Controlled atmosphere storage may lead to local ATP deficiency in apple

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Abstract

A permeation diffusion reaction model was applied to study the internal metabolic gas concentration inside apple fruit cv ‘Kanzi’, ‘Jonagold’ and ‘Braeburn’ under controlled atmosphere (CA) conditions. A new criterion for the local O₂ partial pressure beyond which there is a risk of cell death due to energy shortage was established as the local O₂ partial pressure at which the oxidative ATP production becomes smaller than the maximal ATP production by fermentation. The Michaelis-Menten constant (K_{m,O_2}) of oxidative respiration of apple tissue at 1°C was within 0.13-0.17 kPa. At an O₂ partial pressure of 0.46 to 0.78 times the K_{m,O_2} at a storage temperature of 1°C, energy production for cell maintenance could be still secured in the three cultivars. The effect of natural variability of the maximal respiration rate and tissue gas diffusivities inside apple fruit was further studied by means of a Monte Carlo analysis. The simulations confirm that ‘Jonagold’ has large potential for storage under low O₂ partial pressure, while ‘Kanzi’ and ‘Braeburn’ need to be stored at higher O₂ partial pressure, in line with commercial practices.

Keywords: Controlled atmosphere, anoxia, diffusion, gas transport, modeling, storage.

Introduction

Pome fruit are after harvest often stored under controlled atmosphere (CA) conditions with reduced O₂ and increased CO₂ levels in combination with a low temperature to extend their commercial storage life. In such conditions, gas exchange inside the fruit affects the respiration process. Sub-optimal storage conditions may lead to physiological disorders and loss of product. Browning is such an important physiological disorder that appears in hypoxic storage conditions. It is characterised by internal browning of the flesh, and, at a later stage, the development of cavities. The main hypothesis for explaining the occurrence of browning is that it is caused by anoxia inside the fruit, followed by a switch from respiration to fermentation. The low energy yield of the latter is insufficient for repairing membrane damage, and cell death may result. Although several questions remain, multiple studies support this hypothesis (Peppelenbos et al., 1998; Lammertyn et al., 2000; Ma and Chen, 2003; Veltman et al., 2003; Franck et al., 2007; Pedreschi et al., 2008; Ho et al., 2011; Herremans et al., 2012).

In plant cells, mitochondria are the major sites of O₂ consumption where cytochrome c oxidase is the major terminal oxidase for the respiration. Michaelis–Menten kinetics have been used widely to describe the O₂ dependence of the respiratory process (Millar et al., 1994; Hertog et al., 1998; Lammertyn et al., 2001; Armstrong and Beckett, 2010; Ho et al., 2010; Ho et al., 2012). The low value of the Michaelis-Menten constant (K_m) of cytochrome c oxidase (0.1–0.12 µM, Rawsthorne and LaRue, 1986; 0.14 µM Millar et al., 1994; Armstrong and Beckett, 2010) suggests that the respiration rate would still be close to its saturation level for O₂ concentrations of 1 to 3 kPa that are used in commercial CA. However, diffusion resistances of the fruit skin and cortex tissue have been shown to lead to internal O₂ concentrations that are significantly lower than these external levels (Lammertyn *et al.*, 2003; Franck et al., 2007; Armstrong and Beckett, 2010; Ho et al., 2010 & 2011). As a consequence, hypoxic and even anoxic zones may develop during commercial CA storage and cause fermentation (Gong et al., 2001; Franck et al., 2007).

The critical O₂ level in the storage room is that below which ATP production falls below a critical level and results in loss of cell integrity (Franck et al., 2003 & 2007; Gibbs and Greenway 2003; Huang et al., 2005). While oxidative respiration is very efficient in producing ATP, fermentation is far less efficient (Yearsley et al., 1996).

Different concepts have been reported in the literature for determination of the lower oxygen limit for CA storage. The anaerobic compensation point (*ACP*) was defined as the O_2 concentration level at which the CO_2 production rate is minimal (Boersig et al., 1988; Yearsley et al., 1996). Below the *ACP*, the CO_2 production rate sharply increases with decreasing O_2 concentration due to the dominance of fermentation, while above the *ACP*, the CO_2 production rate increases due to respiration. Another concept uses the respiratory quotient (*RQ*) of CO_2 production to O_2 consumption, which increases as the O_2 level decreases (Cameron et al., 1989; Beaudry, 1993; Gran and Beaudry, 1993; Saenmuang et al., 2012). The fermentative threshold (*FT*) was defined as the O_2 level at which the *RQ* increased to 1.1 to 1.2 times its asymptotic value at high O_2 (Beaudry, 1993; Yearsley et al., 1996). However, the two different approaches for determining the low O_2 limits in fruit mentioned above are empirical and not directly relate to energy shortages inside the fruit under hypoxic conditions (Gibbs and Greenway 2003; Huang et al., 2005).

As there are no noninvasive measurement techniques available for measuring/monitoring respiratory gas concentrations and ATP in fruit during CA storage, mathematical models have been used to study gas transport and energy conversion processes in fruit. Models operating at different spatial scales have been reported, from the macroscale (Mannapperuma et al., 1991; Lammertyn et al., 2003, Ho et al., 2008; Ho et al., 2010) to the microscale level (Ho et al., 2009; Ho et al., 2011, Verboven et al., 2012). Recently, a model for gas exchange in different apple fruit cultivars ('Kanzi', 'Jonagold' and 'Braeburn') has been developed and validated by Ho et al. (2010). This permeation-diffusion-reaction model incorporates both gas transport as well as respiration kinetics, and is solved over the actual shape of the fruit and tissue architecture using the finite element method. With the model, it is possible to predict the local gas concentrations and the production rate of energy inside the fruit for well-defined storage conditions.

The objective of this paper was to extend the permeation-diffusion-reaction model to also predict ATP production, and to use it to evaluate the ATP availability inside apple fruit of different cultivars ('Kanzi', 'Jonagold' and 'Braeburn') stored at a low temperature. 'Jonagold' is a typical commercial cultivar for long term storage at ultra low oxygen (ULO, 1-1.5% O_2) (Saquet et al., 2000), while 'Braeburn' is quite

susceptible for physiological disorders in storage (Gong et al., 2001). ‘Kanzi’ is a recent cultivar (Ho et al., 2010) and has a moderately long storage potential.

Materials and methods

Fruit

Experiments were performed on fruit of three apple cultivars (*Malus × domestica* Borkh., cv. ‘Kanzi’, ‘Braeburn’ and ‘Jonagold’). Fruit were harvested from the orchards of the Experimental Centre of Fruit Growing (pcfruit, Velm, Belgium) in 2010 at the commercial picking date determined by the Flanders Centre of Postharvest Technology. ‘Jonagold’ and ‘Kanzi’ were cooled and stored under controlled atmosphere (CA) conditions of 1% O₂, 2.5% CO₂ and 3% O₂, 0.7% CO₂ at 1°C, respectively. ‘Braeburn’ was cooled and stored for a period of 21 days at 1°C preceding CA storage (3 % O₂, 0.7 % CO₂ at 1°C in air).

