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 CO_2 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 CO_2 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 O_2 levels. At 2 kPa O_2 and 0 kPa CO_2 no CO_2 injury occurred, while the change in hue was minimal, weight loss was minimised, and softening was almost completely inhibited. At such low O_2 levels, no additional benefit was found from raising the levels of CO_2.
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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 (O_2) and elevating levels of carbon dioxide (CO_2), 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 O_2 or CO_2, 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). O_2 requiring processes will be affected straight away through the availability of O_2. 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 CO_2 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 m^3) 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, O_2–free N_2 and foodgrade CO_2 (BOC, Palmerston North, NZ), to create combinations of eight different O_2 levels (0–21 kPa O_2) and four different CO_2 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 CO_2 levels remained constant over time with an average S.E. of ±0.50 kPa CO_2, and the O_2 levels stayed constant with an average S.E. of ±0.32 kPa O_2.

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 CO_2 and depletion of O_2 by about 0.5 kPa. Depending on the MA conditions, this took between 1 and 3 h.
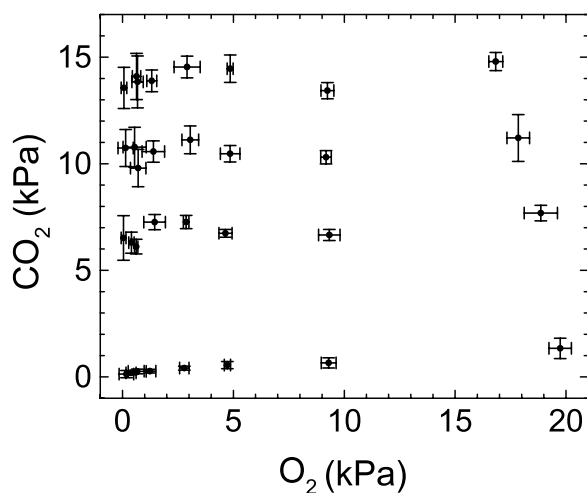

Fig. 1. Matrix of MA generated. The symbols indicate the average O_2 and CO_2 conditions as measured during the weekly routine checks, and the error bars indicate the S.D. of these measurements (eight in total) throughout the 32 days of the experiment.

2.3. Low CO_2 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 CO_2 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 CO_2 (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 O_2 electrode (Citicell C/S type, City Technology Ltd., London, UK) in series with a miniature infrared CO_2 transducer (Analytical Development Company, Hoddesdon, UK), with O_2–free N_2 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.

CO_2 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 CO_2 at 5, 10 or 20 kPa O_2 (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 O_2 consumption (r_{O_2}) increased with increasing levels of O_2 and decreased with increasing levels of CO_2. The fact that the inhibiting effect of CO_2 did not decrease with increasing O_2 levels, indicated an uncompetitive type of CO_2 inhibition (Hertog et al., 1998). The avocado fruit did not show a significant increase in their CO_2 production (r_{CO_2}) as would be expected assuming alcoholic fermentation at low O_2 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% O_2, clearly indicating an in-
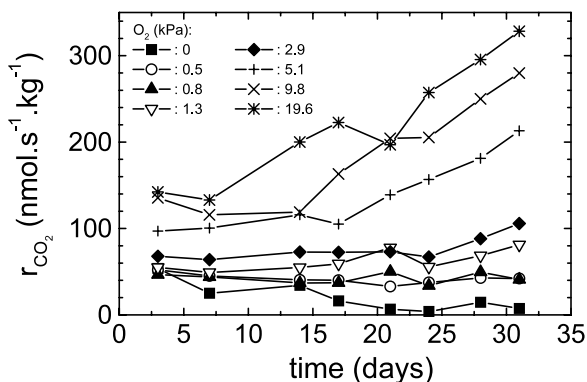

