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Impact of controlled atmosphere scheduling on strawberry and imported avocado fruit

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Abstract

British grown strawberry cv. Sonata and Chilean avocado cv. Hass were exposed to controlled atmospheres (CA) of 15 kPa CO_2 + 5 kPa O_2 (5 °C) and 10 kPa CO_2 + 5 kPa O_2 (5 or 20 °C), respectively, at early, middle or late stages during postharvest storage of avocados and at early and middle stages for strawberries. Real-time respiration rate (RR) was measured during CA storage and regular fruit sampling carried out to assess disease severity, objective colour, ethylene production and firmness. The automated *in situ* set-up used allowed continuous recordings of real-time respiration measurements without disruption to the CA environment. Cold stored strawberry fruit treated for 2.5 d with CA midway through the storage period were firmer and maintained a more vibrant colour despite bursts of increased RR. Furthermore, just 2.5 d of CA was sufficient to extend the shelf-life of strawberries (based on disease incidence) by a further 3 d, as compared to control. Irrespective of timing, RR of avocado stored at 20 °C was reduced while under CA environment; and early CA exposure maintained firmness yet increased the incidence of internal discolouration 7 d after removal from CA. At 5 °C, avocado skin colour and internal discolouration were positively affected by the mid CA treatment. These results are discussed in the context of the targeted use of CA, compared to control, for extending shelf-life, and reducing waste of these two different fruit produces. Furthermore, reducing the length of time required for CA application, which has not

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previously been explored in avocado or strawberries, would potentially be more energy efficient / cost effective.

Keywords: Respiration rate; Food quality; Food waste

1. Introduction

With an ever-increasing global population, new methods to improve food availability and reduce waste have become a priority. One significant cause of food losses occurs when food quality is compromised due to inefficient post-harvest handling, including inappropriate gas storage composition and packaging (Terry et al., 2011).

Strawberries (*Fragaria* x *ananassa* Duch.) and imported avocado (*Persea americana* Mill.) are two high value but contrasting fresh produce types available in the UK, with remarkable differences not only in their physiology but also in their supply chain. In the UK market, a significant proportion of strawberry fruit are grown in the UK so that berries can reach the retailer and consumer within 4-7 d from harvest. Whilst, imported avocado undergo a long transit period (*ca*. one month by boat) from the Southern Hemisphere (viz. South America, South Africa) before arriving at British packing houses, where they can be further stored, ripened and packaged. These two produce types also vary significantly in their physiology and metabolic responses during ripening. For instance, as a climacteric fruit, the respiration rate of avocado can exceed 184 mg kg^{-1} h^{-1} at 20 °C at the onset of ripening (Valle-Guadarrama et al., 2013) coinciding with high levels of ethylene production (≥1180 μg kg^{-1} h^{-1}; Valle-Guadarrama et al., 2013; Basuki et al., 2016). In contrast, strawberry fruit, which are currently classed as non-climacteric, produce lower levels of CO_2 (up to *ca.* 129 mg kg^{-1} h^{-1} at 23 °C) and significantly lower levels of ethylene (Kader 2002; Thompson 2003; Terry et al,

2007). Consequently, these two perishable products provide a useful comparison for gas exchange under different storage conditions including controlled atmosphere (CA).

Low temperature (<5 °C) is widely used to extend strawberry and avocado storability (Kader 2002; Thompson 2003; Castellanos et al., 2017). However, cold storage alone is often not sufficient to store and transport produce such as avocados between intercontinental markets. Furthermore, cold storage of avocado fruit can lead to chilling injury (CI) (Basuki et al., 2016). Optimum CA conditions for normal ripening of avocado are between 2.5 and 5 kPa O_2, and 5 and 7.5 kPa CO_2, yet incidence of CI was not retarded (Basuki et al., 2016). CA environments (e.g. high CO_2) are known to reduce microbial decay (viz. *Botrytis cinerea)* in strawberries (Almenar et al., 2006; Barrios et al., 2014) and stem-end rot (viz. *Botryodiplodia theobromae* and *Lasiodiplodia theobromae*) in avocado (Pesis et al., 2002). However, levels exceeding 15 kPa CO_2 can result in CO_2 injury (Barrios *et al.*, 2014).

Depending on the type of produce, metabolic state can significantly vary throughout storage resulting in different challenges for MAP and CA storage. Use of CA to maintain quality of both strawberry and avocado have been developed, but limitations still exist. The efficacy of CA is affected by avocado maturity and cultivar differences which can lead to a build-up of CO_2 and ethylene if gas exchange is not sufficient (Meir et al., 1997; Hertog et al., 2003). Thus, high levels of ethylene generated during the climacteric increase can hasten ripening and senescence. Therefore, successful storage under CA or MAP would require an environment/packaging with rapid gas exchange (Espinosa-Cruz et al., 2014). Strawberry fruit, on the other hand, despite their lower ethylene and CO_2 levels, are highly perishable and especially prone to postharvest disease; hence market shelf-life is very limited. There is a paucity of up to date research investigating the effects of CA on the respiration rate of strawberry and avocado throughout the supply chain and during post-harvest storage. Furthermore, there is a lack of information on how the application of differential CA

environments during post-harvest life could conserve strawberry and avocado quality. Targeted CA application has previously been found to be as effective as continuous CA to extend shelf-life of onion during cold storage (Chope et al., 2007); however, this has not been explored for avocado or strawberry fruit. Understanding the effects of respiratory gases on fruit physiology is key to the development of optimum packaging to extend post-harvest shelf-life (Castellanos et al., 2017). The objective of this work was to use real time respiration rate technology as a tool to assess physiological changes throughout cold storage and shelf life of both imported avocado and UK-grown strawberry fruit, and to elucidate the impact of physiologically targeted CA on industrially relevant quality attributes (viz. firmness and visual appearance/colour) of both produce types. These findings could provide a better understanding of gas exchange under a CA environment allowing the industry to better maintain quality and reduce waste.

2. Materials and methods

2.1. Plant material

Strawberries cv. 'Sonata' were grown at PR & ON Harrold Farm in Milton, Cambridgeshire and harvested in the early morning on 2nd June 2014. Strawberries were immediately cooled down to 2-5 °C and transported to Cranfield University by midday in an air-conditioned vehicle (1 h). Fruit were immediately transferred to the treatment boxes upon arrival at the Plant Science Laboratory.

Avocado fruit (cv. Hass, size 22) grown at Agricola la Cascada, Chile, were harvested on the 16th October 2013 (20 °C trial) and 4th December 2013 (5 °C trial), and packed at Central Fructicola El Parque, Chile, within 2 d. Avocados were palletised and shipped to the UK at 6 °C under a high nitrogen environment (typically 94% N_2, 4% CO_2 and 2% O_2). The fruit arrived at Worldwide Fruits Ltd. (Pinchbech, Lincs.) approximately one month after harvest: on 19th November and 6th January 2014, respectively. Pre-climacteric avocados (average hue angle:

122 and 120; average firmness: 218 and 215 N, for November and January arrivals, respectively) were transported (2 h) from Worldwide Fruit Ltd. to Cranfield University on 25th November 2013 and 17th January 2014 for 20 °C and 5 °C trials, respectively; and immediately transferred to the treatment boxes (no physiological disorders were observed at arrival).

