The effect of modified atmospheres on the rate of quality change in ‘Hass’ avocado

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Abstract

Gas exchange rates and quality changes of avocados (Persea americana, Mill., cv. ‘Hass’) stored at 7 °C in 32 different modified atmosphere (MA) conditions generated using a flow-through system, were monitored to characterise their functional relationship. An integrated modelling approach was used to link rates of quality loss to the rate of gas exchange. This revealed a close quantitative relationship between the gas exchange of ‘Hass’ avocado fruit on one hand and weight loss, colour change and softening on the other, indicating a direct metabolical link between these processes. High CO2 lowered the respiratory quotient of avocado, an effect which, based on literature, could be explained by a shift from oxidative phosphorylation into the alternative respiratory pathway. In the model approach, the rates of quality degrading processes were linked to oxidative phosphorylation only, assuming this is where ATP was being produced. At high CO2 levels at aerobic conditions, unaccounted weight loss data indicated a considerable volatile production which, however, was not qualitatively characterised. Both weight loss and colour change indicated the involvement of anaerobic processes at low O2 levels. At 2 kPa O2 and 0 kPa CO2 no CO2 injury occurred, while the change in hue was minimal, weight loss was minimised, and softening was almost completely inhibited. At such low O2 levels, no additional benefit was found from raising the levels of CO2.

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1. Introduction

It is generally accepted that modified atmospheres (MA) can inhibit the loss of quality during the postharvest life of a wide range of products (Kader et al., 1989). Although much research has been done to define optimum MA conditions for many fresh food products, the underlying mechanisms for the action of MA are still only superficially understood. Application of MA generally

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involves reducing oxygen levels (O₂) and elevating levels of carbon dioxide (CO₂), thus, reducing oxidative respiration. Parallel to the effect on respiration, the energy needed to support other metabolic processes is affected, as are, consequently, the processes themselves.

Quality attributes, such as texture, generally change with time, as part of the normal ripening metabolism of the product (Tijskens and Polderdijk, 1996). Those developmental changes directly influenced by O₂ or CO₂, or driven by the energy supplied by respiration or fermentation, would all be affected by applying MA conditions. Some of these processes are affected more than others because of the way they depend on atmospheric conditions (Kanellis et al., 1993). Besides inhibiting ripening through inhibition of ethylene production, MA also affects ripening through inhibition of ethylene action (Solomos and Kanellis, 1997). O₂ requiring processes will be affected straight away through the availability of O₂. Although much work has been done on showing the general effects of MA conditions, quantification of the effects on changes in quality attributes is still limited.

Such a relationship between rate of gas exchange and rate of quality loss has been seen in broccoli (Polderdijk et al., 1995), where the rate of discoloration of buds depended on atmospheric composition. Also, deterioration of asparagus spears appeared to be strongly related to its own metabolic rate (Brash et al., 1995). Tijskens (1996) suggested using metabolic rate as a rate index for quality changes and Hertog et al. (1999) applied this approach to explain the effects of MA on spoilage of strawberries.

To increase our understanding of the effect of MA on quality, we have been studying quality changes under MA conditions for a number of important New Zealand export crops. Earlier work on ‘Braeburn’ apples showed that softening and gas exchange had comparable Km values indicating a common kinetic background (Hertog et al., 2001). It also revealed a close quantitative relationship between rate of softening and rate of gas exchange of ‘Braeburn’ apples, indicating that fruit softening is metabolically linked to gas exchange.

The current paper presents results on avocado (Persea americana, Mill., cv. Hass). Although some work has been done on the effect of MA on quality changes in avocado fruit (Meir et al., 1995, 1997) the two have not previously been linked together in a quantitative way. Although the relationship between gas exchange rates and rates of quality loss will be much more complex, and for climacteric fruit should take into account the involvement of ethylene, a first approach is taken by linking the rates of gas exchange directly to the rates of the main quality decay processes in avocado.

2. Material and methods

2.1. Fruit

Export quality ‘Hass’ avocados (P. americana, Mill.) were obtained from one grower from the Auckland region, New Zealand. Fruit were harvested early February 2000 (for the MA experiment), or mid November 2000 (for the low CO₂ treatments), graded, packed and couriered overnight to Massey University. On arrival, fruit were individually labelled, locations for colour and firmness measurements were marked with a circular stamp at both base and stem end, and initial fruit measurements were taken.

