Modelling Respiration of Strawberry (cv. ‘Elsanta’) as a Function of Temperature, Carbon Dioxide, Low and Superatmospheric Oxygen Concentrations

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Keywords: modified atmosphere, respiration, Michaëlis-Menten kinetics, Arrhenius equation, oxygen consumption, carbon dioxide production

Abstract
The effect of oxygen (low and superatmospheric (> 20 kPa) concentrations), carbon dioxide and temperature on the respiration rate of ‘Elsanta’ strawberries, was evaluated. The strawberries were stored in glass jars at three temperatures (2°C, 7°C and 14°C). The jars were flushed with humidified gas mixtures. Two carbon dioxide levels (0 and 20 kPa) were combined with 8 levels of oxygen (0, 2, 5, 20, 50, 60, 80 and 100 kPa). Temperature, carbon dioxide and low oxygen concentrations significantly influenced the respiration rate. The respiration rates at superatmospheric oxygen concentrations (> 20 kPa) were not significantly different from those at 20 kPa O₂.

A model based on Michaëlis-Menten kinetics to describe the respiration rates was constructed, which permits lower respiration rates at lower oxygen concentrations. The influence of temperature was described using an Arrhenius equation, and carbon dioxide was considered as a non-competitive inhibitor of the respiration.

INTRODUCTION
Storing strawberries at superatmospheric oxygen concentrations can control decay caused by Botrytis cinerea and improve colour retention (Wszelaki and Mitcham, 2000). Knowledge of respiration rates at different gas atmospheres is required to determine the gas compositions in modified atmosphere packages. Respiration at oxygen concentrations up to 20 kPa in combination with different levels of CO₂ has been studied for several strawberry cultivars in the past (Li and Kader, 1989; Talasila et al., 1992; Chambroy et al., 1993; Renault et al., 1994; Hertog et al., 1999), but no information on respiration at superatmospheric oxygen concentrations is available. In the current study, O₂ consumption and CO₂ production rates of ‘Elsanta’ strawberries were examined at different temperatures at low and superatmospheric oxygen concentrations with or without carbon dioxide. A Michaëlis-Menten equation was used to model the respiration rates.

MATERIALS AND METHODS
Experimental setup
Strawberries of cultivar ‘Elsanta’ were purchased from a Belgian fruit auction and placed in glass jars (about 40 fruits per jar). The jars were placed in cool rooms at 2, 7 or 14°C and flushed with humidified gas mixtures. Two separate experiments were carried out. In the first experiment, strawberries were stored at 3 different temperatures (2, 7 and 14°C), combined with the following 6 gas mixtures (kPa O₂ : kPa CO₂ : kPa N₂) : 20 : 0 : 80 ; 60 : 0 : 40 ; 100 : 0 : 0 ; 20 : 20 : 60 ; 50 : 20 : 30 ; 80 : 20 : 0. Per treatment
two jars were measured. In the second experiment, 2 temperatures (2 and 7°C) were combined with the following 8 gas mixtures (kPa O₂ : kPa CO₂ : kPa N₂): (0 : 0 : 100); (2 : 0 : 98); (5 : 0 : 95); (20 : 0 : 80); (60 : 0 : 40); (0 : 20 : 80); (20 : 20 : 60); (50 : 20 : 30). After flushing the jars with the gas mixtures for one night, the jars were closed and the headspace composition (O₂, CO₂ and N₂) was measured with a micro gas chromatograph (Chrompack CP 2002, The Netherlands). After 2 to 8 hours, the jars were measured a second time. The concentration changes were used to calculate the O₂ consumption and CO₂ production rates. The jars were flushed again overnight and the measurements were repeated the two following days.

ANOVA

First, we examined the effects of variables other than temperature and gas composition. Because we used different batches of strawberries for the two experiments, the batch effect was examined. This was done using the GLM procedure of the software package SAS/STAT® version 8.2 (SAS Institute Inc., Cary, North Carolina, United States). Consecutively, the same procedure was followed to examine the effect of temperature, oxygen and carbon dioxide concentration on both oxygen consumption and carbon dioxide production rate.

Modelling

To describe the respiration rates of the strawberries at different temperatures and gas conditions, a model based on Michaëlis-Menten kinetics was built. Although this description is a simplification, based on one enzymatic reaction, the relation fits well with experimental respiration data of a large range of vegetable and fruit products (Peppelenbos and van ‘t Leven, 1996; Fonseca et al., 2002). The O₂ consumption rate is given by equation 1.

\[
R_{O_2} = \frac{V_{\text{max O}_2}}{K_m + O_2}
\]  

(1)

\( R_{O_2} \) is the O₂ consumption rate, \( K_m \) is the Michaëlis-Menten constant for O₂, \( V_{\text{max}} \) is the maximum oxygen consumption rate.

