Respiration rate model for mature green capsicum (*Capsicum annum* L.) under closed aerobic atmospheric conditions

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Summary

The respiration rate of green capsicum cv. 'Swarna' fruits harvested at mature green stage were determined under closed system at 10, 15 and 20 ºC temperatures. A simple Michaelis-Menten kinetic model coupled with Arrhenius-type equation, which describes temperature as a function of respiration rate, was used to model the respiration rate of capsicum. The respiration rate model parameters at defined temperature were estimated by fitting the model to the experimental set of data by non-linear regression analysis method. The respiration rate of green mature capsicum was influenced by the temperature. The Arrhenius equation well described the relationship between enzyme kinematics model parameters and temperature. The values of Michaelis-Menten constant for oxygen (Kmo_2) and carbon dioxide ($Kmco_2$) were found to vary from 2.14 to 3.92 and 1.33 to 3.24, respectively at different temperature. Experimental and predicted RRO_2 values for mature green capsicum was found to be ranged from 9.54 to 14.54 and 11.81 to 17.52 mg/kg-h, respectively. Whereas, the experimental and predicted $RRCO_2$ were 20.1 to 38.51 and 22.15 to 39.83 mg/ kg-h, respectively.

Keywords: Capsicum fruit, respiration rate, enzyme kinematic model and validation

Introduction

Modified atmosphere packaging (MAP) is a well-established technology that used for prolonging the shelf-life period of fresh or minimally processed foods. Two aspects involve in the process are the respiration of the products and the gas exchanges through the package materials, both lead to the increase of CO_2 and depletion of O_2 (Deepak and Shashi, 2007). The significance of respiration in extending the shelf-life of fresh fruits and vegetables stems from the fact that there exists an inverse relationship between respiration rate and the shelf-life of the commodity (Lee et al., 1991). The gas constituents inside the package is an important factor affects the shelf life of the product. The enzyme kinetics approach proposed by Lee et al. (1991) for predicting respiration rates of fresh products as a function of O_2 and CO_2 concentrations has been found to be the most suitable theory that making fairly accurate correlations of the respiration rates. The Michaelis-Menten type enzyme kinetic equation has been thoroughly reported in the literature as giving good fits to experimental data on respiration rate of different products (Lee et al. (1996) for tomatoes and fresh cut broccoli, Mclaughlin and Berine (1999) for dry coleslaw mix, Del and Baiano (2006) for minimally processed lettuce, Toshitaka and Daisuke (2004) for leaf lettuce, Liu and Li (2004) for peas). A numerical simulation was generated in this study to investigate the relationship between the respiration rate of the product and the gas composition inside the package under the conditions used by other researchers (Fonseca et al., 2002; Hagger et al., 1992). The present work study the respiration rate of mature green capsicum under different storage conditions and development of a predictable model relating respiration rate to O_2 and CO_2 concentrations and temperature that maybe used in the design of MAP system.

Materials and methods

Fruit material

Capsicum cv. '*Swarna*' fruits having average length 12.2 cm, average diameter 7.3 and average unit fruit weight of 234.53g were manually harvested at mature green stage from PFDC fruit farm of Central Institute of Agricultural Engineering, Nabi-Bagh, Berasia Road, Bhopal for experimentation and study. The capsicums were graded manually to remove damaged, infested and non-uniform fruit. Fruits were selected to insure uniform size, shape and weight. Fruits were immediately shifted to packaging lab for further experimentation.

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Respiration rate measurement of capsicum

The respiration rate measurement of capsicum was done as per the method adopted by Singh et al. (2014b). A closed system was used to measure the respiration rate of capsicum. Manually graded capsicum (1 kg) was filled into air tight glass container of known volume. The container was sealed carefully using vacuum grease. A single hole covered with silicon septum was made in container for measurement of gas concentrations. After sealing, the container was kept at different temperature i.e. 10 °C, 15 °C and 20 °C, at 75% RH in an Environmental chamber (Remi Laboratory Instruments, India; Model: CHM-10). The headspace gas composition was measured and recorded every hour directly by piercing the syringe of the Headspace gas analyser (Systec Instruments Ltd, UK; Model: Gaspace Advance) inside the closed glass chamber. The linear portion of experimental data of gaseous concentration (O2/CO2) was used to calculate the oxygen respiration rate (R_{O2}) and carbon-dioxide respiration rate (R_{CO2}) by using equation 1 and 2 below (Singh et al., 2013):

$$RR_{O_2} = \frac{(P_{O_2}{}^{i} - P_{O_2}) \times V_f}{100 \times W \times (t - t_i)} \quad (1)$$

$$RR_{CO_2} = \frac{(P_{CO_2}{}^{i} - P_{CO_2}) \times V_f}{100 \times W \times (t - t_i)} \quad (2)$$

where:

P^iO_2 = O_2 concentration (%) at the initial time t_i (hours, h)
PO_2 = O_2 concentration (%) at time t (h)
P^iCO_2 = CO_2 concentration (%) at the initial time t_i (hours, h)
PCO_2 = CO_2 concentration (%) at time t (h)
W = total weight of the product (kg)
V_f = free volume inside the glass jar (mL), which is the total volume of the glass jar minus the volume occupied by the product

The volume occupied by the product was calculated by the mass to the density (ρ) of the mature green capsicum (6.17 g/cm^3). The free volume was measured by the water displacement method.

Validation of model

The respiration rate of mature green capsicum predicted by developed mathematical model was verified with experimental respiration rates data observed at 10 °C recommended as storage temperature for capsicum. A sample size of approximately 1 kg was used to measure the respiration rates for verification purpose. The observed respiration rates in terms of oxygen depletion (RRO_2) and carbon-dioxide evolution ($RRCO_2$) data at 10 °C temperature were determined by using Eqs. (1) and (2) respectively and predicted RRO_2 and $RRCO_2$ were obtained by using Eqs. (3) and (4).

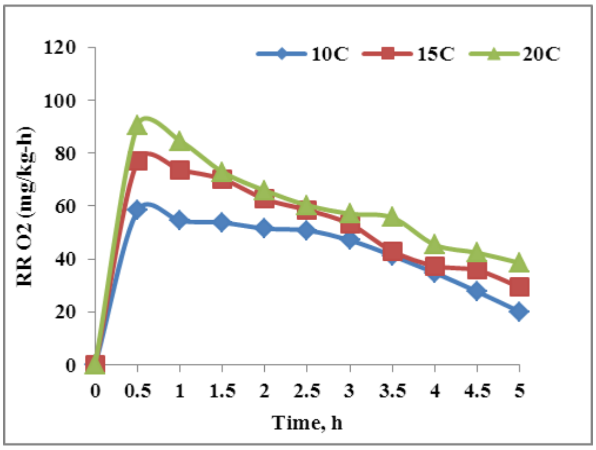
Statistical analysis

The entire set of experiments was replicated twice. Analysis of variance (ANOVA), regression co-efficient (R^2) and standard error for both parameters, were carried out using the SPSS 14.0 software. The developed model was analyzed using GraphPad PRISM® Version 5.00.288 software (GraphPad Software, Inc., CA, USA).

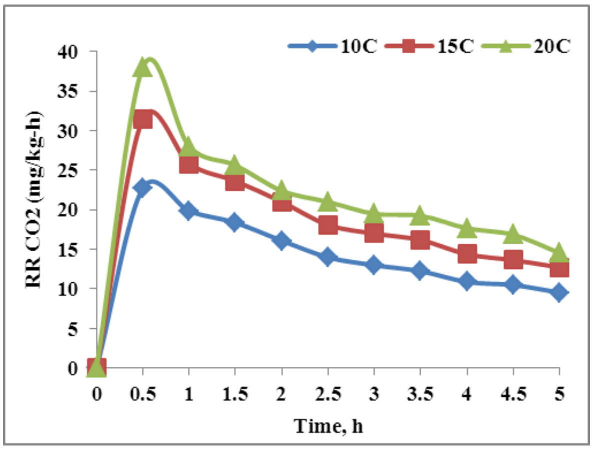
Results and discussions

Effect of time and temperature on rate of respiration

The respiration data corresponding to the different temperature indicated that as the temperature had a significant effect on respiration rates of capsicum fruit as shown in Fig.1. The initial oxygen depletion rates (RRO_2) of fruit ranged from 59.1 to 91.7 mg-O_2/kg- h and CO_2 evolution ($RRCO_2$) ranged from 23.5-36.7 mg-CO_2/kg-h respectively over all the three storage temperatures tested. The rate of respiration was higher at the start of the experiment and gradually declined as the storage period prolonged, before becoming almost constant (Fig. 1). For similar temperature increments, the decrease in respiration rate was 2.93, 2.62 and 2.36 degree folds for O_2 and 2.38, 2.47 and 2.62 degree folds for CO_2 evolution respectively at 10 °C, 15 ºC and 20 °C temperatures.



