ACCEPTED VERSION

Dynamic change in respiration rate of ‘Conference’ pear in DCA storage: Experimental evidence and modeling

H.M. Phan[1], M.L.A.T.M. Hertog[1], B. Verlinden[2], P. Verboven[1], B. Nicolai[1,2,a]

1 KU Leuven, division MebioS, Postharvest Group, Leuven, Belgium
2 Flanders Centre of Postharvest Technology, Leuven, Belgium

Abstract

Controlling respiration is a main target in (dynamic) controlled atmosphere storage, (D)CA, for climacteric fruit. Lowering respiration during (D)CA condition results in the slowing down of physiological metabolism, which retains fruit quality better after a long-term storage period. The Michaelis-Menten enzymatic model is commonly used to describe fruit respiration. However, even in (dynamic) controlled atmosphere storage, ripening still happens, so fruit respiration changes over time. Unfortunately, the Michaelis-Menten-based respiration model cannot capture this dynamic change in respiration.

In this study, an experiment was conducted to measure the respiration rates of ‘Conference’ pears (*Pyrus communis*) during shelf life (18°C in regular air) at harvest and after storage months under various (D)CA conditions at -1°C. Respiration rates were compared across three conditions: pears at harvest, pears after 4 months in DCA storage (in a pilot-scale setting), and pears after 5 months in a mixed storage condition (2 months in CA followed by 3 months in DCA, in an industrial setting). The results showed that once pears were taken out from the storage conditions, pears in the mixed storage condition exhibited the highest respiration rate, while those at harvest had the lowest. Furthermore, during the shelf life period, the respiration rate increased over time. Then, it levelled off and slightly decreased between 8 and 12 days, depending on different storage conditions.

Based on the experimental data, a dynamic respiration model was introduced to capture the change in respiration rate during (D)CA storage conditions. The proposed model was modified from the Michaelis-Menten approach by adding a dynamic variable to represent the synthesis of respiration enzymes. The proposed model demonstrated a good match with experimental data in both CA and DCA conditions, which offers a good prediction of pear respiration rate to improve storage strategies.

Keywords: enzyme kinetics, respiration metabolism, dynamic model, DCA

INTRODUCTION

Respiration is one of the most widely used criteria in postharvest research. Furthermore, controlling respiration is also a target in (dynamic) controlled atmosphere storage. By lowering O_2 level during storage, the respiration is reduced, leading to the slowing down of metabolic pathways inside fruit. As a result, the quality is maintained for a long-term storage period (Mditshwa et al., 2018). Respiration is a complex process including three steps: the glycolysis pathway, the tricarboxylic acid cycle (TCA), and electron transport chains in mitochondria (mETC), each of which involves many enzymatic reactions (Boeckx et al., 2019). Therefore, modeling a complete respiration process is not trivial. By simplifying a complex set of enzymatic reactions in respiration with a single enzyme, the Michaelis-Menten approach is widely used to model respiration in fruit (Hertog et al., 1998). The Michaelis-

[a] E-mail: bart.nicolai@kuleuven.be

Menten approach has been applied to describe the fruit respiration in various studies, such as, tomato (Xiao et al., 2024), pears (Ho et al., 2008), and avocado (Castellanos et al., 2017).

Despite being considered a standard approach to modeling fruit respiration, the Michaelis-Menten-based respiration model overlooks its time dependency in respiration. The model regulates respiration based on O_2 level and temperature (when the Arrhenius law is applied). However, in CA storage, where O_2 and temperature are generally maintained at constant levels, the respiration, as predicted by the Michaelis-Menten model, will be at a fixed rate. However, since the ripening process continues during storage, the respiration rate of the fruit may change over time, as observed in Saquet and Streif, (2017). Therefore, to have better control of fruit physiology and quality, it is vital to have a model that can describe the dynamics of respiration during and after storage periods.

This paper demonstrates that respiration in dynamic controlled atmosphere (DCA) storage can still change compared to the harvest stage. To capture this dynamic of respiration, we propose a simple concept for modeling respiration in 'Conference' pears, Belgium's most profitable fruit. The core idea is to incorporate an enzyme kinetics-based approach, suggesting that respiration is influenced by the production and degradation of enzymes.