Fig. 2. CO_2 production of 'Hass' avocado over time. The fruit were stored for 32 days at 7 °C, 0 kPa CO_2, with a range of O_2 levels (0–20 kPa) as indicated in the legend.
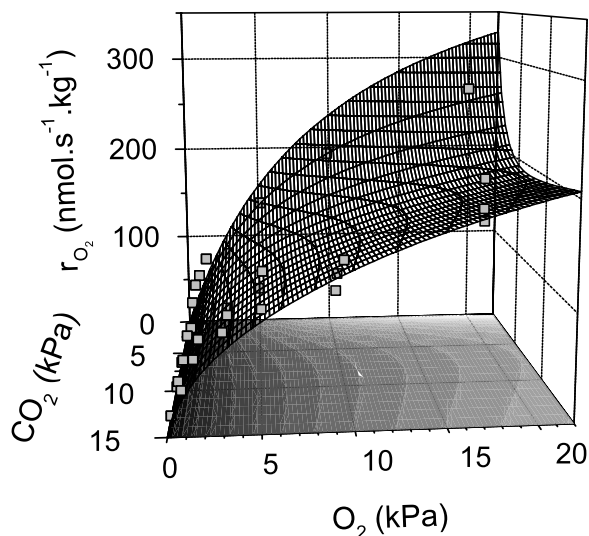
Fig. 3. O_2 consumption of 'Hass' avocado fruit (nmol s^{-1} kg^{-1}) stored at 7 °C as a function of O_2 and CO_2. Data points plotted represent the averaged measured data while the plane represents the model from Eq. (2) using the parameter estimates from Table 1. The contour lines emphasise the effect of CO_2.

Fig. 4. CO_2 production of 'Hass' avocado fruit (nmol s^{-1} kg^{-1}) stored at 7 °C as a function of O_2 and CO_2. Data points plotted represent the averaged measured data while the plane represents the model from Eq. (2) using the parameter estimates from Table 1. The contour lines emphasise the effect of CO_2.
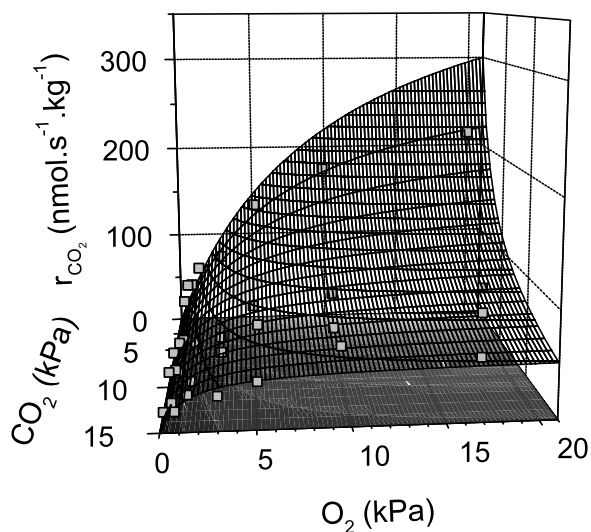
creased anaerobic activity. In spite of not showing an increased r_{CO_2}, the fruit stored at 0 kPa O_2 exhibited clear signs (off odours) of increased volatile production which were not identified. Given the rancid smell, one would expect these volatiles to originate from some part of fatty acid metabolism.

The r_{CO_2} at high CO_2 levels in aerobic conditions was suppressed much more than the r_{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_{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_{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_{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,
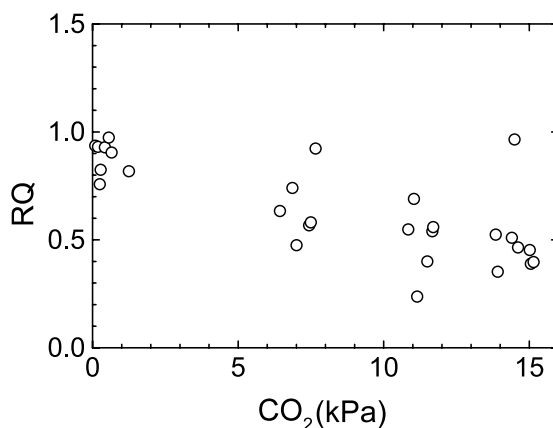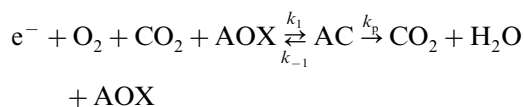

