

Original Research Article

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Modelling the Respiration Rate of Fresh-Cut Pear (*Pyrus communis* L.) Packaged in Modified Atmosphere

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ABSTRACT

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Respiration rate is the important factor involved in creating a modified atmosphere inside a package that will extend the shelf life of fresh fruits and vegetables. Thus, modelling respiration rate of the selected produce is crucial to develop a modified atmosphere packaging (MAP) system. In this study, MAP has been combined with 1% Calcium chloride and 1% citric acid solution. Respiration rates of fresh-cut pear packaged in polypropylene pouches at 8 °C. A mathematical model describing the dynamics of O₂ and CO₂ concentrations inside the MAP package of fresh-cut pear was formulated. It was found that the Michaelis-Menten equation with uncompetitive inhibition kinetic fitted best with the experimental results. The results of the model agreed well with the experimental results with the values of the correlation coefficient, $r^2 > 0.90$. The model could be used to develop a modified atmosphere packaging (MAP) for fresh-cut pear.

Introduction

Pear (*Pyrus communis* L.) is a gently sweet juicy fruit with glitter texture and delicious taste. Pears are a rich source of vitamin C, quercetin and copper, which protect cells from damage by free radicals. Pears are high in dietary fibre, containing 6 g per serving (Reiland and Slavin, 2015). The fruit contains a high amount of pectin, which lowers down the levels of low-density lipoprotein (LDL) and triglycerides thereby reducing the risk of

high cholesterol (Velmurugan and Bhargava, 2013). It possesses multiple medicinal properties such as anti-inflammatory, sedative, anti-pyretic, anti-oxidants, hypolipidemic, hypoglycaemic, anti-ageing, anti-tussive, anti-diarrheal, and hepatoprotective (Parle and Arzoo, 2016).

Respiration of fruits and vegetables is the biochemical process in which sugars and oxygen are converted into carbon dioxide, water, and heat. Controlling respiration is

essential to store produce for a long time. By storing a commodity at low temperature, respiration is reduced and senescence is delayed, thus extending storage life (Halachmy and Mannheim, 1991). Proper control of the oxygen and carbon dioxide concentrations surrounding a commodity is also effective in reducing the rate of respiration. Modified atmosphere packaging (MAP) is a technique used for prolonging the shelf-life of fresh or processed foods by modifying the air surrounding the food in the package to a different composition. Inside packages, O₂ concentration is reduced while CO₂ concentration is increased, causing a reduction in product's respiration rate and a consequent slowing down of senescence and decay phenomena (Das *et al.*, 2006). However, modified atmosphere packaging (MAP) alone does not completely control the post-cutting enzymatic browning of fresh-cut fruits (Gorny *et al.*, 2002). The greatest hurdles to the commercial marketing of fresh-cut fruit products are limited shelf-life due to the browning of cut surface and rapid loss of firmness. Cut surface browning in sliced is caused by the action of polyphenol oxidase (PPO) on phenolic compounds released during the process of cutting (Amiot *et al.*, 1995). Fruit tissue softening during ripening and senescence is a consequence of alterations in cell wall metabolism triggered by ethylene. There are numerous chemical and physical preservation strategies that can be used to reduce enzymatic browning and fruit tissue softening after cutting. A great number of studies have been conducted to avoid browning surfaces on fresh-cut fruits using selected agents such as ascorbic acid, 4-hexylresorcinol, cysteine, N-acetylcysteine and sodium erythorbate (Arias *et al.*, 2008; Dong *et al.*, 2000; Oms-Oliu *et al.*, 2006; Sapers and Miller, 1998; Soliva-Fortuny *et al.*, 2002). Another concern related to the extension of shelf life for fresh-cut fruit is softening, which is primarily due to

enzymatic degradation of the cell wall. Calcium salts, and particularly calcium chloride and lactate, are generally used in combination with browning inhibitors as firmness-maintaining agents in a wide range of cultivars of fresh-cut fruit and vegetables (Alandes *et al.*, 2006).