2.2. Experimental design

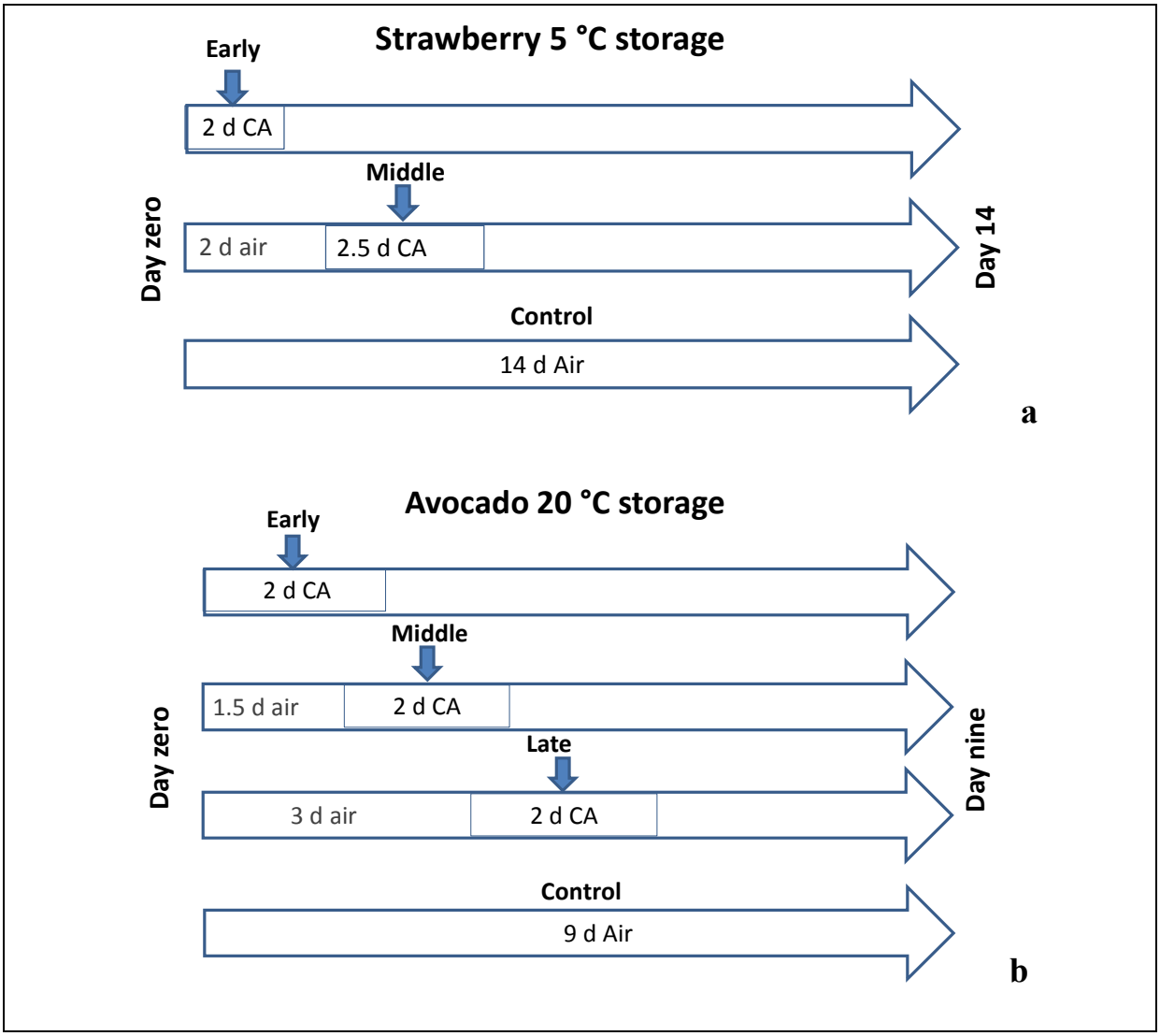
Controlled atmosphere scheduling

Five small plastic containers (punnet) holding *ca*. 400 g of strawberries (18-20 berries) were placed into 12 Lock & Lock 12 L (315 x 230 x 230 mm) polypropylene boxes (L&L Nordic OÜ, Estonia), and further stored at 5°C for 14 d. Storage conditions were: (a) 15 kPa CO_2 + 5 kPa O_2 for 2 d followed by air for the remaining storage period; (b) air for 2 d followed by 15 kPa CO_2 + 5 kPa O_2 for 2.5 d and air for the remaining storage duration; and (c) air only (**Figure 1a**).

For the 20 °C avocado trial, fruit (n = 20) were placed into identical 12 L storage boxes, as described above. CA conditions were 10 kPa CO_2 + 5 kPa O_2. Treatments applied were as follows: (a) CA for 2 d followed by air; (b) 1.5 d of air followed by 2 d of CA and then transferred back to air; (c) air for 3 d followed by CA for 2 d, and then transferred back to air; and (d) air only (**Figure 1b**). For the 5 °C storage trial, the treatments were (a) CA for 5 d followed by air; (b) 5 d of air followed by 5 d of CA, then transferred back to air; (c) air for 10 d followed by CA for 5 d, then transferred back to air; and (d) air only (**Figure 1c**). Avocado fruit were held at 20 °C for 9 d and at 5 °C for 30 d. After 30 d at 5 °C, the avocados were transferred to 20 °C for 4 d to mimic standard shelf-life conditions.

For the strawberry and avocado cold storage experiments (5 °C), and the room temperature (20 °C) avocado trial, the duration of the CA period (2 and 5 d, respectively) was

selected on the basis of desired commercially-relevant increases in shelf-life, in consultation with industry stakeholders.



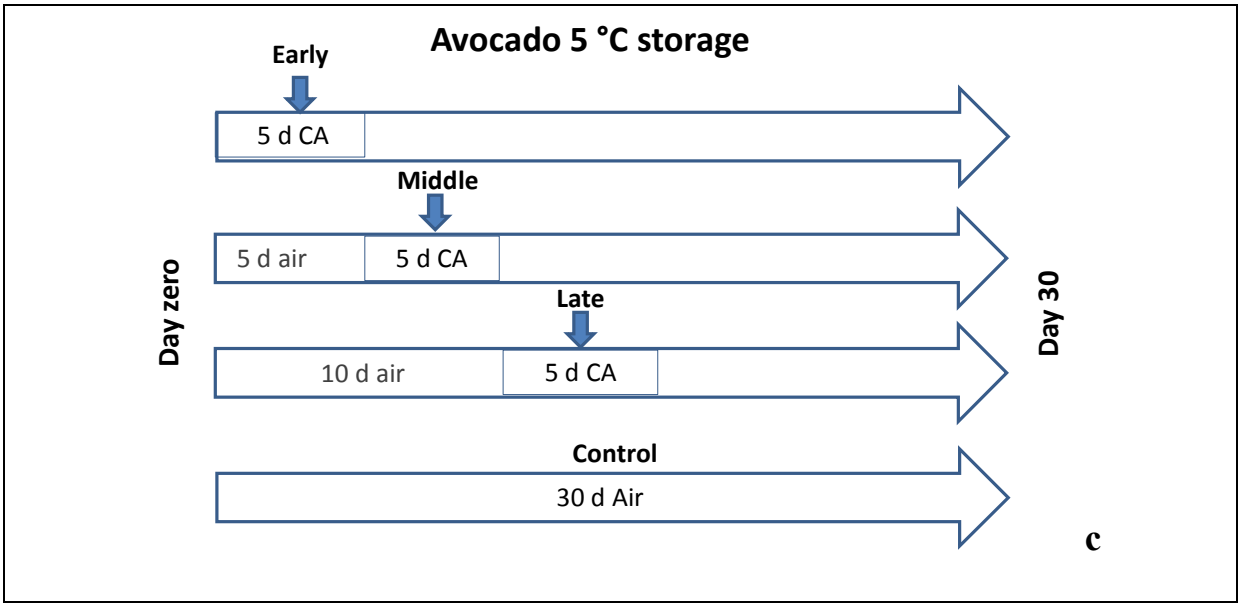


Figure 1. Schematic of controlled atmosphere scheduling for strawberry (a) and imported avocado fruit stored at 5 °C (b) and 20 °C (c) for different time periods (days [d]).

Real-time respiration rate set-up

For each CA condition, four storage containers were connected via PVC tubing to one channel of an ICA6000 (International Controlled Atmosphere Ltd., Paddock Wood, Kent, UK) (CA control system with up to eight channels connected to in-built gas analysers); each channel was split into four, providing a continuous flow (400 mL min^{-1}) to three replicate boxes and one baseline box (empty). The exiting flow was passed directly to a Sable Respirometry System (Model 1.3.8 Pro, Sable Systems International, Las Vegas, NV) and real-time respiration rate measurements (as CO_2 production) continuously recorded (*Section 2.3*). The change in sample weight within each storage container was measured at each sampling point throughout storage to adjust respiration rate calculations, accordingly.

2.3. Physiological measurements

Sample preparation

Fruit were sampled prior to CA treatment application (time zero) and at regular intervals throughout storage, thereafter. At time zero, four individual strawberry fruit (pseudo replicates) were taken per sampling punnet, in triplicate (n = 12 fruit); a total of nine avocado fruit (3 fruit [pseudo replicate] x 3 replicates) were analysed at this point. After time zero, one punnet of strawberries was removed from each storage box after 2, 4.5, 8, 11 and 14 d of cold storage at 5 °C. Physiological measurements, including disease assessment, were performed on four fruit from each punnet. For avocado, three fruit were taken from each storage box on a weekly basis for the fruit stored at 5 °C and three times per week for the avocados at 20 °C (zero, two, four, and seven). For all physiological measurements (e.g. colour, firmness and disease incidence/severity), the mean value from the pseudo replicates (4 strawberries per punnet and 3 avocados from each of the storage boxes) was considered for further statistical analysis.

Respiration rate measurements

Continuous real time respiration rate measurements were acquired using a Sable Respirometry System (model 1.3.8 Pro, Sable Systems International, NV, USA) as described in Collings et al. (2013) with slight modifications. Air from each storage box was passed directly to the system using 'push mode' and measured for 2 min. Automated switching between boxes was achieved using the Sable System MUX flow multiplexer. Sampled air was passed through a SS4-subsampler (Firmware version 2.0), RH-300, CA-10 (Firmware version 1.05) and a FC-10 (Firmware version 3.0) to record flow rate (mL h^{-1}), water vapour pressure, CO_2 (kPa) and O_2 (kPa), respectively. Barometric pressure (BP [kPa]) was simultaneously recorded using the CA-10. Cross contamination between measurements was prevented using empty baseline boxes assigned per gaseous environment (*viz*. air [control] *vs*. CA), which enabled initial O_2 and CO_2 levels to be accurately determined for subsequent respiration rate calculations. Due to lag time and extreme differences in gas composition between air and CA

treatments, baseline boxes were sampled for three consecutive intervals of 2 min allowing equilibration prior to measuring a different CA condition. A 2 min baseline interval was adequate for replicate boxes within treatments. Baseline values were later subtracted from sample measurements to accurately determine respiration rate. Software calculations were performed as described previously in Collings et al. (2013).

