Modelling the respiration rate of mango (cv. Manalagi) during storage under various temperatures and gas compositions

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Abstract. Information on respiration rate of fruit in various temperatures and gas compositions is essential for designing the storage room. The present study aimed to develop respiration rate models of ‘Manalagi’ mango fruit under different temperature and oxygen concentration. Respiration rate was measured with a closed system respirometer at 10, 15 and 28°C of temperature and 3, 10, and 21% of oxygen concentrations. The observed data the used to develop mathematical model based on Michaelis-Menten (MM) with four types of inhibition and an Arrhenius equation. The results indicated that MM models were different for each treatment investigated, among them combination type had the most suitability, however there was no single model which appropriated for all treatments evaluated. Arrhenius equation could be used to describe the effect of temperature on the respiration rate satisfactorily. Three-way repeated measure of statistical analysis confirmed that there was no significant interaction among O2 level, temperature, and measure time in both RO2 and RCO2. However, temperature and O2 concentration of the storage room gave significant effect in both respiration rates of RO2 and RCO2.

1. Introduction
Mango (Mangifera indica L.) is one of tropical fruit that grows well in Indonesia with various varieties. Based on FAO statistical data, Indonesia is the top fourth mango producer in the world [1]. Among numerous local varieties of mango, ‘Manalagi’ is known for its small size, green peel color, and has sweetest taste among other variety. Mango also can be considered as fruit with high nutritional values, its contain lot of vitamin C, carotenoid, and polyphenols [2–4]. Hence, mango is one of the most important fruit for both consumer and horticulture industry worldwide [2,5]. However, mangoes have short shelf life and deteriorate faster during ripening due to its climacteric characteristic [2,6].

In climacteric fruit, ripening process continues even though the fruit has been harvested. Respiration rate is a sign of the metabolism reactions and it is related to the product shelf life [6]. Therefore, controlling the respiration rate may delay the ripening process, which is prolong fruit shelf life and retard deterioration process. Lowering temperature and/or modified the gas composition inside the storage room have been proved to reduce the respiration rate [8,9]. Kader [10] recommended to store mango at 10-13°C with 3-5% O2 and 5-8% CO2 as the optimum conditions to delay ripening process and reduce respiration rate. On the other hand, storage under various temperatures [6,11] and oxygen concentration [2,12] has been used to reduce the respiration rate. Knowing that temperature
and gas composition are the important factor for respiration process, this information can be used to develop models to predict the respiration rate in any storage conditions [7,9]. Nevertheless, no one has yet combined those two factors at the same time to store mango.

Modelling the respiration rate based on the influence of temperature and gas composition is a convenient way to assess the respiratory kinetics of the storage system [13]. Many models have been reported to predict the respiration rate under various storage condition and reviewed by Fonseca et al. [9]. Among the models, Michaelis-Menten (MM) equation has been used to expressed the relationship between enzyme kinetics and substrate concentration (O₂) [7,9,14]. This model tends to fit the experimental data very well [11,14–18]. In another way, Arrhenius equation is used to develop the respiration rate model as the function of temperature. The activation energy (Ea) parameter on Arrhenius equation describes the minimum energy needed for each reaction to occur under the effect of temperature [9]. The lower the Ea value, the faster the reaction occurs. Activation energy values for common fruits and vegetables are in the range of 29.0 to 92.9 kJ/mol [19].

The respiration rate prediction is an essential information for designing the storage room conditions. Although many studies have been reported to the respiration characteristics of mangoes, there is very limited research for local varieties, especially for ‘Manalagi’ mango. Therefore, this study aimed to develop respiration rate models based on Michaelis-Menten equation and Arrhenius equation and to evaluate the suitable model to predict respiration rate of mango cv. Manalagi, under the influence of temperature and gas composition.

2. Materials and methods

2.1. Fruit material
Mango (Mangifera indica L.) fruits of the variety of Manalagi from Situbondo, East Java, Indonesia were used in this study. Fruits were harvested at green-mature state and bought from Giwangan Central Market, Yogyakarta, Indonesia, one day after being harvested. On arrival to the laboratory, the fruit were washed to remove adhering dirt, then the mass and volume of each fruit samples were measured. The fruits were then selected to obtain the uniformity in terms of size and weight (170 – 200 g) of individual fruit to be used as the samples in this research.

