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Studies on the Respiration Rate of Banana Fruit Based on Enzyme Kinetics

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ABSTRACT

Aerobic respiration rates of banana as a function of O_2 and CO_2 concentration at 21°C in closed respirometer is described based on enzyme kinetics. Parameters of three types of Michaelis-Menten equation based on type of inhibition were considered. A Microsoft Visual Basic computer simulation package (MM-Calculator) was developed to calculate parameters. This program estimated regression parameters and the sum of squared errors between observed and predicted values of respiration rates. The proposed software was used for experimental data to find proper Michaelis-Menten parameters. The un-competitive form was found as the best type of inhibition for respiration rate of banana. In addition simulated surface plots for the respiration rates of banana at 21°C with respect to different atmospheres of O_2 and CO_2 using the model constants were presented. Based on simulation curves RO_2 and RCO_2 decreased by decreasing the O_2 and increasing the CO_2 levels and influence of CO_2 concentration on respiratory quotient (RQ) did not observed. Therefore, using both of low O_2 and high CO_2 atmospheres decreased the respiration of banana. In addition, the RQ remained stable and close to unity.

Key Words: Respiration rate; Enzyme kinetics; Michaelis-Menten; Software; Banana

Notations. $KmcCO_2$ Michaelis constant for the competitive inhibition of O_2 consumption by CO_2 (%); KmO_2 Michaelis constant for O_2 consumption (%); $KmuCO_2$ Michaelis constant for the uncompetitive inhibition of O_2 consumption by CO_2 (%); M Mass of fruit, kg; RCO_2 Respiration rate, mL [O_2] kg-1h-1; RO_2 Respiration rate, mL [CO_2] kg-1h-1; t storage time, h; V free volume of the respiration chamber, mL; VmO_2 maximum O_2 consumption rate.

INTRODUCTION

Loss of fresh fruits and vegetables is the main objectives of post harvest technology. Modified atmosphere packaging (MAP) of fresh produce relies on modification of the atmosphere inside the package, which is achieved by the natural interplay between respiration rates of the product and the transfer of gases through the packaging. This atmosphere can potentially reduce respiration rates (Kader *et al.*, 1989; Saltveit, 1997). Ullah *et al.* (2006) reported that high humidity delayed the ripening of bananas and consequently increased their shelf life. The design of a modified atmosphere package strongly depends on respiration process, therefore it is necessary to have an accurate and acceptable equation for respiration rates. Fresh fruits and vegetables are still alive and require oxygen for their metabolism. This metabolism may cause some physical/chemical changes such as weight loss, which reduce value of fruits and vegetables especially at room temperature (Khan & Ahmad, 2005). Different fruits and vegetables have different respiration rates. Respiration rate of fresh produce can be express as O_2 consumption rates and/or CO_2 production rates as given in Eqs. (1) and (2):

$$R_{O2} = -\frac{1}{M}\frac{dV_{O2}}{dt} \quad (1)$$

$$R_{CO2} = \frac{1}{M}\frac{dV_{CO2}}{dt} \quad (2)$$

Where V is the partial volume in headspace of package, R is the respiration rate, which expressed as volume of generated/consumed gas per unit of time (t) and weight of the product (M).

Respiration rate models presented in the literatures are linear, polynomial, exponential and Michaelis-Menten etc. (Beaudry *et al.*, 1992; Beaudry, 1993; Smyth *et al.*, 1998). The optimal condition for controlled atmosphere storage and modified atmosphere packaging depends on the metabolic characteristics of the specific product (Kader *et al.*, 1989; Cameron *et al.*, 1995). Most of the respiration rate models have been reviewed by Fonseca *et al.* (2000). In this research, respiration rate in form of Michaelis-Menten based on inhibition role of carbon dioxide was studied. Although four types of Michaelis-Menten based on inhibition roles are

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available, non-competitive inhibition has been reported only in some papers e.g., by Peppelenbos *et al*. (1993) in fresh mushrooms and by Song *et al*. (1992) in blueberry. On the other hand, McLaughlin and O'Beirne (1999) rejected the non-competitive model for respiration rate.

In this research, developed software (MM-Calculator) was used to find parameters of respiration rate of banana in form of Michaelis-Menten.