Fig. 5. RQ of 'Hass' avocado fruit stored for 32 days at 7 °C as a function of applied CO_2 conditions.

increasing the O_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,a,b,c)), high CO_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_2 production (as observed at low O_2 levels), the increased O_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_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_2 to oxidise the large flow of electrons (e^-) through an intermediate enzyme substrate complex (AC).

$$e^- + O_2 + CO_2 + AOX \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} AC \overset{k_p}{\rightarrow} CO_2 + H_2O + AOX$$

The large flow of e^- is believed to be generated by fermentative pathways induced by the high CO_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_2 consumption by AOX($r_{O_2}^{AOX}$) can be described according to:

$$r_{O_2}^{AOX} = \frac{r_{O_2}^{AOX,max} p_{O_2} p_{CO_2}}{Km_{O_2}^{AOX,max} + p_{O_2} p_{CO_2}}, \begin{cases} r_{O_2}^{AOX,max} = k_p AOX \\ Km_{O_2}^{AOX,max} = \dfrac{k_{-1} + k_p}{k_1} \end{cases}$$

(1)

where p_{O2} and p_{CO2} are the partial pressures for O_2 and CO_2 (both in kPa); $r_{O_2}^{AOX,max}$ is the maximum oxidative O_2 consumption rate by AOX (mol kg^{-1} s^{-1}) unconstrained by O_2 or CO_2; $Km_{O_2}^{AOX}$ (in kPa^2) is the Michaelis constant for the O_2 consumption by AOX. This alternative oxidase is regulated by the presence of CO_2 but does not produce CO_2.

Total O_2 consumption r_{O_2} was assumed to be the combined result of $r_{O_2}^{AOX}$ and O_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_2 inhibition (Hertog et al., 1998). CO_2 production was linked to the oxidative phosphorylation only. This resulted in:

$$r_{O_2}^{OX} = \frac{r_{O_2}^{OX,max} p_{O_2}}{Km_{O_2}^{OX} + p_{O_2}[1 + p_{CO_2}/Kmu_{CO_2}^{OX}]}$$

$$r_{O_2} = r_{o_2}^{OX} + r_{o_2}^{AOX}$$

$$r_{CO_2} = RQ \cdot r_{o_2}^{OX}$$

(2)

where $r_{O_2}^{OX,max}$ is the maximum O_2 consumption rate (mol kg^{-1} s^{-1}) by oxidative phosphorylation unconstrained by O_2; $Km_{O_2}^{OX}$ and $Kmu_{CO_2}^{OX}$ (both in kPa) are the Michaelis constants for respiration and the uncompetitive inhibition of respiration by CO_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-

Table 1
Parameter estimates and their S.E. resulting from the non-linear regression analysis of gas exchange and colour change rates of 'Hass' avocado fruit as a function of O_2 and CO_2

Gas exchange data		Colour change data	
Parameter[a]	Estimate (S.E.)[c]	Parameter[b]	Estimate (S.E.)[c]
$r_{O_2}^{OX,max}$	484 (15)	K_H^f	0.19 (8)
$Km_{O_2}^{OX}$	10.5 (23)	Km_f	0.91 (36)
$Kmu_{CO_2}^{OX}$	1.96 (25)		
$r_{O_2}^{AOX,max}$	334 (51)		
$Km_{O_2}^{AOX}$	389 (76)		
RQ	0.93 (7)		
R^2 (%)[d]	97		98
n	64		32

[a] $r_{O_2}^{max}$ is the maximum oxidative O_2 consumption rate (nmol $kg^{-1}s^{-1}$) unconstrained by O_2; Km_{O_2} and Kmu_{CO_2} (both in kPa) are the Michaelis constants for, respectively, respiration and uncompetitive inhibition of respiration by CO_2; RQ_{OX} is the respiration quotient for oxidative respiration.

[b] k_H^f is the maximum rate of colour change under anaerobic conditions; Km_f is the Michaelis constant for the inhibition of this anaerobic process by O_2.

[c] S.E. are expressed as a percentage of the parameter value.

