Modelling respiration of packaged fresh-cut 'Rocha' pear as affected by oxygen concentration and temperature

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Keywords:

Film permeability

Minimal processing

Modified atmosphere packaging

Pyrus communis

Respiratory quotient

ABSTRACT

Respiration rates were measured in fresh-cut 'Rocha' pear (*Pyrus communis* L.) stored at four temperatures (0, 5, 10 and 15 °C) and with oxygen partial pressures ranging from 0 to 18 kPa. Respiratory quotient and ethanol production were used to determine the fermentation threshold. The oxygen concentration effect on the respiration rate was accurately described using Michaelis–Menten kinetics, without non-competitive inhibition by CO₂, and the effect of temperature on the respiration rate was well modelled by exponential functions. The oxygen level at which respiration was half its maximum (apparent K_{m,O_2}) was similar to or only slightly greater than the fermentation threshold. The narrow range of oxygen between K_{m,O_2} and the fermentation threshold, suggests that modified atmosphere packaging technology has a limited applicability toward extension of the shelf-life of fresh-cut 'Rocha' pear.

Introduction

Respiration is a central process in fruit metabolism: it provides energy and carbon skeletons for the anabolic reactions that occur during maturation and ripening, and for cell maintenance during storage. Besides its physiological importance, knowledge of fruit respiratory parameters possesses a technological relevance, particularly toward development of modified atmosphere packaging (MAP). Fruit respiration rate is affected by a number of factors, among which temperature and oxygen partial pressure exert strong effects (Fonseca et al., 2002). In fresh-cut fruit wounding enhances the respiration rate as part of the healing and ethylene responses (Brecht, 1995).

Fresh-cut processing often uses barrier technologies to modify atmosphere composition, prevent contamination, and reduce water loss (Kader et al., 1989). Since fresh-cut fruit respire, hermetically-sealed barriers used in these convenience products result in a modified atmosphere inside the package. Since MAP can extend the shelf-life and marketing period of fresh-cut fruit (Bai et al., 2001; Martínez-Ferrer et al., 2002) a great deal of effort has been dedicated to develop and test appropriate packages, and to optimize oxygen and carbon dioxide partial pressures inside the pack-

ages (Kader et al., 1989; Fonseca et al., 2002). A number of studies encompassing packaging films and geometries have assessed O₂ concentration versus produce quality, in order to empirically find the best package (Bai et al., 2003; Del Nobile et al., 2007; Soliva-Fortuny et al., 2007; Teixeira et al., 2007). An alternative approach to the trial-and-error, should data be available, is to deduce optimal packaging geometry and film permeability based on the produce respiration rate (Lakakul et al., 1999; Jacxsens et al., 2000), as affected by oxygen concentration and temperature.

Respiration rates of fresh-cut pear were reported for 'd'Anjou', 'Bartlett', 'Bosc' and 'Red Anjou' cultivars stored in air and in 2 kPa oxygen (Gorny et al., 2000). Values range between 0.07–0.22 and 0.27–0.53 mmol CO₂ kg⁻¹ h⁻¹, respectively at 0 and 10 °C, rising to 0.86–1.63 mmol CO₂ kg⁻¹ h⁻¹ at 20 °C. However, detailed information on the effect of oxygen concentration on the respiration of fresh-cut pear at different temperatures is still unavailable.

The impact of MAP on fresh-cut produce has been shown to be effective for some commodities; however benefits have not been demonstrated for fresh-cut pear. Further, the requirement for packaging fresh-cut fruit in sealed packages, to maintain produce safety and quality, will result in a modified atmosphere. Therefore, it is essential to quantify the relationship between oxygen concentration and respiration rate for fresh-cut 'Rocha' pear, stored at different temperatures, and to test respiratory models that would allow the design of MAP for fresh-cut pear at temperatures normally found during processing and distribution.

