Modelling the O$_2$ and CO$_2$ Permeance of Conference Pear

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Abstract

Gas exchange is governed by the combined action of oxygen consumption and carbon dioxide production, and the transport of these gasses by diffusion. The diffusion component is formally neglected and implicitly incorporated in respiration and fermentation parameters of the current Michealis-Menten-based respiration models. The aim was to extend existing modelling approaches by including a diffusion controlled component to quantify the permeance of O$_2$ and CO$_2$. Permeances were estimated using a new gas exchange model which assumes that diffusion can be described by Fick's first Law. Fick's first Law can be applied when only one barrier is present. This was shown to be the case for O$_2$ by O$_2$-electrode measurements. The model estimated the permeance per batch on the basis of external gas exchange measurements, internal and external gas conditions, weight and surface per pear. Traditionally, permeances are not estimated, but calculated directly by the Neon method, using Graham's Law. Permeances estimated using the new model were lower for O$_2$ and CO$_2$ than those found with the Neon method. The lower permeances may be explained by the assumption that the Neon method only assesses the permeance of the skin, while the O$_2$ and CO$_2$ permeances established by using the new model represent all barriers between mitochondria and the external atmosphere. The smaller CO$_2$ permeance found using the new model might be explained by the relatively high pH of the cytosol.

INTRODUCTION

Gas exchange of fruits is governed by the combined action of oxygen consumption (respiration) and carbon dioxide production (respiration and fermentation) on one hand, and the transport of these gases from and to the inside of the cell by diffusion on the other hand. Only scarce information is available on internal gas concentrations in fruits in literature. Considering only external gas conditions in modelling respiration and fermentation of fruits and vegetables implies that the diffusion part of the process is formally neglected and that the diffusion-oriented effects are implicitly incorporated in model parameters. Several barriers exist in fruits for the transport of gases from outside the product into the cells and the opposite direction (e.g. the skin, flesh, cell wall, membranes and cytoplasm), each exerting its own effect on the overall gas exchange rate. The aim of this article is twofold. Firstly, existing modelling approaches on fruit respiration are extended by including a diffusion controlled component resulting in description of the permeance of the pear for O$_2$ and CO$_2$. Secondly, these model-based estimation of the permeances for O$_2$ and CO$_2$ are compared to the traditionally used Neon method (Schotsmans et al., 2002).

MATERIAL AND METHODS

The Gas Exchange/Diffusion Model

Nowadays' general models describing gas exchange of fruits and vegetables are based on external gas conditions, and use a Michaelis-Menten approach. Modelling gas exchange with internal gas conditions may be accomplished by using the existing gas exchange model and exchanging the external for the internal gas conditions. However, the effect of diffusion resistance towards gases of the skin of the fruit should be considered in the gas exchange model when significant differences between internal and external gas conditions are encountered. Under the assumption that there is only one barrier for transport of gasses, namely the skin of the pear, diffusion can be described with Fick's first law (Burg and Burg, 1965). This Law can be rewritten to the gas exchange rate (V_d in mol $kg^{-1}s^{-1}$) due to the diffusion process by converting the mass flux to the diffusion rate per kg pear (M_p) (Eq. 1)

$$V_d = \frac{P \cdot (C_e - C_i) \cdot A}{M_p} \qquad (1)$$

where A represents the surface of the pear (m^2), P the permeance (mol s^{-1} m^{-2} Pa^{-1}), C_i the internal- and C_e the external concentration.

The measured external gas exchange rates (mol $kg^{-1}s^{-1}$) may be described as being the combined result of internal respiration and fermentation with the diffusion exchange rate (Eq. 2-3).

$$V_{O_2}(extern) = V_{O_2}(intern) + \frac{P_{O_2} \cdot (O_{2e} - O_{2i}) \cdot A}{M_p} \qquad (2)$$

$$V_{CO_2}(extern) = V_{co_2}(intern) + \frac{P_{CO_2} \cdot (CO_{2e} - CO_{2i}) \cdot A}{M_p} \qquad (3)$$

where CO_{2i} and O_{2i} are the internal gas partial pressures (Pa), and where P_{O_2} and P_{CO_2} (mol s^{-1} m^{-2} Pa^{-1}) are the permeances for O_2 and CO_2, respectively. The general Michaelis-Menten model for gas exchange due to external respiration and fermentation (Hertog et al., 1998; Hertog et al., 1999) can then be used to describe the internal gas exchange ($VO_2(intern)$ and $VCO_2(intern)$). This enables the description of internal gas exchange in respiration and fermentation parameters without the influence of the diffusion process. There was, however, one adaptation to the existing Michaelis-Menten approach for the internal gas exchange: the RQ_{ox} parameter, the respiration quotient for oxidative respiration, was set to 1.

