | $2.6^{a}$ |
| | | | | Fruit size§ | | Large | $4.0^{b}$ |
| | | | | | | Small | $2.2^{a}$ |

*Means (n = 192, 128, 192, and 48 for atmosphere, duration, fruit size, and the interaction, respectively) for each farm and effect with different letters indicate significant differences ($p \le 0.05$) by the LSD test.

#Means were averaged across the two temperatures, three durations, and two fruit sizes.

§Means were averaged across the two atmospheres, two temperatures, and three durations.

±Means were averaged across the two atmospheres, two temperatures, and two fruit sizes.

&Means were averaged across the three removal times.

## *Internal quality of ripe fruit*

The impact of storage conditions and fruit size on internal quality at ripe varied depending on farm and specific flesh defect (Table 3). In fruit from AT farm, vascular browning was the most severe flesh defect, especially in small fruit, followed by stem end rot, which was more severe in CA-stored compared to Air-stored fruit. In fruit from CH farm, stem end rot was the most severe flesh defect, especially in large fruit stored at 7°C, followed by body rot in large fruit and by vascular browning in CA-stored fruit. When rots were combined, severity was higher in CH-fruit after 14 days of storage (3.3%) compared to 1.8% and 1.5% for storage at 21 and 28 days, respectively . When all flesh defects were combined, fruit size affected fruit from both farms, with higher severity found in small AT-fruit (likely due to the strong effect of vascular browning), and in large CH-fruit (likely due to the strong effect of rots). Storage duration also affected the combined severity of CH-fruit, in line with the effects on combined rots as mentioned above. These effects are likely associated with the fact that CA fruit and 14-days storage generally resulted in a longer ripening time, as shown above, allowing more time at 22°C for stem end rot and vascular browning, a disorder typically associated with stem end rot (Hofman et al., 2013), to develop. Diffuse discolouration was not found in this trial in fruit from either farm .

Accordingly, the proportion of unacceptable fruit, i.e. those with >10% of flesh volume affected by all defects combined, varied depending on farm (Figure 3). In fruit from AT farm, CA storage resulted in a higher proportion of unacceptable fruit compared to air storage, whereas the effects of storage temperature, duration, and fruit size were not significant, so the means presented in Figure 3 for AT farm were

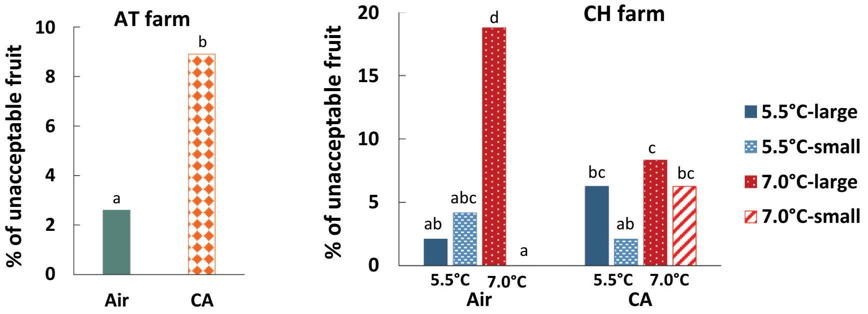

Figure 3. The proportion (%) of unacceptable (>10% of flesh volume affected by defects) 'Shepard' avocado ripe fruit as affected by storage atmosphere (left graph) or by the interaction between storage atmosphere, temperature, and fruit size (right graph). Fruit were sampled from two farms, held under either regular air (Air) or controlled atmosphere (CA; 2% $O_2$ and 5% $CO_2$) for 14, 21, or 28 days at either 5.5 or 7.0°C, before ripening at 22°C. Bars for each graph ($n = 192$ and 48 for AT and CH farms, respectively) with different letters indicate significant differences ($p \le 0.05$) by the LSD test. Means were averaged across the two temperatures, three storage durations, and two fruit sizes (AT farm), or across the three durations (CH farm).

averaged across these three treatment factors. The interactions among all four treatment factors were not significant. In fruit from CH farm, there was a significant interaction between storage atmosphere, temperature, and fruit size, with a higher proportion of unacceptable large fruit that had been Air-stored at 7.0°C, whereas storage at 5.5°C generally resulted in no significant differences between atmospheres or fruit sizes. The means presented in Figure 3 for CH farm were averaged across storage durations, as that treatment factor was not significant.

For the domestic market in Australia, 7°C is generally recommended for storage and transport of green-skinned avocado cultivars (Anon, 2018; Ledger et al., 2011). However, to our knowledge, no previous research had been undertaken on storage of unripe 'Shepard' fruit for longer durations, especially combined with CA. An average storage temperature of 5.5°C during the season, combined with CA or 1-MCP, is reportedly used by South African avocado exporters to reduce the risk of other green-skin cultivars such as 'Fuerte' fruit arriving too soft at European markets (Lütge et al., 2013). The current results with 'Shepard' fruit are novel and suggest that storage of this variety at 5.5°C may be a feasible option for periods of up to 3 weeks, which are typically required for sea freight from Australia to key Asian markets. However, further testing would be warranted, as chilling responses in avocado fruit can vary considerably depending on various factors such as cultivar, growing conditions (as shown in this current work), harvest timing, and fruit maturity (Hofman et al., 2013; Rivera et al., 2017). For example, compared to Air storage, CA storage reduced the severity of diffuse discolouration across two harvest times with distinct fruit maturity levels, whereas that response in 'Edranol' fruit was only found in early harvested fruit (Olivares et al., 2020). However, for vascular browning, CA reduced severity in late harvested 'Fuerte' fruit, but had no impact on 'Edranol' fruit. That study also showed distinct respiration and ethylene production rates during ripening between the two varieties, with CA generally slowing respiration rates and reducing the activity of key enzymes associated with ethylene synthesis. Accordingly, the current results suggest that CA storage could be a safer option to reduce the risk of flesh disorders if more mature 'Shepard' fruit is exported, given that dry matter of fruit sampled in this study averaged 21% (AT farm) and 23% (CH farm).

## Conclusion

The current results under static conditions in the laboratory suggest that Air-storage of 'Shepard' fruit at 5.5°C for up to 21 days may be beneficial compared with the previously recommended 7.0°C. 'Shepard' fruit stored at 5.5°C were generally firmer at removal, slow to ripen, and had less severe flesh rots at ripe than at 7.0°C. Discrete patches on the skin of ripe fruit that could have been exacerbated by chilling did not differ between 5.5 and 7.0°C, and there was no internal diffuse discolouration in either temperature. After 28 days of storage, CA at 2% $O_2$ and 5% $CO_2$ markedly reduced discrete patches on the skin in ripe fruit, with little difference between air and CA after storage for 14 or 21 days. Across all three storage durations, CA storage tended to decrease weight loss at removal and slow ripening compared to air storage. In contrast, CA storage also resulted in ripe fruit with more severe shrivel, stem end rot, and vascular browning, possibly due to longer ripening at 22°C. Compared to small fruit, large fruit at ripe tended to have less severe shrivel and flesh rots, but more severe skin discrete patches and flesh vascular browning. There was also considerable variation between both farms in storage effects on fruit ripening and quality. Given that chilling responses in avocado fruit can vary depending on preharvest factors and maturity at harvest, further testing would be required to better evaluate the risks of loss of external and internal quality under commercial sea freight conditions of 'Shepard' avocado fruit.

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## Data availability statement

The data that support the findings of this study are available from the corresponding author, JRM, upon reasonable request.

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