The CO₂ production rate is given by equation 2.

\[
R_{CO_2} = r_{\text{qox}} R_{O_2} + \left( \frac{V_{mCO_2}}{1 + O_2 / K_{mO_2,f}} \right)
\]  

(2)

\( R_{CO_2} \) is the CO₂ production rate, \( r_{\text{qox}} \) is the respiratory quotient at high oxygen concentrations, \( V_{mCO_2} \) is the maximum specific respiration rate for CO₂, \( K_{mO_2,f} \) is a parameter describing the inhibition on the fermentative metabolism by O₂.

To describe the temperature dependence of \( V_{m,O_2} \) and \( V_{m,CO_2} \), the Arrhenius equation was used (equation 3).

\[
V_{m,i} = V_{m,i,\text{ref}} \exp \left( -\frac{E_a V_{m,i}}{R_{\text{gas}}} \left( \frac{1}{T} - \frac{1}{T_{\text{ref}}} \right) \right), \text{ with } i=O_2 \text{ or } CO_2
\]  

(3)

Where \( V_{m,i} \) is the maximum specific respiration rate of oxygen or carbon dioxide, \( V_{m,i,\text{ref}} \) is the reference maximum specific respiration rate of oxygen or carbon dioxide, \( T_{\text{ref}} \) is the
reference temperature which was set to 283 K (10°C), \( T \) is the temperature, \( E_{act} \) is the activation energy, \( R_{gas} \) is the ideal gas constant.

To model the inhibitive effect of carbon dioxide on the respiration rate, four types of inhibition were evaluated: competitive inhibition (equation 4), uncompetitive inhibition (equation 5), mixed inhibition (equation 6) and non-competitive inhibition (equation 7).

\[
R_{O_2} = \frac{V_{mO_2}O_2}{(K_{mO_2}(1 + CO_2 / K_{mCO_2}) + O_2)}
\]

\( K_{mCO_2} \) is the Michaëlis-Menten constant for competitive inhibition of CO2.

\[
R_{O_2} = \frac{V_{mO_2}O_2}{(K_{mO_2} + O_2(1 + CO_2 / K_{mCO_2}))}
\]

\( K_{mCO_2} \) is the Michaëlis-Menten constant for uncompetitive inhibition of CO2.

\[
R_{O_2} = \frac{V_{mO_2}O_2}{(K_{mO_2}(1 + CO_2 / K_{mCO_2}) + O_2(1 + CO_2 / K_{mCO_2}))}
\]

\( K_{mCO_2} \) is the Michaëlis-Menten constant for competitive inhibition of CO2 and \( K_{mCO_2} \) is the Michaëlis-Menten constant for uncompetitive inhibition of CO2.

\[
R_{O_2} = \frac{V_{mO_2}O_2}{(K_{mO_2} + O_2(1 + CO_2 / K_{mCO_2}))}
\]

\( K_{mCO_2} \) is the Michaëlis-Menten constant for non-competitive inhibition of CO2.

For non-linear regression analyses, the NLIN procedure of SAS/STAT was used. The Levenberg-Marquardt optimisation method was used as non-linear least-square search method. The solutions for the parameters were restricted to positive values. Parameters were considered significant when their approximate 95% confidence interval did not include zero. The models were compared based on their adjusted \( R^2 \) and the Root Mean Squared Error (RMSE).

RESULTS

ANOVA

There was no significant effect of the batch of strawberries. Consequently, the results of the two experiments were pooled for all further analyses.

The oxygen consumption of the strawberries significantly decreased with decreasing temperatures, decreasing oxygen concentrations and increasing carbon dioxide concentrations. The oxygen consumption rates at 2°C in air was 100 nmol.kg\(^{-1}\)s\(^{-1}\). Increasing the temperature to 7°C and 14°C resulted in an increase of the rate with a factor 2 and 3 respectively. Oxygen concentrations between 5 and 100 kPa did not significantly influence the oxygen consumption and carbon dioxide production. At O\(_2\) concentrations below 5 kPa, the oxygen consumption significantly decreased. Also, at O\(_2\) concentrations of
2 kPa, the respiratory quotient (RQ) increased which indicates the occurrence of fermentation. Increasing the carbon dioxide concentration from 0 to 20 kPa caused a significant decrease of the oxygen consumption and carbon dioxide production.

Modelling
The estimated model parameters of the four models are given in Table 1. For the models with the competitive, non-competitive and uncompetitive inhibition term all model parameters were significant. For the model with mixed inhibition term, $K_{m(CO_2)}$ was not significant. Based on the $R^2_{adj}$ and RMSE, the models with uncompetitive and non-competitive inhibition term were fitting the data equally well and gave a better fit than the model with competitive inhibition term. The oxygen consumption and carbon dioxide production rates predicted with the model with non-competitive inhibition term are plotted in Figure 1 (oxygen consumption rates) and Figure 2 (carbon dioxide production rates) together with the experimental results.

