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Research Article

MEASUREMENT OF RESPIRATION RATE OF LIGHT RED STAGE TOMATOES (*Lycopersicon esculentum* Mill.) AND ITS MODELING

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Abstract- Respiration rate of tomatoes at light red stage was measured at several storage temperatures ranging from 10°C to 30°C using closed system respirometer. The study involved the quantification of the effect of time, temperature and gas concentration on the respiration rate of tomatoes and the models were developed, showing the relationship among them. The experimental data were fitted to mathematical models; Peleg model and an uncompetitive inhibition based enzyme kinetics model, for the prediction of respiration rate within the given experimental range. The temperature dependence of the model parameters was established by Arrhenius equation. The suitability of models to predict the respiration rate was examined with the help of relative deviation modulus by calculating the difference between the actual and the predicted respiration rates at 12°C. The difference (relative deviation modulus) between the predicted and the experimental respiration rates was found to be 8.3% and 9.2% for oxygen (O_2) and carbon dioxide (CO_2) respectively respiration expressions for regression model and 7.5% and 8.4% for O_2 and CO_2 for uncompetitive inhibition based enzyme kinetics model, respectively.

Keywords- Tomato, Respiration rate, Peleg model, Enzyme kinetics, Modeling

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Introduction

Tomato is globally the second most important vegetable crop afterthe potato. With an annual production of 18.2 MT during the year 2013, India ranks 2nd contributing around 2.46% in total world production (163 MT) of tomato [4].Tomato is a major contributor of carotenoids (especially lycopene), phenolics, vitamin C and small amounts of vitamin E in daily diets [19]. In order to prolong their availability in the market throughout the year, the perishable commodities like tomato [17, 23] need to be handled and stored with due care. Certain postharvest constraints, including short shelf life due to respiration, moisture loss, ripening and susceptibility to diseases limit their long duration storage and transportation. These physiological and biochemical activities are responsible for the deterioration of the fruits and vegetables, and the deterioration starts right from the moment they are harvested [11].

Researchers have extended the shelf life by studying the influence of altered atmospheres such as modified atmosphere and controlled atmosphere (MA and CA) of the commodity in storage. The basic underlying principle in the above techniques is the careful alteration and manipulation of respiration rate that can help in achieving the extended shelf life of the commodity. Modified atmosphere storage increases the shelf life of the fresh produce by the reducing the moisture loss, browning and senescence by altering the respiration rate and thereby increasing the shelf life. Depending on the specific requirements, MA and CA can extend market availability of the produce up to the next harvest [16].

A number of models were proposed by researchers for the prediction of respiration rate of various fruits and vegetables, but the Peleg model and uncompetitive inhibition based enzyme kinetics were the commonly used [2, 5, 13, 16]. The majority of the models has been developed by either considering the respiration rate as a function of the gas concentration or the time elapsed [3, 5, 9, 13, 15, 22, 24]. Yang and Chinnan [24] studied the effect of gas concentration and storage time on the respiration rates of tomato. The effect of temperature was not considered in their models. Among the models, the most theoretically based model is the Michaelis–Menten equation, which assumes that the process of respiration is a one limiting enzymatic reaction with oxygen being the substrate. Model parameters are suitably modified for temperature dependence based on linear interpolation or using an Arrhenius type of relationship [6, 12]. Hence, it is difficult to formulate the model, which includes all the factors that influence the respiration rate.

Since factors like mechanical damage, ageing, maturity stage at harvest, product type and variety, temperature, gas composition affects the respiration rate of fresh produce; the respiration rate needs to be quantified for each fruit and vegetable product, due to the specificity of the model parameters [8, 25, 26]. It is required to take all these factors into consideration in order to correctly predict the respiratory behavior of any commodity. The measurement and modeling of respiration rate kinetics of tomatoes (cv. Roma) at the breaker stage have been reported [10] with respect to temperature only. However, the respiration rate of fresh produce greatly varies with the gas concentration and also with the stage of maturity and variety of the produce. Singh *et al*., 2013 investigated the respiratory behavior of turning stage mature tomato, but they have not studied the kinetic modeling of the respiration rate. The literature that provides some information regarding the respiratory behavior of tomatoes, but its kinetic modeling in the light red stage (USDA color scale 5) of tomatoes is scarce.