2.2. MA

Thirty-two PVC containers (volume = 0.0135 m³) were packed on the same day with 19 fruit each (average fruit weight of 198 g) and stored at an air temperature of 7 °C, using a range of different MA. A separate sample of 30 fruit was stored in its original box in the same cold room for control measurements during the experiment. The main purpose of the air-stored control fruit was to indicate for how long the experiment in the closed PVC containers had to last to generate maximum differences between the treatments applied, with the air-stored control fruit representing the fastest ripening scenario. Based on the observations on the control fruit the experiment was finished after 32 days of storage.
Fruit were stored for 32 days using a flow-through system. A matrix of 32 different MA gas mixtures was generated by mixing flows of dry air, O2–free N2 and foodgrade CO2 (BOC, Palmerston North, NZ), to create combinations of eight different O2 levels (0–21 kPa O2) and four different CO2 levels (0–15 kPa; Fig. 1). Before entering the PVC containers, the gas mixtures were humidified by bubbling through jars with water resulting in ±98% RH. The flow rate was controlled at 0.15 l min\(^{-1}\).

The MA conditions were held constant throughout the whole time span of the experiment (Fig. 1). The CO2 levels remained constant over time with an average S.E. of ±0.50 kPa CO2, and the O2 levels stayed constant with an average S.E. of ±0.32 kPa O2.

Gas conditions inside the containers were checked weekly by removing a sample using 100 μl glass syringes and analysing them as described below. At the same time, respiration rates of the fruit were measured by temporarily closing the tubes to allow accumulation of CO2 and depletion of O2 by about 0.5 kPa. Depending on the MA conditions, this took between 1 and 3 h.

### 2.3. Low CO2 treatments

Five containers were packed on the same day with 20 fruit each (average fruit weight of 240 g) and stored at an air temperature of 7 °C, applying CO2 levels ranging from 0 to 5 kPa. Fruit were stored for 52 days using a flow-through system. The gas mixtures were generated by mixing flows of dry air with foodgrade CO2 (BOC, Palmerston North, NZ). Before entering the containers, the gas mixtures were humidified by bubbling through jars with water resulting in ±98% RH. The flow rate was controlled at 0.15 l min\(^{-1}\). Gas conditions inside the containers were checked weekly by removing a sample using 100 μl glass syringes and analysing them as described below.

### 2.4. Gas analysis

All gas samples were analysed using an O2 electrode (Citicell C/S type, City Technology Ltd., London, UK) in series with a miniature infrared CO2 transducer (Analytical Development Company, Hoddesdon, UK), with O2–free N2 as carrier gas (flow rate 35 ml min\(^{-1}\)). Output signals were linear over the range applied and analysed using HP integrators (Hewlett-Packard, model 3396A). Commercially prepared standards were used for calibrating the gas analysers. All samples were collected in duplicates through the two sampling ports of the containers. When duplicates differed by more than 0.1 kPa, new samples were taken and the system was checked for possible errors until consistent results could be obtained. Standard gases were routinely used to check for possible drift in the signal.

### 2.5. Fruit measurements

All fruit stored under MA conditions were measured at 20 °C for initial and final (after 32 days of storage) weight, firmness and colour. The separate batch of control fruit was monitored for firmness and colour on a weekly basis. Firmness and colour were measured at both base and stem end on pre-marked spots.

Firmness was measured non-destructively using a HandyHit (model 500-800, Fujihira Industry Co.
Ltd., Japan), which has been proven useful for the assessment of avocado (Inoue and Tateishi, 2000) and kiwifruit firmness (Burdon et al., 1999). The Handyhit measures the elastic deformation of the tissue when compressed for 1 mm with a 6 mm diameter flat-ended plunger. The analogue gauge of the HandyHit device gives a reading between 0 (firm) and 100 (soft) corresponding with a compression force of, respectively, 800 g (or more) and 500 g (or less). The sensitivity of the device is such that it will only start to respond at the stage the fruit start to turn to black. Colour was measured as hue angle (H) using a chromameter (model CR-200, Minolta, Osaka, Japan) calibrated to a green plate.