Objective colour, ethylene production and firmness

At each sampling point, objective colour was assessed using a Minolta colorimeter (CR-400 series, Konica Minolta Inc., Japan) where three readings were taken around the equator of the fruit (Meyer et al., 2008). Internal (avocado fruit only) and external photographs were taken at every sampling point using an SLR digital camera (model K100D, Pentax, Japan).

Real time endogenous ethylene levels were recorded for 10 min intervals in 'continuous flow' mode using an ethylene analyser device (Sensor Sense B.V., Nijmegen, the Netherlands) capable of measuring down to 0.3 nL L^{-1}. Ethylene produced by avocado and strawberry fruit were analysed at every sampling point using a photoacoustic laser-based ethylene detector (EDT-300 real time ethylene analyser). The detector worked in conjunction with a valve control box allowing connection of up to six sampling cuvettes and a catalyser that enabled removal of ethylene or other hydrocarbons present in the atmosphere. At each sampling day, fruit samples were placed in glass cuvettes (with an empty jar as control) at a constant air flow of 4 L h^{-1}. Ethylene production (nL h^{-1}) was calculated by automatic integration with EDT-software and final measures expressed in terms of sample weight (ng kg^{-1} h^{-1})

Firmness was measured using a uniaxial testing machine (model 5542, Instron, Norwood, MA, USA) equipped with a calibrated 500 or 5 N load cell, depending on the maximum loads recorded on the test day. One measurement was taken on the equatorial region

of each fruit (skin was removed for avocado) (Landahl *et al.*, 2009). Cylindrical probes of 8 and 4 mm diameter were used to measure maximal force at penetration (estimation of firmness [N]) at a speed of 100 mm min^{-1} for avocado and strawberry, respectively.

Bruise and disease incidence and severity (strawberry only)

Disease incidence (%) was estimated as percentage of diseased fruit (over the total number of fruit) found in a punnet. Both incidence of bruising and severity (%) was assessed based on four fruit selected per replicate, at random; bruise severity was recorded as percentage of the total fruit surface affected.

Internal discolouration (avocado only)

Incidence of internal flesh discolouration (%) was recorded for all samples removed from storage, at each sampling point, according to Woolf et al. (2005).

2.4. Statistical analysis

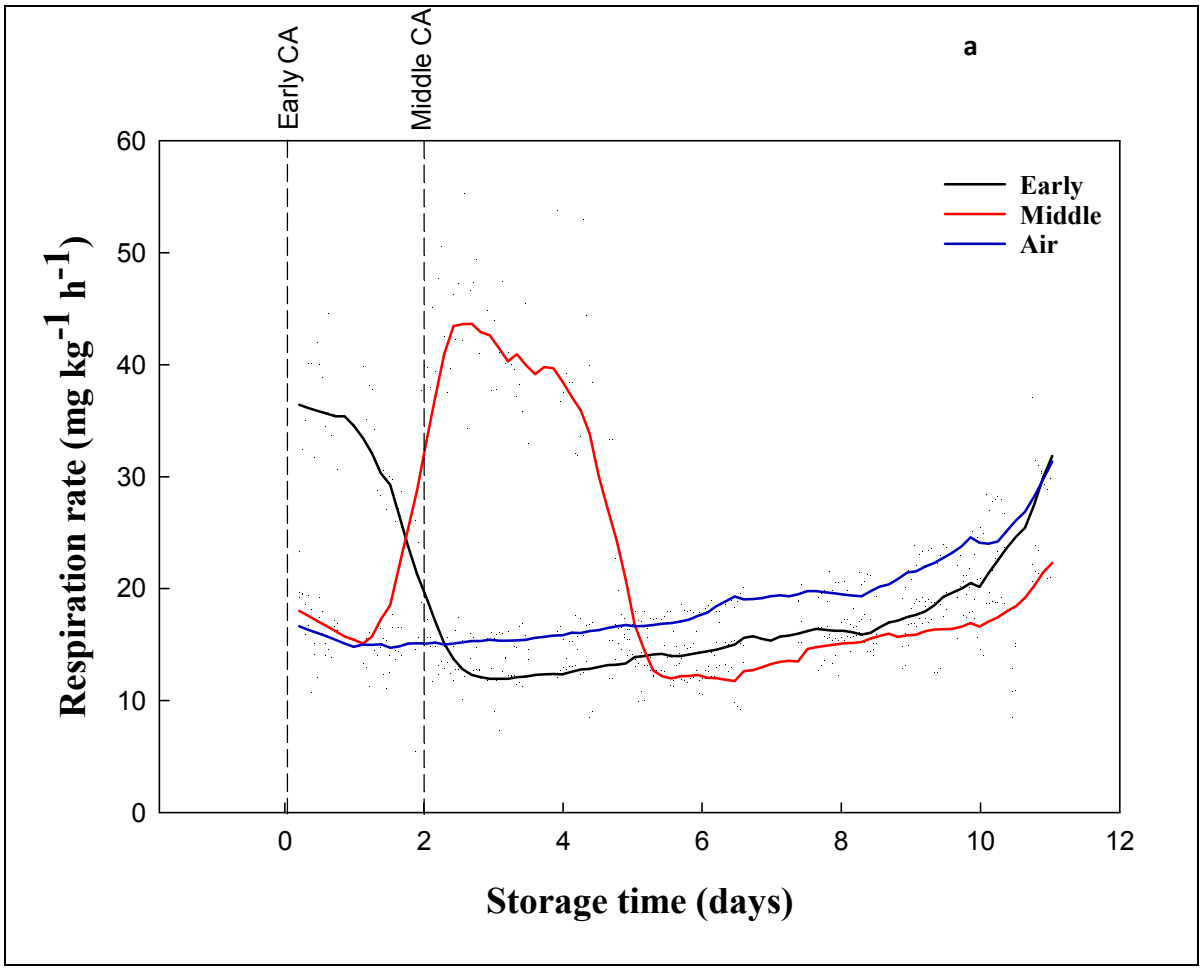
Data were statistically analysed using Genstat for Windows version 16 (VSN International Ltd, Herts., UK). Analysis of variance (ANOVA) was used to identify the main effects of CA environment and storage time, and the interactions between these factors to a probability of $P < 0.05$ unless otherwise stated. Least significant difference values (LSD $P_{0.05}$) are displayed. For avocado fruit, data was analysed without the shelf-life (20 °C) time points, when no significant differences were observed for the 5 °C trial and 20 °C shelf-life combined In this case, two Least Significant Differences (LSD) values are provided (5 °C and 5 – 20 °C).

3. Results

3.1. Respiration rate

Differential CA timings applied to strawberry and avocado during storage caused fluctuations in the real-time respiration rate (RR). Data was smoothed (using the 'running

median algorithm' in SigmaPlot version 13.0, Systat Software, Inc.) and each measurement on the graph is representative of true variability (**Figure 2**).