(a)



(b)

Fig. 1. Respiration rate RRO_2 (a) and $RRCO_2$ (b) for capsicum at 10, 15 and 20 ºC temperatures

Modeling respiration using enzyme kinetic model (Michaelis-Menten)

A Michaelis-Menten type model is the mostly used respiration rate model based on enzyme kinetics describing the respiratory behavior of perishable produces. A simple Michaelis-Menten kinetic model (Eqs.3) (Lee et al., 1991) and Arrhenius-type equation which describes temperature as a function of RR (Waghmare et al., 2013; Iqbal et al., 2009; Torrieri et al., 2010) for both RR_{O2} and RR_{CO2} was applied in model fitting as presented in Eqs. (4) and (5) below:

$$R = \frac{V_m \, y^0_{O_2}}{K_m \left(1 + \frac{y^0_{CO_2}}{K_i}\right) y^0_{O_2}} \quad (3)$$

where R is the respiration rate of the produce in ml/kg-h; *Vm* is the maximum rates of O_2 consumption for the produce in ml/kg-h; *km* is the Michaelis-Menten constant in CO_2 evolution in kPa; K*i* is the inhibition constant in CO_2 evolution in kPa; P^i_{CO2} and P^i_{O2} are the percent volumetric concentrations of CO_2 and O_2 in the container, respectively.

$$RR_{O_2} = R_{O_{2,ref}} \times e^{[[-E_a, O_2/R](1/T - 1/T_{ref})]} \quad (4)$$

$$RR_{CO_2} = R_{CO_{2,ref}} \times e^{[[-E_a, CO_2/R](1/T - 1/T_{ref})]} \quad (5)$$

where RR_{O2} and RR_{CO2} are respiration rate (mg/kg-h) of the commodity at temperature T (K), $RR_{O2,\, ref}$ and $RR_{CO2,\, ref}$ are the initial respiration rate (mg/kg-h) of the commodity at reference temperature ($T_{ref,}$ K), R is the universal gas constant (0.008314 kJ/kg-mol), E_a (O_2/CO_2) is activation energy (kJ/mol).

Based on this, a secondary model was developed by substituting RO_2 and RCO_2 in Eqs. (1) and (2), with Eqn (4) and (5) resulting Eqs (6) and (7) respectively.

$$P_{O_2} = P_{O_2}{}^{i} - \left[RR_{O_{2,ref}} \times e^{[[-E_a, O_2/R](1/T - 1/T_{ref})]}\right] * \frac{W}{V_f} * (t - t_i) \times 100 \quad (6)$$

$$P_{CO_2} = P_{CO_2}{}^{i} - \left[RR_{CO_{2,ref}} \times e^{[[-E_a, CO_2/R](1/T - 1/T_{ref})]}\right] * \frac{W}{V_f} * (t - t_i) \times 100 \quad (7)$$

where P^iO_2 = initial O_2 concentration, P^i CO_2 = initial CO_2 concentration, t is the elapsed time (h) during RR measurement and parameter estimates of $RR_{O2,\ ref}$, $RR_{CO2\ ref}$, E_a, O_2 and E_a, CO_2 were estimated using GraphPad Prism 5 (GraphPad Software, Inc., CA, USA).