Statistical Analysis

Experimental data on external gas exchange rates were analysed statistically using the non-linear regression routine of Genstat 5 (release 3.2, Lawes Agricultural Trust, Rothamsted Experimental Station, UK). The set of gas exchange data was analysed using the model formulation of Eqs. 2-3. For the analysis the gas exchange data were analysed treating measured internal and external gas partial pressure and surface measurements simultaneously as independent variables, and O_2 consumption and CO_2 production rates as dependent variables (multi-response, multi-variate, non-linear regression analysis).

Plant Material

Pears (Pyrus communis L. cv. Conference) were harvested in Randwijk, The Netherlands, on 18 September 2001, the optimal picking date for long-term Controlled

Atmosphere (CA) storage, established by using the Streif index. Until the experiments started (about one month after harvest), fruits were stored in crates at 0°C wrapped up in plastic to avoid water loss. Weight per pear was determined prior to the start of the experiments.

External Gas Exchange Measurements

Pears were subjected to 0, 0.5, 1, 2.5, 6 and 21 kPa O_2 combined with 0, 5 or 10 kPa CO_2 (20 °C, 95% RH). Individual fruits were enclosed in 2-L cuvettes, which were connected to a flow-through system as described in Veltman et al. (1999). Every condition was applied in duplicate or in eight-fold (in case of 0-5, 6-5 and 21-5; O_2-CO_2). Gas exchange rates were determined after the pears had been subjected to these conditions for two days using the method of De Wild et al. (1999). Hereafter, the cuvettes were reconnected to the flow-through system for at least two hours to remove accumulated CO_2 before internal gas was extracted from the same, individual pears.

Internal Gas Measurements

For determination of CO_2 and O_2 partial pressures in the intercellular spaces of pear tissue the method of Saltveit (1982) was used. Fig. 1 shows the experimental set-up. After 1 min at 0.35 atm the maximum amount of gas was extracted from a pear.

Surface Measurements by Computer Imaging

Under the assumption that a pear is rotationally symmetrical and mathematically convex, a pear can be approximated by a finite series of cylinders with an equal, small thickness and a varying radius. The height of every cylinder was one pixel, and the diameter of it was determined in pixels. Next, for each cylinder from stem to flower the outer surface was determined, and the outer surfaces of all cylinders were summed, obtaining the estimation of the fruit's surface. To obtain the surface area in m^2, calibration took place using pictures with known dimensions.

O_2-Electrode Measurements

O_2 profiles in the aqueous phase of the pear were determined as described in Smid et al. (1993) with an O_2-electrode, which was mounted to a motor-driven micro-manipulator (Märzhäuser MM33), and stepwise, perpendicularly pierced into the belly of a pear (at the largest diameter of the fruit). O_2 profiles were determined in a cooled room, at 5 °C. The electrode was calibrated using nitrogen and air saturated tap water. O_2-saturated tap water (5 °C) contains 398.5 µM O_2. (Microscal Measurements, The Hague, The Netherlands).

Neon Measurements

The method described by Peppelenbos and Jeksrud (1998) to determine skin resistance for O_2 and CO_2 by using Ne (7 ml per cuvette, 10 h incubation) was applied. Pears were, after loading them with Ne, quickly transferred to an empty, clean cuvette. Before the fruit was transferred from the first to the second cuvette the Ne partial pressure in the first cuvette was determined. After transfer to the second cuvette the Ne efflux was monitored (determined after 0, 80, 160 and 240 s) by GC.

RESULTS AND DISCUSSION

O_2 in the Aqueous Phase of a Pear

Although O_2 concentrations in the aqueous phase of the pear were not included in the model, the absence of an O_2 profile in the aqueous phase would also exclude a gradient in the intercellular spaces, which makes the choice of using Fick's first law plausible. To check this assumption, O_2 concentrations were determined using an O_2 electrode that was pierced into the fruit flesh of a pear. The small needle-like tip crushed the cells and O_2 concentrations, on a cell level, were averaged. No gradient in the aqueous

phase could be observed for 3 pears at 5 °C and 3 pears at 20 °C in ambient air. The O_2 level in the pear cortex tissue was about 50% of the maximal O_2 solubility (in pure water), i.e. around 200 μM. The absence of O_2 gradients indicates that the assumption of free diffusion may be warranted. CO_2 concentrations in the aqueous phase of the fruit were not determined because no reliable CO_2 electrode is available.