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Nomenclature

A	surface area (cm ²)	R	universal gas constant (0.0083144 kJ mol ⁻¹ K ⁻¹)
E_a	activation energy (kJ mol ⁻¹)	RMSE	root mean square error
FT	fermentation threshold (kPa)	R_{O_2}, R_{CO_2}	respiration (consumption, production) rate (mmol kg ⁻¹ h ⁻¹)
K_{m,O_2}	Michaelis–Menten constant for the pO_2 pkg at half of $R_{O_2}^{max,T}$ (kPa)	$R_{O_2}^{max,T}$	Michaelis–Menten constant for maximal value of R_{O_2} (mmol kg ⁻¹ h ⁻¹)
K_{mn,CO_2}	Michaelis–Menten constant for the non-competitive CO ₂ inhibition	RQ	respiratory quotient
l	thickness (cm)	T	temperature (K)
M	mass (kg)	Subscripts	
p	partial gas pressure (kPa)	atm	outside the package
P	permeability (mmol cm cm ⁻² h ⁻¹ kPa ⁻¹)	pkg	inside the package

Materials and methods

Plant material

Pears (*Pyrus communis* L. 'Rocha') were harvested in two consecutive years, 2006 and 2007, at commercial maturity (65 N firmness), from orchards located in the Western Region of Portugal (39°11' N, 9°08' W). 'Rocha' pear has a long storage life and raw material is available for fresh-cut processing for up to ten months. Pears used in 2006 had been stored for 6 months at −0.5 °C, 93–95% RH, and 2.5 kPa O₂ plus 0.7 kPa CO₂. Fruits processed in 2007 had been stored at −0.5 °C and 93–95% RH in air for 5 weeks. Fruits removed from storage were allowed to ripen and were processed when the flesh firmness reached 47–49 N, since partially ripe pears are the most suitable for fresh-cut processing (Gorny et al., 2000; Soliva-Fortuny et al., 2004).

Fresh-cut preparation and packaging

Whole fruits were sanitized with 150 µL L⁻¹ NaClO for 2 min, rinsed with tap water and air dried. Pears were cut in wedges with 5–10 mm of thickness, without skin removal. The fresh-cut pear wedges (30–265 g) were packed in low-density polyethylene (LDPE) (Dow Chemical Company, Midland, MI) pouches (15 × 15 cm or 20 × 20 cm), that were hermetically-sealed using an impulse heat sealer. The film thickness used in the experiments ranged from 27.0 to 102.1 µm.

Package size, film thickness, and fruit mass were varied to intentionally develop a range of oxygen concentrations (18–0.1 kPa). A gas sampling septum, consisting of a dab of silicone sealant (GE[®] Silicone, NY) on a short strip of electrical tape (Scotch[®], St. Paul, MN), was attached to each pouch.

Respiration rate determination

A permeable system using LDPE of known permeability was used to obtain the respiratory rate of the enclosed produce. Rates of O₂ uptake (R_{O_2}) and CO₂ production (R_{CO_2}), and respiratory quotient ($RQ = R_{CO_2}/R_{O_2}$), were calculated once steady-state O₂ and CO₂ partial pressures were achieved inside the packages, using the following equations (Beaudry et al., 1992; Joles et al., 1994; Lakakul et al., 1999):

$$R_{O_2} = \frac{P_{O_2} \times A}{l} \times \frac{(pO_{2 \text{ atm}} - pO_{2 \text{ pkg}})}{M} \quad (1)$$

$$R_{CO_2} = \frac{P_{CO_2} \times A}{l} \times \frac{(pCO_{2 \text{ pkg}} - pCO_{2 \text{ atm}})}{M} \quad (2)$$

where R_{O_2} and R_{CO_2} denote respiration rates (mmol kg⁻¹ h⁻¹) of O₂ and CO₂, P_{O_2} and P_{CO_2} denote O₂ and CO₂ permeabilities

(mmol cm cm⁻² h⁻¹ kPa⁻¹), A is the film area (cm²), l is the film thickness (cm), $pO_{2 \text{ atm}}$, $pO_{2 \text{ pkg}}$, $pCO_{2 \text{ atm}}$ and $pCO_{2 \text{ pkg}}$ are O₂ and CO₂ partial pressures (kPa) outside (atm) and inside (pkg) the package, and M is the produce mass (kg).