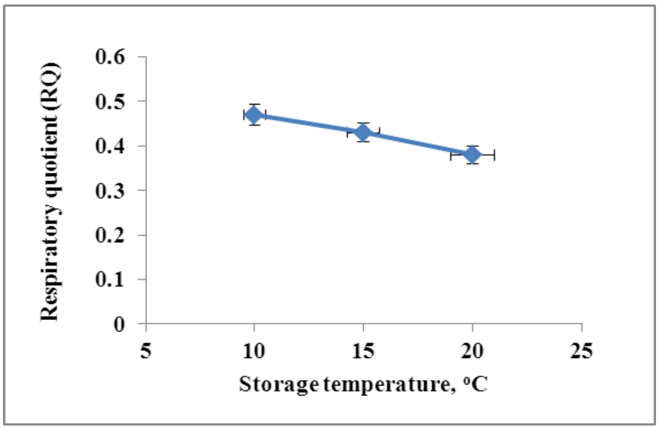


Fig. 2. Respiration quotient of mature green capsicum changes with storage temperature

Influence of respiratory quotient

A change in the respiratory quotient at different temperature was shown in Fig 2. The respiratory quotient exhibits slight minor fluctuations during the initial stage of respiration rate experiments. The respiratory quotient (RQ), as determined from the ratio of CO_2 produced to the O_2 consumed, ranged from 0.38 ± 0.04 to 0.47 ± 0.17 for mature green capsicum (Fig. 3). At a given temperature condition, RQ was found varying insignificantly with the time under aerobic condition. A gradual decrease in RQ value was observed with rising temperature. This phenomenon is corresponding to some other fresh produce reported by Fonseca et al. (2002) for avocado, banana, apple, lemon, potato and tomatoes. RQ is less than unity; the O_2 consumption was always higher than the oxidative CO_2 production.

Influence of the temperature

The respiration rate of green mature capsicum was influenced by the temperature. The activation energies for kinematic parameters, namely *V*m, and *K*m were determined and the effect was expressed as Arrhenius-type relations in the defined temperature range for modified atmosphere packaging of mature green capsicum. The values of Vmo_2 and $Vmco_2$ were 44.32 to 49.48 mg/kg-h and 53.47 to 62.27 mg/kg-h, at 10-20 °C temperature, respectively (Table. 1). Similarly an increasing trend in Km_{CO2} value was noticed between temperature ranges from 283 to 293 ºK. The values of Kmo_2 and $Kmco_2$ were 2.14 to 3.92 and 1.33 to 3.24, at 10-20 °C temperature, respectively. The higher value of Km_{CO2} indicates the stronger influence of temperature dependence on respiration kinetics. Similar effects of a decrease in respiration rate with reduced temperature were observed by Singh et al. (2014) in tomato, Singh et al. (2013) in guava and Singh et al. (2012a) for mango stored at varied temperatures (5-35 °C) and analysis of variance shows the effect of time and temperature on respiration rates.

Table 1. Enzyme kinetic model (Michaelis-Menten) parameters [Eqs. (4) and (5)]* along with constants and standard deviation depicting the effect of time and temperature dependence on respiration rate of mature green capsicum

Model equation	Model parameters	RRO_2			$RRCO_2$		
		283°K	288 °K	293 °K	283°K	288 °K	293 °K
Enzyme kinetic and Arrhenius type model	$R_{ref,}$ mg/kg-h	46.00±0.46	47.00±0.25	49.00±0.73	58.00±0.74	59.00±0.47	62.00±0.94
	E_a, kJ/mol	42.20±0.10	43.40±0.40	45.80±0.70	57.80±2.01	59.20±1.90	63.20±2.20
	Vm (Maximum O_2 inhibition) mg/kg-h	44.32±0.20	45.83±0.30	49.48±0.80	53.47±0.40	56.73±0.10	62.27±0.50
	Km Michaelis-Menten constant	2.14	3.28	3.92	1.33	2.73	3.24
	K_i (Inhibition constant)	1.83	1.24	1.32	1.46	2.24	2.34
	R^2	97.34	96.38	98.37	96.28	95.39	98.39