Combinations of modified atmosphere packaging (MAP) and chemical treatment have been successfully studied to increase the shelf-life of various fruit such as strawberry (Aguayo *et al.*, 2006), litchi (Sivakumar and Korsten, 2006), banana (Vilas- Boas and Kader, 2006), apple (Rocculi *et al.*, 2004) and fresh-cut pear (Sapers and Miller, 1998).

The objectives of this study were developing a suitable model for determining the respiration rate of fresh-cut pear and to find out the combined effect of chemical treatment with MAP on the respiration rate of freshly cut pear.

Materials and Methods

Sample preparation

The fresh William Bartlett variety pears were purchased from the local fruit market in Kharagpur. The pears were stored in the refrigerator for 3 hours at 0°C to assure its freshness. The selected quantity of pears was washed by running tap water, dried by cotton and peeled by peeler manually. Then each pear was cut into 7-8 wedges using a sharp knife. Then the cut pears were dipped in water to avoid frequent surface browning by contact of air. After that, each wedge of pear dried by tissue paper dipped in a chemical solution (1% citric acid + 1% calcium chloride which was previously prepared) for 5 minutes. Then samples were removed from the container and put in a glass plate. Pear slices were subjected to four different treatments: (1) Map + Treated -(1% citric acid + 1% CaCl₂) and

stored in MAP at 8°C, (2) Treated -(1% citric acid + 1% CaCl₂) and stored at room temperature and regular atmosphere, (3) MAP + Untreated -No chemical treatment and stored in MAP at 8°C, (4) Untreated - No chemical treatment and stored at room temperature and regular atmosphere. The samples of all groups were replicated three times and stored for 8 days.

Packaging material

Pear wedges were packaged in polypropylene (PP) pouches of size 12 × 20 cm from inside and 0.025 mm thickness (Nath *et al.*, 2012). The gas permeability of packaging material was 2660cc μm m⁻² h⁻¹ for O₂ and 14958cc μm m⁻² h⁻¹ for CO₂ at 1 atm.

Respiration data

The respiration data of samples in MAP were estimated by sealed chamber technique (Forcier *et al.*, 1987). A special type of container (respirometer) made out of acrylic sheet was fabricated for measurement of the rate of O₂ utilized and CO₂ produced (Plate 1). The dimensions and volume of the container were 23.5 × 18 × 13.5 cm, and 5600 ml, respectively. The container was filled one with treated and another one with untreated pear such that the free volume was minimum. Then the container was sealed and kept in a refrigeration chamber at a pre-set temperature (8°C). The concentrations of O₂ and CO₂ were measured using a gas analyzer (PBI; Dansensor, Ringsted, Denmark) after every 8 hours. The procedure was repeated three times for both treated and untreated pear. The concentrations of O₂ and CO₂ were recorded till the CO₂ concentration reaches 18%.

Modelling of respiration rate

Respiration rates can be measured by observing the concentration of O₂

consumption or CO₂ production per unit weight of the fruit per unit time. Let the head space inside the container was V (mL) and the weight of fruit kept in the container was W (kg). At time θ_i, the concentrations of O₂ and CO₂ were y_i and z_i, respectively and after time θ_{i+1}, the concentrations of O₂ and CO₂ changed to y_{i+1} and z_{i+1}, respectively. Therefore, the rates of O₂ consumption, R_y (mL kg⁻¹ h⁻¹) and of CO₂ production, R_z (mL kg⁻¹ h⁻¹) at a given temperature were calculated using the following Equations (1) and (2) as given by Kays (1991):

$$R_y = \frac{V}{W} \left(\frac{y_i - y_{i+1}}{\theta_{i+1} - \theta_i} \right) \quad (1)$$

$$R_z = \frac{V}{W} \left(\frac{z_{i+1} - z_i}{\theta_{i+1} - \theta_i} \right) \quad (2)$$

The experimental respiration rates for O₂ consumption and CO₂ were calculated by putting the respiration data obtained from respirometer in Equations (1) and (2).