DISCUSSION
Several authors previously reported on the influence of temperature, carbon dioxide and oxygen concentrations up to 20 kPa on the oxygen consumption and/or carbon dioxide production of different cultivars of strawberries (Li and Kader, 1989; Talasila et al., 1992; Chambroy et al., 1993; Renault et al., 1994; Hertog et al., 1999). In this study, we additionally examined the effect of superatmospheric oxygen concentrations.

Kader and Ben-Yehoshua (2000), reported that exposure to superatmospheric O2 concentrations may stimulate, have no effect, or reduce rates of respiration and ethylene production, depending on the commodity, maturity and ripeness stage, O2 concentration, storage time and temperature, and concentrations of CO2 and C2H4 present in the atmosphere. We found that superatmospheric oxygen concentrations had no effect on the oxygen consumption and carbon dioxide production of ‘Elsanta’ strawberries. Respiration rates at oxygen concentrations above 20 kPa were comparable to those at 20 kPa. Consequently, a Michaëlis-Menten type model is still applicable when including respiration rates at high oxygen concentrations.

Renault et al. (1994) found no effect of oxygen concentrations between 2 and 20 kPa on the oxygen consumption of ‘Selva’ strawberries. This is in agreement with our results and is reflected in a small $K_{m(O_2)}$-value of 1.2 kPa. Small changes in oxygen concentration between 0 and 2 kPa will result in large changes in the oxygen consumption. The $K_{m(O_2)}$-value estimated by Hertog et al. (1999) for ‘Elsanta’ strawberries was 2.63 % and thus higher than in our experiments.

At the low oxygen concentrations, fermentative metabolism occurs. This is reflected in a higher respiratory quotient resulting from a higher carbon dioxide production compared to oxygen consumption. In our experiments the Michaëlis-Menten constant for fermentation is equal to 0.14 kPa, which is rather low.

The estimated RQ from our experiments is 0.66 which is low in comparison to the results of Hertog et al. (1999) and Renault et al. (1994) where it is 0.91 and 1 respectively. Reason could be that CO2 is retained in the fruit. According to Renault et al. (1994)

About the effect of carbon dioxide on strawberry respiration, some controversy exists. Some authors found no effect of CO2 (Hertog et al., 1999) on the respiration whereas others did (Li and Kader, 1989; Talasila et al., 1992). In our study, there was significant inhibitive influence of 20% kPa CO2. This influence however, is inferior to the temperature
effect and the effect of very low oxygen concentrations. The inhibition effect of carbon

dioxide was included in the Michaëlis-Menten model. We could not make a distinction

between uncompetitive or non-competitive inhibition based on the goodness-of-fit

parameters $R^{2}_{adj}$ and RMSE. The $R^{2}_{adj}$ (0.96) and RMSE (15) were equal for the two

inhibition types. As a comparison, we fitted a model without CO$_2$-inhibition term. This

resulted in a clearly lower $R^{2}_{adj}$ (0.89) and higher RMSE (22). Including a CO$_2$-inhibition

term in the model thus strongly improves the model.

The maximum oxygen consumption rate at a reference temperature of 10°C was

equal to 242 nmol.kg$^{-1}$.s$^{-1}$. Chambroy et al. (1993), Renault et al. (1994) and Hertog et al.

(1999) obtained similar results of 250, 210 and 270 nmol. kg$^{-1}$.s$^{-1}$ respectively.

The effect of temperature on the maximum oxygen consumption is reflected in the

activation energy $E_{a/Vm,O_2}$ and was 64 kJ/mol. This value is in good agreement with the

estimate obtained by Hertog et al. (1999) and Chambroy et al. (1993) which was 74 and 65

kJ/mol respectively.

CONCLUSIONS

The effect of oxygen (low and superatmospheric concentrations), carbon dioxide (0

and 20 kPa) and temperature (2, 7 and 14°C) on the oxygen consumption and carbon

dioxide production of ‘Elsanta’ strawberries, was evaluated. Temperature, carbon dioxide

and oxygen concentrations below 5 kPa significantly influenced the respiration rate.

A model based on Michaëlis-Menten kinetics to describe the respiration rates was

constructed. The influence of temperature was described using an Arrhenius equation, and

carbon dioxide was considered as a non-competitive inhibitor of the respiration. The model

described the oxygen consumption and carbon dioxide production of the strawberries very

well.