Permeation-diffusion-reaction model of gas exchange in the fruit

The permeation-diffusion-reaction model of gas exchange that we developed earlier (Ho et al., 2008; 2010) was used. In this approach, tissues are considered to be homogeneous continuum materials. The effect of microstructural features (porosity and tortuosity) on gas transport is incorporated in the apparent value of the tissue properties. The model distinguishes three distinct tissues, namely skin, outer and inner cortex (Fig. 1; Ho et al., 2010) and uses the actual geometry of the fruit to calculate the spatial profiles of internal gas concentrations of O₂, CO₂ and N₂, as a result of gas exchange, respiration and fermentation.

Gas concentration gradients are the driving force for gas exchange. Differences in diffusion rates of the different gasses lead to total pressure gradients that cause convective exchange as described by Darcy's law. The model thus contains permeation, diffusion and reaction terms:

$$\alpha_i \frac{\partial C_i}{\partial t} + \nabla \cdot (\mathbf{u} C_i) = \nabla \cdot D_i \nabla C_i + R_i \quad (1)$$

with α_i the gas capacity of the component i (O₂, CO₂ and N₂) of the tissue (Ho et al., 2006; 2010), D_i (m² s⁻¹) the apparent diffusion coefficient of the tissue, $\mathbf{u}$ (m s⁻¹) the

126 apparent permeation velocity vector, R_i ($\text{mol m}^{-3} \text{s}^{-1}$) the reaction term of the gas
 127 component i related to O_2 consumption or CO_2 production, ∇ (m^{-1}) the gradient
 128 operator, and t (s) the time.

129 The gas capacity α_i is defined as (Ho et al., 2006):

$$130 \quad \alpha_i = \varepsilon + (1 - \varepsilon) \cdot R \cdot T \cdot H_i = \frac{C_{i,tissue}}{C_{i,g}} \quad (3)$$

131 where ε is the porosity of tissue, $C_{i,g}$ (mol m^{-3}) and $C_{i,tissue}$ (mol m^{-3}) are the
 132 concentration of the gas component i in the gas phase and the tissue, respectively. The
 133 concentration of the compound in the liquid phase of fruit tissue normally follows
 134 Henry's law represented by constant H_i ($\text{mol m}^{-3} \text{kPa}^{-1}$). R ($8.314 \text{ J mol}^{-1} \text{K}^{-1}$) is the
 135 universal gas constant and T (K) the temperature. As O_2 and CO_2 diffuse at a different
 136 rate through the tissue, pressure gradients causing permeation transport may result.
 137 Permeation through the tissue due to a pressure gradient is described by Darcy's law
 138 (Geankoplis, 1993):

$$139 \quad \mathbf{u} = -\frac{K}{\mu} \nabla P = -\frac{K \cdot R \cdot T}{\mu} \nabla \left(\sum C_i \right) \quad (4)$$

140 with K (m^2) the permeation coefficient; P (Pa) the pressure and μ (Pa.s) the viscosity
 141 of the gas. The relation between gas concentration and pressure is assumed to follow
 142 the ideal gas law ($P = CRT$).

143 A non-competitive inhibition model (Peppelenbos et al., 1996; Hertog et al., 1998;
 144 Lammertyn et al., 2001; Ho et al., 2010) is used to describe consumption of O_2 by
 145 respiration:

$$146 \quad R_{\text{O}_2} = -\frac{V_{m,\text{O}_2} \cdot C_{\text{O}_2}}{\left(K_{m,\text{O}_2} + C_{\text{O}_2} \right) \cdot \left(1 + \frac{C_{\text{CO}_2}}{K_{mn,\text{CO}_2}} \right)} \quad (5)$$

147 with V_{m,O_2} ($\text{mol m}^{-3} \text{s}^{-1}$) the maximum oxygen consumption rate, C_{O_2} (mol m^{-3}) the O_2
 148 concentration, C_{CO_2} (mol m^{-3}) the CO_2 concentration, K_{m,O_2} (mol m^{-3}) the Michaelis-
 149 Menten constant for O_2 consumption, K_{mn,CO_2} (mol m^{-3}) the Michaelis-Menten constant
 150 for non-competitive CO_2 inhibition, and R_{O_2} ($\text{mol m}^{-3} \text{s}^{-1}$) the O_2 consumption rate of
 151 the sample.

The equation for production rate of CO₂ consists of an oxidative respiration part and a fermentative part (Peppelenbos et al., 1996):

$$R_{CO_2} = -r_{q,ox} \cdot R_{O_2} + \frac{V_{m,f,CO_2}}{\left(1 + \frac{C_{O_2}}{K_{m,f,O_2}}\right)} \quad (6)$$

with V_{m,f,CO_2} (mol m⁻³ s⁻¹) the maximum fermentative CO₂ production rate, K_{m,f,O_2} (mol m⁻³) the Michaelis-Menten constant of O₂ inhibition on fermentative CO₂ production, $r_{q,ox}$ the respiration quotient at high O₂ concentration, and R_{CO_2} (mol m⁻³ s⁻¹) the CO₂ production rate of the sample. At the fruit surface the following boundary condition is assumed:

$$C_i = C_{i,\infty} \quad (2)$$

with the index ∞ referring to the gas concentration of the ambient atmosphere.

The continuum gas exchange model was numerically solved using the finite element method (Comsol 3.5, Comsol AB, Stockholm). For further details, the reader is referred to Ho et al. (2010).

Gas exchange properties and respiration parameters

Model parameters are defined in Table 1 and their values that were taken from the literature are listed in Table 2. While values of the model parameters were available for 20°C (Ho et al, 2010), this was not the case for 1°C and additional experiments were, therefore, carried out to estimate them.

The gas transport properties of the fruit tissue were measured using optical probes described in Ho et al. (2010). The resulting values of the properties are given in Table 2 for the skin, outer and inner cortex of each apple cultivar, and assuming that the values are independent of temperature (Ho et al., 2010) (more details are given in Figure 1 and Table 2).