When the variation of y and z with θ is expressed by a continuous functional relationship, the Equations (1) and (2) can be expressed as

$$R_Y = \frac{V}{W} \left(-\frac{dy}{d\theta} \right) \quad (3)$$

$R_z = \frac{V}{W} \left(-\frac{dz}{d\theta} \right)$ (4) where dy and dz, respectively are the concentration differences of O₂ and CO₂ within the time difference between two gas measurements dθ.

It was assumed that the respiration rate reached a stable condition when Equation (1) was verified for:

$$R_y(\theta) - R_y(\theta - d\theta) \leq \pm 0.05 \quad (5)$$

The experiment was performed at a given temperature with three replications.

Inside a hermetically sealed container, the variation of y and z as a function of θ was observed by Hagger *et al.*, (1992) as per the following relationships:

$$y = 0.21 - \left(\frac{\theta}{a_y \theta + b_y} \right) \quad (6)$$

$$z = \left(\frac{\theta}{a_z \theta + b_z} \right) \quad (7)$$

where 0.21 is the initial value in a fraction of O₂ in atmospheric air; a_y , b_y , a_z and b_z are constants and θ is the storage time in h. After finding $dy/d\theta$ and $dz/d\theta$ from Equations (6) and (7) and putting them into Equations (3) and (4) we get,

$$R_y = \frac{v}{w} \left(\frac{b_y}{(a_y \theta + b_y)^2} \right) \quad (8)$$

$$R_z = \frac{v}{w} \left(\frac{b_z}{(a_z \theta + b_z)^2} \right) \quad (9)$$

Using Equations (8) and (9) the values of R_y and R_z at different values of θ were obtained from the data available for pear kept inside hermetically sealed container. At different values of θ , values of y , z , R_y and R_z were computed from Equations (6), (7), (8) and (9), respectively. The values of R_y and R_z were then related to the values of y and z by using regression equations.

Considering that CO₂ acts as a respiration inhibitor, the effect of CO₂ on the product respiration can be described by the uncompetitive inhibition (McLaughlin and O'Beirne, 1999). The maximum respiration rate is not much influenced at high CO₂ concentration. At high levels of CO₂ concentration (17-18%), however, the respiration mechanism changes from aerobic to the anaerobic pathway (Mahajan, 2001). Hence Michaelis-Menten enzyme kinetics equation with uncompetitive inhibition (Lee *et al.*, 1991) was used to develop a model for

predicting the respiration rate of fresh cut pear. Equations (10) and (11) express the uncompetitive inhibition mechanisms for the respiration process in terms of O₂ consumption and CO₂ production rate, respectively. The model has three parameters viz., R_m , K_m , and K_i for both O₂ consumption and CO₂ production.

$$R_y = \frac{R_{my} y}{K_{my} + \left(1 + \frac{z}{K_{iy}}\right) y} \quad (10)$$

$$R_z = \frac{R_{mz} y}{K_{mz} + \left(1 + \frac{z}{K_{iz}}\right) y} \quad (11)$$

Where R_m denotes the maximum rates ($\text{mLkg}^{-1} \text{h}^{-1}$), K_m denotes the Michaelis-Menten constant and K_i denotes the inhibition constant. The model parameters were determined using the experimental respiration data using MS-EXCEL software.