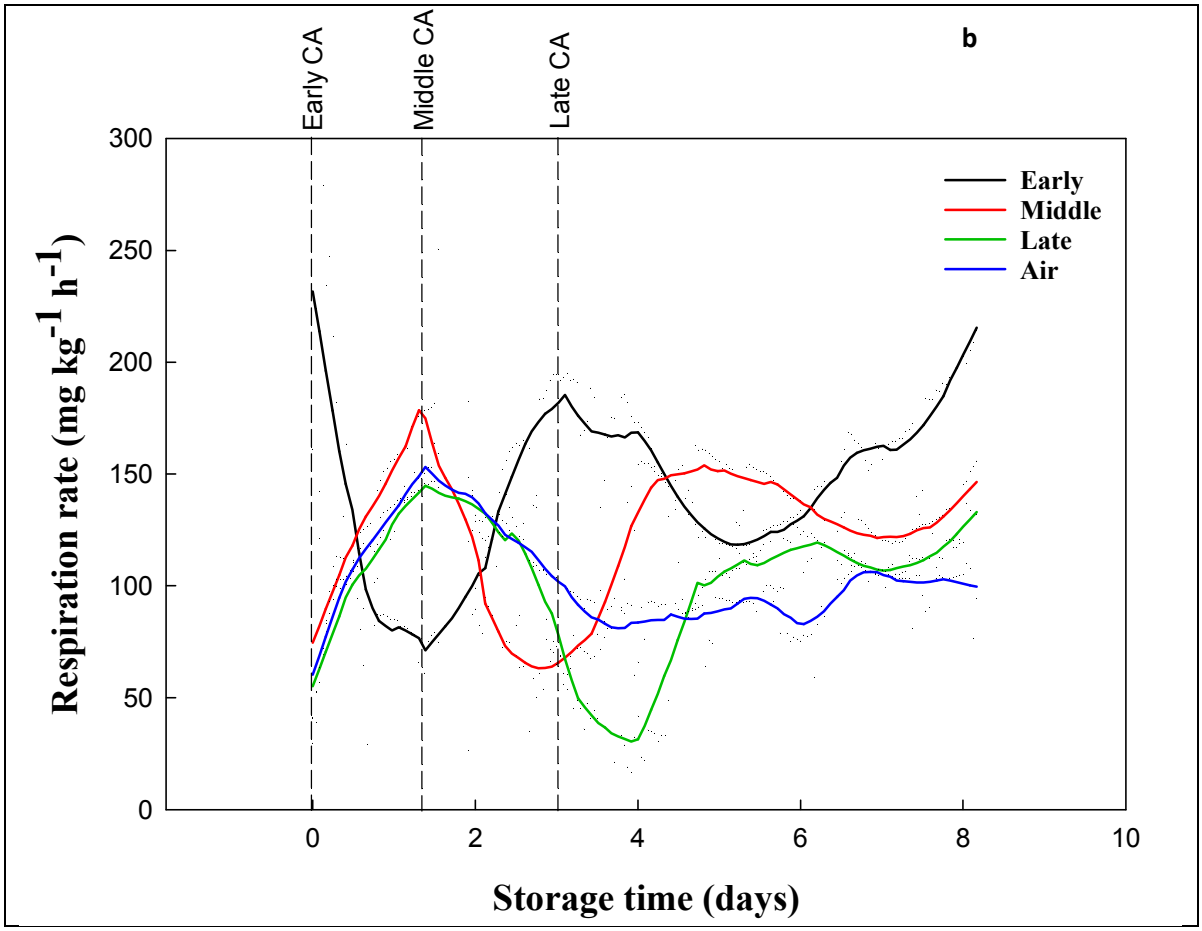


Figure 2. Real-time respiration rate (CO_2 production in mg kg^{-1} h^{-1}) of strawberry and avocado subjected to CA at different timings throughout storage; [**a**] applied CA (15 kPa CO_2 + 5 kPa O_2) for strawberry: 11 d at 5 °C (2 d CA [early], 2.5 d after 2 d of air [middle] and air [control]); [**b**] applied CA (10 kPa CO_2 + 5 kPa O_2) for avocado: 9 d at 20 °C (2 d CA [early], 2 d CA after 1.5 d of air [middle], 2 d CA after 3 d of air [late] and air [control]). Dots represent raw data points; lines represent smooth fit curves.

Real time respiration rate measurements for strawberry revealed a transient increase in CO_2 production on transferral to the CA (15 kPa CO_2 + 5 kPa O_2) environment, but transferral back to an air environment caused respiration rates to decrease back to control levels (**Figure 2a**). In contrast, a steady increase in respiration occurred for air stored samples with no clear increase noted.

For avocado, the respiration rate at 20°C in CA (10 kPa CO_2 + 5 kPa O_2) was lower after exposure to CA but values rapidly increased when they were removed from CA (**Figure 2b**). This is shown by the rapid decrease in respiration (*ca*. from 228 to 76 mg kg^{-1} h^{-1}) in early CA-treated fruit between 0 and 20 h before values increased again up to *ca*. 171 mg kg^{-1} h^{-1}. For middle and late CA-treated avocados, respiration followed a similar trend to the controls (as CA had not yet been applied) where values reached up to *ca*. 142.5 mg kg^{-1} h^{-1} before decreasing to *ca*. 95 mg kg^{-1} h^{-1}. During this rise and fall, application of CA at middle and late timing resulted in respiration rates falling more rapidly, reaching lower values (*ca.* 74 and 38 mg kg^{-1} h^{-1}, respectively) compared to the control. As with early CA-treated avocado, the respiration rate increased after removal from CA storage with values returning to similar levels as that recorded at the time of initial CA application. Early CA-treated fruit also experienced a rise and fall in respiration as was observed in the control, but this was delayed by *ca*. 48 h.

Discrete respiration rate measurements (at each sampling day) were performed on avocados stored at 5°C (data not shown). Early CA-treated avocado fruit were found to have significantly higher RR (40 – 47 mg kg^{-1} h^{-1}) compared to the control (23 – 32 mg kg^{-1} h^{-1}) after 20 d up to the end of cold storage (30 d); whereas mid and late CA-treated fruit had similar RR to the control.

3.2. Ethylene production

Ethylene production of middle CA-treated strawberries remained constant, and was the lowest, throughout storage (*ca.* 83 -142 ng kg^{-1} h^{-1}) at 5 °C when compared to those exposed to early CA environment and the control (**Figure 3**). After 8 d, there was a significant increase in ethylene production in both early CA-treated and control fruit; the mean ethylene values at the end of storage were *ca.* 427 *vs*. 954 ng kg^{-1} h^{-1}, respectively.

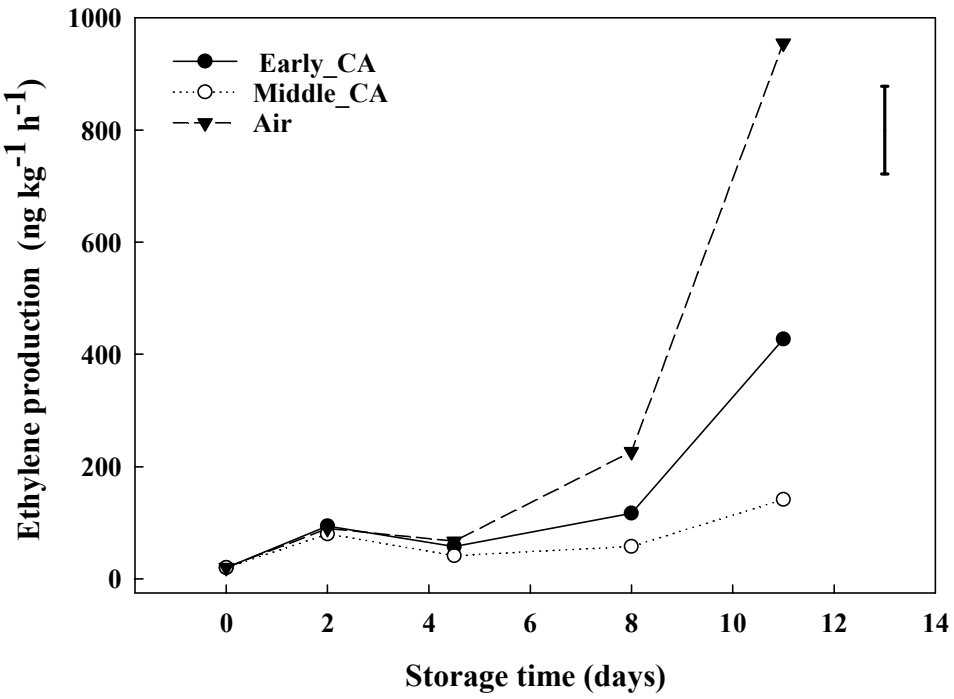


Figure 3. Ethylene production (ng kg^{-1} h^{-1}) of strawberries subjected to CA at different timings throughout storage for 11 d at 5 °C (2 d initial 15 kPa CO_2 5 kPa O_2 [early], 2.5 d 15 kPa CO_2 5 kPa O_2 after 2 d of air [middle] and air [control]). LSD bar ($P < 0.05$) for the interaction treatment x sampling day is shown.

Ethylene production of avocado was found to increase significantly (2-fold) in the first 2 d of storage and during the final 2 d at 20 °C. There was only a significant difference in ethylene production after 3 d storage at 20 °C where the early CA-treated fruit had higher ethylene production 5096.0 ng kg^{-1} h^{-1} (3.71 log10) than middle CA 106.5 ng kg^{-1} h^{-1} (2.03 log10) and late CA 294.5 ng kg^{-1} h^{-1} (2.47 log10) treated fruit, but not compared to the control 951.4 ng kg^{-1} h^{-1} (2.98 log10) (**Figure 4a**). The sharp increase in ethylene production towards the end of storage occurred earlier (at day 5) for early CA-treated avocado compared to the control and mid and late CA-treated fruit (at day 7).

