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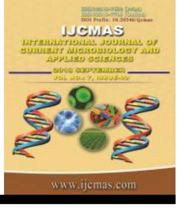

Original Research Article

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Respiration Rate of Fresh Bengal Gram Kernels

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ABSTRACT

Keywords

Respiration rate, Enzyme kinetics, Michaelis-Menten, Fresh Bengal gram kernels

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The respiration rates of fresh Bengal gram kernels as a function of O_2 and CO_2 concentration at 30^0 C in closed system is studied on the basis of enzyme kinetics. Parameters of three types of Michaelis-Menten equation based on type of inhibition were considered. GraphPad PRISM software was used to find parameters of respiration rate of fresh Bengal gram kernels inform of Michaelis-Menten. The competitive type of inhibition was found as the best type for respiration rate of fresh Bengal gram kernels. Respiration rate and gas exchange through the package material are the processes involved in creating a modified atmosphere inside a package that extends shelf life of fresh Bengal gram kernels.

Introduction

The basic principle in the wake of modified atmosphere packaging involves manipulation of respiration rate of the stored produce. However, since respiration rate is dependent upon factors like storage temperature and composition of storage atmosphere, a mathematical approach to predict the respiration rate under given condition would be an immense help in both design and process control of storage systems. Experimental data were generated at ambient temperature (30^0C) using closed system method.

Respiration can be defined as the metabolic process that provides energy for plant biochemical processes. It is the process by which the stored organic materials (carbohydrates, protein and fats) are broken down into simple end products with the release of energy. Oxygen is used during this process and carbon dioxide is produced. This results in hastening of senescence, reduced food value for the consumer, loss of flavor and salable weight (Kader, 1992). Respiration is accompanied by release of heat according to the following chemical reaction (Lee *et al.*, 1991; Nikhane, 2011).

$$C_6H_{12}O_6 + 6O_2 \Longrightarrow 6CO_2 + 6H_2O + 2816kJ \quad (1)$$

The significance of respiration in extending the shelf-life of fresh fruit and vegetables stems from the fact that there exists an inverse relation between reparation rate and the shelf-life of the commodity. Respiration rate, which is commonly expressed as rate of O_2 consumption and/or CO_2 production per unit weight of the commodity, reflects the metabolic activity of the fruit/vegetables tissue in the form of biochemical changes associated with ripening/senescence. Because of decrease in respiration rate during storage is beneficial to maintaining the quality, the accurate measurement of respiration rate has become the important factor in food research.

Shelf-life and respiration rate

In general, there is an inverse relationship between respiration rates and postharvest-life of fresh commodities. The higher the respiration rate, the more perishable, i.e. shorter postharvest-life, the commodity usually is. Respiration plays a major role in the postharvest life of fresh commodities because it reflects the metabolic activity of the tissue that also includes the loss of substrate, the synthesis of new compounds, and the release of heat energy.

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 and 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 Equations (1) and (2):

$$R_{O_2} = \frac{\left(y^{ti}_{O_2} - y^{tf}_{O_2}\right) V_v}{100 x M x \left(t^f - t^i\right)} \quad (2)$$

$$R_{CO_2} = \frac{\left(y^{tf}_{CO_2} - y^{ti}_{CO_2}\right) V_v}{100 x M x \left(t^f - t^i\right)} \quad (3)$$

Where V_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 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, GraphPad PRISM® Version 5.00.288 software (GraphPad Software, Inc., USA) was used to find parameters of respiration rate of fresh Bengal gram kernels inform of Michaelis-Menten.

Materials and Methods

In this research, six types of Michaelis-Menten have been discussed. Respiration rates of fresh Bengal gram kernels were measured at 30°C using closed system. Software was used to find parameters of Michaelis-Menten. This program calculates needed parameters

(K_m, V_m, $K_{mc_{CO_2}}$&$K_{mu_{CO_2}}$) based on type of inhibition (no inhibition, competitive inhibition & uncompetitive inhibition).

This software was used for experimental data to find proper Michaelis-Menten parameters. The competitive form of Michaelis-Menten was found as the best respiration rate for fresh Bengal gram kernels.