CO2 injury was scored by estimating the percentage surface area affected and the depth of the injury of individual fruit. The injury consisted of round dark brown patches clearly distinctive against the green peel. At the late stages of ripening the patches became less obvious against the then purple-black colour of the peel. The flesh underneath these patches stayed firmer than the surrounding tissue. The percentage surface area affected was estimated using the following six classes: 0, 0–10, 10–25, 25–50, 50–75, 75–100 with increasing weights from 0 to 5. The depth of the injury was scored by cutting the fruit and scoring the depth of the injury according to the following four classes: 0, 0–5, 5–10 and 10–20 mm, assigning weights from 0 to 3. The final injury index was calculated as the product of the weighted average for both indices.

2.6. Data analysis

All data collected were expressed according to the units proposed by Banks et al. (1995). Data were analysed statistically with the iterative non-linear regression routine of STATISTICAL ANALYSIS SYSTEM (SAS software, version 6.11, SAS institute Inc., Cary, NC, USA). The non-linear equations were applied directly, without transformation to data or equations.

3. Results and discussion

3.1. Gas exchange

Under most of the MA conditions applied, gas exchange rates stayed constant throughout the experimental storage period. The fruit stored at 0 kPa CO2 at 5, 10 or 20 kPa O2 (Fig. 2), showed an increased rate of gas exchange, indicating a climacteric rise in the metabolic activity of these fruit between 7 and 14 days storage. For the statistical analysis of the gas exchange data, the data averaged for the whole storage period were used to link the overall metabolic activity to the overall quality changes that occurred throughout the whole storage period. The averaged gas exchange data (plotted symbols from Figs. 3 and 4) showed typical Michaelis Menten type behaviour. The rate of O2 consumption \( (r_{O2}) \) increased with increasing levels of O2 and decreased with increasing levels of CO2. The fact that the inhibiting effect of CO2 did not decrease with increasing O2 levels, indicated an uncompetitive type of CO2 inhibition (Hertog et al., 1998). The avocado fruit did not show a significant increase in their CO2 production \( (r_{CO2}) \) as would be expected assuming alcoholic fermentation at low O2 levels (Fig. 4).

Yahia (1993) found that avocados showed an increased production of ethanol and acetaldehyde, and activity of anaerobic enzymes after 1 day exposure to 0.5% O2, clearly indicating an in-

![Fig. 2. CO2 production of ‘Hass’ avocado over time. The fruit were stored for 32 days at 7 °C, 0 kPa CO2, with a range of O2 levels (0–20 kPa) as indicated in the legend.](image)
creased anaerobic activity. In spite of not showing an increased $r_{\text{CO}_2}$, the fruit stored at 0 kPa O$_2$ exhibited clear signs (off odours) of increased volatile production which were not identified. Given the rancid smell, one would expect these volatiles to originate from some part of fatty acid metabolism.

The $r_{\text{CO}_2}$ at high CO$_2$ levels in aerobic conditions was suppressed much more than the $r_{\text{O}_2}$ (Fig. 4) resulting in a reduced respiratory quotient (RQ; Fig. 5). This change in RQ depending on CO$_2$ (Fig. 5) might be explained by the fruit recycling its respiratory CO$_2$ by phosphoenolpyruvate carboxylase fixing CO$_2$ as malate (Blanke, 1991). In this case ATP production would be related to the total $r_{\text{O}_2}$. Under the assumption that gas exchange rates can be linked to the rates of quality change processes, as the former is providing energy for the latter, the inhibiting effects of MA on $r_{\text{O}_2}$ (Fig. 3) should be relative to the inhibiting effects of MA on colour change (Fig. 8B) and firmness loss (Fig. 9B). Prior analysis showed this was not the case. The rate of softening and colour change was much further suppressed than would be expected based on the suppression of the overall $r_{\text{O}_2}$. One could also argue whether the phosphoenolpyruvate carboxylase fixing capacity could be large enough to explain the observed drop in RQ as it will ultimately stop as a maximal amount of malate accumulates (Blanke, 1991).