Three replicates of each combination of film thickness, film area, and fruit mass were stored at 0, 5, 10 and 15 °C.

Film permeability assessment

Film permeability to O₂ (P_{O_2}) and CO₂ (P_{CO_2}) was measured using an isostatic method (Beaudry et al., 1992; Gavara et al., 1996). The permeation cell contained two external receiver chambers separated from a middle donor chamber by film samples. The middle chamber was initially flushed (100 mL min⁻¹) with pure N₂ to purge receiver chamber of oxygen. Once receiver chamber oxygen levels were reduced below detection limits, a mixture of 72 kPa O₂ and 29 kPa CO₂ was supplied to the donor chamber. The receiver gas composition was sampled (100 µL), using a glass syringe, at 10 min intervals and analysed via sequential O₂ and CO₂ analyzers, using a paramagnetic O₂ detector (Series 1100, Servomex Co., Sussex, UK) and an infrared CO₂ detector (ADC 255-MK3, Analytical Development Co., Hoddesdon, UK), respectively, and connected in series (Lakakul et al., 1999). The diffusion rate of gases through the film was used to calculate the permeability coefficients, at 0.6, 10, 21 and 23 °C, in controlled temperature chambers. The Arrhenius equation was used to fit the temperature influence on permeability (Beaudry et al., 1992; Joles et al., 1994; Cameron et al., 1995; Lakakul et al., 1999). The pre-exponential factor and activation energies were estimated via regression analysis, after linearization (Beaudry et al., 1992): $\ln(P_i) = -E_a/RT + \ln(P_{0,i})$, where P_i is permeability to O₂ or CO₂ (mmol cm cm⁻² h⁻¹ kPa⁻¹), $P_{0,i}$ is the pre-exponential factor, E_a is activation energy (kJ mol⁻¹), R is ideal gas constant (kJ mol⁻¹ K⁻¹) and T is temperature (K).

O₂, CO₂, and ethanol determination

Headspace gas composition (O₂ and CO₂) in individual packages was monitored daily using a gas analyzer, as described above, until steady-state was attained. The ethanol present in the headspace was sampled at steady-state and determined using a gas chromatograph system (HP-6890, Hewlett Packard, USA), equipped with a mass spectrometry detector.

Experimental data modelling

Respiration rate (R_{O_2}) was described as a function of O₂ partial pressure by a Michaelis–Menten model (Eq. (3)), as suggested by Lee et al. (1991):

$$R_{O_2} = \frac{R_{O_2}^{\max,T} \times pO_2 \text{ pkg}}{K_{m,O_2} + pO_2 \text{ pkg}} \quad (3)$$

where $R_{O_2}^{\max,T}$ is the maximal value of R_{O_2} ($\text{mmol kg}^{-1} \text{ h}^{-1}$), $pO_2 \text{ pkg}$ is the oxygen partial pressure inside packages (kPa) and K_{m,O_2} (apparent K_m) is the $pO_2 \text{ pkg}$ at half of $R_{O_2}^{\max,T}$ (kPa).

A non-competitive CO_2 inhibition model (Peppelenbos et al., 1996) was also fitted to the data (Eq. (4)), to account for the possible effect of pCO_2 on O_2 consumption (Kerbel et al., 1988):

$$R_{O_2} = \frac{R_{O_2}^{\max,T} \times pO_2 \text{ pkg}}{(K_{m,O_2} + pO_2 \text{ pkg}) \times (1 + pCO_2 \text{ pkg}/K_{mn,CO_2})} \quad (4)$$

where K_{mn,CO_2} is the Michaelis–Menten constant for the non-competitive CO_2 inhibition.