ACKNOWLEDGEMENTS

This research has been supported by the Belgian Ministry of Small Enterprises,

Traders and Agriculture as part of the project S-6056, and by the Institute for the Promotion

of Innovation by Science and Technology in Flanders (IWT-Vlaanderen, project CO-

020803).

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Tables
Table 1: Parameter estimates with approximate 95% confidence limits, R^2_{adj} and RMSE for Michaëlis-Menten type models with carbon dioxide effect included as competitive, uncompetitive, mixed or non-competitive inhibition term. Temperature effect is included in all four models using an Arrhenius equation.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Competitive</th>
<th>Uncompetitive</th>
<th>Mixed</th>
<th>Non-competitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>V_m,O2ref (nmol/kg.s)</td>
<td>233 ± 5</td>
<td>242 ± 5</td>
<td>242 ± 4</td>
<td>242 ± 4</td>
</tr>
<tr>
<td>KmO2 (kPa)</td>
<td>0.9 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>EaVm,O2 (kJ/mol)</td>
<td>63 ± 2</td>
<td>64 ± 2</td>
<td>64 ± 2</td>
<td>64 ± 17</td>
</tr>
<tr>
<td>V_mCO2ref (nmol/kg.s)</td>
<td>176 ± 43</td>
<td>175 ± 36</td>
<td>175 ± 36</td>
<td>175 ± 36</td>
</tr>
<tr>
<td>KmO2f (kPa)</td>
<td>0.14 ± 0.12</td>
<td>0.14 ± 0.09</td>
<td>0.14 ± 0.09</td>
<td>0.14 ± 0.09</td>
</tr>
<tr>
<td>EaVm,CO2 (kJ/mol)</td>
<td>65 ± 20</td>
<td>65 ± 17</td>
<td>65 ± 17</td>
<td>65 ± 17</td>
</tr>
<tr>
<td>r_qox (-)</td>
<td>0.66 ± 0.02</td>
<td>0.66 ± 0.02</td>
<td>0.66 ± 0.02</td>
<td>0.66 ± 0.02</td>
</tr>
<tr>
<td>KmCO2 (kPa)</td>
<td>1.5 ± 0.4</td>
<td>-</td>
<td>50 ± 186</td>
<td>-</td>
</tr>
<tr>
<td>KmCO2 (kPa)</td>
<td>-</td>
<td>51 ± 5</td>
<td>53 ± 8</td>
<td>-</td>
</tr>
<tr>
<td>K_mnCO2 (kPa)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>53 ± 5</td>
</tr>
<tr>
<td>R^2_{adj}</td>
<td>0.94</td>
<td>0.96</td>
<td>0.96</td>
<td>0.96</td>
</tr>
<tr>
<td>RMSE</td>
<td>18</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>
Figures

Fig. 1: Measured (symbols) and modelled (equation 3 and 7) (lines) oxygen consumption rates (nmol.kg\(^{-1}\).s\(^{-1}\)) of ‘Elsanta’ strawberries as a function of oxygen concentration (kPa) at different temperatures and carbon dioxide concentrations.

Fig. 2: Measured (symbols) and modelled (equation 2 and 3) (lines) carbon dioxide production rates (nmol.kg\(^{-1}\).s\(^{-1}\)) of ‘Elsanta’ strawberries as a function of oxygen concentration (kPa) at different temperatures and carbon dioxide concentrations.
Modélisation de la respiration de la fraise en fonction de la température, de la concentration en dioxyde de carbone et en oxygène superatmosphérique.

Mots clés : atmosphère modifiée, respiration, cinétiques de Michaëlis et Menten, loi d'Arrhénius, consommation d'oxygène, production de dioxyde de carbone.

Résumé
Cet article étudie l'effet de concentrations faibles et superatmosphériques (>20 kPa) en oxygène, de la concentration en dioxyde de carbone et de la température sur la vitesse de respiration de fraises 'Elsanta'. Les fraises étaient stockées dans des pots en verre maintenus à trois températures (2°C, 7°C et 14°C) et remplis de mélanges de gaz humides : deux niveaux de concentration en CO₂ (0 et 20 kPa) ont été combinées à 8 niveaux de concentration en O₂ (0, 2, 5, 20, 50, 60, 80 et 100 kPa). La température, le taux de dioxyde de carbone et de faibles concentrations en oxygène influencent significativement la vitesse de respiration ; en revanche les vitesses de respiration à des taux d'oxygen superatmosphérique ne sont pas significativement différentes de celles observées à 20 kPa d'O₂.

Un modèle décrivant la vitesse de respiration basé sur les cinétiques de Michaëlis et Menten a été construit pour reproduire de faibles vitesses de respiration à de faibles concentrations en oxygène. L'influence de la température a été décrite en utilisant la loi d'Arrhénius, le dioxyde de carbone a été considéré comme un inhibiteur non compétitif de la respiration.