MATERIAL AND METHODS

Harvest and storage experiment

'Conference' pears (Pyrus communis) were harvested on September 5, 2023, from a commercial orchard in Bierbeek, Belgium (COBB) and transported to VCBT. The fruit was randomized, precooled at -1 °C (RA) for 21 days, and divided into three groups. The first group was stored at 18 °C in regular air (RA) for 14 days, with respiration and ethylene emission measured every two days using 10 fresh pears. The second group was stored at VCBT's pilot plant. The fruit stored at VCBT was separated into two main storage types: CA conditions (during 6-8 months) with various combinations of temperature (-1 and 4 °C) and oxygen (1-3-5 kPa O_2), and DCA (during 4 months, DCA4M). Finally, the other was stored in an industrial storage (COBB) at -1 °C with 2 months in 3 kPa O_2 and 3 months in DCA. After storage, both were held at 18 °C in regular air (RA) for 12–18 hours before starting the respiration measurement over 2 weeks, with 7 pears per sampling day.

Michaelis-Menten experiment

This experiment assessed respiration rates across various O_2 and temperature conditions to calibrate the static Michaelis-Menten model. It involved pears at harvest and those stored in DCA4M (February 2024) and COBB (March 2024). Since the shelf-life experiment was conducted first, pears taken out from DCA4M and COBB were maintained at -1 °C in 3 kPa O_2 to wait for the 2-week shelf-life measurement to finish. For measurement, individual pears were placed in glass jars. The jars were flushed with predetermined O_2 levels at specific temperatures for 36–40 hours for fruit adaptation. Table 1 shows the condition set-up used for the Michaelis-Menten experiment.

Table 1. A condition set (●) combined between temperature (°C) and oxygen (kPa) for the Michaelis-Menten experiment

Condition	0 kPa	2 kPa	5 kPa	RA
-1 °C	●	-	-	●
4 °C	●	-	-	●
12 °C	●	-	-	●
18 °C	●	●	●	●

Respiration measurement

Respiration rates were measured using a standard protocol (Ho et al., 2018). Each pear was weighed and placed in a 1.7 L airtight jar at the specified temperature. The initial O_2 and CO_2 levels were recorded with a gas analyzer (CheckMate II, PBI, Dansensor, Denmark). After a set period, a second measurement was taken. Gas concentrations were calculated using the ideal gas law, taking into account temperature, jar volume, and pressure.

Concept development of a dynamic respiration model

The dynamic model of fruit respiration started from modifying the typical Michaelis-Menten form (Hertog et al. 1998) by adding an enzyme kinetic scheme to describe a production of enzyme during ripening. To describe the change in the respiration enzyme during ripening, a scheme of enzyme biosynthesis is proposed, as outlined in equations (R1-3). This scheme mainly focuses on the dynamics of oxidative respiration, assuming a constant fermentation rate. Here, we assume that there is a limited resource of precursor (Pre) in the presence of O_2, which will be converted to protein (Pro). The protein is then transformed into the enzyme [E], while the enzyme can be degraded into amino acids over time.

$$\text{Pre} + nO_2 \rightarrow \text{Pro} + nO_2 \tag{R1}$$

$$\text{Pro} \rightarrow \text{E} \tag{R2}$$

$$\text{E} \rightarrow \text{amino acids} \tag{R3}$$

Based on this scheme, a set of differential equations for enzyme kinetics was derived. The Arrhenius law was used to describe the dependency of the rate constant and maximum respiration rates. A complete model of dynamic respiration was calibrated in two steps. First, the parameters in the typical Michaelis-Menten part of the dynamic model were estimated using the measured data from the Michaelis-Menten experiment. The rate constants in the reaction scheme (R1-3) were subsequently estimated by the shelf-life measurements after certain storage periods in VCBT. For validation, the fruit stored in COBB was used. The model calibration and validation were implemented using OptiPa, a MATLAB-based software developed within our group (Hertog et al., 2007).

RESULTS AND DISCUSSION

Respiration changes from the Michaelis-Menten viewpoint

The Michaelis-Menten model for respiration, was used to calibrate the Michaelis-Menten experiment at harvest, DCA4M, and COBB. Each dataset was used to estimate $R_{max,O2,ref}$ (maximum O_2 consumption rate at a reference temperature of 283 K) separately, while the other parameters, considered as generic parameters, were estimated from all datasets. The estimated $R_{max,O2,ref}$ values for harvest, DCA4M and COBB are shown in Figure 1. It can be seen that $R_{max,O2,ref}$ (at harvest) is 73.12 ± 2.79 µmol $m^{-3}s^{-1}$, which is lower than those at DCA4M (91.25 ± 3.23 µmol $m^{-3}s^{-1}$) and at COBB (120.30 ± 4.01 µmol $m^{-3}s^{-1}$). Therefore, there is an increase in respiration in DCA after 4 months, from harvest. The $R_{max,O2,ref}$ of COBB is the highest since these pears were stored at -1 °C in 3 kPa O_2 for 2 months before switching to DCA. It demonstrates that DCA inhibits respiration change better than CA storage.