Variation of O₂ and CO₂ concentration inside modified atmosphere package

Let the concentrations (mL) of O₂ and CO₂ inside the package are y and z , respectively. Similarly, y_a and z_a are the concentrations (mL) of O₂ and CO₂ in atmospheric air, respectively. For the transfer of oxygen from atmospheric air through packaging material into the package space, following generalized equation was applied:

The rate of O₂ entry into package space - Rate of O₂ accumulation = Rate of O₂ accumulation inside package space

$$\text{That is, } A_p k_y (y_a - y) - W_p \times R_y = V_e \times \left(\frac{dy}{d\theta} \right) \quad (12)$$

$$\text{or } \left(\frac{dy}{d\theta} \right) = - \left(\frac{W_p}{V_e} \right) R_y + \left(\frac{A_p k_y}{V_e} \right) (y_a - y) \quad (13)$$

where $\frac{dy}{d\theta}$ is the rate of change of O₂ concentration within the package at θ storage time, W_p (kg) is the weight of fruit stored inside the packaging material, V_e is the

headspace inside the packaging material (mL), R_y ($\text{mL kg}^{-1} \text{h}^{-1}$) is the respiration rate of fruit for O_2 , A_p (m^2) is the surface area of packaging material through which O_2 and CO_2 permeates, k_y [$\text{mLh}^{-1}\text{m}^{-2}$ (concentration difference of O_2 infraction) $^{-1}$] is the O_2 permeability of packaging material and t is the thickness of the packaging material. Similarly, the transfer rate for CO_2 from inside to outside of packaging material can be written as:

The rate of CO_2 generated by fruit - Rate of CO_2 leaving out of package space by fruit = Rate of accumulation CO_2 inside package space.

That is,

$$W_p \times R_z - A_p k_z (z_a - z) = V_e \left(\frac{dz}{d\theta} \right) \quad (14)$$

$$\text{or} \left(\frac{dz}{d\theta} \right) = \left(\frac{W_p}{V_e} \right) R_z - \left(\frac{A_p k_z}{V_e} \right) (z_a - z) \quad (15)$$

where, $\frac{dz}{d\theta}$ is the rate of change of CO_2 concentration within the package at θ storagetime, R_z ($\text{mL kg}^{-1} \text{h}^{-1}$) is the respiration rate of fruit for CO_2 , A_p (m^2) is the surface area of the packaging material through which CO_2 permeation takes place.

K_z ($\text{mLh}^{-1}\text{m}^{-2}$ (concentration difference of CO_2 infraction) $^{-1}$) is the CO_2 permeability of packaging material and using regression coefficient, simultaneous solution of Equations (13) and (15) by numerical means the variation of oxygen concentration y and carbon dioxide concentration z inside the package with a time of storage θ were calculated.

Results and Discussion

Respiration rate

The respiration data obtained from closed system respirometer are shown in Figure 1.

The respiration rates of treated and untreated fresh-cut pear are shown in Table 1. It was found that the decrease in concentration of O_2 was almost proportional to the increase in CO_2 concentration with storage period. Similar results were reported by Mangaraj and Goswami (2011) for guava.

Modelling of respiration rate

A model based on principles of enzyme kinetics and a regression model was developed to predict the respiration rate of fresh-cut pear at any combination of O_2 and CO_2 concentrations.

Prediction of respiration rate based on experimental data using regression analysis

Instantaneous O_2 consumption and CO_2 production rates were obtained by plotting gas concentrations versus time and measuring the slopes from linear regression line and substituting the values of $(dy/d\theta)$ and $(dz/d\theta)$ in Equations (3) and (4). Regression function is often used to fit the data of gas concentration versus time and the respiration rate at the given time is determined from the first derivative of the regression function (Kang and Lee, 1998). By using the generated respiration data, a non-linear regression analysis was done to fit O_2 and CO_2 concentrations at different storage times.

The regression coefficient a_y , b_y and a_z , b_z of equations (6) and (7) and correlation coefficients (r^2) of both the sample are shown in Table 2. Respiration rate was calculated using equations (8) and (9).

The respiration rate as predicted by equations (8) and (9) was found to be decreased with the time due to depletion of O_2 and accumulation of CO_2 inside respirometer in both conditions. Similar observation was reported by Mangaraj *et al.*, (2014) for guava.