Estimation of $R_{O_2}^{\max,T}$ and K_{m,O_2} , based on experimental data, was performed by non-linear regression using the Levenberg–Marquardt method. The estimates obtained at each temperature were used to describe the temperature dependence of $R_{O_2}^{\max,T}$ and K_{m,O_2} . In addition, the Michaelis–Menten models were fitted to the whole data set at all temperatures ($n = 129$ in 2006 and $n = 154$ in 2007). In both models, $R_{O_2}^{\max,T}$ was an exponential function of temperature, and K_{m,O_2} could be modelled as either an exponential function of temperature or a constant, whereas K_{mn,CO_2} was always a constant (Table 1). The predictions of respiratory parameters, $R_{O_2}^{\max,T}$ and K_{m,O_2} , at 0, 5, 10 and 15 °C, generated by solving the equations depicted on Table 4, were used to calculate activation energies, through an Arrhenius plot (Eqs. (5) and (6)), and the temperature coefficient (Q_{10}).

$$\ln(R_{O_2}^{\max,T}) = \ln(R_{O_2}^{\max,0}) - \frac{E_a}{R} \left(\frac{1}{T} \right) \quad (5)$$

$$\ln(K_{m,O_2}) = \ln(K_{m,O_2}^0) - \frac{E_a}{R} \left(\frac{1}{T} \right) \quad (6)$$

where $R_{O_2}^{\max,T}$ is the maximal value of R_{O_2} ($\text{mmol kg}^{-1} \text{ h}^{-1}$), $R_{O_2}^{\max,0}$ and K_{m,O_2}^0 are the pre-exponential factors, K_{m,O_2} (apparent K_m) is the $pO_2 \text{ pkg}$ at half of $R_{O_2}^{\max,T}$ (kPa), E_a is activation energy (kJ mol^{-1}), R is ideal gas constant ($\text{kJ mol}^{-1} \text{ K}^{-1}$) and T is temperature (K).

The accuracy of parameters estimation was given by the ratio of the standard error to value estimate. The likelihood of the fit was ascertained by the root mean square error (RMSE) (Yang and Chinan, 1988).

All statistical analyses were performed using the software package SPSS for Windows v.16.0 (SPSS, Chicago, IL).

Results and discussion

Film permeability

Permeability to O_2 and CO_2 and their temperature dependence are depicted in Fig. 1. The predicted permselectivity ($\beta = P_{CO_2}/P_{O_2}$)

Table 2

Equations used to predict permeability as a function of temperature (K) and activation energies for the films used in the experiments.

Predicting equation ($\text{mmol cm cm}^{-2} \text{ h}^{-1} \text{ kPa}^{-1}$)	E_a (kJ mol^{-1})	R^2
$P_{O_2} = 0.067 \times e^{[-4423/T]}$	$E_{aO_2} = 36.8$	0.930
$P_{CO_2} = 0.118 \times e^{[-4153/T]}$	$E_{aCO_2} = 34.5$	0.923

Table 3

Experimental rates of O_2 uptake and CO_2 production, respiratory quotient ($RQ = R_{CO_2}/R_{O_2}$) and fermentation threshold (FT) for fresh-cut 'Rocha' pear stored at various temperatures.

Temperature (°C)	R_{O_2} range ($\text{mmol kg}^{-1} \text{ h}^{-1}$)	R_{CO_2} range ($\text{mmol kg}^{-1} \text{ h}^{-1}$)	RQ^a	FT (kPa)
0	0.02–0.06	0.04–0.08	1.37 ± 0.02	0.75
5	0.03–0.18	0.07–0.22	1.38 ± 0.03	0.25
10	0.04–0.49	0.13–0.60	1.26 ± 0.02	0.75
15	0.09–0.95	0.23–1.19	1.18 ± 0.01	0.88

^a RQ are the mean ($\pm$ SE) of the experimental values measured at oxygen concentrations ranging from 18 kPa to the fermentation threshold (Fig. 3).