Another explanation might be that because of the high CO$_2$ levels, the mitochondrial respiration had shifted to the alternative respiratory pathway,
increasing the O\textsubscript{2} consumption as a result of up-regulation of alternative oxidase proteins. Lange and Kader (1997a, b, c) showed this effect for both isolated avocado mitochondria and whole fruit, and related this to the ripening stage of the fruit. This alternative non-phosphorylating respiratory pathway allows recycling of metabolic intermediates when the energy charge is high by bypassing oxidative phosphorylation (Lambers, 1982). According to Lange and Kader (1997a, b, c), high CO\textsubscript{2} in avocado could trigger both this alternative respiratory pathway and anaerobic pathways at the same time. Assuming the triggered anaerobic pathway is one that mainly results in volatile production without extra CO\textsubscript{2} production (as observed at low O\textsubscript{2} levels), the increased O\textsubscript{2} consumption due to up-regulation of the alternative oxidase could explain the observed reduction in RQ. ATP production would be related to just that part of \( r_{O_2} \) used in the oxidative phosphorylation which is relative to the observed \( r_{CO_2} \).

The data were analysed using Michaelis Menten type gas exchange models using the uncompetitive type of CO\textsubscript{2} inhibition (Hertog et al., 1998). Assuming the change in RQ was due to activation of the alternative respiratory pathway, the gas exchange model was extended to include this phenomenon. A simplified pathway was assumed, describing how an alternative oxidase (AOX) is regulated by the presence of CO\textsubscript{2} to oxidise the large flow of electrons (e\textsuperscript{-}) through an intermediate enzyme substrate complex (AC).

\[
e^{-} + O_{2} + CO_{2} + AOX \xrightarrow{k_{1}} AC \xrightarrow{k_{-1}} CO_{2} + H_{2}O + AOX
\]

The large flow of e\textsuperscript{-} is believed to be generated by fermentative pathways induced by the high CO\textsubscript{2} levels and is assumed not to be rate limiting. The active complex AC is assumed to be in steady state with its constituents. The steady state reaction is characterised by its forward and backward reaction rates \( k_{1} \) and \( k_{-1} \). The reaction rate for the final reaction step is given by \( k_{p} \). For this case, O\textsubscript{2} consumption by AOX (\( r_{O_{2}}^{AOX} \)) can be described according to:

\[
\begin{align*}
\frac{r_{O_2}^{AOX}}{Km_{O_2}^{AOX} + p_{O_2}P_{CO_2}} & = \frac{r_{O_2}^{AOX,max}}{Km_{O_2}^{AOX,max} + p_{O_2}P_{CO_2}} \\
\frac{r_{CO_2}}{k_{-1} + k_{p}} & = k_{p}AOX
\end{align*}
\]

where \( p_{O_2} \) and \( p_{CO_2} \) are the partial pressures for O\textsubscript{2} and CO\textsubscript{2} (both in kPa); \( r_{O_{2}}^{AOX,max} \) is the maximum oxidative O\textsubscript{2} consumption rate by AOX (mol kg\textsuperscript{-1} s\textsuperscript{-1}) unconstrained by O\textsubscript{2} or CO\textsubscript{2}; \( Km_{O_2}^{AOX} \) (in kPa\textsuperscript{2}) is the Michaelis constant for the O\textsubscript{2} consumption by AOX. This alternative oxidase is regulated by the presence of CO\textsubscript{2} but does not produce CO\textsubscript{2}.

Total O\textsubscript{2} consumption \( r_{O_2} \) was assumed to be the combined result of \( r_{O_{2}}^{AOX} \) and O\textsubscript{2} consumption by the normal oxidative phosphorylation (\( r_{O_{2}}^{OX} \)) as described by the standard Michaelis Menten type gas exchange model using the uncompetitive type of CO\textsubscript{2} inhibition (Hertog et al., 1998). CO\textsubscript{2} production was linked to the oxidative phosphorylation only. This resulted in:

\[
r_{O_2} = r_{O_{2}}^{OX} + r_{O_{2}}^{AOX}
\]

\[
r_{CO_2} = RQ \cdot r_{O_2}^{OX}
\]

where \( r_{O_{2}}^{OX,max} \) is the maximum O\textsubscript{2} consumption rate (mol kg\textsuperscript{-1} s\textsuperscript{-1}) by oxidative phosphorylation unconstrained by O\textsubscript{2}; \( Km_{O_2}^{OX} \) and \( Km_{CO_2}^{OX} \) (both in kPa) are the Michaelis constants for respiration and the uncompetitive inhibition of respiration by CO\textsubscript{2}, respectively. The parameter estimates are given in Table 1. The simulated model values using these parameter estimates are represented by the planes in Figs. 3 and 4.