Gas Extraction

The internal O_2 and CO_2 partial pressures in pears stored under various conditions were determined. Fig. 2 shows the O_{2e} versus the O_{2i} and CO_{2i} partial pressures. The general trend is that O_{2i} was always lower than O_{2e}, and that for pears stored at increasing O_{2e} the difference between internal and external partial pressure increased. The same trend was found by Dadzie et al. (1996) for two apple cultivars. A difference with these experiments with apples, however, is that some pears showed almost no difference between internal and external gas conditions over a range of O_2 partial pressures. For pears preserved in the presence (5 and 10 kPa) or in the absence of CO_2, the accumulation of CO_2 internally was considerable, and the CO_{2i} partial pressure is increased with an offset of about 2%, independently of CO_{2e}.

Application of the Gas Exchange/Diffusion Model

In the gas exchange models used to describe external gas exchange the RQ_{ox} parameter, the respiration quotient for oxidative respiration, is assumed to be a constant factor independent of partial pressures describing the. In literature it is shown that this value is often estimated smaller than, but close to 1 (Hertog et al. 1998, Peppelenbos, 1996). This implies that the CO_2 production is partly originating from glucose and partly from other sources resulting in less CO_2 production. It is known that in pome fruits next to glucose, malate (Metlitskii et al. 1983) is important as substrate for CO_2 production. Malate will generate more CO_2 per O_2 compared to glucose (1.33 instead of 1). So, it seems strange that the RQ_{ox} parameter allways is estimated smaller than 1. We propose that the RQ_{ox} parameter has, up till now, been used to describe the influence of diffusion process on the external gas exchange. As we have formulated the contribution of the diffusion process to the externally measured gas exchange (Eq. 2-3), there is no need for the RQ_{ox} parameter.

Table 1 shows the permeances for O_2 and CO_2, leaving out all the other respiration and fermentation parameters. Respiration and fermentation parameters were comparable with those found by Hertog et al. (1998) for apple and chicory. All parameters were obtained in one optimisation using multi-response multivariate non-linear regression analysis.

Comparison with the Neon Based Permeance Measurements

Resistance to Ne was converted to permeance for O_2 and CO_2 using Graham's law, following the method described in Peppelenbos and Jeksrud (1998) (Table 1). Although literature on permeance measurements of pears is scarce, the measurements reported by Amarante et al. (2001) using O_2 instead of Ne, are within the range found for this batch of Conference pears. Amarante et al. (2001) reported O_2 and CO_2 permeance measurements for Bartlett (600, 580 pmol s^{-1} m^{-2} Pa^{-1}), Packham's Triumph (250, 150 pmol s^{-1} m^{-2} Pa^{-1}) and Doyenne du Comice (350, 350 pmol s^{-1} m^{-2} Pa^{-1}) pears.

PO_2 and PCO_2 for the batch of Conference pears obtained from the analysis (Table 1) are considerable different from Ne-derived O_2 and CO_2 permeances. This difference may be explained partly by the inert nature of Ne. Ne measurements may be regarded as indicator of only the permeance due to physical properties of the pear skin. The O_2 and CO_2 permeances estimated with the model may be regarded as the permeances due to all the barriers between mitochondria, the place where respiration occurs, and the external atmosphere (Rajapakse, 1990). This means O_2 and CO_2 have to pass more barriers than Ne. The analysis estimated a lower permeance for CO_2 than for O_2. When determining the CO_2 permeance, the solubility of CO_2 in the cytoplasm must be considered. The pH of the

464

cytosol of pears is normally between 7 and 7.5 (Nanos and Kader, 1993). At this pH the majority of the CO_2, (80-90%, Woldendorp and Zijlstra, 1985) is present in the bicarbonate form. Bicarbonate not only diffuses much slower because it is a bigger molecule, it is also a charged molecule which makes it difficult to pass membranes.