at 0 °C was 4.7 and slightly lower (4.4) at 20 °C. These permselectivities are similar to previous published results by Joles et al. (1994) (4.8 at 20 °C and 4.9 at 0 °C) and by Beaudry et al. (1992) (4.4 at 25 °C and 5.2 at 0 °C). The activation energy for P_{O_2} was 36.8 kJ mol^{-1} (Table 2), a value similar to those reported elsewhere (Beaudry et al., 1992; Joles et al., 1994; Lakakul et al., 1999), but slightly below the 40–50 kJ mol^{-1} range claimed for LDPE films used in MAP (Cameron et al., 1995). Activation energies for P_{CO_2} were 34.5 kJ mol^{-1} (Table 2), which were essentially identical to the values 35.0 kJ mol^{-1} (Lakakul et al., 1999) and 35.5 kJ mol^{-1} (Beaudry et al., 1992; Joles et al., 1994).

MAP design requires knowledge of permeability coefficients in the range of temperatures found during processing and marketing. Film suppliers usually provide O_2 transmission rate determined at a single temperature (e.g. 23 °C), often outside the temperature range in the cold chain. Using these values to predict O_2 concentration inside packages at 5 °C, without correction of film O_2 permeability, would lead to gross overestimation.

Effect of oxygen concentration and temperature on respiration rate

Fruit at the same maturity stage were harvested in two consecutive years and stored for 6 months or 5 weeks, in 2006 and 2007, respectively. Despite the differences in storage durations, respiratory parameters did not differ significantly between years, likely because fruit were processed and analysed with similar maturities, as assessed by flesh firmness.

Table 1

Models used to describe the effects of oxygen concentration and temperature on the respiration rate of fresh-cut 'Rocha' pear.

Model label ^a	Model equation	Model parameters		
		$R_{O_2}^{\max,T}$	K_{m,O_2}	K_{mn,CO_2}
MM	$R_{O_2} = \frac{R_{O_2}^{\max,T} \times pO_2 \text{ pkg}}{K_{m,O_2} + pO_2 \text{ pkg}}$	$a \times e^{(b \times T)}$	$q \times e^{(r \times T)}$	–
MM _k	$R_{O_2} = \frac{R_{O_2}^{\max,T} \times pO_2 \text{ pkg}}{K_{m,O_2} + pO_2 \text{ pkg}}$	$a \times e^{(b \times T)}$	Constant	–
MMNC	$R_{O_2} = \frac{R_{O_2}^{\max,T} \times pO_2 \text{ pkg}}{(K_{m,O_2} + pO_2 \text{ pkg}) \times (1 + pCO_2 \text{ pkg}/K_{mn,CO_2})}$	$a \times e^{(b \times T)}$	$q \times e^{(r \times T)}$	Constant
MMNC _k	$R_{O_2} = \frac{R_{O_2}^{\max,T} \times pO_2 \text{ pkg}}{(K_{m,O_2} + pO_2 \text{ pkg}) \times (1 + pCO_2 \text{ pkg}/K_{mn,CO_2})}$	$a \times e^{(b \times T)}$	Constant	Constant

^a MM: Michaelis–Menten equation (Eq. (3)); MMNC: Michaelis–Menten equation with non-competitive inhibition by CO_2 (Eq. (4)). MM_k and MMNC_k have constant K_{m,O_2} .

Table 4Models predictions of $R_{O_2}^{max,T}$, K_{m,O_2} and K_{mn,CO_2} as a function of temperature (K) and models corresponding accuracies.

Model ^a	$R_{O_2}^{max,T}$ (mmol kg ⁻¹ h ⁻¹)	K_{m,O_2} (kPa)	K_{mn,CO_2} ^b (kPa)	R^2	RMSE	Q_{10} $R_{O_2}^{max,T}$	E_a ^c $R_{O_2}^{max,T}$	E_a ^c K_{m,O_2}
MM	$2.40 \times 10^{-26} \times e^{0.2057T}$	$4.26 \times 10^{-30} \times e^{0.2387T}$	–	0.963	0.043	7.8	134.2	155.8
MM _k	$3.84 \times 10^{-24} \times e^{0.1877T}$	2.07	–	0.927	0.057	6.5	122.4	–
MMNC	$1.38 \times 10^{-24} \times e^{0.1917T}$	$1.28 \times 10^{-30} \times e^{0.2427T}$	41.99 (77%)	0.966	0.039	6.8	125.0	158.4
MMNC _k	$2.43 \times 10^{-19} \times e^{0.1497T}$	1.27	14.26 (42%)	0.948	0.048	4.4	97.5	–