*T_{ref}: Reference temperature, 283 ºK

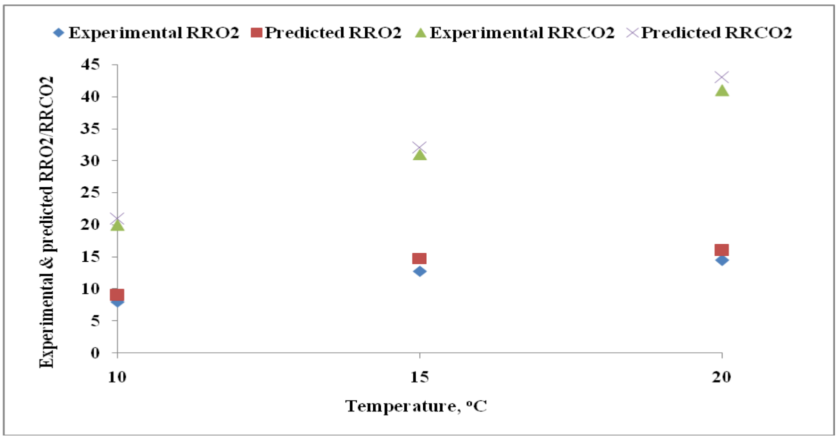


Fig. 3. Effect of the temperature on experimental and predicted (a) RRO2 and (b) RRCO2 of mature green capsicum stored at 10, 15 and 20 °C

In Fig. 3, respiration rates in terms of O_2 consumption and CO_2 production are functions of storage time and temperature. A significant effect of time and the interaction between time and temperature on respiration rate ($p < 0.05$) was noticed. Both the respiration rates (RRO_2 and $RRCO_2$) decreased for capsicum during the storage period under aerobic condition for 5 h. The selected produce achieved steady state condition and an-aerobic condition was achieved, hence respiration rates were determined for up-to 5 h. Respiration rates (RRO_2 and $RRCO_2$) of green mature capsicum decreased significantly during the experiment storage period at 10-20 °C. Similar decreases in respiration rates of guava (Singh et al., 2013), for tomato (Singh et al., 2014) and for chickpea sprouts (Singh et al., 2012b) were obtained at 10 °C during storage period under aerobic conditions in closed system. In all the cases, the RRO_2 was higher as against $RRCO_2$. Similar finding were also reported by Singh et al. (2012a) for chickpea sprouts. The influence of both storage period and temperature with their interaction on the respiration rate of produce was significant. The scatter plot shows a good relationship between experimental and predicted respiration rate values of capsicum under study. There was slight variation between experimental and predicted (modeled) values at all temperatures. This could be due to slight fluctuation in storage temperature, product behavior and moisture loss of the product which led to high variability of experimental data obtained between the various trials. The developed mathematical model would be suitably used in selecting type of packaging material, pack size and weight, size and number of perforations for development of packaging system for mature green capsicum.

Model validation

The experimental data of respiration rate of mature green capsicum predicted by using the Arrhenius type model [Eqs. (6) and (7)] was verified with modeled data generated by software. Experimental and predicted RRO_2 values for mature green capsicum was found to be ranged from 9.54 to 14.54 and 11.81 to 17.52 mg/kg-h, respectively. Whereas, the experimental and predicted $RRCO_2$ were 20.1 to 38.51 and 22.15 to 39.83 mg/ kg-h, respectively (Fig. 3). The developed model parameters Vm, Km, R_{ref}, Ea, and R^2 for Arrhenius model was presented in Table 1. A mathematical model tested at 10 °C showed good agreement between the experimental and predicted data (Fig. 3). Hence, the developed model based on enzyme kinematics could be used to predict the respiration rate of mature green capsicum by considering length of storage period and temperature during distribution to super markets and also for design of suitable packaging system.

Conclusions

It was observed that the respiration rates were found to be decreasing with storage time. The respiration rates were also found to be increasing with increasing storage temperature. It was also observed that, the respiration rate RRO_2 remained higher than the respiration rate $RRCO_2$ giving steady-state respiration quotient values of 0.38 to 0.47 at temperature ranging from 10-20 °C for mature green capsicum. The capability of developed Michaelis-Menten kinetic model in tracking the gas composition revealed a substantial effect of produce respiration models on the predicted values. The values of Michaelis-Menten constants (Km_{O_2} and Km_{CO_2}) were 2.14 to 3.92 and 1.33 to 3.24, at 10-20 °C temperature, respectively. The higher value of Km_{CO2} indicates the stronger influence of temperature dependence on respiration kinetics. The verification of developed model for the prediction of in-pack gaseous consumption and its judgment with the experimental value, showed a near proximity.

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