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Tables

Table 1. Permeances for O_2 and CO_2

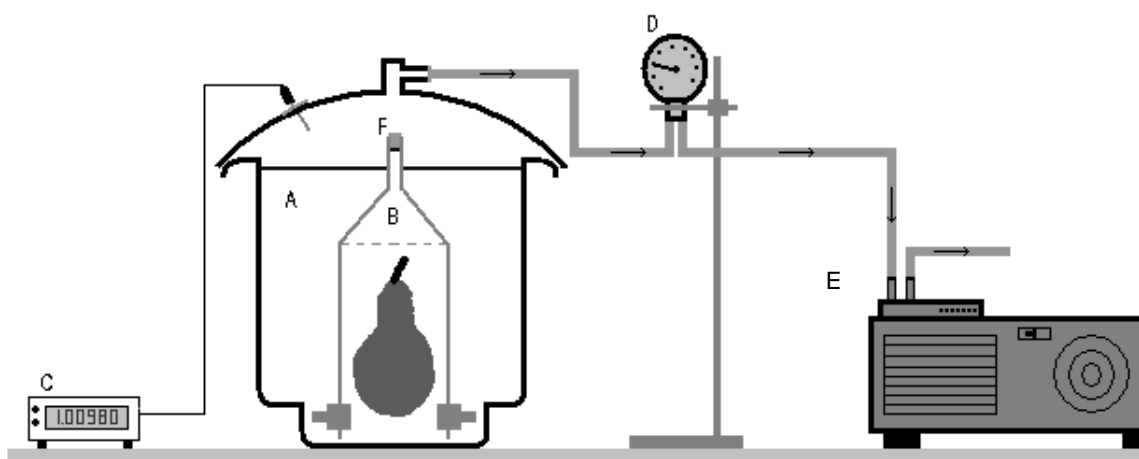| Parameter	Model		Neon	
	estimate	s.e.	average	s.e.
PO_2 (pmol s^{-1} m^{-2} Pa^{-1})	164.2	20.1	722	30
PCO_2 (pmol s^{-1} m^{-2} Pa^{-1})	85.7	17.3	615	25
R^2_{adj} (%)	90.5			
N	102		16	

Figures

Fig. 1. Set-up for gas extraction. A pump (E) was used to vacumise desiccator (A). The pressure was lowered to 0.35 atm, which was checked with an electronic manometer (Druck PDI 265, (C)). Gas from the fruit was collected in funnel that was placed upside-down (B). After the pressure in the desiccator was normalised the collected gas mixture in the bell jar (B) was investigated by stinging a needle in septum (F), which was directly connected to a GC.
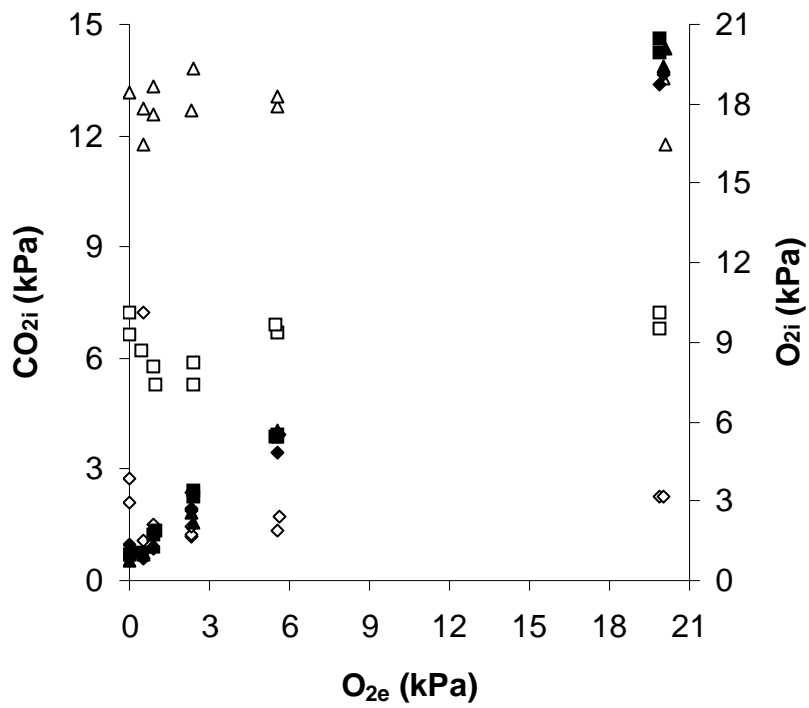
Fig. 2. Externally applied O_2 partial pressure (O_{2e}) versus internal CO_2 partial pressure (CO_{2i}) for externally applied CO_2 partial pressures of 0 (◊), 5 (□) and 10 (Δ) kPa on the first y-axis. The second y-axis shows the accompanying internal O_2 partial pressures (O_{2i}) for externally applied CO_2 partial pressures of 0 (♦), 5 (■) and 10 (▲) kPa.