^a MM – Michaelis–Menten model; MMNC – Michaelis–Menten model with non-competitive inhibition by CO₂.^b Values within parenthesis indicate parameter accuracy (ratio of standard error to estimated value).^c Activation energies (E_a) of respiratory parameters were calculated using Arrhenius plots (Eqs. (5) and (6)). **Fig. 1.** Effect of temperature on film permeability to O₂ (solid symbols) and CO₂ (open symbols). Lines based on predicting equations presented in Table 2. Inset: Arrhenius plot of gases permeabilities. **Fig. 2.** Effect of steady-state O₂ partial pressure and storage temperature on the rate of O₂ uptake of 'Rocha' pear slices in sealed LDPE packages at 0 °C (□), 5 °C (◆), 10 °C (○) and 15 °C (▲) fitted with a Michaelis–Menten model.

Gas equilibrium concentration inside packages was reached by 4–5 days at 15 °C, 6–8 days at 10 °C, 7–10 days at 5 °C, and 10–11 days at 0 °C. The oxygen uptake rate increased with pO_2 pkg in a manner consistent with saturation kinetics (Fig. 2).

Respiration rates of fresh-cut 'Rocha' pear are shown in Table 3. These rates, expressed as CO₂ production and obtained between 0.1 and 18 kPa O₂, were similar to the range of value reported in air and in 2 kPa O₂ for other fresh-cut pear varieties at 0 or 10 °C (Gorny et al., 2000).

The respiratory quotient (RQ) for aerobic respiration of fresh-cut 'Rocha' pear stored at various temperatures ranged between 1.2 and 1.4 (Table 3), consistent with the usage of organic acids

as major respiratory substrates (Fonseca et al., 2002). RQ values reported in the literature for whole 'Bartlett' pear varied from 0.8 at the initial climacteric to 1.4 at fully ripe stage (Biale, 1950). RQ values pertaining to intact 'Conference' pear lie between 0.7 and 0.8 (de Wild and Peppelenbos, 2001; Lammertyn et al., 2003). The reported RQ for fresh-cut 'Conference' pear above 1.7 after 1 day following processing (Soliva-Fortuny et al., 2007) are likely an analytical artefact or else a clear sign of fermentation.

Respiratory quotients and ethanol levels responded similarly to steady-state oxygen partial pressure (pO_2) (Fig. 3), and either variable may be used to determine the lower limit for aerobic respiration (Joles et al., 1994; Cameron et al., 1995). 'Fermentation threshold', the sudden increase in RQ or headspace ethanol concentration occurring when pO_2 dropped below a certain level, ranged between 0.3 and 0.9 kPa (Table 3). At elevated temperatures fermentation occurred at higher pO_2 (Beaudry et al., 1992; Lakukul et al., 1999), as observed for 5 up to 15 °C (Table 3). Fermentation threshold of whole 'Bartlett' pears varied between 0.3 and 1.7 kPa, at temperatures between 0 and 25 °C (Kader et al., 1989). Temperature has a small influence on CO₂ and O₂ diffusion in 'Conference' pear as demonstrated by small activation energies obtained by Ho et al. (2006). Since the higher demand for O₂ at elevated temperatures cannot be compensated by diffusion, fermentation occurs at higher pO_2 (Beaudry et al., 1992).