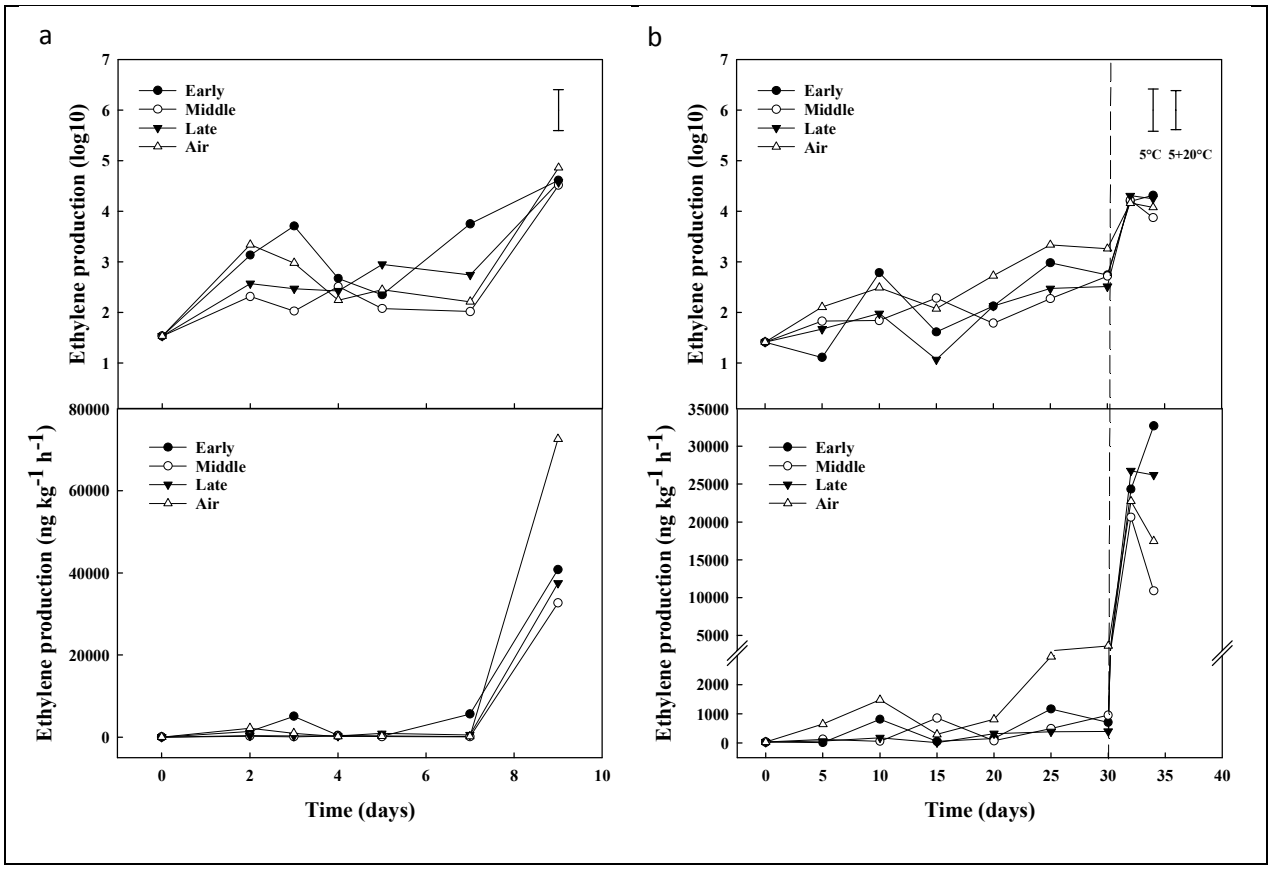


Figure 4. Ethylene production of avocado fruit subjected to CA (10 kPa CO_2 + 5 kPa O_2) at different timings throughout storage for [**a**] 9 d at 20 °C (2 d initial CA [early], 2 d CA after 1.5 d of air [middle], 2 d CA after 3 d of air [late] and air [control]) [**b**] 30 d at 5 °C (5 d initial CA [early], 5 d CA after 5 d of air [middle], 5 d CA after 10 d of air [late] and air [control]) then transferred to 20 °C for a further 4 d (shelf life; dashed line). LSD bars ($P < 0.05$) for the interaction treatment x sampling day are shown.

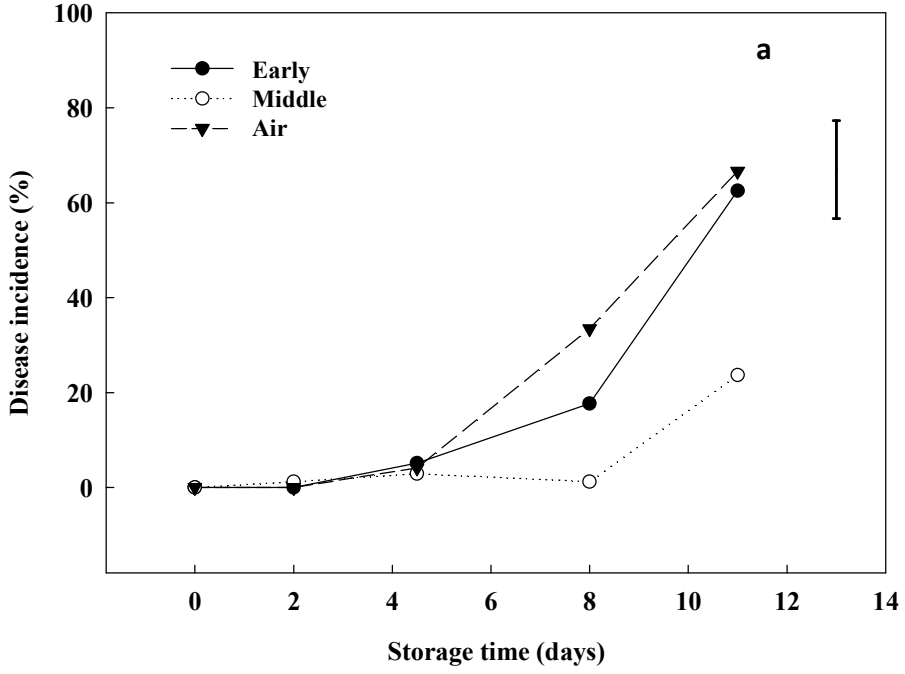
During cold storage of avocado at 5 °C, ethylene production (log10) steadily increased with no rapid increases observed following CA treatments (**Figure 4b**). Air stored avocado had higher overall ethylene values throughout storage, whereas middle CA-treated fruit had significantly lower ethylene production 3790.7 ng kg^{-1} h^{-1} (3.58 log10) compared to control

5559.9 ng kg^{-1} h^{-1} (3.75 log10) after 20 d. No differences in ethylene production were observed during the 4 d shelf-life at 20 °C following 30 d at 5 °C.

3.3. Bruise and disease incidence and severity

Based on a disease incidence threshold of 2 % (i.e. which equates to approximately one fruit with signs of damage/disease in 45), the time to first indication of disease was 8 d for the controls and the early CA-treated strawberries, and 11 d for the middle CA-treated fruit. Controls were also found to have the highest disease incidence (approximately 40 – 90 %) from 8 to 11 d (**Figure 5a**); whereas application of CA (15 kPa CO_2 + 5 kPa O_2) midway through storage resulted in the lowest disease incidence with values significantly lower (7 %) compared with the control (26.0 %). In contrast, an early CA application followed by air did not have any effect on the incidence of disease as values were similar to control.

Bruise severity sharply increased after day 5 (up to 40 % of affected surface area) in control strawberries versus those <2 % for CA-treated fruit. Both CA-treated groups (early and middle) had similar bruise severity; this was lower (*ca.* 5 – 10 %) than control fruit, which were highly affected by day 11 in cold storage (40 %) (**Figure 5b**).



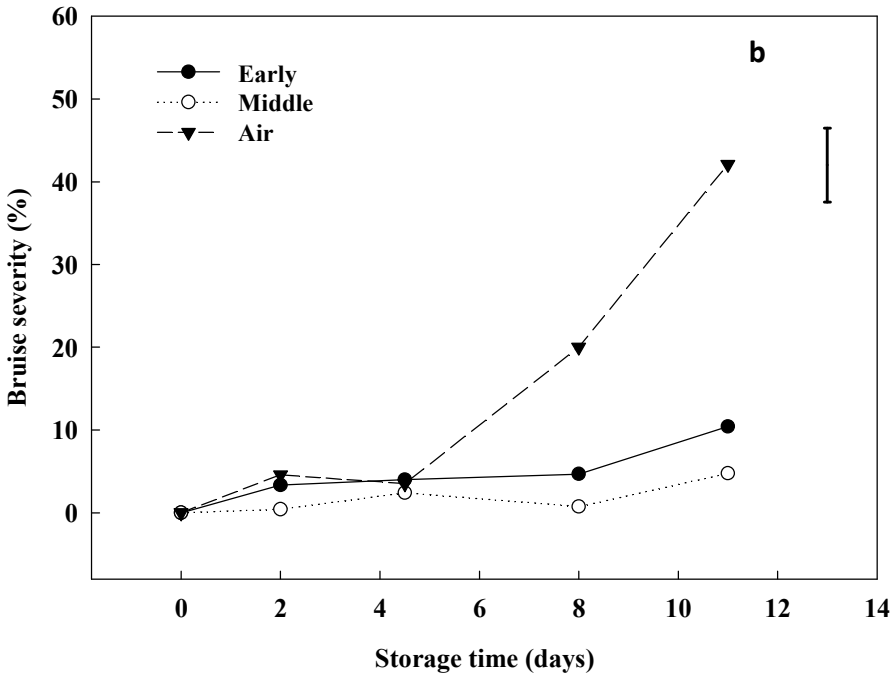


Figure 5. [**a**] Disease incidence (per punnet) and [**b**] severity (of affected area) of strawberry fruit stored under CA at different timings throughout storage for 11 d at 5 °C (2 d initial 15 kPa CO_2 5 kPa O_2 [early], 2.5 d15 kPa CO_2 5 kPa O_2 after 2.5 d of air [middle] and air [control]). LSD bars ($P < 0.05$) for the interaction treatment x sampling day are shown.