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 modelling 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 $KmuCO_2$. Conesa *et al.*, (2007) studied the respiration rates of fresh-cut bell peppers under diverse high and low O_2 levels, with or without 20 kPa CO_2, at 2, 7 and 14 ^{0}C. Menon *et al.*, (2008) summarized the modelling of respiration rate of green mature mango under the aerobic conditions.

Accurate measurement of respiration rate is an important aspect in designing and operating systems like controlled and modified atmosphere storage. Dash *et al.*, (2009) suggested that accurate measurement of respiration rate is an important aspect to the success of design and operational features of technique like controlled and modified atmosphere storage.

The closed or static system was used for measurement of respiration rate i.e. to estimate the kinetics of O_2 consumption and CO_2 evolution by fresh Bengal gram kernels. Kernels of known weight and volume were placed in a pet jar of 900 ml volume, for each measurement of respiration rate in terms of O_2 and CO_2 gases as shown in figure 1. An impermeable cover was mounted tightly on top of each jar. To prevent gas leakage, the top cover was glued and then adhesive tape was pasted on the joint to make it further tight. Each jar was provided with 5mm diameter opening at center of the top cover of the jar. 10mm diameter septum was plugged on this opening. The sample jars were placed at room temperature, at 5±1^{0}C and at 0±1^{0}C. The respiration of the fresh Bengal gram would change the gaseous concentration inside the containers. These altered concentrations were recorded after specified intervals until a steady state was reached. A steady state is considered when no difference in the gaseous concentration between two successive intervals is observed.

For each experiment, the volume of sample (V_s) filled in the impermeable container was determined using the relation

$$V_s = \frac{W_s}{P_b} \quad (4)$$

Where,

V_s= Volume of sample
W_s= Weight of sample
P_b= Mean density of Bengal gram

The total inside volume (V_t) of the impermeable container used for respiration experiment was measured. The density of fresh Bengal gram kernels was determined by tolune displacement method through the evaluation of true volume of known mass of fresh Bengal gram kernels. The void volume

(V_v) for each experiment was determined by using the relationship

$$V_v = V_t - V_s \quad (5)$$

The headspace was continuously monitored to determine the O_2 and CO_2 concentrations using a gas analyzer (Quantek Instrument – Model 902D, Dual Trak). The gaseous concentrations obtained in percent are to be required to be converted into partial pressure using the relation that 1atmospheric pressure (101.325kPa) corresponds to 100% gaseous composition in the atmosphere but as a simplification under the present study has been assumed 100% gaseous composition equivalent to 100 kPa.

The rate of O_2 and CO_2 evolution has been calculated using the following formulae (Ratti *et al.*, 1996; Jacxsens *et al.*, 1999; Fonesca *et al.*, 2002; Song *et al.*, 2002).

$$R_{O_2} = \frac{\left(p^{in}_{O_2} - p^{f}_{O_2}\right)V_v}{100xWx\left(t^f - t^i\right)} \quad (6)$$

$$R_{CO_2} = \frac{\left(p^{f}_{CO_2} - p^{in}_{CO_2}\right)V_v}{100xWx\left(t^f - t^i\right)} \quad (7)$$

Where,

R_{CO_2}: Rate of CO_2 evolution, ml.kg^{-1}.h^{-1}

R_{O_2}: Rate of O_2 consumption, ml.kg^{-1}.h^{-1}

$p^{in}_{O_2}$: Initial partial pressure of O_2 inside film package, kPa,

$p^{f}_{O_2}$: Final partial pressure of O_2 inside film package, kPa,

$p^{in}_{CO_2}$: Initial partial pressure of CO_2 inside film package, kPa,

$p^{f}_{CO_2}$: Final partial pressure of CO_2 inside film package, kPa,

V_v: void volume of container, ml,

W: weight of fresh Bengal gram kernels inside the container, kg,

t^i: initial time, h, and

t^f: final time, h.