Dedicated jar experiments were carried out to determine the respiration parameters of each cultivar at low temperature and in CA conditions. Respiration experiments were performed at 1°C in closed jars of 1.7 mL containing two apples with approximately

400g apple per jar. To determine V_{m,O_2} and V_{m,f,CO_2} , respiration rate measurements were carried out at 20 and 0 kPa O_2 combined with 0 kPa of CO_2 at 1°C. The initial gas mixtures were measured when the jars were closed. The headspace was analysed again after 24 h. The O_2 consumption and CO_2 production rates were calculated from the difference in gas concentration and the time lag between the two measurements. The values of K_{m,O_2} and K_{m,f,O_2} of apple tissue may depend on temperature (Ho et al., 2011). It is difficult to measure K_{m,O_2} and K_{m,f,O_2} of apple tissue at 1°C using the method presented in Ho et al. (2010) because the low respiration rates and corresponding long measurement times introduce large errors in the fitting procedure. Furthermore, very small and accurate values of O_2 levels are difficult to control accurately for such purpose. To determine K_{m,O_2} and K_{m,f,O_2} of tissue at 1°C, we therefore followed an alternative approach. Two different gas conditions (4 kPa O_2 , 0 kPa CO_2 and 0 kPa O_2 , 0 kPa CO_2) were generated using an in house built mixing panel equipped with mass flow controllers (Brooks Instrument, The Netherlands). The composition of the mixtures was measured by means of a gas analyser (Checkmate II, PBI Dansensor, Denmark). The gas analyser has an accuracy of $\pm 0.1\%$ and $\pm 0.5\%$ of the O_2 reading and CO_2 reading, respectively. The analyser was calibrated against calibrated mixtures (Air products N.V., Belgium). For each gas condition, four jars containing two apples each were connected in series and flushed with conditioned air for at least 2 days. Then the jars were closed and the O_2 and CO_2 gas partial pressures changes with time were measured by the gas analyser. The gas percentages were converted to partial pressures by multiplying with the measured total pressure (DPI 142, GE Druck, Germany). The gas partial pressure was converted to molar concentration according to the ideal gas law, and from this the O_2 consumption and CO_2 production rates were calculated and expressed in mol per volume of sample (m^3 fresh volume of sample) and per unit time (s). The K_{m,O_2} and K_{m,f,O_2} of the tissues were determined by fitting the continuum model to the measured respiration data of the intact fruit by using an iterative least squares estimation procedure written in Matlab (The Mathworks, Inc., USA).

Critical O_2 level based on energy supply criteria

Energy supply rates in normal and anoxic conditions have been reported by Gibbs and Greenway (2003). The ATP synthesis rate has been shown to be at least 3 times lower in anoxia compared with normoxia (Gibbs and Greenway, 2003).

Aerobic respiration requires O_2 in order to generate energy. From an energy point of view, when the O_2 concentration in the cell is sufficiently high, pyruvate produced by the glycolysis process is oxidised in the mitochondria by the Krebs cycle (Buchanan et al., 2000). The energy produced by this process is stored in ATP. The rate of ATP production from the oxidative respiration $R_{ATP,o}$ of nutrient metabolisation can thus be written as follows:

$$R_{ATP,o} = f_o \cdot r_{q,ox} \cdot R_{O_2} = f_o \cdot r_{q,ox} \cdot \frac{V_{m,O_2} \cdot C_{O_2}}{(K_{m,O_2} + C_{O_2}) \cdot \left(1 + \frac{C_{CO_2}}{K_{mn,CO_2}}\right)} \quad (7)$$

where f_o is the stoichiometric coefficient of the ATP production due to the oxidative respiration. Assuming glucose is the main nutrient for respiration, one glucose molecule can yield 38 ATP molecules and 6 CO_2 molecules when respiration mainly follows the cytochrome *C* oxidase pathway (Buchanan et al., 2000). In this case, f_o is 38/6=6.333. Some authors (Gibbs and Greenway, 2003; Edwards et al., 2012) assumed the ATP: O_2 ratio equal to 5.

In the absence of O_2 in the cell, pyruvate remains in the cytoplasm and is converted to mainly ethanol and CO_2 (Buchanan et al., 2000). Here, we assume that the energy production due to fermentation ($R_{ATP,f}$) is proportional to the rate of fermentative CO_2 production by the fruit tissue:

$$R_{ATP,f} = f_f \frac{V_{m,f,CO_2}}{1 + \frac{O_2}{K_{m,f,O_2}}} \quad (8)$$

where f_f is the stoichiometric coefficient for ATP production due to the fermentation. Fermentation of one mole of glucose would produce 2 moles of ATP and 2 moles of CO_2 (Buchanan et al., 2000). Therefore, f_f is equal to 1 for glucose.

We propose now that oxidative respiration can be considered as dominant when $R_{ATP,o}$ is larger than the maximal rate of ATP production by fermentation. By virtue of equation (8) this happens when

$$R_{ATP,o} \geq f_f \cdot V_{m,f,CO_2} \quad (9)$$

as the denominator of equation (8) is always larger than one. Neglecting the relatively small inhibition effect of the CO_2 concentration on respiration, substitution of Eq. (7) and Eq. (8) into constraint (9) yields a condition for the oxygen concentration:

$$C_{O_2} \geq C_{O_2}^* \quad (10)$$

with

$$C_{O_2}^* = \frac{f_f \cdot V_{m,f,CO_2} \cdot K_{m,O_2}}{f_o \cdot V_{m,O_2} \cdot r_{q,ox} - f_f \cdot V_{m,f,CO_2}} \quad (11)$$

Hence, $C_{O_2}^*$ is defined as the critical O_2 level of tissue where the ATP production rate due to the oxidative respiration is equal to maximal ATP production rate by fermentation, or in other words the O_2 concentration where the ATP production by oxidative respiration dominates the ATP production over that by fermentation.

The value of the new energy-based criterion $C_{O_2}^*$ was compared to the *ACP* and *FT* thresholds.

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251 *Sensitivity analysis*

A sensitivity analysis was performed to study how sensitive the computed output I of the model is with respect to small changes in model parameters P . A high value of the relative sensitivity of a parameter indicates that the particular predicted model output is highly influenced by a small change in that parameter value. The relative sensitivity $S_{I,P}$ the predicted I with respect to parameter P was defined as follows:

$$S_I = \frac{\partial I / I}{\partial P / P} \cong \frac{I_{P+\Delta P} - I_{P-\Delta P}}{2\Delta P} \frac{P}{I_P} \quad (12)$$

The perturbation of the parameters ΔP was taken as 10% of the value of P which was used for simulation. The minimal O_2 ($C_{O_2,min}$) and maximal CO_2 ($C_{CO_2,max}$) concentrations inside the fruit computed from the continuum model were the target model outputs to be considered in the analysis.

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Stochastic analysis of biological variation

For the most sensitive parameters, a Monte Carlo analysis was performed to study the effects of biological variability. Hereto we generated 5000 random parameter sets and for each set we solved the model equations. As from the sensitivity analysis it appeared that V_{m,O_2} , V_{m,f,CO_2} and the gas diffusivities of skin, cortex and outer cortex were the most important parameters, we only considered these parameters to be random and kept all other parameters fixed. For the random number generation we assumed that all parameters were normally distributed. Further, V_{m,O_2} and V_{m,f,CO_2} were assumed to be perfectly correlated (correlation coefficient equal to one) as they share a common pathway (glycolysis). Similarly, the tissue diffusivities of O_2 , CO_2 and N_2 of skin, cortex and outer cortex were also assumed to be perfectly correlated as they are all determined by the same tissue microstructure (Ho et al., 2011).