Verification of the regression model

The respiration rates of fresh-cut pear predicted through regression model shown in

Equations (8) and (9) were verified with experimental respiration rates calculated using Equations (1) and (2).

Table.1 O₂ consumption rate (R_y), CO₂ production rate (R_z) and respiratory quotient

Sample	R _y (mL kg ⁻¹ h ⁻¹)	R _z (mL kg ⁻¹ h ⁻¹)	Respiratory Quotient (R _z /R _y)
Treated pear	7.844	6.97	0.89
Untreated pear	8.78	7.90	0.90

Table.2 Regression coefficients for O₂ consumption and CO₂ production

Sample	Regression coefficients For O ₂ consumption		r ²	Regression coefficients For CO ₂ production		r ²
	a _y	b _y		a _z	b _z	
Treated pear	6.922	624.075	0.9993	7.0012	811.967	0.9997
Untreated pear	6.658	486.126	0.9988	5.5014	698.839	0.9998

Table.3 Model parameters of enzyme kinetics for treated and untreated fresh-cut pear

Sample		R _m (mL kg ⁻¹ h ⁻¹)	K _m (% O ₂)	K _i (% CO ₂)	r ²
Treated pear	O ₂	64.77	55.6448	0.7295	0.989
	CO ₂	42.15	46.0486	1.1344	0.992
Untreated pear	O ₂	35.62	16.1176	1.6461	0.990
	CO ₂	34.40	50.7237	2.7996	0.996