3.4. Objective colour and internal discolouration

Calyx and flesh colour in strawberry fruit was remained similarly during storage with slight differences between CA treatments (data not shown). Overall L* of strawberry flesh was lowest for air treated fruit (42.45) whereas the middle CA-treated strawberries had higher L* (44.76). Both CA-treated fruit were more vivid compared to control, as indicated by overall higher C* values (53 *vs*. 50, respectively). There was a significant interaction between treatment and time for strawberry calyx h°. At the end of the storage, both CA-treated fruit had similar h° values, while h° for control strawberries significantly decreased (106 *vs*. 111) at the end of storage indicating a less green calyx.

During avocado storage at 20 and 5 °C, a slow decrease in colour values (h°, L* and C*) was observed, indicative of ripening (**Figure 6a**). At 20 °C, late CA-treated avocado appeared to be in a less ripened state compared to those stored in air as the h° values were higher on days 2 (before CA applied), 7 and 9. This also coincided with higher overall values for L* and C* (brighter and intense colour). In contrast, early CA-treated fruit appeared to be in a more advanced stage of ripeness as indicated by lower h°, L* and C* values (less green, darker and duller in appearance), from day 5 to 9, compared to the air stored fruit. Application of CA after 1.5 d (middle treatment) showed similar values to the controls.

Avocado treated with mid and late CA and subsequently stored at 5 °C appeared to be less ripe compared to control on days 20 to 30 as indicated by higher h° and overall higher values for C* and L* (**Figure 6b**). In contrast, early CA-treated fruit experienced a more rapid drop in h° with values similar to control on most days during storage except on days 20 and 30. However, once all CA samples were transferred to shelf life conditions (4 d at 20 °C), colour became similar to controls.

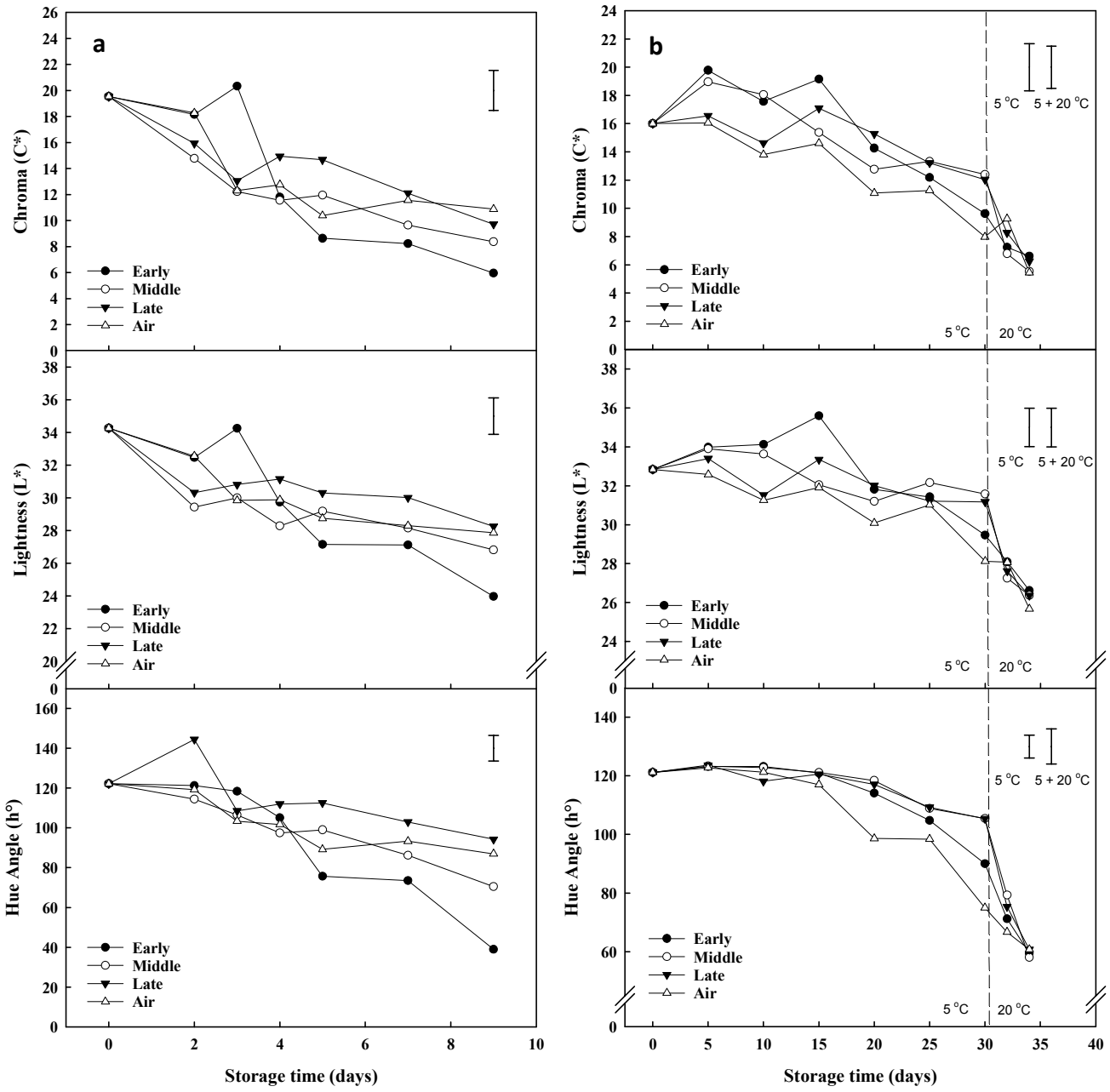


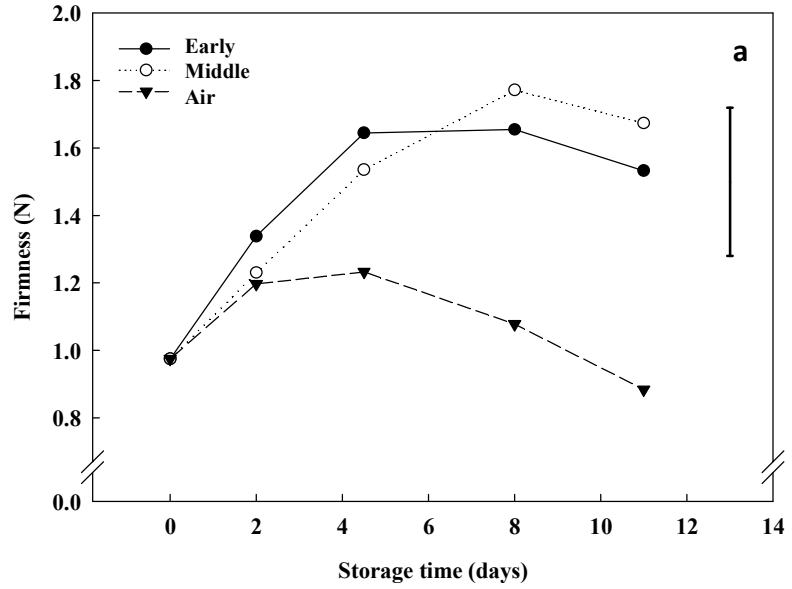
Figure 6. Objective colour (L*, C*, h°) of avocado fruit subjected to CA (10 kPa CO_2 + 5 kPa O_2) at different timings throughout storage for [**a**] 9 d at 20 °C (2 d initial CA [early], 2 d CA after 1.5 d of air [middle], 2 d CA after 3 d of air [late] and air [control]) [**b**] 30 d at 5 °C (5 d initial CA [early], 5 d CA after 5 d of air [middle], 5 d CA after 10 d of air [late] and air [control]) then transferred to 20 °C for a further 4 d (shelf life). LSD bars ($P < 0.05$) for the interaction treatment x sampling day are shown.