In the present study, effect of time, temperature and concentration of gases were compared to verify the effect of each one of these parameters on the respiration rate. The present study is targeted particularly on tomatoes (*Lycopersicon esculentum cv*. Vaishali) at light red stage and aimsto determine the effect of

temperature and gas concentration on the respiratory behaviour and to develop regression models for the prediction of respiration rate of fresh tomatoes as a function of O_2 and CO_2 concentration storage temperature and time.

For the sake of simplicity and to prevent confusion, the model parameters for the enzyme kinetics model may be mentioned as V_m, K_m and K_i when mentioned in their generalized forms instead of writing all their forms separately, such as V_{mO2}, V_{mCO2ox} and V_{mCO2f} for V_m and K_{mO2} and K_{mCO2} for K_m and K_{iO2}, K_{iCO2ox}. And K_{iCO2f} until mentioned otherwise.

Materials and Methods

Respiration kinetics of tomatoes (Lycopersicon esculentum Mill.*)*

Tomato fruits (USDA color scale 5 for tomatoes) were purchased from the local market and were washed to remove adhering dirt before using for the experiment. The tomatoes procured were of uniform size, mass and maturity (depending on color of tomatoes) and varied from 0.052 ± 0.008 m in circumference and 80.36 ± 13.37 g in weight (measurements were made for 10 representative samples). The physico-chemical parameters like moisture content – 91.43 ± 1.02%, total soluble solids (TSS) – 2.86 ± 0.06°Bx, titratable acidity – 3.05 ± 0.04%, color (L*, a*, b) – 41.74 ± 3.16, 25.25 ± 1.23, 29.28 ± 2.25 and pH – 4.03± 0.06 were evaluated before conducting experiments to ensure the same maturity levels.

Experiments were conducted at five different storage temperatures (10°C, 15°C, 20°C, 25°C and 30°C) using closed system respirometer 2, 16, 25]. Tomatoes were kept in respirometer, which were kept in a controlled test chamber with a tolerance limit of ± 0.5°C. Gas composition of respirometer was analyzed at regular intervals depending on the storage temperature of tomatoes. The sampling interval was varied depending on the rate of respiration at different temperatures, as at higher temperature the rate of evolution of CO_2 and consumption of O_2 was faster, therefore, the sampling was done at shorter intervals and vice versa [26]. The headspace analyzer comprises of a zirconium sensor for O_2 determination and an infrared detector to detect CO_2. The change in gas concentration was measured until the CO_2 and O_2 concentrations reached 18% and reached 0%, respectively. Responses (in triplicates) namely R_{CO2}, R_{O2} and RQ were studied with respect to the changes in temperature and time.

Modeling and data analysis

The experimental respiration rate was calculated from the concentration difference, mass of produce and free volume of the chamber; the respiration rates in terms of O_2 and CO_2 at a given temperature were expressed using the [Eq-1-3] [11].

$$R_{CO_2} = \left[\frac{\left(G_{CO_2}\right)_{t+1} - \left(G_{CO_2}\right)_t}{\Delta t}\right]\frac{V_{fr}}{W} \quad [1]$$

$$R_{O_2} = \left[\frac{\left(G_{O_2}\right)_t - \left(G_{O_2}\right)_{t+1}}{\Delta t}\right]\frac{V_{fr}}{W} \quad [2]$$

$$RQ = \frac{R_{CO2}}{R_{O2}} \quad [3]$$

Where, R_{O2} is the O_2 consumption rate, mL O_2 kg^{-1} h^{-1}; R_{CO2} is the CO_2 evolution rate, mL CO_2 kg^{-1} h^{-1}; G_{O2} and G_{CO2} are the gas concentrations of O_2 and CO_2, respectively; t is the storage time in hours; Δt is the time difference between two gas measurements; V_{fr} is the free volume of the respiration chamber in mL and W is the mass of the fruit in kg. Mass of tomatoes and free volume of respirometer taken during the experiment were 0.5678 ± 0.05 kg and 1262 ± 67 mL, respectively. The volume of tomatoes was measured by water displacement method and free volume was calculated by subtracting the volume of tomatoes from the total volume of respirometer.