Results

Respiration kinetics of different cultivars in low temperature CA conditions

The estimated values of V_{m,O_2} and V_{m,f,CO_2} at 1°C are shown in Table 3. These values are one order of magnitude lower than the corresponding values at 20°C reported by Ho et al. (2010). V_{m,O_2} ranged from $9.37 \times 10^{-6} \text{ mol m}^{-3} \text{ s}^{-1}$ to $1.25 \times 10^{-5} \text{ mol m}^{-3} \text{ s}^{-1}$ for the different cultivars. ‘Jonagold’ showed the lowest value of all cultivars. V_{m,f,CO_2} ranged from $1.52 \times 10^{-5} \text{ mol m}^{-3} \text{ s}^{-1}$ to $2.34 \times 10^{-5} \text{ mol m}^{-3} \text{ s}^{-1}$. V_{m,f,CO_2} was larger than V_{m,O_2} for all three cultivars.

The estimated values of K_{m,O_2} and K_{m,f,O_2} are given in Table 3 and were obtained by fitting the permeation-diffusion-reaction model to the measured gas profiles in the jar experiments (Figure 2). The fit of the overall respiration of intact apple predicted by the model to measured values is good. The K_{m,O_2} (0.13 to 0.171 kPa) and K_{m,f,O_2} (0.012 to 0.028 kPa) values are small, indicating that fermentation is initiated at a local O_2 concentration that is much smaller than the ambient O_2 concentration at which fermentation is usually observed to start in CA storage (typically 3-5 kPa).

Internal gas concentration profiles in CA conditions

Simulation results of the O₂, CO₂ and N₂ distribution inside the fruit are shown in Fig. 3. The CA storage conditions were 1 kPa O₂, 2.5 kPa CO₂ and 1°C for ‘Jonagold’; 2 kPa O₂, 2.5 kPa CO₂ and 1°C for ‘Kanzi’ and 2.5 kPa O₂, 2.5 kPa CO₂ and 1°C for ‘Braeburn’, respectively. Due to the diffusion resistance of the tissues, concentration gradients are established inside the apples. A decrease of the O₂ partial pressure and an increase of CO₂ partial pressure towards the center of the fruit are observed. A steep gradient is predicted over the skin. This is due to the low gas diffusion properties of the skin compared to those of cortex tissue. The concentration gradient in the cortex was the most shallow in ‘Jonagold’, while the steepest gradient occurred in ‘Braeburn’. This is expected because gas diffusivity and permeability of cortex tissue increase from ‘Braeburn’ over ‘Kanzi’ to ‘Jonagold’, while the maximum respiration rates increase from ‘Jonagold’, ‘Kanzi’ to ‘Braeburn’. The statistical significance of these differences is analysed below.

Critical oxygen limit of the tissue of different cultivars in low temperature CA

Using a ratio of ATP:O₂ of 6.333 proposed by Buchanan et al. (20000), the calculated value of $C_{O_2}^*$ was equal to 5.7×10^{-2} , 6.5×10^{-2} and 5.6×10^{-2} kPa for ‘Kanzi’, ‘Jonagold’ and ‘Braeburn’, respectively (Table 4). The normalised oxygen consumption and carbon dioxide production rates are plotted as a function of the O₂ partial pressure in Figure 4, and the values of K_{m,O_2} and $C_{O_2}^*$ are indicated. The value of $C_{O_2}^*$ is very similar for the different cultivars, in between those of K_{m,f,O_2} and K_{m,O_2} and lower than the minimum of the CO₂ production rate (ACP). Some authors (Gibbs and Greenway, 2003; Edwards et al., 2012) assumed ATP:O₂ equal to 5. Using this value, the estimated $C_{O_2}^*$ values of ‘Kanzi’, ‘Jonagold’ and ‘Braeburn’ were 9.23×10^{-2} , 9.89×10^{-2} and 7.83×10^{-2} kPa, respectively. The anaerobic compensation point (ACP), and fermentation threshold (FT) of the cortex tissue were calculated and compared to $C_{O_2}^*$ (Table 4). We found that the ACP is close to the values of $C_{O_2}^*$ when ATP:O₂ equals 5 while the FT is always larger than the $C_{O_2}^*$ for different cultivars.

The calculated ATP level (the ratio of the ATP production rate to its maximal value) at the critical point was 0.397 to 0.485 for different cultivars. These values are equal or larger than the ATP level requirements for cellular maintenance under hypoxia

found in literature. The ratio of the ATP production rate in anoxic conditions to that in normal respiration in rice coleoptiles was reported to be 0.13-0.29 at 30°C (Colmer et al., 2001) or 0.1-0.4 at 28°C (Edwards et al., 2012). Zhang and Greenway (1994) found this ratio of 0.1-0.25 in red beet tissue at 25°C.

Effect of atmospheric O₂ level in low temperature CA storage on the minimal O₂ concentration in the fruit

The model was applied to compute the smallest O₂ ($C_{O_2,min}$) and largest CO₂ ($C_{CO_2,max}$) partial pressure inside the fruit corresponding to different storage conditions. The predicted $C_{O_2,min}$ was then compared to $C_{O_2}^*$ and is shown as a function of the O₂ partial pressure of the storage atmosphere at 1°C in Figure 5. For ‘Jonagold’ at 1 kPa O₂, $C_{O_2,min}$ (0.21 kPa) was considerably larger than $C_{O_2}^*$ (9.2×10^{-2} kPa) indicating sufficient energy supply for maintaining cell integrity. However, at the same storage O₂ partial pressure, the values of $C_{O_2,min}$ of ‘Kanzi’ (0.013 kPa) and ‘Braeburn’ (0.0086 kPa) were lower than $C_{O_2}^*$ (9.89×10^{-2} for ‘Kanzi’ and 7.83×10^{-2} kPa for ‘Braeburn’). The commercially used storage O₂ partial pressures of 2 kPa and 2.5 kPa for ‘Kanzi’ and ‘Braeburn’, respectively, provide much safer levels of $C_{O_2,min}$ equal to 0.218 kPa for ‘Kanzi’ and 0.22 kPa for ‘Braeburn’. Clearly, while $C_{O_2}^*$ is very similar for the three cultivars, due to differences in tissue diffusion resistance different atmospheres are required for safe storage of the fruit.