2.2. Equipments
The experiment started by constructing respirometer apparatus to measure the changes of O₂ and CO₂ concentrations. The respiration rate was determined with a closed system respirometer method. The container of the respirometer used a glass chamber of 5 mm thick and 3300 ml of total volume. The chamber was covered with a metal plate (25x25x3 mm) which was equipped with a thermo-hygrometer sensor, mini fan, and inlet-outlet holes for gas flushing. Wooden plate was used as the base of the respirometer. In the use, the container was positioned between the top cover plate and the wooden base, then to strengthen the construction four metal rod tightened with the nuts equipped at the four corners of the plate (Figure 1). The inlet-outlet holes were covered with rubber sheets and vinyl tape. The respirometer was previously tested to ensure no air leakage before it was used in the experiment.

2.3. Gas exchange measurement
The following research evaluate the respiration rate of mango in three different storage temperatures of 10, 15, and 28 C and three different O₂ concentrations of 3, 10, and 21%. To create the desired low O₂ concentration in the respirometers, it was flushed by N₂ at the beginning of the storage time until reach the O₂ level required. While for 21% O₂ concentration, it was directly used ambient air condition. The desired O₂ concentration was only gave at the first time of the storage period and it would decrease naturally due to the respiration process of the stored mango. Two mangoes were stored in each respirometer for each O₂ concentration investigated, then those respirometers were put in the
cold storage at 15°C and 10°C, while the other respirometers were put at the ambient room temperature (28°C).

![Figure 1. Schematic picture of closed system respirometer.](image)

The depletion of O₂ and evolution of CO₂ concentration was measured periodically for every 6 hours (first day), 12 hours (second day), and 24 hours in the next days, for totally 21 days. The changes in the headspace gases were measured by using Gas Analyzer O₂ and CO₂ (Quantek, Model 902D Dual Trak), these values were then used to calculate the respiration rate of the stored mango.

2.4. Modelling and data analysis

2.4.1. Respiration rate. Respiration rates based on O₂ (RO₂) and CO₂ (RCO₂) were calculated by using the changes of O₂ and CO₂ over time. Equations (1) and (2) were used to calculate RO₂ and RCO₂ respectively.

\[ R_{O_2} = \frac{y_{O_2}^{f} - y_{O_2}^{i}}{100 M (t_f - t_i)} \times V_f \]  
\[ R_{CO_2} = \frac{y_{CO_2}^{f} - y_{CO_2}^{i}}{100 M (t_f - t_i)} \times V_f \]

where, subscripts O₂ and CO₂ refer to gas O₂ and CO₂, R = respiration rate in ml (O₂) or (CO₂).kg⁻¹ h⁻¹, y = concentration of gas in %, V_f = free volume of respirometer in ml, M = mass of the product inside chamber in kg, and t_i and t_f = initial and final time of a certain period in hour.

2.4.2. Respiration rate models. Modelling the respiration rate of RO₂ were carried out under the effect of various gas compositions and temperatures. Following models were used to describe the RO₂ of mango during storage. For convenience the data beyond aerobic respirations were considered as unchanged.

Model 1. Assuming there was no any CO₂ inhibition during respiration process and known as Michaelis-Menten simple equation.

\[ R_{O_2} = \frac{a \times y_{O_2}}{\phi + y_{O_2}} \]  

Model 2. The Michaelis-Menten with competitive inhibition, considering the role of CO₂ as an inhibitor on the respiration process, it occurs when both the inhibitor and the substrate (O₂) compete for the same active site of the enzyme [9]. The following equation used to describe Michaelis-Menten competitive type.

\[ R_{O_2} = \frac{a \times y_{O_2}}{\phi \times (1 + y_{CO_2}) \times y_{O_2}} \]  

Model 3. The Michaelis-Menten equation with uncompetitive type of inhibition, where in this model CO₂ reacted with the enzyme substrate complex [11,20].
\[ R_{O_2} = \frac{\alpha \cdot y_{O_2}}{\phi + (1 + \frac{y_{O_2}}{\gamma}))} \]  

(5)

Model 4. The CO₂ inhibition might occur at the same time in both competitive and uncompetitive reactions, this mechanism describes with the following equation, and known as Michaelis-Menten combination type.

\[ R_{O_2} = \frac{\alpha \cdot y_{O_2}}{\phi + (1 + \frac{y_{O_2}}{\gamma})) + y_{CO_2}(1 + \frac{y_{CO_2}}{\gamma_u}))} \]  

(6)