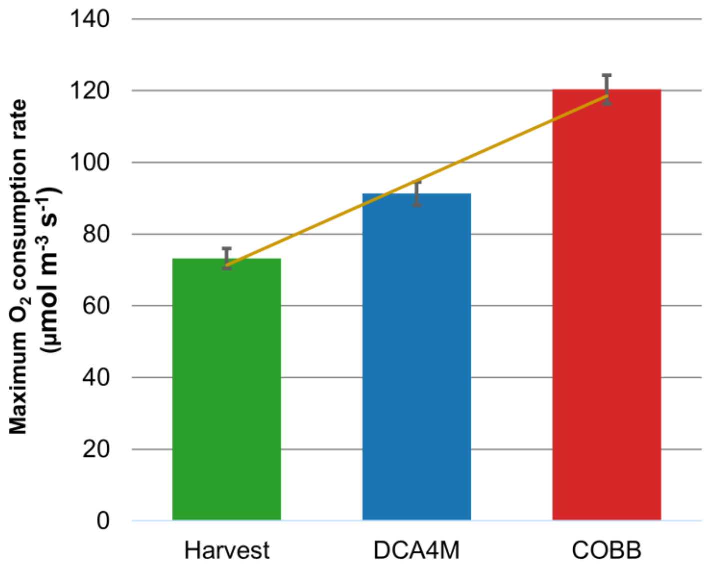

Figure 1. Maximum O_2 consumption rate ($R_{max,O2,ref}$, μmol m^{-3}s^{-1}) at a reference temperature of 283 K estimated from Michaelis-Menten experiment for different periods: Harvest, DCA at -1 °C after 4-month storage (DCA4M), and industrial storage at -1 °C in 3 kPa O_2 for 2 months, then switching to DCA for 3 months (COBB). The error bar represents the standard error from the parameter estimation of the typical Michaelis-Menten respiration model.

The dynamic model of respiration

The dynamic model of pear respiration was calibrated using shelf-life measurements from different datasets: at harvest, after CA storage periods in VCBT (unpublished data), and DCA4M. The model was then validated using shelf-life measurements after industry storage (COBB). For the calibration dataset, the model can explain up to 70 % of the variance, while the R^2_{adj} in the validation dataset is around 0.5. Since the O_2 consumption rate and CO_2 production rate in shelf-life seem close to each other, Figure 2 only shows the simulation and measured data of O_2 consumption rate for the calibration set (Harvest and DCA4M) and the validation set (COBB). The simulation acceptably fits with the measured data. However, the model appears to overpredict the respiration rate from day 8 for DCA4M (the calibration set) and from day 4 for COBB (the validation set), as the respiration rate seems to level off and then decrease slightly in shelf-life. Still, the mean absolute percent error is very low, at approximately 13 %. Furthermore, it can be observed that the respiration dynamics change differently across various shelf-life periods. At harvest, the respiration rate appears to have a longer lag phase compared to that after DCA4M and COBB. However, the model still captures this dynamic change.