Fresh-cut produce generally need lower O₂ levels than whole fruits (Beaudry, 2000), due to absence of skin or less cuticle that could restrict gas diffusion (Burg and Burg, 1965; Schotsmans et al., 2003; Ho et al., 2006). The minimum O₂ concentration tolerated by mature intact green 'Bartlett' pear at 25 °C is 1.6 to 1.7 kPa, and by 'Passe Crassane' pear cell cultures is 1.1 to 1.3 kPa (Boersig et al., 1988). The lower oxygen limit reported for sliced pear is 0.5 kPa at 0–5 °C (Beaudry, 2000; Gorny, 2001). Our results (Table 3) indicate that fresh-cut 'Rocha' pear should be packaged with pO_2 higher than 0.8 kPa at 0 °C, 0.3 kPa at 5 °C, 0.8 kPa at 10 °C and 0.9 kPa at 15 °C to avoid fermentation. Consistent with the fermentation thresholds reported herein for 'Rocha' pear, 2.5 kPa O₂ was considered acceptable for fresh-cut 'Conference' pear hold at 4 °C, but 0 kPa O₂ induced visual tissue damage (Soliva-Fortuny et al., 2007).

Modelling the effects of oxygen and temperature on respiration rate

The respiratory parameters $R_{O_2}^{max,T}$, K_{m,O_2} and K_{mn,CO_2} were estimated individually at each temperature, and their temperature dependence subsequently studied with the models presented in Table 1.

Exponential functions gave the best fit of $R_{O_2}^{max,T}$ in both experiments using the estimates of each temperature ($R^2 > 0.98$, not shown). The temperature dependence of $R_{O_2}^{max,T}$ has been analysed according to the Arrhenius' law (Hertog et al., 1998; Jaxsens et al., 2000; Lammertyn et al., 2001; Ho et al., 2008). The rise in maximal rate of O₂ uptake with temperature was equivalent to Q_{10} values of 6.5–7.8 for data obtained in 2007 (Table 4). Q_{10} values of 3.5–4.1 obtained in 2006 (not shown) are similar to those re-

Fig. 3. Respiratory quotient (A) and headspace ethanol (B) as a function of steady-state O_2 of packaged fresh-cut 'Rocha' pear.

ported for other fresh-cut pear varieties (Gorny et al., 2000). Similarly Q_{10} of fresh-cut vegetables may range from 2.8 to 8.0 (Jacxsens et al., 2000). $E_{a,RO_2, \max, T}$ of whole 'Conference' pear ranged from 64.6 kJ mol⁻¹ (Lammertyn et al., 2001) to 80.2 kJ mol⁻¹ (Ho et al., 2006); these values are only 48% to 86% of $E_{a,RO_2, \max, T}$ found in this study for fresh-cut 'Rocha' pear (Table 4). Respiration activation energy of common intact produce range between 50 and 89 kJ mol⁻¹ (Exama et al., 1993; Fonseca et al., 2002), and can be as high as 135.9 kJ mol⁻¹ in fresh-cut vegetables (Jacxsens et al., 2000).

Hertog et al. (1998) examined the temperature dependence of apparent K_m according to the Arrhenius' law and concluded that this variable can be treated as a constant. Non-competitive models published for intact 'Conference' pear (de Wild et al., 1999; Lammertyn et al., 2001, 2003; Ho et al., 2006, 2008) did not make K_{m,O_2} and K_{m,CO_2} temperature-dependent. A constant K_{m,O_2} was obtained for ten fresh-cut vegetables (Jacxsens et al., 2000) and packaged raspberry fruit for the temperature range from 0 to 20 °C (Joles et al., 1994), while for blueberry a temperature effect on K_{m,O_2} was found (Song et al., 1992; Cameron et al., 1994). Ho et al. (2006) indicated for cortex tissue of 'Conference' pear a K_{m,O_2} of 1.16 kPa, similar to K_{m,O_2} found for fresh-cut 'Rocha' pear (Table 4). Cytochrome c oxidase, which is believed to be the rate determining enzyme in the respiratory pathways, has a high affinity to oxygen ($K_{m,O_2} = 0.10$ – 0.15 kPa) and its activity does not change during hypoxia, when $pO_2 < 0.25$ kPa (Nanos et al., 1994). However, diffusion limitations can lead to a much higher value of K_{m,O_2} in intact pear (6.2 kPa) than that in protoplasts (≈ 0.007 kPa), and even higher than in the case of mitochondrial respiration (Lammertyn et al., 2001). Differences in the reported values of K_m can be accounted for by the joint effect of respiration at the cellular level and gas diffusion through the pear tissue and skin (Lammertyn et al., 2003).