There is only a limited amount of literature on the gas exchange rates to which we can compare our results. Most work on gas exchange rates of avocado was done by Eaks (1976, 1978, 1980, 1983) but covered mainly rates at 20 °C after exposure to different chilling temperatures. However, our results seem to fit within the common knowledge on avocado, confirming its relatively high metabolic activity as compared with, for instance, apple or tomato, which have gas ex-
change rates five times lower at 7 °C (Hertog et al., 1998). The postulated mechanism from Eq. (2) was able to explain 97% of the observed variation in gas exchange rates and is consistent with literature data on the effects of elevated CO₂ on alternative oxidase activity.

3.2. Weight loss

During the 32 days of the experiment, fruit lost about 0.4–1.8% of their initial weight, depending on the MA conditions applied (Fig. 6). Weight loss mainly consists of water loss through transpiration and carbon loss through gas exchange (Maguire et al., 2001).

The metabolic change observed in the gas exchange data resulting in an RQ of 0.5 (for each mole of O₂ consumed, only half a mole of CO₂ is being released) implied fruit would have gained net weight at high CO₂ treatments. As this was not observed the fruit must have lost weight through other means.

Based on the modelled gas exchange and estimates of transpiration assuming an average water vapour permeance of avocado fruit of 60 nmol s⁻¹ m⁻² Pa⁻¹ (Johnston and Banks, 1998) and an average relative humidity of 98%, the expected weight losses at 0 kPa CO₂ could be largely explained (Fig. 6). However, avocado fruit developed larger weight losses at low O₂ levels and at increased CO₂ levels than was expected based on transpiration and gas exchange rates. The only possible way to explain this gap in the mass balance is by assuming a certain volatile production at both low O₂ levels (induced by anaerobic conditions) and increased CO₂ levels (in aerobic conditions). The volatiles generated under both conditions do not necessarily have to be the same.

Ethylene could be a possible candidate for the volatile produced at increased CO₂ levels at aerobic conditions. However, there is no reason why ethylene would only be produced at increased CO₂ levels and not at 0 kPa CO₂ especially as high CO₂ is known to completely inhibit ethylene production of preclimacteric fruit (Lange and Kader, 1997a). Also, if the weight loss unaccounted for was to be completely attributed to

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (S.E.)</th>
<th>Parameter</th>
<th>Estimate (S.E.)</th>
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</thead>
<tbody>
<tr>
<td>( r_{O_2}^{OX, \text{max}} )</td>
<td>484 (15)</td>
<td>( K_{H} )</td>
<td>0.19 (8)</td>
</tr>
<tr>
<td>( K_{mO_2}^{OX} )</td>
<td>10.5 (23)</td>
<td>( K_{mO_2} )</td>
<td>0.91 (36)</td>
</tr>
<tr>
<td>( K_{muO_2}^{OX} )</td>
<td>1.96 (25)</td>
<td>( K_{mO_2}^{AOX} )</td>
<td>3.29 (76)</td>
</tr>
<tr>
<td>( RQ )</td>
<td>0.93 (7)</td>
<td>( R^2 ) (%)</td>
<td>97</td>
</tr>
<tr>
<td>( n )</td>
<td>64</td>
<td>32</td>
<td></td>
</tr>
</tbody>
</table>

\( a \) \( r_{O_2}^{max} \) is the maximum oxidative O₂ consumption rate (nmol kg⁻¹ s⁻¹) unconstrained by O₂; \( K_{mO_2} \) and \( K_{muO_2} \) (both in kPa) are the Michaelis constants for, respectively, respiration and uncompetitive inhibition of respiration by CO₂; \( RQ_{OX} \) is the respiration quotient for oxidative respiration.

\( b \) \( K_{H} \) is the maximum rate of colour change under anaerobic conditions; \( K_{mO_2} \) is the Michaelis constant for the inhibition of this anaerobic process by O₂.

\( c \) S.E. are expressed as a percentage of the parameter value.

\( d \) \( R^2 \), percentage variance accounted for; \( n \), number of observations.