Estimation of respiratory behavior

The dependence of the rate of respiration on O_2 concentration has been widely expressed by Michaelis - Menten type equation which is the simplest enzymatic kinetic mechanism. This model is a simplification that tends to fit the experimental data very well, being based on one limiting enzymatic reaction in which the substrate is O_2. The dependence of the respiration rate on the O_2 and CO_2 concentrations has been described assuming mixed inhibition caused by CO_2using the enzymatic kinetics model for combined type of inhibition proposed by Peppelens and Van't Leven (1996) for the respiration of fresh produce as follows;

$$R_{O_2} = \frac{V_{mO_2} x P^{in}_{O_2}}{K_{mO_2}\left(1+\frac{P^{in}_{CO_2}}{K_{mcCO_2}}\right)+P^{in}_{O_2}\left(1+\frac{P^{in}_{CO_2}}{K_{muCO_2}}\right)} \quad (8)$$

Where,

R_{O_2}: Rate of O_2 consumption, ml.kg^{-1}.h^{-1}

$p^{in}_{O_2}$: Initial partial pressure of O_2 inside film package, kPa,

$p^{in}_{CO_2}$: Initial partial pressure of CO_2 inside film package, kPa,

V_{mO_2}: Maximum oxygen consumption rate, ml kg^{-1} h^{-1},

K_{mcCO_2}: Michaelis-Menten constant for competitive inhibition of O_2 consumption by CO_2, %,

K_{mO_2}: Michaelis-Menten constant for oxygen consumption, % and

K_{muCO_2}: Michaelis-Menten constant for uncompetitive inhibition of O_2 consumption by CO_2, %.

The equation 8is a comprehensive and flexible relationship describing basically all types of inhibition (competitive, uncompetitive and mixed) on the rates of O_2 consumption. When inhibition constant $K_{muCO_2}$is infinite, the inhibition is competitive and when inhibition constant $K_{mcCO_2}$is infinite then the inhibition becomes uncompetitive. However, when both of the inhibitions constant are infinite, there develops a condition called “No inhibition”.

Another possibility of finite but unequal values of $K_{mcCO_2}$and K_{muCO_2}has been described as combined or mixed inhibition. Mixed inhibition (Copeland, 2000) encompasses a broad range of behavior and for unambiguous interpretation has been further sub-divided into three types: predominantly competitive, non-competitive and predominantly uncompetitive (Table 1).

The experimentally determined respiration rates and partial pressures of O_2 and CO_2 were subsequently used to estimate the enzyme kinetics model parameters. Non-linear regression analysis was carried out using the measured values of R_{O_2}, R_{CO_2}, $P_{O_2}^{in}$ and $P_{CO_2}^{in}$ to estimate the values of model parameters for O_2 consumption and CO_2 evolution rates of fresh Bengal gram kernels.

Results and Discussion

Respiratory behavior of fresh Bengal gram kernels

The variation in the head space partial pressure ($p_{O_2}^{in}$and $p_{CO_2}^{in}$) were measured for the impermeable container containing fresh Bengal gram kernels maintained at room temperature, 0^0 and 5^0C. The container containing fresh Bengal gram kernels were maintained for 4 h. The $p_{O_2}^{in}$value decreases with respect to time in this case whereas corresponding $p_{CO_2}^{in}$ increases with time as shown in figure 2 and 3. Throughout the respiration study, both O_2 and CO_2 partial pressures remained within the aerobic range and no fermentation was observed for fresh Bengal gram at ambient conditions.

The calculated values of rates of oxygen consumption and carbon dioxide evolution for the same intervals were plotted for fresh Bengal gram kernels. The $p_{O_2}^{in}$ and $p_{CO_2}^{in}$ values remain relatively higher initially, mainly because of initial environmental and respiration adjustments. However as the time progressed, R_{O_2} and R_{CO_2} values kept on decreasing. The partial pressure becomes constant after 3.5 to 4 hr. The respiration rate for O_2 consumption was higher than the rates of CO_2 evaluation as shown in figure 2 and 3. The steady state respiration rates for O_2 consumption and CO_2 evaluation for fresh Bengal gram kernels are presented as in table 2.

The predicted equation for rate of oxygen O_2 consumption and CO_2 evolution have been predicted for this storage condition with R^2 value more than 0.99.