Sensitivity of minimal oxygen concentration in the fruit to respiration and gas exchange parameters

A sensitivity analysis was performed to investigate how $C_{O_2,min}$ and $C_{CO_2,max}$ changed with varying model parameters. The relative sensitivity of $C_{O_2,min}$ and $C_{CO_2,max}$ was calculated for each model parameter separately and this was repeated for the three different cultivars. To simulate commercial practices, different reference storage gas atmospheres (1 kPa O₂ and 2.5 kPa CO₂ for ‘Jonagold’; 2 kPa O₂ and 2.5 kPa CO₂ for ‘Kanzi’; 2.5 kPa O₂ and 0.7 kPa CO₂ for ‘Braeburn’) were applied; the

temperature was set to 1°C. The results are shown in Table 5. A large absolute sensitivity of $C_{O_2,\min}$ for V_{m,O_2} was observed. For the three different cultivars, the relative sensitivity values of $C_{CO_2,\max}$ were low. In addition, the O_2 diffusivity of the different tissues also has a strong effect on the $C_{O_2,\min}$. As they determine the diffusion rate of oxygen, their relative sensitivity is positive.

Monte Carlo analysis

The sensitivity analysis indicated that V_{m,O_2} and the O_2 diffusivity of the different tissues affected the minimal O_2 concentration in the fruit the most (see Table 5). When the variation in these parameters is high, the minimal O_2 concentration may become smaller than $C_{O_2}^*$.

V_{m,O_2} and V_{m,f,CO_2} are likely to be proportional to the initially available enzyme concentration (Hertog et al., 1998), and hence depend on fruit maturity. Fruit maturity, an important post-harvest storage factor, is inherently affected by biological variation. To study the effect of variation of maturity in a batch of fruit on the risk of anoxia during CA storage, Monte Carlo simulations were carried out for the three cultivars. Because V_{m,O_2} and V_{m,f,CO_2} are correlated, from Eq. 11 it follows that $C_{O_2}^*$ is not sensitive to variations of these parameters either and can be considered as relatively stable for each cultivar. The lowest O_2 concentration ($C_{O_2,\min}$) inside the fruit is shown in Figure 6. The histograms of $C_{O_2,\min}$ are in general skewed to the right. The computed $C_{O_2,\min}$ (3.8×10^{-4} -0.46 kPa) of ‘Jonagold’ has a smaller variation than that of ‘Kanzi’ (0.0015-0.91 kPa) and ‘Braeburn’ (0.0184-0.74 kPa), but has a larger proportion of values in the lower range. Under commercial storage conditions, $C_{O_2,\min}$ is in 7.9%, 7.2% and 4.9% of the cases lower than $C_{O_2}^*$ for ‘Jonagold’, ‘Kanzi’ and ‘Braeburn’, respectively, indicating that maturity and diffusivity variations may result in disorder-inducing conditions.

Discussion

In controlled atmosphere storage, the gas exchange model predicts very low oxygen concentrations inside fruit that may result into a switch from respiration to fermentation that may eventually cause cell death. Michaelis-Menten kinetics has been widely used to describe the oxygen consumption and carbon dioxide production rates in fruit (Hertog et al., 1998; Lammertyn et al., 2001; Armstrong and Beckett, 2010). In this model, respiration and fermentation are modeled as continuous processes across the entire range of oxygen and carbon dioxide levels, without considering a ‘switch’ mechanism. According to Michaelis-Menten kinetics, respiration decreases and fermentation increases gradually with decreasing oxygen concentration. Therefore, it is relatively difficult to determine a critical level of oxygen concentration below which the risk of storage disorders increases significantly. Several threshold criteria have been presented in the literature based on interpreting measured profiles of consumption and production rates. Here a more mechanistic approach has been introduced based on the actual ATP production rates through respiration and fermentation. The critical O₂ level was found to agree well with the concept of *ACP* but not with that of *FT*.

Physiological disorders in fruit such as browning of tissue are indeed believed to be caused by an imbalance in the energy metabolism in the cells due to too low O₂ or too high CO₂ concentration (Rawyler et al. 2002; Saquet et al., 2003; Franck et al., 2007). A reduction in ATP production leads to membrane damage and decompartmentalisation (Rawyler et al. 2002). Zhang and Greenway (1994) suggested that tissue of red beet may adapt to low oxygen concentrations to reduce the energy requirements for maintenance. The energy requirements for maintenance in anoxia-tolerant tissues is in between 2.3 and 8 fold lower in hypoxia than in air (Gibbs and Greenway, 2003, see Table 4). The ratio of ATP production rate at $C_{O_2}^*$ was 0.4 to 0.485 their maximal value for the three cultivars, which agrees with that range.

The critical oxygen level can be interpreted with respect to the parameters of the respiration and fermentation kinetics. The value of K_{m,O_2} of different apple tissues at 1°C ranged from 0.13 to 0.171 kPa for ‘Kanzi’, ‘Jonagold’ and ‘Braeburn’ and was in good agreement with values found in literature (0.14 kPa O₂ at 1°C; Lammertyn et al., 2001) and that from microscale simulations (Ho et al., 2011). These values are much larger than that of cytochrome c oxidase, the terminal oxidase in plant respiration,

which has been measured in artificial media (0.10 - 0.12 μM , Rawsthorne and LaRue, 1986; 1 μM , Taiz and Zeiger, 1993; 0.14 μM , Millar et al., 1994). The K_{m,O_2} value of intact apples, on the other hand, is much larger than that of tissue found here (Peppelenbos and Van't Leven, 1996; Hertog et al., 1998). The reason is that K_{m,O_2} depends strongly on the spatial scale as it encompasses diffusion resistance effects that depend on the scale. The K_{m,O_2} of apple tissue of different apple cultivars is almost twice as high as the critical oxygen level based on energy considerations.

The inhibition effect by O_2 on fermentation is characterized by K_{m,f,O_2} , which was for the first time determined accurately for apple tissue at low temperature (1°C). A high variation of K_{m,f,O_2} values was reported previously due to limitations in measurement accuracy (Ho et al., 2008 & 2010). Predicted values of K_{m,f,O_2} in this study ranged from 0.012 to 0.028 kPa for the three apple cultivars. The K_{m,f,O_2} values were thus one order of magnitude smaller than those of K_{m,O_2} (0.13 to 0.171 kPa) at 1°C . Likewise, values of K_{m,f,O_2} of intact fruit were also smaller than that of K_{m,O_2} (Hertog et al., 1998; Lammertyn et al., 2003). K_{m,f,O_2} was 3 to 7 times smaller than the critical oxygen level based on energy considerations.

The critical oxygen level of the tissue was shown to be independent of the maximal respiration and fermentation rate of the fruit, and the resulting values for the different cultivars were very similar. However, due to differences in diffusion resistance of the different cultivars, these levels translated into different minimal O_2 partial pressures for each cultivar (Figure 3), explaining why the optimal storage conditions for these cultivars differ. 'Braeburn' in particular has a high risk for browning at CA storage conditions of 1.5% O_2 , 1.2% CO_2 and 0°C (Saquet et al., 2000; Gong et al., 2001), confirming our findings.