MATERIALS AND METHODS

In this research, only three types of Michaelis-Menten have been discussed. Respiration rates of banana were measured at 21°C using closed system. In addition, a Visual Basic program was developed to find parameters of Michaelis-Menten. This program calculates needed parameters (Km, Vm, $KmcCO_2$ & $KmuCO_2$) based on type of inhibition (no inhibition, competitive inhibition & uncompetitive inhibition). Executable file of this program is available at: http://www.UMA.ac.ir/Heydari/MM-Calculator.exe. This software was used for experimental data to find proper Michaelis-Menten parameters. The uncompetitive form of Michaelis-Menten was found as the best respiration rate for banana.

Michaelis-Menten kinetics. Chevillotte (1973) introduced michaelis–menten kinetics to describe respiration rate. Lee *et al.* (1991) included uncompetitive inhibition by CO_2 and tested the model on cut broccoli. Peppelenbos and Van't Leven (1996) evaluated four types of inhibition for modeling the influence of CO_2 levels on O_2 consumption of fruits and vegetables as compared to no influence of CO_2. They introduced an equation to describe the O_2 consumption rate (RO_2) as inhibited by CO_2 both in a competitive and in an uncompetitive way. Hertog *et al.* (1998) described and discussed multiple faces of the formulation for the combined types of inhibition of O_2 consumption by CO_2 depending on the value of the parameters $KmcCO_2$ and $KmuCO_2$. The related mathematical equations for no inhibition, competitive inhibition and uncompetitive inhibition are presented in equation of 3, 4 and 5, respectively.

$$R_{O_2} = \frac{Vm_{O_2}.[O_2]}{Km_{O_2} + [O_2]} \quad (3)$$

$$R_{O_2} = \frac{Vm_{O_2}.[O_2]}{Km_{O_2}.(1+\frac{[CO_2]}{Kmc_{CO_2}}) + [O_2]} \quad (4)$$

$$R_{O_2} = \frac{Vm_{O_2}.[O_2]}{Km_{O_2} + [O_2].(1+\frac{[CO_2]}{Kmu_{CO_2}})} \quad (5)$$

Where $[O_2]$ and $[CO_2]$ are oxygen and carbon dioxide concentrations (%), Vm_{O2} the maximum O_2 consumption rate, Km_{O2} the Michaelis constant for O_2 consumption (%), Kmc_{CO2} the Michaelis constant for the competitive inhibition of O_2 consumption by CO_2 (%) and Kmu_{CO2} the Michaelis constant for the uncompetitive inhibition of O_2 consumption by CO_2 (%). All of these equations are non-linear, therefore it is necessary to use a mathematical technique to find related parameters to each equation.

Mathematical method. Equation of 3, 4 and 5 can be change in linear forms and can considered as general form of below:

$$\frac{1}{R_{O_2}} = a + b.\frac{1}{[O_2]} + c.[CO_2] + d.\frac{[CO_2]}{[O_2]} \quad (6)$$

Therefore, the Eq. 6 is considered as the regression model. Several estimation methods are available for estimating the parameters of linear and non-linear regression models. The most popular method for estimating the regression parameters of the models (a, b, c & d in Eq. 6) is the least squares method, where the sum of squared errors between observed values and predicted values is minimized (Dodge, 1992; Rao & Toutenburg, 1999; Chatterjee *et al.*, 2000).

The best approximation involves finding parameters of the models to minimize sum of square errors. The proposed mathematical method has no need to iteration processes to find parameters. Cramer's rule is a convenient way to use determinants to solve a system of n linear equations in n unknowns. Michaelis-Menten parameters (Km, Vm, $KmcCO_2$ & $KmuCO_2$) will be found easily based on a, b and c or d constants. The procedure to find parameters of carbon dioxide production is similar to the procedure used for oxygen consumption.

Software description. In this research, a Microsoft Visual Basic computer package (MM-Calculator) using Object Linking and Embedding (OLE) technique is developed. OLE technique easily exchanges data between source code of software and Micro soft Excel (as spreadsheet & drawing tool). Respiration rate is considered in forms of Michaelis-Menten. The MM-Calculator can be downloaded and used easily (http://www.UMA.ac.ir/Heydari/MM-Calculator.exe). This comprehensive and graphic program computes parameters of three types of Michaelis-Menten equation of respiration rate. Respiration rates data can easily be entered into this software. This program is very user friendly as summarized below:

(1). To insert the new data, double click on the table and enter the respiration rates data.

(2). Choose the needed form of Michaelis-Menten respiration rate.