Fig. 6. Weight loss of ‘Hass’ avocado fruit stored for 32 days at 7 °C as a function of the MA conditions applied. Weight loss is expressed as a percentage of the initial weight. Plotted symbols represent the measured data while the lines represent the weight loss expected based on modelled gas exchange and transpiration rates. A negative value indicates weight gain. The difference between modelled and measured resembles the unaccounted carbon loss due to volatile production.
ethyleneproduction, this would imply an average ethylene production during the 32 days of storage at 15 kPa CO₂ of about 250 nmol s⁻¹ kg⁻¹ which is about 100 times the literature values generally found for avocado fruit in their climacteric phase (Eaks, 1978, 1980, 1983). In other words, assuming average values, ethylene production could only explain 1% of the observed gap in the mass balance and some other volatile must have been responsible for the observed gap in the mass balance.

3.3. Colour

Avocado fruit ripened to different degrees, as measured by change of colour, with differing MA conditions (Fig. 7). Generally the stem end coloured faster than the base of the fruit (results not shown), but the responses to the applied MA conditions were similar. The results will be discussed using the data coming from the stem end of the fruit. The largest change in hue was observed at high O₂ and low CO₂ levels (Fig. 7). The minimum change in hue was observed around 1–2 kPa O₂, corresponding to where the minimum weight loss was observed (Fig. 6). Colour change at low O₂ levels is apparently also driven by anaerobic processes not visible from the normal gas exchange (Figs. 3 and 4). The observed response of colour change to MA conditions was comparable to that observed by Meir et al. (1995), although they did not report an increased colour change under anaerobic conditions as they did not measure below 3 kPa O₂.

Just as the rate of softening of apple depended on the MA conditions via the energy provided by the gas exchange (Hertog et al., 2001), we would expect the rate of colour change to depend on the MA conditions. With apple softening, Hertog et al. (2001) assumed that softening was linear over the short time interval the fruit were monitored. For the current work on avocado this assumption is not valid, and the complete non-linear behaviour of colour change with time needs to be taken into account (Fig. 8A).

Hertog (2002) applied the following model to colour change of avocado describing hue (H in °) during time (t in days):

\[ H_t = H_{+\infty} + \frac{H_{-\infty} - H_{+\infty}}{1 + e^{k_H \cdot H_{+\infty} - H_{-\infty}}/(H_0 - H_{+\infty})}, \]

\[ H_{-\infty} = (1 + C) \cdot H_0 \]  

with the two asymptotic hue values (\(H_{+\infty}\) and \(H_{-\infty}\) both in °) at plus and minus infinite time, the rate constant \(K_H\) (day⁻¹) and the initial hue value \(H_0\), \(H_{-\infty}\), was defined relative to \(H_0\) through the batch specific parameter \(C\). The rate constant for storage in air at 7 °C was \(k_{H}^{RA} = 0.213\) per day, while \(H_{+\infty}\) was set at 35° (Hertog, 2002). For the control fruit from Fig. 8A, \(C\) was estimated to be 0.00194, with \(H_0\) set to the initial measured hue value of 122.2°.

Depending on the MA conditions under which the fruit were stored, we would expect the rate constant \(k_H\) to vary depending on the gas exchange rates. However, comparing Fig. 7 with Fig. 4 showed that the increased colour change at low O₂ levels cannot be explained by increased anaerobic activity in terms of \(r_{CO₂}\). There was also a clear difference between the normal colour change under aerobic and the defective colour change under anaerobic conditions; while the fruit turned purple black under aerobic conditions due to normal anthocyanin formation, they turned greyish green under anaerobic conditions. This indicated that a different process governed the colour change under anaerobic conditions. To describe
colour change of avocado fruit, the rate constant \( k_H \) was therefore linked to the relative metabolic rate (Tijskens, 1996; Hertog et al., 1999) describing the aerobic colour change process. As the \( r_{O_2}^{AOX} \) was assumed not to result in any ATP production, the aerobic colour change process was linked to \( r_{O_2}^{OX} \) only. The colour change model was further extended with an anaerobic term analogous to the term generally applied to describe fermentative CO\(_2\) production (Peppelenbos et al., 1996). This resulted in the following equation for the rate of colour change as a function of the MA conditions:

\[
k_H = k_{RA}^{OX,MA} r_{O_2}^{OX,RA} + \frac{k_{RA}^{OX} O_2}{1 + O_2/K_{m_H}}
\]  

(4)

with \( r_{O_2}^{OX,MA} \) the O\(_2\) consumption rate by oxidative phosphorylation at certain MA conditions; \( r_{O_2}^{OX,RA} \) the O\(_2\) consumption rate by oxidative phosphorylation in regular air; \( k_{RA}^{OX} \) the maximum rate of colour change under anaerobic conditions and \( K_{m_H} \) the Michaelis constant for the inhibition of this anaerobic process by O\(_2\).