Fig.1 Changes in O₂ and CO₂ concentration with storage time inside respirometer

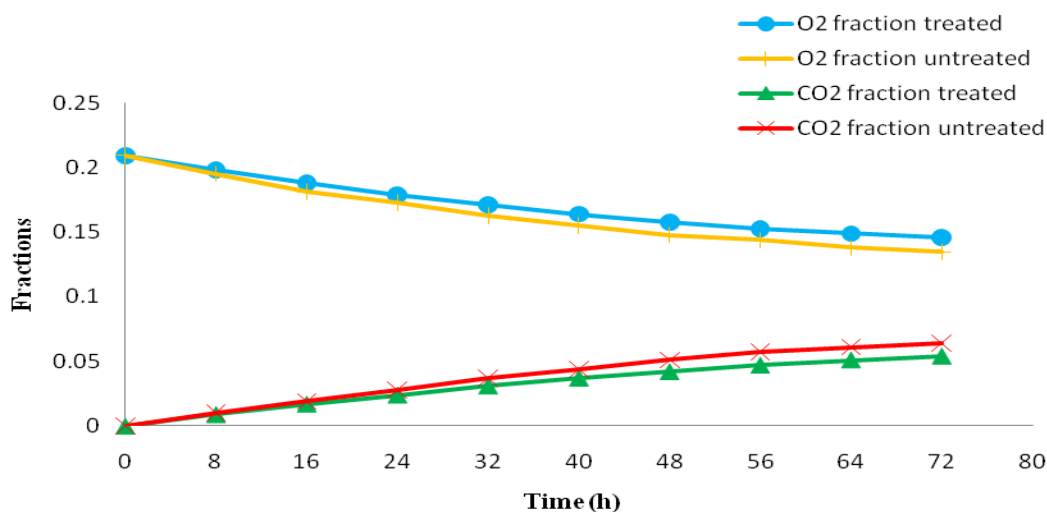


Fig.2 Experimentally estimated and predicted respiration rates for treated pear

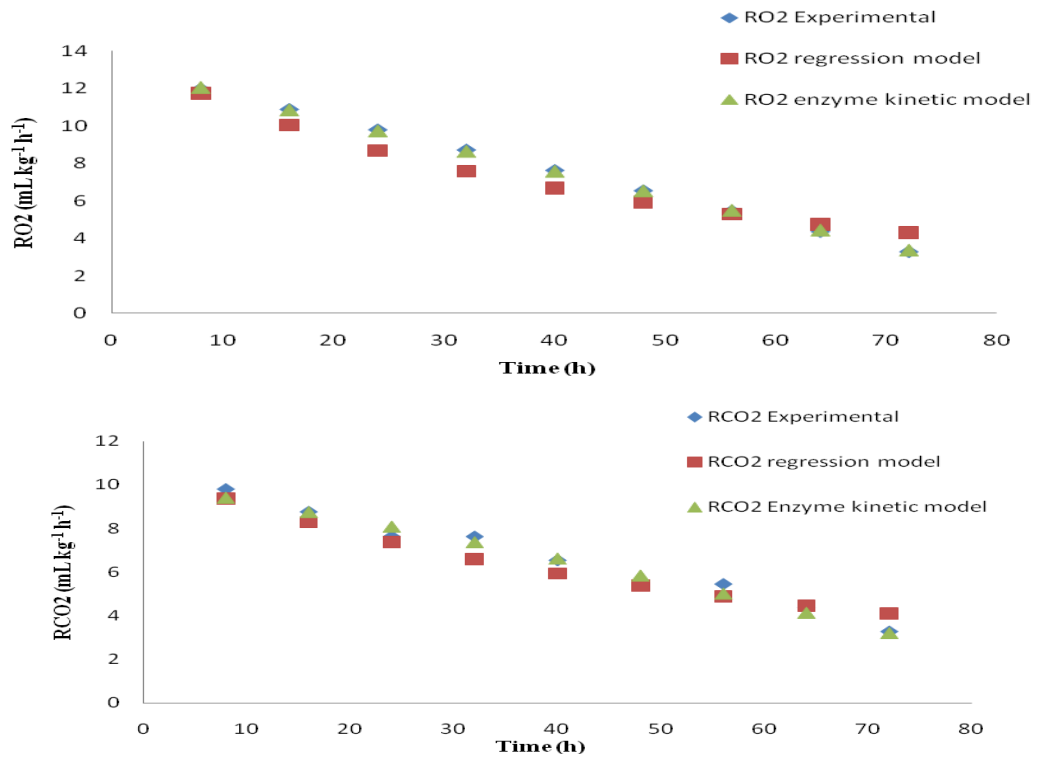


Fig.3 Experimentally estimated and predicted respiration rates for untreated pear