[d] R^2, percentage variance accounted for; n, number of observations.

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 CO_2 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 O_2 consumed, only half a mole of CO_2 is being released) implied fruit would have gained net weight at high CO_2 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 CO_2 could be largely explained (Fig. 6). However, avocado fruit developed larger weight losses at low O_2 levels and at increased CO_2 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 O_2 levels (induced by anaerobic conditions) and increased CO_2 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 CO_2 levels at aerobic conditions. However, there is no reason why ethylene would only be produced at increased CO_2 levels and not at 0 kPa CO_2 especially as high CO_2 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
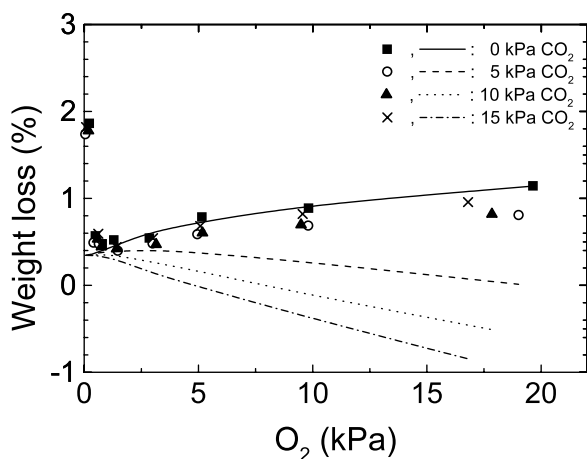
Fig. 6. Weight loss of 'Hass' avocado fruit stored for 32 days at 7 °C as a function of the MA conditions applied. Weight loss is expressed as a percentage of the initial weight. Plotted symbols represent the measured data while the lines represent the weight loss expected based on modelled gas exchange and transpiration rates. A negative value indicates weight gain. The difference between modelled and measured resembles the unaccounted carbon loss due to volatile production.

ethylene production, this would imply an average ethylene production during the 32 days of storage at 15 kPa CO_2 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 O_2 and low CO_2 levels (Fig. 7). The minimum change in hue was observed around 1–2 kPa O_2, corresponding to where the minimum weight loss was observed (Fig. 6). Colour change at low O_2 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
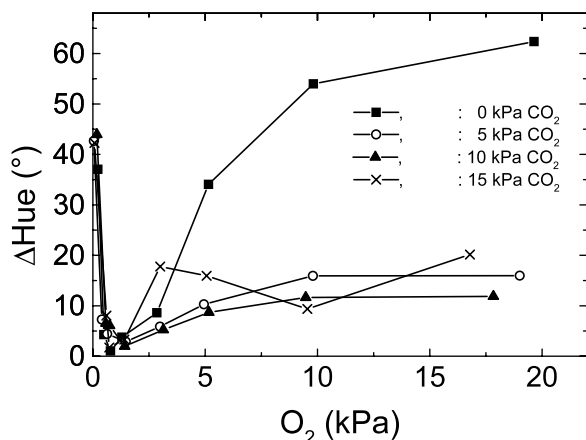
Fig. 7. Change in hue (°) of 'Hass' avocado fruit stored for 32 days at 7 °C as a function of the MA conditions applied. The colour was measured at the stem end of the fruit.

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 O_2.

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):