In equation (3-6), \( \alpha, \phi, \gamma \) were the parametric model of the Michaelis-Menten equation, where, \( \alpha = \) maximum respiration rate in ml.kg⁻¹ h⁻¹, \( \phi = \) dissociation constant in %O₂, and \( \gamma = \) inhibition constant in subscript c and u denoted the type of MM competitive and uncompetitive (respectively) in %CO₂. Equation (3-6) were changed into linear forms as shown in equation (7-10), then parameters of the model were estimated by using linear regressions.

\[ \frac{1}{R_{O_2}} = \frac{\phi}{\alpha \cdot y_{O_2}} + \frac{1}{\alpha} \]  

(7)

\[ \frac{1}{R_{O_2}} = \frac{\phi}{\alpha \cdot y_{O_2}} + \frac{\phi \cdot y_{CO_2}}{\alpha \gamma \cdot y_{O_2}} + \frac{1}{\alpha} \]  

(8)

\[ \frac{1}{R_{O_2}} = \frac{\phi}{\alpha \cdot y_{O_2}} + \frac{\phi \cdot y_{CO_2}}{\alpha \cdot y_{O_2}} + \frac{1}{\alpha} \]  

(9)

\[ \frac{1}{R_{O_2}} = \frac{\phi}{\alpha \cdot y_{O_2}} + \frac{\phi \cdot y_{CO_2}}{\alpha \cdot y_{O_2}} + \gamma_{CO_2} + \frac{1}{\alpha} \]  

(10)

Model 5. Temperature dependency of the respiration rate was analyzed using Arrhenius equation as shown below [6,9].

\[ k = A \times \exp \left( -\frac{E_a}{R \cdot T} \right) \]  

(11)

Where \( k = \) rate constant, in this study \( k \) was the respiration rate of O₂, \( A = \) pre-exponential factor, \( E_a = \) activation energy (J.mol⁻¹), \( R_c = \) universal gas constant of 8.314 J.mol⁻¹ K⁻¹, \( T = \) absolute temperature (K).

2.5. Model validation and statistical analysis

The validation of the experimental data to all Michaelis-Menten models were evaluated using the coefficient of determination (\( R^2 \)), sum square error (SSE), root mean square error (RMSE), and reduced chi-square (\( \chi^2 \)). These parameters could be calculated using following equations:

\[ R^2 = 1 - \frac{\sum_{i=1}^{N}(X_{pre,i} - X_{exp,i})^2}{\sum_{i=1}^{N}(X_{pre,i})^2} \]  

(12)

\[ SSE = \frac{1}{N} \sum_{i=1}^{N}(X_{exp,i} - X_{pre,i})^2 \]  

(13)

\[ RMSE = \left[ \frac{1}{N} \sum_{i=1}^{N}(X_{exp,i} - X_{pre,i})^2 \right]^{1/2} \]  

(14)

\[ \chi^2 = \frac{\sum_{i=1}^{N}(X_{exp,i} - X_{pre,i})^2}{N - z} \]  

(15)

Where, \( X_{exp} = \) experimental data of RO₂, \( X_{pre} = \) predicted data of RO₂, \( N = \) number of the observations, \( z = \) number of constants. Model with the highest \( R^2 \) and the lowest SSE, RMS, and \( \chi^2 \) was chosen as the best model for its goodness of fit [21,22].

On the other hand, the interaction between treatment factors (temperature and O₂ concentration) and respiration rate was analyzed using three way repeated measures with O₂ concentration (O1 = 21%, O2 = 15%, O3 = 3%), temperature (T1 = 28°C, T2 = 15 °C, T3 = 10 °C), and time (time-1 = 0 week, time-2 = first week, time-3 = second week, time-4 = third week) as a factor. The means of RO₂ and RCO₂ were compared using Duncan Multiple Range Test (DMRT) at a significance level of 5%. All the statistical analysis was conducted by using SPSS ver. 20 software.
3. Results and discussion

3.1. Changes in gas concentration and respiration rate

The changes in the gas composition of O\textsubscript{2} and CO\textsubscript{2} concentration during storage are shown in Figure 2. O\textsubscript{2} concentration decreased over time, conversely the concentration of CO\textsubscript{2} increased along with the storage time. The storage temperature influenced behavior of gases changes for both O\textsubscript{2} and CO\textsubscript{2}. It was observed that lowering the temperature might reduce the consumption of O\textsubscript{2} and the production of CO\textsubscript{2}, as it was also occurred for other mango varieties, e.g. ‘Amrapali’ mango [11], ‘Tommy Atkins’ mango [6], and other fruits like banana [15] and guava [17]. These patterns were also showed on the respiration rate of RO\textsubscript{2} and RCO\textsubscript{2} changes (Figure 3). The graph showed that stored mango with low O\textsubscript{2} level could inhibited the respiration rate of RO\textsubscript{2}, but the same was not true for RCO\textsubscript{2}. It was possibly because a lower O\textsubscript{2} concentration triggered anaerobic respiration, wherein the CO\textsubscript{2} increased significantly mainly at room temperature at the initial period of storage time.