The colour data were analysed by applying this integrated approach combining Eqs. (2)–(4) using the parameter values already established, and estimating the two additional parameters \( k_{RA}^{OX} \) and \( K_{m_H} \). Over 98% of the variation in the final colour of the MA stored avocado fruit was explained by the model (Fig. 8B; Table 1).

3.4. Firmness

On average, the control fruit stored in air (Fig. 9A) did not soften sufficiently to reach a firmness of 100. Generally, the stem end softened faster than the base of the fruit (results not shown), but the responses to the applied MA conditions were similar. The results will be discussed using the data coming from the stem end of the fruit.

In analogy to the colour data, a simple logistic model was used to describe the firmness data in function of time:

\[
F(t) = \frac{F_0 + \left( F_{+\infty} - F_0 \right) e^{-k_F t}}{1 + e^{-k_F t} \left( F_{+\infty} - F_0 \right) / F_0}
\]  

(5)

with the asymptotic firmness values \( F_{+\infty} = 100 \) at plus infinite time, the rate constant \( k_F \) (per day) and the initial firmness value \( F_0 \) (in HandyHit units). Analysing the control data with this model (Fig. 9A) resulted in values for the rate constant \( k_F \) valid for air conditions at 7 °C of \( k_F^{RA} = 0.1633 \) per day and a value for \( F_0 \) of 0.36.

The same approach applied to the colour data was followed to predict the firmness data after storage at the different MA conditions by making the rate of softening \( k_F \) dependent on the relative metabolic rate according to:
This time no additional anaerobic term was added, as fruit did not show increased softening under anaerobic conditions (Fig. 9B). It needs to be emphasised that in this approach to describe softening at MA conditions, the model was not fitted to the data but the model was applied using the estimated parameter values on the softening of the air stored control fruit, assuming the rate of softening of the MA stored fruit depended on the relative metabolic rate according Eq. (6).

We realise that the model used to describe softening is a crude simplification of the whole softening process ignoring all details on the involvement of ethylene and a whole range of enzymes. However, the message that can be learned from this simplified approach is that MA was inhibiting the rate of softening to the same extend as it was inhibiting the metabolic rate as expressed by \( r_{O_2}^{OX} \).

The results (Fig. 9B) showed a good agreement between measured and predicted values with the model explaining about 91% of the observed variation. The observed response of softening under MA conditions was comparable with the observations of Meir et al. (1995) of increasing inhibition of fruit softening with decreasing levels of \( O_2 \) and increasing levels of \( CO_2 \).

3.5. \( CO_2 \) injury

Depending on the MA conditions applied, the fruit coming out of storage showed varying levels of what we believe was \( CO_2 \) injury (Fig. 10).

The injury was similar to that reported by Meir et al. (1995), however, our fruit showed some different responses to the applied MA conditions. Meir et al. related the injury to either high \( CO_2 \) (8 kPa) or low \( O_2 \) (3 kPa) levels, but injury did not occur at high \( CO_2 \) in combination with high \( O_2 \) (21 kPa) levels. In the current experiment, fruit indeed showed increasing injury with increasing \( CO_2 \) levels, but no reduction at 21 kPa \( O_2 \) (Fig. 10). In contrast, the injury level was reduced with lowering levels of \( O_2 \). The fruit stored at 0 kPa \( CO_2 \) also showed increased injury levels at \( O_2 \) levels below 2 kPa. At 0 kPa \( O_2 \) this injury never occurred, but fruit were unacceptable because of their general anaerobic state.

The results contrast with earlier work on ‘Fuerto’ avocado stored for up to 60 days under 2 kPa \( O_2 \) and 10 kPa \( CO_2 \) that incurred less injury than control fruit (Allwood and Cutting, 1994).