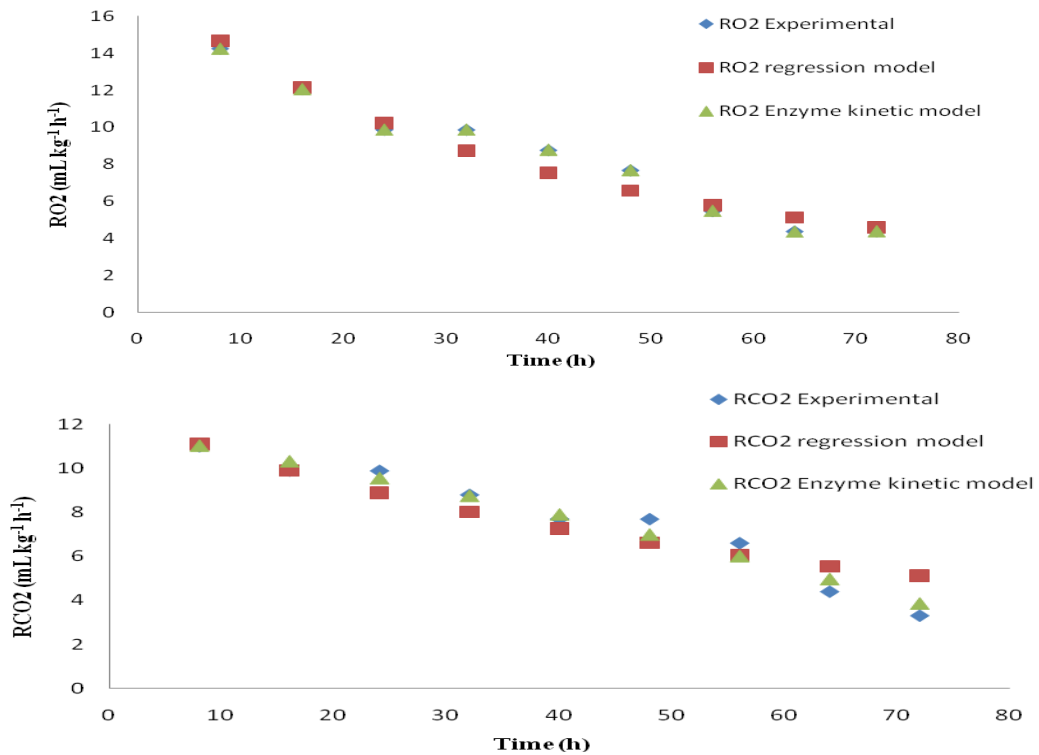


Fig.4 Change in gaseous composition inside the package for treated cut-pear

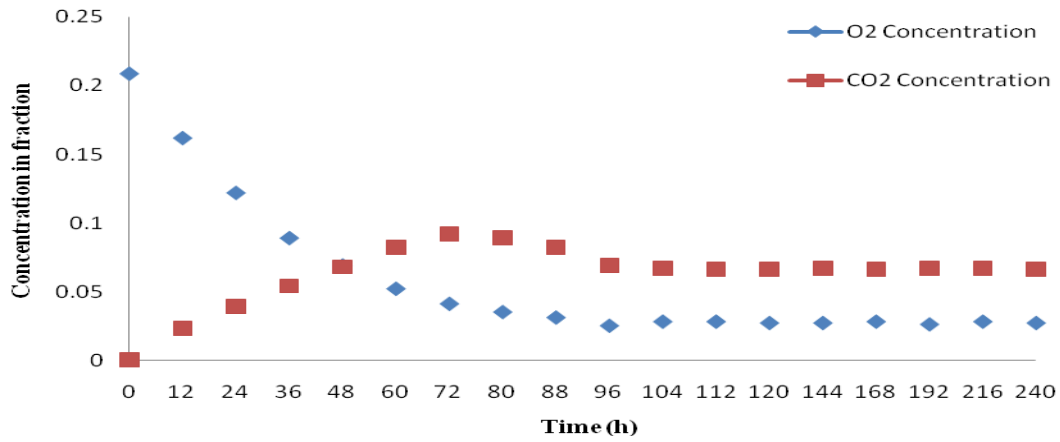


Fig.5 Change in gaseous composition inside the package for untreated cut-pear

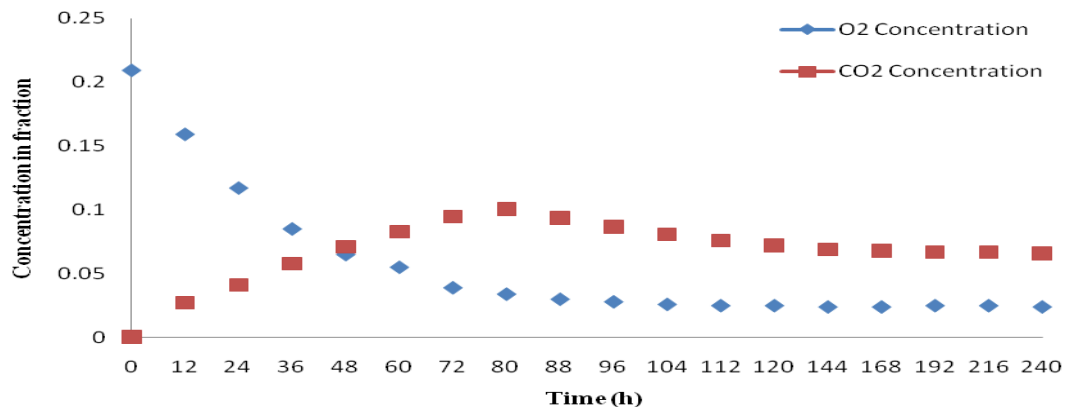


Plate.1 Measurement of respiration data for Fresh-cut pears



The experimental and predicted respiration rates for treated and untreated fresh-cut pear at different time intervals are shown in Figure 2 and 3, respectively. The mean relative deviation moduli between predicted and those of experimentally determined respiration rates for treated were found to be 9.35 % and 6.96 % for O₂ consumption and CO₂ evolution for untreated 8.77% and 9.53 % for O₂ consumption and CO₂ evolution respectively. This suggests that the respiration rates predicted by the regression model are in reasonably good agreement with experimentally determined respiration rates for cut-pear.

Prediction of respiration rates based on enzyme kinetics model

Multiple linear regression analysis was done to obtain the parameters of enzyme kinetics model such as R_m, K_m, K_i. In equations (10) and (11), dependent variables such as the rate of respiration (R_y) or (R_z) were obtained from equations (8) and (9), respectively. The independent variables such as O₂ concentration (y) and CO₂ concentration (z) were obtained through experiments as shown in Figure 1. The model parameters of the uncompetitive inhibition enzyme kinetics as shown in equations (10) and (11) were calculated from the coefficients of multiple linear regression analysis. The model parameters and coefficients of determination (r²) for both the sample is shown in Table 3. By using the model parameters and equations (3) and (4), respiration rates for both the sample predicted for different combinations of O₂ and CO₂ concentrations as shown in Figure 2 and 3. The mean relative deviation moduli between predicted and those of experimentally determined respiration rates were found to be 3.31 % and 6.68% for O₂ consumption and CO₂ evolution respectively. This suggests that the respiration rates predicted by the enzyme-kinetic model at

different time intervals were fairly good agreement with experimental respiration rates.