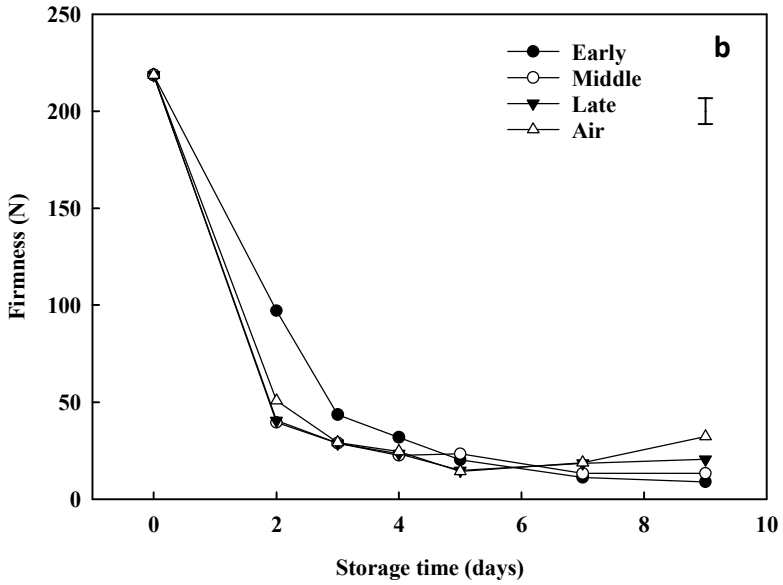
3.5. Firmness

Strawberry firmness tended to increase during or following CA treatment whereas there was no significant difference in firmness throughout storage for the control fruit (**Figure 7a**). During the first 2 d of storage, no significant difference was found for any of the treatments (*ca.* 1.2-1.3 N). However, from day 4 onwards, early CA-treated fruit were significantly firmer (by up to *ca*. 0.6 N) compared to control for the remainder of the storage period; while middle CA-treated fruit were significantly firmer from day 8 onwards (by up to *ca*. 0.8 N).

Initial firmness values for pre-climacteric avocado fruit were *ca*. 220 N before rapidly decreasing during 2 d of storage at 20 °C (**Figure 7b**). The firmness at time zero was in accordance with those reported by Meyer and Terry (2010) and Meyer et al. (2017) on pre-climacteric 'Hass' avocado. Early CA-treated avocado fruit were firmer (*ca*. 97 N) than the rest of the evaluated fruit (*ca*. 40-50 N) after 2 d at 20 °C (**Figure 7b**). By day 4, there were no longer significant differences among treatments and control. When stored at 5 °C, the early CA treatment was the only application that significantly resulted in better overall retained firmness (114.8 N) compared with the control (97.3 N). Firmness for the other two CA treatments was statistically similar to the control (data not shown).

Firmness of pre-climacteric avocado fruit (prior to CA application) harvested in December (5 °C trial) was similar (*ca*. 225 N) to that observed for avocados harvested one month earlier (trial at 20 °C). Moreover, early CA-treated fruit showed the highest firmness up to 20 d of cold storage, compared to the control, after which no significant differences were found between treatments under cold storage or ripening period (**Figure 7c**).


Early
Middle
Air
a
Firmness (N)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.0
0
2
4
6
8
10
12
14
Storage time (days)


Early
Middle
Late
Air
b
Firmness (N)
250
200
150
100
50
0
0
2
4
6
8
10
Storage time (days)

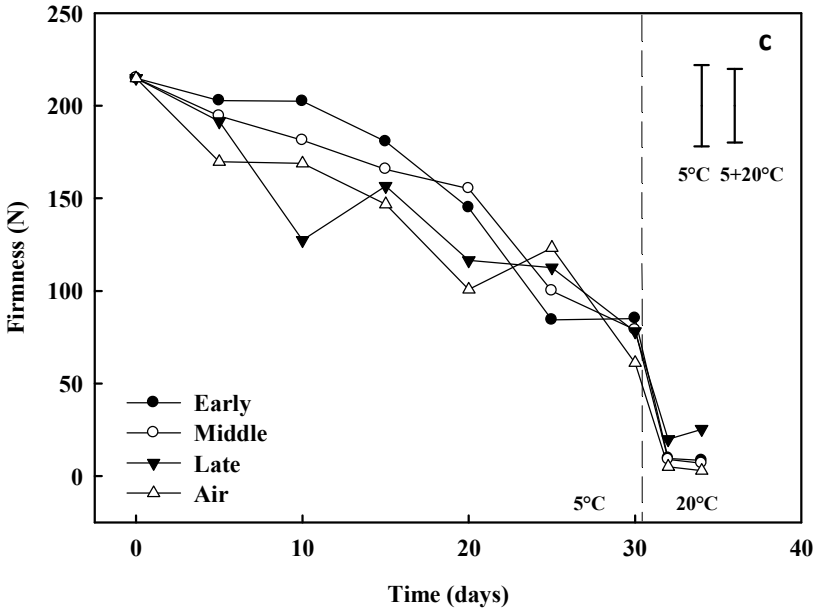


Figure 7. Firmness (N) of [**a**] strawberry fruit stored under CA (15 kPa CO_2 + 5 kPa O_2) at different timings throughout storage for 11 d at 5 °C (2 d CA followed by air [early], 2.5 d CA after 2 d of air [middle] and air only [control]); [**b**] avocado subjected to CA (10 kPa CO_2 + 5 kPa O_2) at different timings throughout storage for 9 d at 20 °C (2 d CA [early], 2 d CA after 1.5 d of air [middle], 2 d CA after 3 d of air [late] and air only [control]); [**c**] avocado subjected to CA (10 kPa CO_2 + 5 kPa O_2) at different timings throughout storage for 30 d at 20 °C followed by 4 d at 20 °C (5 d CA [early], 5 d CA after 5 d of air [middle], 5 d CA after 10 d of air [late] and air only [control]). LSD bars ($P < 0.05$) for the interaction treatment x sampling day are shown.

3.6. Discolouration

Internal discolouration assessed at day 5 and 7 of storage at 20 °C was found to be similar in CA-treated avocados compared to control (**Table 1**). Contrastingly, after 9 d of storage at 20 °C, early CA-treated avocados had significantly higher % discolouration compared to the control and mid/late CA treatments. For avocado stored at 5 °C, minimal discolouration (*ca.* 4%) was observed after 5 d of cold storage (data not shown). Only at 20 d

were significant differences noted between the middle CA-treated fruit which had significantly lower discolouration compared to the other treatments and control (**Table 1**). However, from 25 d of storage at 5 °C, no differences in avocado discolouration were observed between treatments.

Table 1. Discolouration (% of total internal cut area) of avocado subjected to CA (10 kPa CO_2 + 5 kPa O_2) at different timings throughout storage for 9 d at 20 °C (2 d CA [early], 2 d CA after 1.5 d of air [middle], 2 d CA after 3 d of air [late] and air only [control]); for 30 d at 20 °C followed by 4 d at 20 °C (5 d CA [early], 5 d CA after 5 d of air [middle], 5 d CA after 10 d of air [late] and air only [control]). Values in brackets are square root transformations for statistical purposes. LSD ($P < 0.05$) = 1.502 relates to transformed values only.

Storage temperature	**CA Treatment**	***After 5 d storage***	***After 7 d storage***	***After 9 d storage***
20°C	**Early CA**	0.00 (*0.00*)	3.33 (*1.47*)	22.22 (*4.58*)
	Middle CA	5.00 (*1.65*)	1.67 (*1.04*)	2.78 (*1.61*)
	Late CA	5.00 (*1.65*)	1.58 (*0.88*)	0.00 (*0.00*)
	Air	1.11 (*0.61*)	4.44 (*1.22*)	0.78 (*0.70*)
	LSD	1.502		
5°C	**CA treatment**	***After 20 d storage***	***After 25 d storage***	***After 30 d storage***
	Early CA	21.67	48.89	51.11
	Middle CA	5.56	29.44	35.28
	Late CA	33.33	25.78	42.44
	Air	29.44	38.89	41.67
	LSD	25.23		

4. Discussion

4.1. Advantages of *in situ* real time respiration rate measurements under CA conditions

The automated *in situ* set-up used herein allowed continuous recordings of real-time respiration measurements without disruption to the CA environment. This was deemed essential for obtaining more accurate and practically useful information comparable to real-life industry conditions. In addition, physiological effects were not influenced by handling or changes in temperature and/or CA. This is better than other flow through set-ups which are considered to be insufficiently accurate for measuring low respiration rates (Fonseca et al., 2002); in the present study, the CO_2 detectors used were capable of accurately measuring down to 1 μL L^{-1}. Furthermore, multiple chambers were connected to the set-up allowing replicates to be stored under different CA conditions for simultaneous analyses in cycles, providing real time data sets with no disruptions over time. These data points could be plotted and smoothed to provide informed changes in respiration rate every hour under specific CA conditions.