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

$$H_{-\infty} = (1 + C) \cdot H_0 \tag{3}$$

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 O_2 levels cannot be explained by increased anaerobic activity in terms of r_{CO_2}. 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
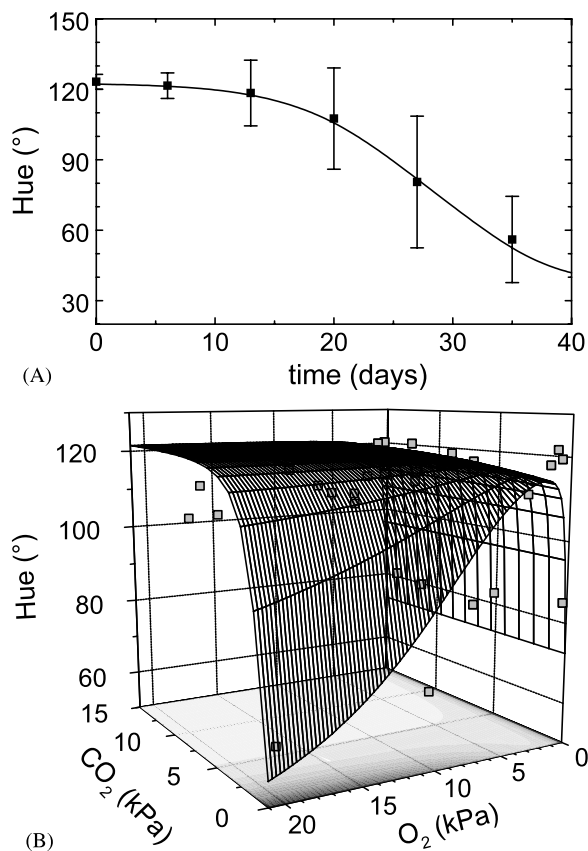
(A)

(B)

Fig. 8. Hue colour (°) of the stem end of 'Hass' avocado fruit stored at 7 °C with (A) representing the observed time course for the control fruit and (B) the final hue of the MA stored fruit as a function of the MA conditions applied. The plotted symbols represent the average of the measured data with the error bars representing the S.D. due to fruit-to-fruit variation. The line from (A) is fitted using the model from Eq. (3) while the plane (B) represent the final colour prediction based on the modelled gas exchange rates (Eqs. (2)–(4)).

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:

$$k_H = k_H^{RA} \frac{r_{O_2}^{OX,MA}}{r_{O_2}^{OX,RA}} + \frac{k_H^f}{1 + O_2/Km_f} \qquad (4)$$

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_H^f the maximum rate of colour change under anaerobic conditions and Km_f 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_H^f and Km_f. 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:

$$F(t) = \frac{F_{+\infty}}{1 + e^{-k_F \cdot t} \cdot (F_{+\infty} - F_0)/F_0} \qquad (5)$$

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:
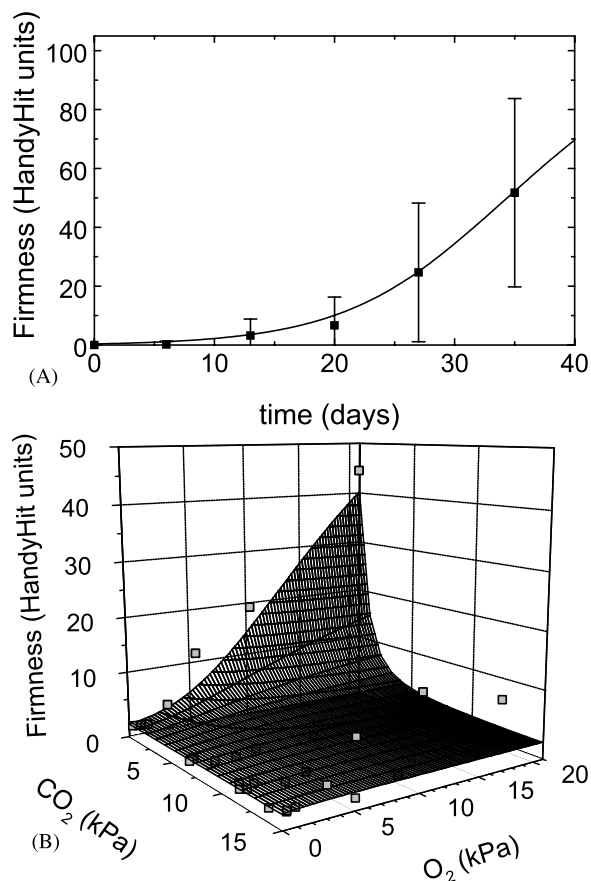
Fig. 9. Firmness (HandyHit units) of the stem end of 'Hass' avocado fruit stored at 7 °C with (A) representing the observed time course for the control fruit and (B) the final firmness of the MA stored fruit as a function of the MA conditions applied. The plotted symbols represent the average of the measured data with the error bars representing the S.D. due to fruit-to-fruit variation. The line from (A) is fitted using the model from Eq. (5) while the plane (B) represent the final firmness prediction based on the modelled gas exchange rates (Eqs. (2), (5) and (6)).