Postharvest storage behaviour is inherently affected by the omnipresent biological variation. In spite of efforts of sorting and grading the product at the different stages in the post-harvest chain, one will always have to deal with more or less heterogeneous batches (Hertog et al., 2009). Respiration is the main reason for reducing the O_2 concentration in the fruit; it is affected by the maturity stage of fruit (Bulens et al., 2012). The observed variability was considerably larger for 'Kanzi' and

‘Braeburn’ than for ‘Jonagold’, which resulted in wider variability range of the minimum oxygen levels for these cultivars. The oxygen diffusivity of skin and cortex is the second most important parameter determining internal oxygen levels. Little is known so far on how diffusivity values change as a function of growth conditions. Schotsmans et al. (2003) found no change in diffusivity of O₂ or CO₂ in pear tissue during maturation. Tissue diffusivity strongly depends on the tissue microstructure, in particular the morphology of airspaces in between the cells (Verboven et al., 2008; Pham et al., 2009; Ho et al., 2010 & 2011). How microstructure is affected by growth conditions and maturity is yet unknown, but Schotsmans et al. (2004) found that intercellular spaces of cortex region of Braeburn and Jonica apples increased during storage in CA conditions. The effects of optimal CA and browning inducing storage conditions on ‘Braeburn’ apple microstructure have recently been shown (Herremans et al., 2012). The variability of tissue diffusivity was high: the 95% confidence interval was between 25% and 70% of the average value, for both skin and cortex tissue. The skin in particular is the major barrier for gas diffusion (Figure 3), and its large variability was shown to have important consequences on the stochastic distribution of the minimum oxygen level. ‘Jonagold’ skin showed the largest variability, which resulted in a significant amount of cases with very low minimum oxygen levels that were below the critical threshold. This seems somewhat contradictory to practical experience which shows that ‘Jonagold’ rarely or never develops internal storage disorders. Future research should be directed to experimentally investigate anoxia in relation to ATP levels and tissue breakdown to verify and further explain the findings in this paper.

Conclusion

Gas exchange in different apple cultivars (‘Kanzi’, ‘Jonagold’ and ‘Braeburn’) under CA conditions was investigated by a gas transport model incorporating respiration kinetics. The model was extended to predict ATP production. The occurrence of anoxic zones in the fruit was investigated. The Michaelis-Menten constant (K_{m,O_2}) of oxidative respiration of apple tissue at 1°C was 0.13-0.17 O₂ kPa while fermentation becomes important at a very low O₂ levels. Below an O₂ partial pressure of $C_{O_2}^*$ (46 to 78% of the K_{m,O_2} value), the reduction of the ATP production may cause tissue

damage. The effect of storage O₂ partial pressures on the risk of fermentation inside the fruit was calculated. ‘Jonagold’ can be stored at for low O₂ partial pressure, while high O₂ partial pressures are required for ‘Kanzi’ and Braburn in CA storage.

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620

621 Table 1. List of symbols

Symbols	Units	Definition
ACP		Anaerobic compensation point
$ATP_{o/f}$		Ratio of ATP production by oxidation to fermentation.
$C_{i,g}$	mol m^{-3}	Concentration of the gas component i in the gas phase
$C_{i,tissue}$	mol m^{-3}	Concentration of the gas component i in the tissue
C_{CO_2}	mol m^{-3}	CO_2 concentration
$C_{CO_2,max}$		Maximal CO_2 concentrations inside the fruit
C_{O_2}	mol m^{-3}	O_2 concentration
$C_{O_2,min}$	mol m^{-3}	Minimal O_2 concentrations inside the fruit
$C_{O_2}^*$	mol m^{-3}	O_2 partial pressure at which the energy production due to oxidative respiration is equal to the maximal ATP production by fermentation
D_i	$(\text{m}^2\text{s}^{-1})$	Apparent diffusion coefficient of the tissue of gas i
FT		Fermentation threshold, defined as the O_2 level at which the RQ increased to 1.1 to 1.2 times its asymptotic value at high O_2
f_f		Stoichiometric coefficient for ATP production due to the fermentation
f_o		Stoichiometric coefficient of the ATP production due to the oxidative respiration
H_i	$\text{mol m}^{-3} \text{ kPa}^{-1}$	Henry's constant
I		Computed output from the model
K	m^2	Permeation coefficient

K_{m,f,O_2}	mol m^{-3}	Michaelis-Menten constant of O_2 inhibition on fermentative CO_2 production
K_{mn,CO_2}	mol m^{-3}	Michaelis-Menten constant for non-competitive CO_2 inhibition
K_{m,O_2}	mol m^{-3}	Michaelis Menten constant for O_2 consumption
P		Model parameter
R	$\text{J mol}^{-1} \text{K}^{-1}$	Universal gas constant ($8.314 \text{ J mol}^{-1} \text{K}^{-1}$)
$R_{ATP,f}$	$\text{mol ATP m}^{-3} \text{s}^{-1}$	Rate of ATP production from the oxidative respiration
$R_{ATP,o}$	$\text{mol ATP m}^{-3} \text{s}^{-1}$	Rate of ATP production from the fermentation
R_{CO_2}	$\text{mol m}^{-3} \text{s}^{-1}$	CO_2 production rate
R_i	$\text{mol m}^{-3} \text{s}^{-1}$	Reaction term of the gas component i related to O_2 consumption or CO_2 production
R_{O_2}	$\text{mol m}^{-3} \text{s}^{-1}$	O_2 consumption rate
RQ		Ratio of CO_2 production to O_2 consumption
r_{ATP}		Ratio of actual ATP production in anoxic conditions to the maximal value at saturation
r_f		Ratio of fermentation rate in anoxic conditions to maximal fermentation rate with glucose as main substrate for respiration
r_o		Ratio of oxidative respiration rate in anoxia to saturated respiration rate
$r_{q,ox}$		Respiration quotient
$S_{I,P}$		Relative sensitivity of the predicted I with respect to parameter P
T	K	Temperature

t	s	Time.
$\mathbf{u}$	(m s ⁻¹)	Apparent permeation velocity vector
V_{m,f,CO_2}	mol m ⁻³ s ⁻¹	Maximal fermentative CO ₂ production rate
V_{m,O_2}	mol m ⁻³ s ⁻¹	Maximal O ₂ consumption rate
α_i		Gas capacity of the component i (O ₂ , CO ₂ and N ₂) of the tissue
μ	Pa.s	Viscosity of gas
ε		Porosity of tissue
∇	m ⁻¹	Gradient operator
Subscript		
CO_2		Carbon dioxide
i		Inner cortex
N_2		Nitrogen
O_2		Oxygen
o		Outer cortex
$skin$		Skin tissue