Table.1 Types of inhibition based upon the value of inhibition constants

Values of inhibition constants	Type of inhibition	Equation for estimation of O_2 consumption rate
K_{mcCO_2} **=8,** K_{muCO_2} **=8**	No inhibition Michaelis-Menten kinetics	$R_{O_2} = \frac{V_{mO_2} x P_{O_2}^{in}}{K_{mO_2} + P_{O_2}^{in}}$
$K_{muCO_2} = \infty$	Competitive inhibition	$R_{O_2} = \frac{V_{mO_2} x P_{O_2}^{in}}{K_{mO_2}\left(1 + \frac{P_{CO_2}^{in}}{K_{mcCO_2}}\right) + P_{O_2}^{in}}$
$K_{muCO_2} = \infty$	Uncompetitive inhibition	$R_{O_2} = \frac{V_{mO_2} x P_{O_2}^{in}}{K_{mO_2} + P_{O_2}^{in}\left(1 + \frac{P_{CO_2}^{in}}{K_{muCO_2}}\right)}$
$K_{mcCO_2} = K_{muCO_2}$	Non-competitive inhibition	$R_{O_2} = \frac{V_{mO_2} x P_{O_2}^{in}}{(K_{mO_2} + P_{O_2}^{in}) x \left(1 + \frac{P_{CO_2}^{in}}{K_{muCO_2}}\right)}$
$K_{mcCO_2} < K_{muCO_2}$	Mixed inhibition Predominantly competitive	$R_{O_2} = \frac{V_{mO_2} x P_{O_2}^{in}}{K_{mO_2}\left(1 + \frac{P_{CO_2}^{in}}{K_{mcCO_2}}\right) + P_{O_2}^{in}\left(1 + \frac{P_{CO_2}^{in}}{K_{muCO_2}}\right)}$
$K_{mcCO_2} > K_{muCO_2}$	Mixed inhibition Predominantly uncompetitive	$R_{O_2} = \frac{V_{mO_2} x P_{O_2}^{in}}{K_{mO_2}\left(1 + \frac{P_{CO_2}^{in}}{K_{mcCO_2}}\right) + P_{O_2}^{in}\left(1 + \frac{P_{CO_2}^{in}}{K_{muCO_2}}\right)}$

Table.2 Predicted equations and coefficient of determination for partial pressure

Partial pressure (kPa)	Equation predicted	R^2
O_2	$y = 0.00018x^2 - 0.07239x + 21.84462$	0.994
CO_2	$y = -0.00005x^2 + 0.03517x + 0.00645$	0.999

Table.3 Steady-rate of oxygen consumption (Ro_2) and carbon dioxide evolution (Rco_2) at ambient temperature

Respiration rate(ml $kg^{-1}h^{-1}$)	
(Ro_2)	180.8572
(Rco_2)	125.7766

Table.4 Predicted equations and coefficient of determination values for respiration rate

Respiration rate (ml $kg^{-1}h^{-1}$)	Equation predicted	R^2
(Ro_2)	$y = 0.00017x^2 - 0.69980x + 271.41080$	0.992
(Rco_2)	$y = 0.00044x^2 - 0.73203x + 214.41919$	0.998

Table.5 Enzyme kinetics model parameters and type of inhibition for fresh Bengal gram kernels at ambient storage condition

V_{mO_2} (ml kg/h)	K_{mO_2} (kPa)	$K_{mc_{CO_2}}$ (kPa)	Alpha, (a)	$K_{mu_{CO_2}} = axK_{mc_{CO_2}}$ (kPa)	Types of inhibition	R^2
306.5	$3.144x10^{-7}$	$9.159x10^{-8}$	$5.104x10^{23}$	$4.67x10^{16}$	competitive	0.96

Fig.1 Set up for determining respiration rate of fresh Bengal gram kernels

Fig.2 Partial pressures of oxygen and carbon dioxide in the container headspace during closed system experiment for fresh Bengal gram kernels at ambient temperature