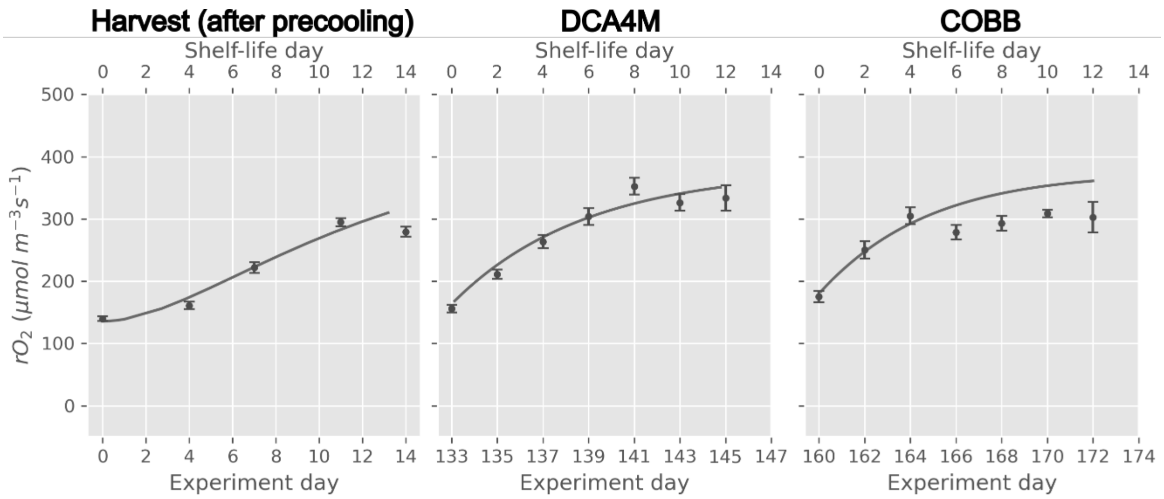

Figure 2. The simulation (solid line) by the dynamic respiration model and the measured data (scatter point) of the O_2 consumption rate in the shelf-life condition (18 °C and regular air) at Harvest, DCA at -1 °C after 4-month storage (DCA4M), and industrial storage at -1 °C in 3 kPa O_2 for 2 months, then switching to DCA for 3 months (COBB). The error bar represents the standard error of measured data, based on 10 fruit replicates for Harvest and 7 for either DCA4M or COBB. The primary x-axis stands for the experiment day (absolute day) counting from harvest, while the secondary x-axis stands for the shelf-life day (relative day) for the shelf-life experiment.

CONCLUSIONS

This paper demonstrates that respiration is dynamically changing even during DCA storage, which results in changes in respiration rates once the fruit is commercialized under the shelf-life condition. This highlights the need for a predictive model of respiration dynamics to enhance decision-making for postharvest managers. A concept of respiration dynamics is proposed in this paper. The dynamic model of respiration derived from this concept was calibrated using measured data on respiration during shelf life after the fruit was removed from various (D)CA conditions after specific storage periods, and then validated against measured data on respiration during shelf life after an industrial storage period. The model shows a good match with the measured data, with a mean absolute percent error of only 13%. Consequently, the model is promising for improving storage management in (D)CA storage.

ACKNOWLEDGEMENTS

This research received funding from the European Union's Horizon 2020 programme under grant agreement No. 101036588, European food chain supply to reduce GHG emissions by 2050 (ENOUGH). All opinions, conclusions, and interpretations presented are solely those of the authors and do not reflect those of the European Union. The EU and other granting authorities bear no responsibility for the content provided.

Literature cited

Boeckx, J., Pols, S., Hertog, M.L.A.T.M., Nicolaï, B.M., 2019. Regulation of the Central Carbon Metabolism in Apple Fruit Exposed to Postharvest Low-Oxygen Stress. Front. Plant Sci. 10. https://doi.org/10.3389/fpls.2019.01384

Castellanos, D.A., Mendoza, R., Gavara, R., Herrera, A.O., 2017. Respiration and ethylene generation modeling of "Hass" avocado and feijoa fruits and application in modified atmosphere packaging. International Journal of Food Properties 20, 333–349. https://doi.org/10.1080/10942912.2016.1160921

Hertog, M.L.A.T.M., Peppelenbos, H.W., Evelo, R.G., Tijskens, L.M.M., 1998. A dynamic and generic model of gas exchange of respiring produce: the effects of oxygen, carbon dioxide and temperature. Postharvest Biology and Technology 14, 335–349.

Hertog, M.L.A.T.M., Verlinden, B.E., Lammertyn, J., Nicolaï, B.M., 2007. OptiPa, an essential primer to develop models in the postharvest area. Computers and Electronics in Agriculture 57, 99–106. https://doi.org/10.1016/j.compag.2007.02.001

Ho, Q.T., Hertog, M.L.A.T.M., Verboven, P., Ambaw, A., Rogge, S., Verlinden, B.E., Nicolaï, B.M., 2018. Down-regulation of respiration in pear fruit depends on temperature. Journal of Experimental Botany 69, 2049–2060. https://doi.org/10.1093/JXB/ERY031

Ho, Q.T., Verboven, P., Verlinden, B.E., Lammertyn, J., Vandewalle, S., Nicolaï, B.M., 2008. A continuum model for metabolic gas exchange in pear fruit. PLOS Computational Biology 4, e1000023. https://doi.org/10.1371/JOURNAL.PCBI.1000023

Mditshwa, A., Fawole, O.A., Opara, U.L., 2018. Recent developments on dynamic controlled atmosphere storage of apples—A review. Food Packaging and Shelf Life 16, 59–68. https://doi.org/10.1016/j.fpsl.2018.01.011

Saquet, A.A., Streif, J., 2017. Respiration rate and ethylene metabolism of ‘jonagold’ apple and ‘conference’ pear under regular air and controlled atmosphere. Bragantia 76, 335–344. https://doi.org/10.1590/1678-4499.189

Xiao, H., Verboven, P., Tong, S., Pedersen, O., Nicolaï, B., 2024. Hypoxia in tomato (Solanum lycopersicum) fruit during ripening: Biophysical elucidation by a 3D reaction–diffusion model. Plant Physiology 195, 1893–1905. https://doi.org/10.1093/plphys/kiae174