The models in which there was a temperature dependence of K_{m,O_2} were only marginally better than those where K_{m,O_2} was constant (Table 4). Michaelis–Menten and non-competitive Michaelis–Menten models were able to explain more than 93% of the total variability of the data and either model accurately predicted the experimental results (RMSE < 0.06). However, the non-competitive Michaelis–Menten model provided an estimate of K_{m,CO_2} with low accuracy (Table 4). In addition, the K_{m,CO_2} values were very high (14.3–42.0 kPa), indicating a low inhibitory effect of CO_2 on O_2 consumption (Ho et al., 2008).

To discriminate between different types of inhibition it is necessary to know O_2 consumption at two or more different CO_2 concentrations and at three O_2 concentrations (Peppelenbos et al., 1996). Since our data did not satisfy these requirements, it is not possible to assume an influence of CO_2 on O_2 consumption. However, given the accuracy of the simpler Michaelis–Menten model and the indications of minimal CO_2 influence, the use of simpler model is preferred in this case (Fig. 2).

Respiratory responses to low oxygen and implications for MAP

Respiratory behavior as a function of oxygen concentration provides a basis to deduce the potential benefits of using MAP technologies in a given commodity. Low oxygen atmospheres could be useful if a sizeable reduction in respiration rate (e.g. 50%) can be reached without the induction of fermentation (Beaudry, 2000). In cases where K_{m,O_2} is much higher than fermentation threshold, a reduction in pO_2 slows down metabolic activities without an increase in the RQ and fermentative compounds. The range of pO_2 between K_{m,O_2} and fermentation threshold is termed 'safe working atmosphere' (Beaudry, 2000). On the other hand, if K_{m,O_2} is lower than the fermentation threshold any attempt to reduce O_2 could

Table 5

Michaelis–Menten model (MM) respiratory parameters for fresh-cut 'Rocha' pear stored at various temperatures.

Temperature (°C)	Fermentation threshold (kPa)	K_{m,O_2} (kPa)	Safe working atmosphere (kPa)
0	0.75	0.07	No
5	0.25	0.24	0.01
10	0.75	0.79	0.04
15	0.88	2.59	1.71

lead to fermentation. Fresh-cut 'Rocha' pear had a very narrow (0.01–1.71 kPa) safe working atmosphere at the temperatures tested (Table 5). Low oxygen atmospheres did not offer any reduction in metabolic activity without the danger of inducing anaerobiosis, especially between 0 and 10 °C, temperatures normally found during storage and marketing. These data are consistent to the findings compiled by Gorny (2001), who also reported a poor efficiency of modified atmosphere for fresh-cut pear at 0–5 °C.

In conclusion, respiration of fresh-cut 'Rocha' pear as function of oxygen concentration and temperature can be accurately predicted through Michaelis–Menten kinetics without inhibition by CO₂. These data provide a foundation whereby sealed packages can be designed to avoid fermentative metabolism of fresh-cut 'Rocha' pear. However, given the respiratory kinetics of fresh-cut 'Rocha' pear, it is anticipated that low oxygen packaging is of little or no benefit in slowing down metabolism in this convenience produce.

Acknowledgements

This work was supported by Fundação para a Ciência e a Tecnologia (Portugal) via project grant PTDC/AGR-ALI/66144/2006 and the PhD grant SFRH/BD/22628/2005 to M.H. Gomes. The authors are grateful to Filipe Silva (CPF, Bombarral, Portugal) for providing pears.

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