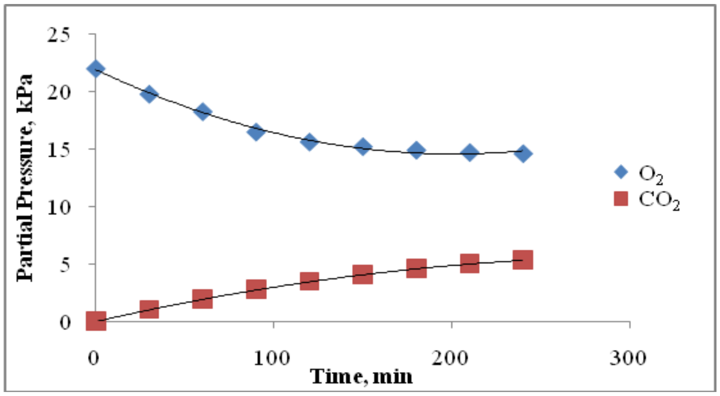
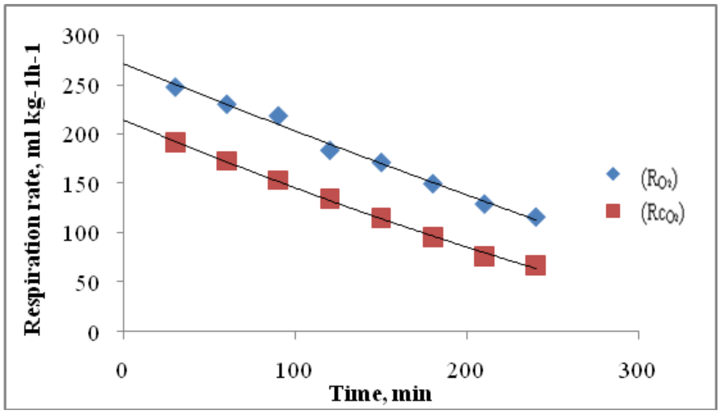


Fig.3 Oxygen consumption rate and carbon dioxide evolution rate in the container headspace during the closed system experiment for fresh Bengal gram kernels at ambient temperature



Enzyme kinetics model parameters and type of Inhibition

The enzyme kinetics parameters viz., V_{mO_2}, K_{mO_2} and K_{muCO_2} as determined by the nonlinear analysis of respiration data of fresh Bengal gram kernels on the basis of enzyme kinetics equation for mixed or combined inhibition, have been given in Table 5.

Alpha determines mechanism. Its value determines the degree to which the binding of inhibitor changes the affinity of the enzyme for the substrate. Its value is always greater than zero. When Alpha=1, the inhibitor does not alter binding of substrate to the enzyme, and the mixed-model is identical to competitive inhibition. When alpha is very small (but greater than zero), binding of the inhibitor enhances substrate binding to the enzyme, and the mixed model becomes nearly identical to an uncompetitive model.

Since the values of K_{muCO_2} tends to infinity and alpha is very large in case of fresh Bengal gram indicating that the fresh Bengal gram kernels exhibits predominantly competitive type of inhibition during its respiration at ambient temperatures. As per enzyme theory, during competitive type of inhibition, the inhibitor (CO_2 in this case) binds reversibly to the same site as the substrate (O_2), so its inhibition can be entirely overcome by using very high concentration of O_2. But in case of uncompetitive type of inhibition, inhibitor (CO_2) binds with equal infinity to the enzyme, and the enzyme O_2 complex. The inhibition is not surmountable by increasing substrate concentration. Because the enzyme O_2 complex is stabilized, it takes less O_2 to get to half maximal activity.

The idea of respiratory inhibition by CO_2 was first supported by simple explanation, i.e., that CO_2 was a sample of the respiration process and, caused simple feedback inhibition. Another hypothesis considered that CO_2 had a controlling effect on mitochondrial activity, including citrate and succinct oxidation. Kader (1989) considered that elevated CO_2 might affect the Krebs cycle intermediates and enzymes. Others considered that CO_2 might inhibit ethylene sample on rather than having a direct effect on the respiration process.

At ambient, under steady state the rate of oxygen consumption for fresh Bengal gram kernels was 180.85 ml $kg^{-1}h^{-1}$ and rate of carbon dioxide evolution was 125.77 ml $kg^{-1}h^{-1}$.

The inhibition constants obtained by non-linear analysis of the respiration data showed that respiration of fresh Bengal gram kerenels was subjected to competitive type of inhibition at ambient storage conditions.

It was found that $Ro_2$and Rco_2reduced by decreasing the O_2 and increasing the CO_2 levels. Therefore, use of both of low O_2 and high CO_2 atmospheres can decrease the respiration of fresh Bengal gram kernels.

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