‘Hass’ avocado stored at 5 °C and 2 kPa \( O_2 \) benefited from increased levels of \( CO_2 \) (2.5 kPa) to...
suppress flesh discoloration (Faubian et al., 1992) although increasing CO$_2$ to 10 kPa resulted in increased discoloration again.

Based on our results we can not conclude whether the CO$_2$ injury was the direct result of the high CO$_2$ levels or an indirect effect because of injury due to the volatiles induced by the high CO$_2$ levels. A conservative recommendation to prevent quality losses due to high CO$_2$, would be to keep the CO$_2$ level during storage of ‘Hass’ avocado below 5 kPa.

3.6. Low CO$_2$ treatment

To examine the effect of low levels of CO$_2$ a second experiment was completed, measuring firmness and hue over time during storage at 7 °C and applying CO$_2$ levels ranging from 0 to 5 kPa (Fig. 11). The data clearly showed how an increasing level of CO$_2$ increasingly inhibited both the rate of softening and the rate of colour change.

The results were compared with the models established above. Given the large time difference between the two experiments (February vs. November of the same year) and the potential difference in harvest maturity, the batch-dependent parameters were estimated again, while all other parameters were kept at their previously estimated values. In spite of this potential maturity difference we assumed that the underlying principles did not change.

For colour, $H_0$ was set to the measured initial hue of 127.4°, while $C$ was estimated to be 0.00254 explaining 99% of the observed variation. For firmness, $F_0$ was estimated to be 0.0892 explaining 83% of the observed variation.

The results clearly show that the same principles applied; the relationship established for the high CO$_2$ levels was also able to explain the observed responses to the range of low CO$_2$ levels applied. It also confirmed that the rates of colour change and softening were related to the $rO_2^{OX}$ only and not the overall $rO_2$.

![Fig. 11. Firmness (A) and hue (B) of ‘Hass’ avocado fruit stored for 52 days at 7 °C at CO$_2$ levels ranging from 0 to 5 kPa. Firmness and hue were measured at the stem end of the fruit. Plotted symbols represent the measured data while the lines represent the predictions based on the modelled gas exchange rates.](image)
4. Optimal MA conditions

Control fruit stored in air and the 0 kPa CO₂ stored fruit at the higher O₂ levels reached eating quality at the end of the 32 days of storage, exhibiting normal ripening characteristics. Fruit stored at 0 kPa O₂ were unacceptable because of their general anaerobic state. The fruit stored at 10–15 kPa CO₂ were mostly unacceptable because of the incurred CO₂ injury and their firm state. The remaining fruit would need an additional shelf life to reach eating quality.

Taking all the different aspects together, the optimum MA conditions for the studied batch of avocado fruit stored for 32 days at 7 °C was around 2 kPa O₂ and 0 kPa CO₂. Under these conditions no CO₂ injury occurred (Fig. 10), the change in hue was minimal (Fig. 7), weight loss was minimised (Fig. 6), and softening was almost completely inhibited (Fig. 9B). At such low O₂ levels, no additional benefit was found from raising the levels of CO₂.

However, it would go too far to recommend these conditions as the optimal storage conditions for avocado fruit as too many variables were not covered. This work was done for only one temperature, so the temperature dependencies of all the involved processes have not been characterised, and results can not be easily extrapolated to other temperatures. Even though one would assume that the underlying principles are the same for different avocado cultivars, one should also realise that different cultivars can respond in different extents to the same variables as is illustrated by the positive effect of 10 kPa CO₂ on ‘Fuerto’ avocado (Allwood and Cutting, 1994).

Due to the relative high storage temperature of 7 °C, chilling injury did not occur in the current experimental setup. As MA is known to interact with chilling injury (Pesis et al., 1994) certain combinations of low temperature storage might be feasible as long as the proper MA conditions are applied to prevent chilling injury.

As if this is not enough, considerable variation can be expected between batches due to maturity differences at harvest. As long as the maturity dependent batch specific parameters can be identified and quantified at harvest, the developed models can be used to predict quality aspects of particular batches instead of only giving a general indication for the average ‘Hass’ avocado fruit.

Assuming the above mentioned issues have been addressed, well founded recommendations can be made for the storage of a particular batch of avocado fruit taking into account characteristics of both the product and the intended postharvest chain.

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