Figure 2. The changes of O\textsubscript{2} and CO\textsubscript{2} concentrations in all treatment during storage

Figure 3. Respiration rates of mango over certain period of time in all treatments investigated

3.2. Parameter estimation for Michaelis-Menten models

The Michaelis-Menten’s model parameter and their corresponding adjusted determination coefficient (R\textsuperscript{2}) were present in the Table 1. It was observed that the parameter values were vary for each treatment. The low values of \( \phi \) indicated a higher affinity for substrate (O\textsubscript{2}) to bond with enzyme, therefore O\textsubscript{2} had a significant effect on the respiration rate. Conversely, higher dissociation constant showed that the enzymes were most likely to bond with CO\textsubscript{2} [6]. The parameters obtained were then used to develop MM models and used to calculate the predicted respiration rate.

3.3. Evaluation of Michaelis-Menten models

Respiration rates calculated using developed models were evaluated with R\textsuperscript{2}, SSE, RMSE, and \( \chi^2 \) to find the best fit between respiration rate prediction and experimental data. Figure 4 depicts the example of the validation graph for observation and prediction data. The best respiration rate models for each treatment are shown in Table 2 with their evaluation values. Each treatment showed a different goodness of fit with the Michaelis-Menten models. The best models were different for each treatment investigated, among them combination type had the most suitability, however there was no single model which appropriated for all treatments evaluated. As this difference was affected by CO\textsubscript{2}, it indicated that the CO\textsubscript{2} has an important role during respiration process.
Table 1. Michaelis-Menten parameters for simple, competitive, uncompetitive, and combination types

| Model - parameters | 21% O₂ | 10% O₂ | 5% O₂ |
|--------------------|--------|--------|-------|
|                    | 10°C   | 15°C   | 28°C  | 10°C   | 15°C   | 28°C  | 10°C   | 15°C   | 28°C  |
| MM-simple          | -0.54  | -1.06  | -1.86 | 1.59   | 5.88   | -59.17| 0.06   | -1.30  | 0.38  |
| q                  | -19.30 | -19.92 | -16.96| 0.91   | 10.14  | -22.75| -1.78  | -8.40  | -0.58 |
| R²                 | 0.9359 | 0.8787 | 0.8783| 0.7389 | 0.0422 | 0.0104| 0.5182 | 0.0472 | 0.5976|
| MM-competitive Yc  | -9.00  | 431.18 | -14.62| -17.50 | -17.94 | 33.11 | 87.94  | -20.17 | 38.49 |
| R²                 | 0.9488 | 0.8285 | 0.9380| 0.8601 | 0.8908 | 0.9136| 0.4771 | 0.2225 | 0.9900|
| MM-uncompetitive Yc| -63.34 | -3045.29| -20.28| -72.82 | -79.31 | 12.59 | 54.69  | -30.97 | -18.23|
| R²                 | 0.9093 | 0.8294 | 0.9684| 0.6501 | 0.9016 | 0.9807| 0.9373 | 0.2620 | 0.9743|
| MM-combination Yc | -13.97 | -175.79| -0.87 | -73.17 | 926.56 | 7.61 | 683.63 | 260.92 | -40.50|
| R²                 | 0.9449 | 0.8161 | 0.9658| 0.8555 | 0.8876 | 0.9769| 0.9373 | 0.4246 | 0.9948|

Figure 4. Observed and predicted RO₂ stored at (a) 21%,10°C, (b) 10%, 10°C and (c) 3%,10°C ; ○ observed data; ● MM-simple; ◊ MM-competitive; ♦ MM-uncompetitive; Δ MM-combination