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623

624 Table 2. Gas transport properties parameters of model (Ho et al., 2010).

Parameters	Unit	‘Jonagold’	‘Kanzi’	‘Braeburn’
$D_{O_2,skin}$	$10^{-9} \text{ m}^2 \text{ s}^{-1}$	0.19	0.31	0.15
$D_{CO_2,skin}$	$10^{-9} \text{ m}^2 \text{ s}^{-1}$	0.31	0.98	0.95
$D_{N_2,skin}$	$10^{-9} \text{ m}^2 \text{ s}^{-1}$	0.3	0.44	0.12
K_{skin}	10^{-17} m^2	0.59	0.27	0.59
$D_{O_2,i}$	$10^{-9} \text{ m}^2 \text{ s}^{-1}$	10.10	2.73	1.73
$D_{CO_2,i}$	$10^{-9} \text{ m}^2 \text{ s}^{-1}$	35.10	18.10	10.60
$D_{N_2,i}$	$10^{-9} \text{ m}^2 \text{ s}^{-1}$	18.00	3.48	0.84
$K_{r,i}$	10^{-17} m^2	92.3	6.94	2.25
$D_{O_2,o}$	$10^{-9} \text{ m}^2 \text{ s}^{-1}$	10.10	5.05	3.14
$D_{CO_2,o}$	$10^{-9} \text{ m}^2 \text{ s}^{-1}$	35.10	25.0	14.1
$D_{N_2,o}$	$10^{-9} \text{ m}^2 \text{ s}^{-1}$	18.09	9.40	7.18
$K_{r,o}$	10^{-17} m^2	92.3	6.94	2.25

625 Subscript i and o indicating inner and outer cortex, respectively. The symbols and their
626 meaning are listed in Table 1.

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628

629 Table 3 Respiration model parameters of apple cortex tissue at 1°C.

	‘Jonagold’	‘Kanzi’	‘Braeburn’
V_{m,O_2} (mol m ⁻³ s ⁻¹)	(9.37±0.22)×10 ⁻⁶	(1.18±0.102)×10 ⁻⁵	(1.25±0.10)×10 ⁻⁵
V_{m,f,CO_2} (mol m ⁻³ s ⁻¹)	(1.52±0.08) ×10 ⁻⁵	(2.34±0.17)×10 ⁻⁵	(1.78±0.21)×10 ⁻⁵
K_{m,O_2} (kPa)	0.167±0.046	0.127±0.036	0.1709±0.079
K_{m,f,O_2} (kPa)	0.024±0.07	0.012±0.005	0.028 ±0.016
K_{m,CO_2} (kPa) ⁽¹⁾	163	168	80
$r_{q,ox}$	0.91	0.91	0.9

630 ±: Standard error

631 ⁽¹⁾: Ho et al. (2010)

632

633

Table 4 Low O₂ limits and rates of ATP production, oxidative respiration and fermentation for different apple cultivars at 1°C

Lower Oxygen limit criteria		‘Jonagold’	‘Kanzi’	‘Braeburn’
$C_{O_2}^{* (1)}$	O ₂ (kPa)	6.53×10^{-2}	6.71×10^{-2}	5.64×10^{-2}
	r_{ATP}	3.57×10^{-1}	3.98×10^{-1}	3.30×10^{-1}
	$ATP_{o/f}$	3.72	6.59	3.01
	r_o	2.81×10^{-1}	3.46×10^{-1}	2.48×10^{-1}
	r_f	2.69×10^{-1}	1.52×10^{-1}	3.32×10^{-1}
$C_{O_2}^{* (2)}$	O ₂ (kPa)	9.23×10^{-2}	9.89×10^{-2}	7.83×10^{-2}
	r_{ATP}	4.30×10^{-1}	4.85×10^{-1}	3.97×10^{-1}
	$ATP_{o/f}$	4.85	9.24	3.80
	r_o	3.56×10^{-1}	4.38×10^{-1}	3.14×10^{-1}
	r_f	2.06×10^{-1}	1.08×10^{-1}	2.63×10^{-1}
<i>ACE</i>	O ₂ (kPa)	1.22×10^{-1}	8.40×10^{-2}	1.19×10^{-1}
	r_{ATP}	4.81×10^{-1}	4.53×10^{-1}	4.58×10^{-1}
	$ATP_{o/f}$	7.21	7.28	8.69
	r_o	4.22×10^{-1}	3.98×10^{-1}	4.10×10^{-1}
	r_f	1.64×10^{-1}	1.25×10^{-1}	1.90×10^{-1}
<i>FT</i>	O ₂ (kPa)	5.30×10^{-1}	3.40×10^{-1}	5.40×10^{-1}
	r_{ATP}	7.76×10^{-1}	7.43×10^{-1}	7.75×10^{-1}
	$ATP_{o/f}$	49.3	48.8	49.0
	r_o	7.60×10^{-1}	7.28×10^{-1}	7.60×10^{-1}
	r_f	4.33×10^{-2}	3.41×10^{-2}	4.93×10^{-2}

The symbols and their meaning are listed in Table 1.

⁽¹⁾ Assuming an ATP:O₂ of 6.33 for oxidative respiration (Buchanan et al., 2000).

⁽²⁾ Assuming an ATP:O₂ of 5 for oxidative respiration (Gibbs and Greenway, 2003; Edwards et al., 2012) .

642 Table 5 Relative sensitivity of $C_{O_2,\min}$ and $C_{CO_2,\max}$ in the fruit for different model parameters. Symbols are defined in Materials and
643 Methods section.