(3). Press the Run button; the needed parameters based on selected form of Michaelis-Menten and summation of square errors will appear.

(4). Press the plot button to export data to micro soft excel and to compare experimental and predicted respiration rates.

Windows 95/98/2000/XP computers with minimal specifications can run this program. This software needs microsoft excel 2003 to be installed on computer to input data and draw the graphs. The program was checked for its functionality in windows XP operation system. Based on installed components on the user's computer, msvbvm60.dll file may be needed to run this software, which can be downloaded from: http://www.UMA.ac.ir/Heydari/msvbvm60.dll.

Respiration rates of banana were measured as a function of O_2 and CO_2 concentrations by using the closed system method. This method is faster and requires a less complex set up than the continuous flow system. Bananas were obtained from local markets. These samples placed in airtight glass jars and contained air as initial gas atmosphere. Gas samples were taken periodically through an airtight septum and analyzed, using OXYBABY V O_2/CO_2 (WITTGAS Co. Ltd., Germany) gas analyzer, after calibration with standard gases. By following the O_2 and CO_2 concentrations over time, the respiration rates of samples expressed as O_2 consumed/CO_2 produced (kg h). Temperature of laboratory was fixed in 21±1ºC. In Fig. 1, sample, respirometer and gas analyzer are shown.

Statistical analysis. The least squares method was used to find the values of regression coefficients and Cramer's rule was used to solve a system of n linear equations in n unknowns. All mathematical calculations were done under microsoft visual basic environment. In this used technique, there is no need to use other statistical programs like SPSS or MATLAB.

RESULTS

Experimental data analysis. Average weights of banana and free volumes of respirometer taken for generating respiration data are 283.5 g and 759.33 mL, respectively. The resultant regression equations for O_2 consumption and CO_2 evolution with related correlation coefficients (R2) were derived using Gong and Corey (1994) method to determine gas composition versus time (Table I). The change of rate of gas concentrations was determined from the first derivative of the regression functions. The regression functions describe the experimental data very well (R2 > 0.998). Experimental respiration rates derived using Eq. 1 and 2 are shown in Fig. 2.

Software results. Gas compositions and respiration rates data were entered into MM-Calculator. Base on calculated squared errors for three types of inhibitions, it was found that uncompetitive inhibition is the best form of Michaelis-Menten equation. Constants for O_2 consumption and CO_2 evolution are presented in Table II.

DISCUSSION

The proposed software can be used for the enzymatic reactions. Relative errors between experimental respiration rates and predicted respiration rates were calculated by MM-Calculator for RO_2 and RCO_2 are presented in Fig. 3. As a result, MM-Calculator can predict experimental respiration rates with accepted accuracy. Simulated surface plot for the respiration rates of banana at 21°C with respect to different atmospheres of O_2 and CO_2 using the model constants were presented in Fig. 4. Based on simulation curves, RO_2 and RCO_2 decreased with a decrease in the O_2 and an increase in the CO_2 levels in closed system.

Table I. Values of regression coefficient by using Gong and Corey method

O_2 composition=	**0.0033 t2 - 0.6357 t + 20.349**	**R2 = 0.9993**
CO2 composition=	-0.0063 t2 + 0.7308 t + 0.877	R2 = 0.9983

Fig. 1. Method for the measurement of respiration rate of banana

Fig. 2. Respiration rates of banana measured at 21°C at different O_2 and CO_2 concentrations

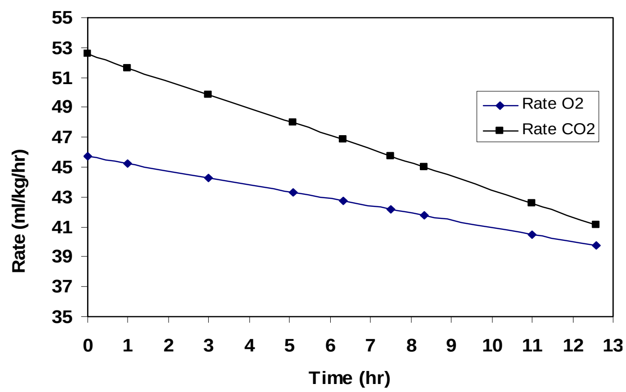


Fig. 3. Relative errors between experimental respiration rates and predicted respiration rates by MM-Calculator