Table 2. Best fit of Michaelis-Menten’s model for all treatment

| Treatments            | Model          | SSE  | RMSE | χ²   | R²  |
|-----------------------|----------------|------|------|------|-----|
| O₂ 21%, 28°C          | MM-uncompetitive | 1.813| 1.347| 3.626| 0.990|
| O₂ 21%, 15°C          | MM-combination  | 0.713| 0.844| 1.069| 0.879|
| O₂ 21%, 10°C          | MM-combination  | 0.130| 0.361| 0.178| 0.960|
| O₂ 10%, 28°C          | MM-competitive  | 0.929| 0.964| 1.393| 0.982|
| O₂ 10%, 15°C          | MM-combination  | 0.101| 0.318| 0.159| 0.914|
| O₂ 10%, 10°C          | MM-competitive  | 0.037| 0.193| 0.051| 0.951|
| O₂ 3%, 28°C           | MM-combination  | 0.141| 0.376| 0.424| 0.995|
| O₂ 3%, 15°C           | MM-combination  | 0.016| 0.128| 0.033| 0.814|
| O₂ 3%, 10°C           | MM-uncompetitive | 0.174| 0.417| 0.347| 0.997|

3.4. Arrhenius equation

Table 2 shows the activation energy and pre-exponential factor for RO₂ of mango which was stored at different O₂ concentration. By using linear form of equation 12, then plotting ln values of RO₂ against absolute temperature, the value of Ea could be found as the slope of the straight line and the pre-exponential factor was the intercept. Ea in 10% and 3% O₂ were higher than in normal atmosphere, this indicated that RO₂ in lower O₂ level occurred slower than in the ambient temperature. The Ea values found in this research were in the range for fruits and vegetable [19]. However, comparing the Ea value of mango cv. Palmer from the research conducted by Agudelo (± 70 kJ/mol), this was only
about a half. It was possibly caused by the differences in temperature range and mango variety used in the research [6].

Table 3. Arrhenius parameters for RO₂ of ‘Manalagi’ mango

| Arrhenius Parameter | O₂ concentration |
|--------------------|-----------------|
|                    | 21% | 10% | 3% |
| Ea (kJ/mol)        | 34.535 | 49.888 | 44.353 |
| A (gram/h)         | 9.969 x 10⁶ | 2.425 x 10⁹ | 7.322 x 10⁷ |

3.5. Statistical analysis results

The 3-way repeated measures analysis resulted that the interaction among O₂ level, temperature, and time was not found for RO₂ nor RCO₂. Nevertheless, each dependent factor, which is O₂ concentration and temperature, gave a significant effect of both respiration rate of RO₂ and RCO₂. The means of RO₂ and RCO₂ during storage were compared by using DMRT (Table 3). According to O₂ concentration in the storage room, it could be found that the smallest respiration rates was for the lowest O₂ concentration in the storage room and the largest was for the higher O₂ concentration in the storage room (p<0.05). While according to the storage temperature, the respiration rates both for RO₂ and RCO₂ at 10°C and 15°C were not significant different, while those two were different with respiration rate at 28°C (p<0.05). This follows the Van’t Hoff rules where the respiration rate generally increase two to three times for every 10°C rise in temperature [8,9,17].

Table 4. DMRT analysis for RO₂ and RCO₂ means during storage

| Respiration rate | O₂ concentration | Storage temperature |
|-----------------|-----------------|---------------------|
| RO₂ (ml O₂/kg h) | O3 (3%) | 1.652 ± 0.435a  | T3 (10°C) | 2.930 ± 0.435a |
|                 | O2 (10%) | 4.057 ± 0.435b  | T2 (15°C) | 3.080 ± 0.435a |
|                 | O1 (21%) | 7.954 ± 0.435c  | T1 (28°C) | 7.654 ± 0.435b |
| RCO₂ (ml CO₂/kg h) | O1 (3%) | 6.419 ± 0.963a | T2 (15°C) | 5.599 ± 0.936a |
|                 | O2 (10%) | 7.694 ± 0.963a | T3 (10°C) | 6.688 ± 0.936a |
|                 | O3 (21%) | 12.828 ± 0.936b | T1 (28°C) | 14.653 ± 0.936b |

a,b,c different superscripts within the same column corresponding to a factor indicate that the means differ significantly (p≤0.05), for a specific respiration rate.

4. Conclusion

The respiration rate of mango was successfully measured by using a closed system respirometer. The lower the temperature, the slower the respiration rate (RO₂ and RCO₂). Low O₂ level affected RO₂ and RCO₂ in the opposite ways. RO₂ and RCO₂ were not significantly affected by the interaction of the treatments. However, each factor shows a significant effect for both respiration rate. The best models were different for each treatment investigated, among them combination type had the most suitability, however there was no single model which appropriated for all treatments evaluated. Arrhenius equation could be used to describe the effect of temperature on the respiration rate satisfactorily.

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