Statistical analysis

The experimental respiration data were subjected to analysis of variance (ANOVA) to establish the effect of temperature on the respiration rate of tomatoes ($p > 0.05$).

Model 1

Experimental respiration data were used to perform nonlinear regression analysis (OriginPro 8.5) to fit O_2 concentration and CO_2 concentration at different storage time periods. Slope of the plot between gas concentration and time, theoretically gives the respiration rate [26]. However, this method is not recommended since data sets have large experimental variations [16]. Therefore, a regression function [Eq-4 and 5] was used to fit the gas concentration data versus time and the rate of change of gas concentration was determined from the first derivative of the regression functions as outlined in [Eq-6 and 7].

$$G_{CO_2} = \frac{t}{(at+b)} \quad [4]$$

$$G_{O_2} = 0.21 - \left[\frac{t}{at+b}\right] \quad [5]$$

GCO_2 and GO_2 are the gas concentrations of CO_2 (%) and O_2(%), respectively, t is the time in hour, a(h%$^{-1}$) is a Peleg rate constant and b(%$^{-1}$) is a Peleg capacity constant. The rate of change of gas concentration was determined from the first derivative of the regression functions as outlined in [Eq-6 and 7] and then substituted in [Eq-8 and 9] to calculate the respiration rate of tomatoes at any given conditions with the experimental range.

$$\frac{dG_{CO_2}}{dt} = -at(at+b)^{-2} + (at+b)^{-1} \quad [6]$$

$$\frac{dG_{O_2}}{dt} = at(at+b)^{-2} - (at+b)^{-1} \quad [7]$$

$$R_{CO_2} = \frac{d\left[G_{CO_2}\right]}{dt}\frac{V_{fr}}{W} \quad [8]$$

$$R_{O_2} = -\frac{d\left[G_{O_2}\right]}{dt}\frac{V_{fr}}{W} \quad [9]$$

A similar model was applied to respiration data of banana [2] and for apples [14] and other products [2, 5, 16, 25]. According to the previous studies, the values of model parameters at given temperature could hitherto be predicted by linear interpolation of the values. Therefore, linear regression analysis was performed on the values of model parameters at different temperatures and the equations found can be used to calculate the value of model parameters (a and b for both CO_2 evolution and O_2 consumption) at given temperatures.

Model 2

A model based on uncompetitive inhibition based enzyme kinetics model [Eq-10 and 11] was also fitted to the experimental data [13] Peppelenbos and Van't Leven, 1996). Although there are other models as well based on enzyme kinetics like competitive inhibition based and non-competitive inhibition based, this model was chosen on the basis of consideration that CO_2 acts as a respiratory inhibitor. But the production of CO_2 is attributed to two processes - a fermentative part, which is inhibited at high O_2 concentrations, and an oxidative part, almost negligible at very low O_2 concentrations [6] Lammertyn et al. 2003; Ho et al. 2010). The first part in [Eq-11 and 13] is CO_2 production by oxidative respiration and the second part is by the fermentation. The linearized forms [Eq-12 and 13] of the [Eq-10 and 11] were fitted to the gas concentrations at different storage time using multiple linear regression analysis (OriginPro 8.5).