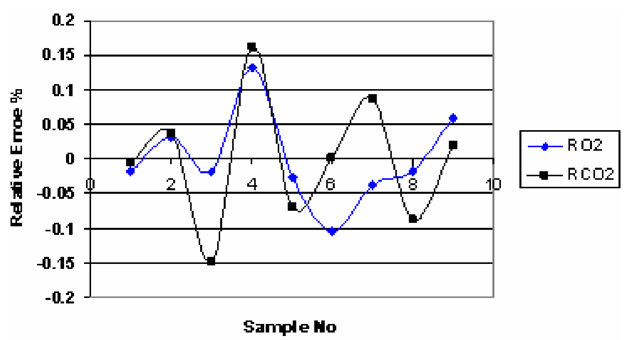
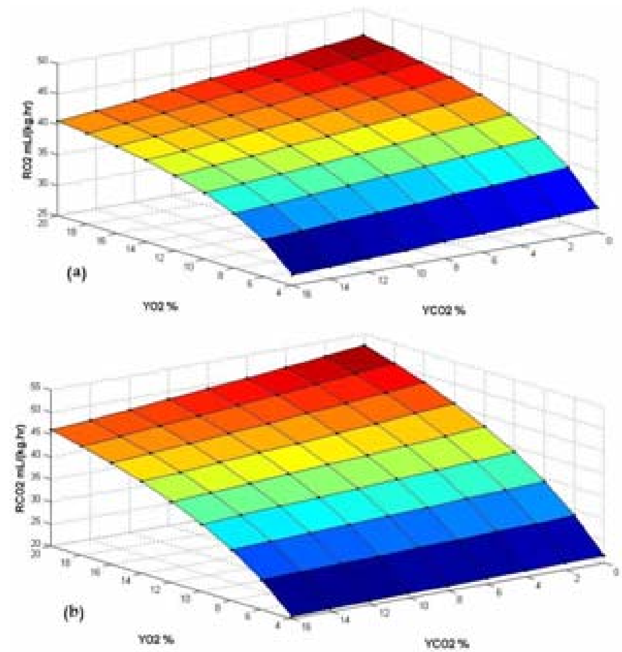


Table II. Uncompetitive Michaelis-Menten constants for O_2 consumption and CO_2 evolution

Form of Michaelis-Menten	Vm (ml kg^{-1} h^{-1})	Km(% O_2)	Ki(%CO_2)	Sum of square errors
Uncompetitive O_2 consumption	53.798	3.427	102.672	6.546E-3
Uncompetitive CO_2 evolution	81.953	11.124	70.491	1.596E-2

Fig. 4. Simulated surface plot for the respiration rates of banana at 21°C with respect to different atmospheres of O_2 and CO_2 (a) O_2 consumption rate, RO_2, (mL kg^{-1} h^{-1}) (b) CO_2 production rate, RCO_2, (mL kg^{-1} h^{-1})



Therefore both of low O_2 and high CO_2 atmospheres decreased the respiration of banana. Iqbal *et al.* (2009) were reported similar behavior for the influence of gas composition on the respiration rate of shredded carrots.

The respiratory quotient (RQ) is normally assumed to be equal to 1.0 if the metabolic substrates are carbohydrates. The total oxidation of 1 mol of hexose consumes 6 mol of O_2 and produces 6 mol of CO_2. Normal RQ values range from 0.7 to 1.3 (Kader, 1987). Renault *et al.* (1994) justified an RQ value of 1.0 for strawberries. Generated data of respiratory quotient of banana at 21°C with respect to different atmospheres of O_2 and CO_2 using the model constants were studied statistically. The values of the RQ remained stable and close to unity when O_2 concentration remained above 9%. This implied that carbohydrates were consumed as substrate for respiration process respiration. The calculated R_2 for RQ as function of O_2 and CO_2 concentrations were 0.0004 and 0.9678, respectively. This revealed that the RQ was considerably influenced by O_2 but not CO_2 concentration. It is possible to evaluate the factors affecting the RQ in works in which determinations of O_2 consumption and CO_2 production rates were made. Jurin and Karel (1963) did not observe an influence of CO_2 concentration on RQ for apples, but Beaudry (1993) observed an increase in RQ under high CO_2 concentrations in blueberries.

CONCLUSION

It was found that RO_2 and RCO_2 decreased by decreasing the O_2 and increasing the CO_2 levels and influence of CO_2 concentration on RQ was not observed. Therefore, use of both of low O_2 and high CO_2 atmospheres can decrease the respiration of banana.

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