Effect of modified Atmospheric packaging on chemically treated and untreated cut-pear

Headspace O₂ and CO₂ compositions of both the samples were measured. The level of O₂ and CO₂ concentration maintained by respiration of commodity and permeability of packaging film is shown in Figure 4 for treated pear and Figure 5 for untreated pear. Under all the packaging treatments, initially, a rapid decrease in O₂ and a corresponding increase in CO₂ concentrations were observed on the first day to the fifth day, which may be attributed to the initial adjustment and high respiratory behaviour of fruits in the transient state of equilibrium as well as the permeability of the packaging film. For both the samples equilibrium of gases established on fifth days of storage. The maximum decrease in O₂ was 2.5% in 96 h then slightly increased and maintained equilibrium to 2.7% in 112 h. For CO₂, maximum concentration increase was 8.9% in 80 h and then decreased and maintained equilibrium to 6.6% in 112 h depends on permeability. Similarly, for untreated pear sample, the maximum decrease in O₂ was observed to be 3.4% in 144 h and maintained equilibrium to 2.4% in 144 h and for CO₂ maximum decreases to 10.1% in 80 h and then decreased and maintained equilibrium to 6.8% in 144 h throughout the storage period.

In conclusion, the respiration rates were found to decrease with storage time. The respiration rate of fresh-cut pear was well described by a Michaelis–Menten model. The effect of O₂ and CO₂ concentration on respiration rate was found to fit well with the uncompetitive inhibition enzyme kinetics for both the sample. The mean relative deviation moduli between predicted and those of

experimentally determined respiration rates for treated were found to be 9.35 % and 6.96 % for O₂ consumption and CO₂ evolution for untreated 8.77% and 9.53 % for O₂ consumption and CO₂ evolution respectively. Based on the results of the investigation it maybe concluded that polypropylene film can be used to maintain the proper gaseous composition inside modified atmospheric packaging for both freshly cut-pear chemically treated and untreated pear. The model can successfully be used to develop a modified atmosphere package.

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