4.2. Differential effect of CA on metabolic and physiological changes during storage

The metabolic rate of strawberry and avocado fruit was found to be differentially affected by CA timing depending on the produce type. Strawberry fruit experienced bursts in respiration rate while subjected to CA environments. This is in contrast to the literature which suggests that high CO_2 levels reduce strawberry respiration rates (Fonseca et al., 2002; Almenar et al., 2006). Continuous monitoring under a CA environment enabled the detection of physiological changes throughout storage. Thus, the transient increase in RR detected immediately upon exposure to CA for both strawberry and pre-climacteric avocado may not have been captured using conventional methods, where respiration rate is normally recorded in a discrete way and after fruit acclimatisation following removal from a CA environment. An enhanced respiration rate typically accompanies high ethylene production (Merchante et al., 2013); yet this was not replicated for (non-climacteric) strawberry CA-treated fruit compared to the control. It is hypothesized, that the potential increase in ethylene preceding the peak in CO_2 production may have occurred while under CA storage and therefore prior to the ethylene

measurements. Moreover, the effects of CA on physiological changes have been shown to vary depending on the strawberry cultivar which could also differentially affect metabolic rate (Watkins et al., 1999). This phenomena may explain the observed discrepancies in the literature. Reduced exposure time to high CO_2 levels may benefit strawberry fruit quality since fermentation products (acetaldehyde, ethanol and ethyl acetate) can accumulate after just one day of storage under high CO_2 (20 kPa) (Watkins et al., 1999; Fernandez-Trujillo et al., 2007). Therefore, strawberry storage under high CO_2 for short time periods may benefit the preservation of strawberry quality.

Contrastingly, RR of avocado stored at 20°C was reduced while under CA conditions as reported previously (Meir et al., 1995; Hertog et al., 2003). This reduction in metabolic rate was observed through quality indices, such as delayed softening in the early CA-treated fruit. Nevertheless, when CA exposure was removed, respiration returned to similar levels prior to the start of CA. Pre-treating avocado with high levels of CO_2 for 48 h prior to cold storage (2-5 °C) has previously been shown to delay ripening as well as reduce incidence of chilling injury (CI) (Thompson, 2003). In contrast, exposing avocado to 3 % O_2 and 97 % nitrogen for 24 h (at 17 °C) diminished CI, and reduced RR and ethylene production during subsequent storage at 2 °C (Pesis et al., 1994). There have been few studies centred on the impacts of CA on avocado, with the majority focused towards extending pre-climacteric storage life (Meir et al., 1995; Prabath et al., 2013; Basuki et al., 2016). However, the underlying mechanisms responsible for the observed metabolic changes during CA are not yet fully understood.

Strawberry and pre-climacteric avocado appeared to react similarly upon initial placement under CA conditions, where fruit showed a stress response reflected as a substantial increase in CO_2 production. This behaviour differs from post-climacteric avocados, where the respiration decreased from the moment they were subjected to CA. This agrees with previous studies, where the application of abiotic treatments induced stress responses in pre-climacteric

tomatoes and mango leading to increases in stress-related gene expression and respiration rate, respectively (Rothan et al., 1997; Rees et al., 2012).

Lower ethylene levels have been reported to reduce senescence and delay ripening in avocado (Meyer and Terry, 2010). Changes in metabolic rate are not widely assessed during CA storage as many studies focus on physiological changes during ripening. Basuki et al. (2016) measured respiration and ethylene during ripening at 20 °C following removal from CA. However, real time changes in metabolic rate during CA could provide additional information on the storability of particular batches of produce. For ethylene production, higher levels were recorded on day 3 during 20°C in early CA-treated avocado fruit, but in general, values were similar to the controls. For avocados stored at 5°C, the beneficial effect of targeted mid CA treatment is supported by lower ethylene production whilst preventing an increase in RR.

4.3. Effect of CA timing on ripening and senescence

Despite rapid bursts in respiration following CA treatment, CA applied mid-way through strawberry storage was shown to maintain a more vibrant flesh colour, whilst calyx colour remained greener compared to control. Previous studies have reported lighter fruit colour after storage at high CO_2 levels (20 kPa) when applied at the beginning of storage (Watkins et al., 1999). Furthermore, after 8 d storage at 5 °C and regardless of application time, strawberries treated with CA (15 kPa CO_2 + 5 kPa O_2) were firmer compared with the control samples. Many studies have previously demonstrated an increase in strawberry fruit firmness following pre-treatments with high CO_2 prior to storage (Watkins et al., 1999; Harker et al., 2010; Hwang et al., 2012). For example, Harker et al. (2010) applied high CO_2 (10 kPa) (O_2 levels were not given) to strawberries cv. Pajaro for an initial 3 d before transferral to atmospheric levels (3 weeks at 0 °C). The firmness was enhanced by high CO_2 treatment which was suggested to be due to changes in apoplast pH enhancing cell-to-cell adhesion. The

mechanism for increased firmness was further investigated by Hwang et al. (2012), who suggested that this was caused by pectin polymerisation mediated by calcium. Disease incidence in strawberry was also affected by the timing of the CA, which has not previously been examined. A continuous CA treatment of high CO_2 (15 kPa for 5 or 14 d at 5°C) alone was shown to be effective at reducing *Botrytis* rots (Wskelaki and Mitcham, 2003). However, the present study showed that only 2.5 d of CA was sufficient to extend the shelf-life of strawberries (based on disease incidence) by a further 3 d.

Lower ethylene and respiration rate values in avocado CA stored fruit could indicate reduced sensitivity to cold storage (5 °C), since an increase in RR after storage is postulated to be triggered by chilling and coincides with incidence of CI; symptoms of which include unusual changes/patterns in respiration rate and ethylene production, as well as failure to soften and ripen normally after cold storage (Basuki et al., 2016). However, despite normal fruit softening, internal discolouration of the mesocarp was observed in all samples, including the control, towards the end of cold storage. Therefore, CA storage did not hasten CI at 5°C. Increases in ethylene production during avocado storage at 5°C was also observed at the end of storage, by which time, changes in colour and firmness were already evident. Increases in ethylene could therefore have been attributed to the occurrence of internal discolouration at the end of storage.

Application of early CA to avocado during storage (at 20 °C) was found to delay the climacteric peak in respiration which coincided with higher firmness levels, an indication that these fruit were less ripe; however, this was not reflected in colour measurements which showed these fruit were darker in colour. It is possible that the higher ethylene values recorded at day 3 in early CA-treated fruit only, may have hastened colour change; since avocado colour change is reported to be highly sensitive to ethylene (Meyer and Terry, 2010). However, together with the fact that firmness was maintained and percentage of internal discolouration

of early CA-treated avocados was the highest among treatments, early CA treatment appeared to hasten sensitivity to CI. Previous work has also reported higher incidences of mesocarp discolouration in CA-stored avocados compared to regular air storage (Eksteen et al., 1998).

Mesocarp discolouration was more severe in samples subjected to extended storage at 5°C for 30 d (total storage time as 8 weeks [including transportation period]) compared to samples immediately stored at 20 °C (total storage time 5 weeks). According to Woolf et al. (2005), 7 weeks is considered a long storage time and can be a significant challenge in terms of quality management. While, 4 weeks is not uncommon for exports from the Southern to Northern Hemisphere, disorders such as mesocarp discolouration can become a problem. Incorporating targeted CA was not found to reduce incidence of CI (mesocarp discolouration) and therefore did not extend the storage period beyond 3 additional weeks.

Application of different CA scheduling regimes during cold storage was also found to maintain colour. In addition, delaying the start of CA storage (*viz*. mid CA) was more effective in slowing colour change associated with ripening and reducing incidence of mesocarp discolouration up to 20 d of storage compared to early- and late-CA. Similar findings were reported on onion where delayed CA was just as beneficial at preventing sprout growth as continuous CA (Chope et al., 2007). However, for the experiment herein, additional shelf life assessments at this time point would be required to confirm potential commercial benefits of targeted CA mid-way through cold storage.

5. Conclusions

In conclusion, these results have shown that the targeted application of CA for just 2.5 d mid-way through storage at 5 °C (2.5 d; 15 kPa CO_2 + 5 kPa O_2 after 2 d in air) increased strawberry shelf-life by 3 d (based on disease incidence). Furthermore, CA reduced endogenous ethylene production, and resulted in lighter, more vivid and firmer fruit suggesting

a reduction in senescent decline. This has implications for modified atmosphere packaging of strawberry fruit where challenges lie in maintaining a desired atmosphere throughout shelf-life.

The real-time respiration results for avocado fruit suggest that CA delays the climacteric peak and in general reduced ethylene production. The commercial application of early CA in avocado packaging suggests that shelf life could be extended by 2 d based on firmness. Conversely, storage of avocados at 5 °C would benefit from targeted mid-CA application as shown by better maintenance of colour and lower chilling injury incidence. It is evident that better scheduling CA treatments could be a more cost effective method for industry by, for example, reducing the amount and control of controlled respiratory gases required, yet effectiveness may be limited by the postharvest storage period. Further research into the optimum targeting of CA duration and concentration are needed to further enhance storage shelf-life and reduce postharvest losses.

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Impact of controlled atmosphere scheduling on strawberry and imported avocado fruit

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