	‘Jonagold’			‘Kanzi’			‘Braeburn’		
Parameters	Value	$S_{C_{O_2,\min},P}$	$S_{C_{CO_2,\max},P}$	Value	$S_{C_{O_2,\min},P}$	$S_{C_{CO_2,\max},P}$	Value	$S_{C_{O_2,\min},P}$	$S_{C_{CO_2,\max},P}$
$D_{O_2,o}$ ($m^{-2}s^{-1}$)	1.01×10^{-8}	4.50×10^{-1}	3.52×10^{-3}	5.05×10^{-9}	1.08	6.11×10^{-3}	3.28×10^{-9}	1.34	2.38×10^{-2}
$D_{CO_2,o}$ ($m^{-2}s^{-1}$)	3.51×10^{-8}	3.82×10^{-4}	-1.85×10^{-2}	2.50×10^{-8}	3.08×10^{-3}	-3.62×10^{-2}	1.73×10^{-8}	2.81×10^{-3}	-1.31×10^{-1}
$D_{N_2,o}$ ($m^{-2}s^{-1}$)	1.80×10^{-8}	1.92×10^{-3}	5.74×10^{-4}	9.40×10^{-9}	1.87×10^{-2}	1.5×10^{-3}	7.51×10^{-9}	2.62×10^{-2}	2.54×10^{-3}
$D_{O_2,i}$ ($m^{-2}s^{-1}$)	1.01×10^{-8}	3.31×10^{-1}	2.47×10^{-4}	2.73×10^{-9}	1.66	6.46×10^{-3}	1.73×10^{-9}	1.16	9.95×10^{-3}
$D_{CO_2,i}$ ($m^{-2}s^{-1}$)	3.51×10^{-8}	4.09×10^{-4}	-1.05×10^{-2}	1.81×10^{-8}	-2.66×10^{-4}	-3.55×10^{-2}	1.34×10^{-8}	-3.03×10^{-4}	-6.22×10^{-2}
$D_{N_2,i}$ ($m^{-2}s^{-1}$)	1.81×10^{-8}	9.51×10^{-4}	2.95×10^{-4}	1.04×10^{-9}	3.31×10^{-3}	3.45×10^{-4}	8.40×10^{-10}	2.43×10^{-3}	2.76×10^{-4}
$D_{O_2,skin}$ ($m^{-2}s^{-1}$)	1.90×10^{-10}	1.21	1.48×10^{-2}	3.10×10^{-10}	1.10	6.67×10^{-3}	1.50×10^{-10}	1.82	4.07×10^{-2}
$D_{CO_2,skin}$ ($m^{-2}s^{-1}$)	3.10×10^{-10}	4.27×10^{-3}	-1.16×10^{-1}	9.80×10^{-10}	5.00×10^{-3}	-5.69×10^{-2}	9.50×10^{-10}	-7.42×10^{-4}	-1.51×10^{-1}
$D_{N_2,skin}$ ($m^{-2}s^{-1}$)	3.00×10^{-10}	4.83×10^{-3}	1.61×10^{-3}	4.40×10^{-10}	1.83×10^{-2}	1.55×10^{-3}	1.20×10^{-10}	2.50×10^{-2}	1.39×10^{-3}

V_{m,O_2} (mol m ⁻³ s ⁻¹)	-7.50×10 ⁻⁶	-2.01	1.01×10 ⁻¹	-1.18×10 ⁻⁵	-3.87	9.91×10 ⁻²	-1.25×10 ⁻⁵	-4.39	2.36×10 ⁻¹
V_{m,f,CO_2} (mol m ⁻³ s ⁻¹)	1.30×10 ⁻⁵	-7.35×10 ⁻⁴	2.11×10 ⁻²	2.35×10 ⁻⁵	-2.44×10 ⁻⁴	5.57×10 ⁻³	1.78×10 ⁻⁵	-1.35×10 ⁻⁴	2.79×10 ⁻²
K_{m,O_2} (mol m ⁻³)	0.073477	6.73×10 ⁻¹	-3.26×10 ⁻²	0.057064	6.74×10 ⁻¹	-1.52×10 ⁻²	0.075	9.54×10 ⁻¹	-4.87×10 ⁻²
K_{m,CO_2} (mol m ⁻³)	56.647	-4.43×10 ⁻²	2.23×10 ⁻³	79.89	-5.94×10 ⁻²	1.50×10 ⁻³	56.64	-3.35×10 ⁻²	1.79×10 ⁻³
K_{m,f,O_2} (mol m ⁻³)	0.0106	-6.84×10 ⁻⁴	1.96×10 ⁻²	0.0053	-2.40×10 ⁻⁴	5.44×10 ⁻³	0.012	-1.28×10 ⁻⁴	2.63×10 ⁻²
$r_{q,ox}$	0.91	-4.19×10 ⁻³	1.20×10 ⁻¹	0.91	-7.25×10 ⁻³	1.18×10 ⁻¹	0.9	-1.58×10 ⁻³	3.10×10 ⁻¹

644 Subscripts *i* and *o* indicate inner and outer cortex, respectively.

645 Simulation conditions: ‘Jonagold’ at 1 kPa O₂, 2.5 kPa CO₂ and 1°C; ‘Kanzi’ at 2 kPa O₂, 2.5 kPa CO₂ and 1°C; ‘Braeburn’ at 2.5

646 kPa O₂, 0.7 kPa CO₂ and 1°C.

647

648 Figure 1 Apple geometry (adapted from Ho et al., 2010) with different tissues as
649 described by Ho et al. (2010).

650

Figure 2. Normalised oxygen consumption ($R_{O_2} / V_{m,O_2}$) and CO_2 production ($R_{CO_2} / V_{m,O_2}$) rate of intact apple fruit as a function of the ambient O_2 partial pressure at $1^\circ C$. (a), (b) and (c) show the results for 'Jonagold', 'Kanzi' and 'Braeburn', respectively. Solid (—) and dashed (---) lines represent the predicted O_2 consumption and CO_2 production rates while closed symbols (o) and (*) indicate the corresponding measurements.

660 Figure 3 Simulated O₂, CO₂ and N₂ partial pressure distributions in intact fruit at
661 commercial CA conditions. (a) 'Jonagold' at 1 kPa O₂, 2.5 kPa CO₂ and 1°C; (b) 'Kanzi'
662 at 2 kPa O₂, 2.5 kPa CO₂ and 1°C; (c) 'Braeburn' at 2.5 kPa O₂, 2.5 kPa CO₂ and 1°C.
663 Color bars indicate gas partial pressure (kPa).
664

666 Figure 4. Normalised predicted oxygen consumption ($R_{O_2}/V_{m,O_2}$) and carbon dioxide
 667 production ($R_{CO_2}/V_{m,O_2}$) of apple tissue as a function of O₂ partial pressure at 1°C. (a), (b)
 668 and (c) show the results of ‘Jonagold’, ‘Kanzi’ and ‘Braeburn’, respectively. The solid
 669 (—) and dashed (- -) lines represent the O₂ consumption and CO₂ production of tissue,
 670 respectively. Vertical solid lines (—) indicate K_{m,O_2} while vertical dashed lines (- -)
 671 represent $C_{O_2}^*$.

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674

675 Figure 5 Predicted $C_{O_2,min}$ as a function of the ambient O_2 partial pressure at $1^\circ C$. Solid
 676 (—), dashed (- -) and dashed- dot (- ·) curves predictions for 'Jonagold', 'Kanzi' and
 677 'Braeburn', respectively. Horizontal solid (—), dashed (- -) and dashed- dot (- ·) lines
 678 indicate the $C_{O_2}^*$ of 'Jonagold', 'Kanzi' and 'Braeburn', respectively.

679

681 Figure 6. Histogram of $C_{O_2,min}$ at commercial CA conditions computed with Monte Carlo
 682 simulations at 1 kPa, 2.5 kPa CO_2 and 1°C for 'Jonagold' (a); 2 kPa O_2 and 2.5 kPa CO_2

683 and 1°C for ‘Kanzi’ (b); 2 kPa, and 2.5 kPa, 0.7 kPa CO₂ and 1°C for ‘Braeburn’ (c).
684 Vertical dashed lines (- -) represent $C_{o_2}^*$.