$$k_F = k_F^{RA} \frac{r_{O_2}^{OX,MA}}{r_{O_2}^{OX,RA}} \tag{6}$$

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 gas exchange data in combination with 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
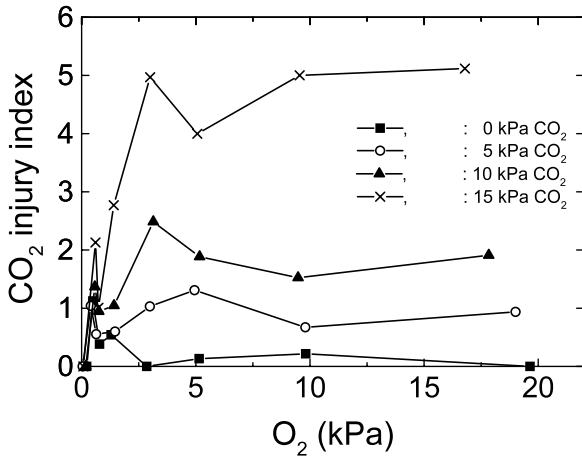
Fig. 10. CO_2 injury of 'Hass' avocado fruit after 32 days storage at 7 °C as a function of the MA conditions applied. The index was based on the surface area and the depth of underlying pulp affected.

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 $r_{O_2}^{OX}$ only and not the overall r_{O_2}.
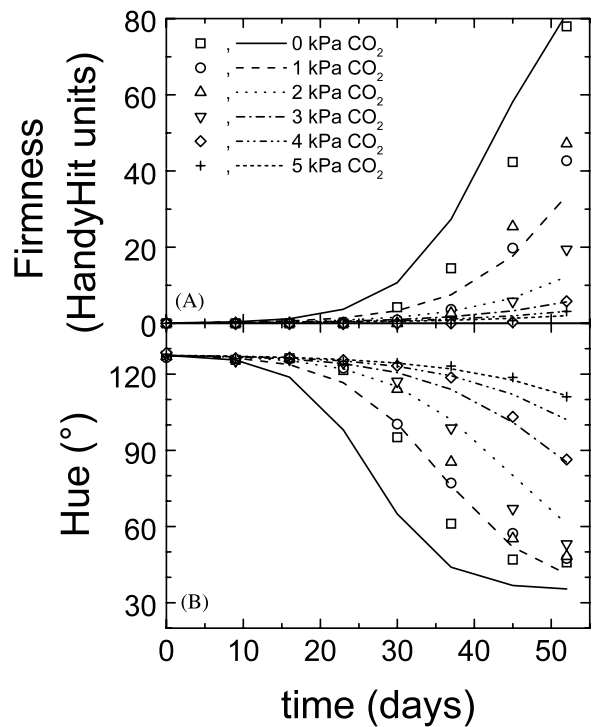

Fig. 11. Firmness (A) and hue (B) of 'Hass' avocado fruit stored for 52 days at 7 °C at CO_2 levels ranging from 0 to 5 kPa. Firmness and hue were measured at the stem end of the fruit. Plotted symbols represent the measured data while the lines represent the predictions based on the modelled gas exchange rates.

4. Optimal MA conditions

Control fruit stored in air and the 0 kPa CO_2 stored fruit at the higher O_2 levels reached eating quality at the end of the 32 days of storage, exhibiting normal ripening characteristics. Fruit stored at 0 kPa O_2 were unacceptable because of their general anaerobic state. The fruit stored at 10–15 kPa CO_2 were mostly unacceptable because of the incurred CO_2 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 O_2 and 0 kPa CO_2. Under these conditions no CO_2 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 O_2 levels, no additional benefit was found from raising the levels of CO_2.

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 extends to the same variables as is illustrated by the positive effect of 10 kPa CO_2 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.

Acknowledgements

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