$$R_{O_2} = \frac{V_{m(O_2)} \times G_{O_2}}{K_{m(O_2)} + \left[1 + \frac{G_{CO_2}}{K_{i(O_2)}}\right] G_{O_2}} \quad [10]$$

$$R_{CO_2} = \left[\frac{V_{m(CO_2)} G_{O_2}}{K_{m(CO_2)} + \left(1 + \frac{G_{CO_2}}{K_{i(CO_2)}}\right) G_{O_2}}\right] + \left[\frac{V_{m(CO_2)f}}{\left(1 + \frac{G_{CO_2}}{K_{i(O_2)f}}\right)}\right]$$

or

$$R_{CO_2} = RQ \times RO_2 + \frac{V_{m(CO_2)f}}{\left(1 + \frac{G_{CO_2}}{K_{i(O_2)f}}\right)} \quad [11]$$

$$\frac{1}{R_{O_2}} = \frac{1}{V_{m(O_2)}} + \left(\frac{K_{m(O_2)}}{V_{m(O_2)}} \times \frac{1}{G_{O_2}}\right) + \left(\frac{1}{K_{i(O_2)} \times V_{m(O_2)}} \times G_{CO_2}\right) \quad [12]$$

$$\frac{1}{R_{CO_2}} = \left[\frac{1}{V_{m(CO_2)}} + \left(\frac{K_{m(CO_2)}}{V_{m(CO_2)}} \times \frac{1}{G_{O_2}}\right) + \left(\frac{1}{K_{i(CO_2)} \times V_{m(CO_2)}} \times G_{CO_2}\right)\right] + \left[\frac{1}{V_{m(CO_2)f}} + \left(\frac{1}{V_{m(CO_2)f} \times K_{i(O_2)f}} \times G_{CO_2}\right)\right] \quad [13]$$

Where, $V_{m(O2)}$ is the maximum O_2 consumption rate; $V_{m(CO2)}$ and $V_{m(CO2)f}$ are the maximum CO_2 production rates by oxidative respiration and fermentation (mL.kg^{-1}.h^{-1}) respectively; K_{mO2} and K_{mCO2} are the Michaelis-Menten constants for O_2 consumption and CO_2 evolution (%O_2), respectively; and K_{iO2} and K_{iCO2} are the inhibition constants for O_2 consumption and CO_2 evolution (%CO_2), respectively, $K_{i(O2)f}$ is the inhibition constant of O_2 on fermentative CO_2 production rate (%CO_2); G_{CO2} and G_{O2} are gaseous concentrations of CO_2 and O_2 (%). The model parameters, thus obtained were correlated at different temperatures using Arrhenius equation [2, 16]. Therefore, the Arrhenius equation was used in its linear form to overcome the high correlation introduced, as a result of exponentiation of the reciprocal of the absolute temperature, which otherwise, makes the estimation of parameters difficult [16,26].

In order to further minimize the high correlation among the parameters of the Arrhenius equation, reparameterization was carried out as suggested by Schwaab and Pinto [21]. The reparameterization can be attained by involving a new temperature term, T_{ref} into the Arrhenius equation, which is the reference temperature and is the average temperature of analyzed experimental range. The proper selection of the reference temperature [Eq-14] allows estimation of uncorrelated parameters and simultaneous improvement of the precision of the parameter estimates in problems involving a single kinetic constant [21].

$$R_m = R_p \exp\left[\frac{-E_a}{R} \times \left(\frac{1}{T_{abs}} - \frac{1}{T_{ref}}\right)\right] \quad [14]$$

R_m is the generalized form of model parameters (i.e. V_m, K_m, *and* K_i), R_p is respiration pre-exponential factor, E_a is the activation energy (kJ.g^{-1}.mol^{-1}), R is the universal gas constant (kJ g^{-1} mol^{-1} K) and T_{abs} is the absolute temperature (K) and T_{ref} is the reference temperature (K).

Verification of the Models

It is important to validate suitability of the developed models for prediction of the respiration rate within the experimental domain, which was examined by calculating the difference between the actual and the predicted respiration rates using the relative deviation modulus [Eq-15]. Since, the experiments were conducted from 10°C to 30°C, validation of the model was conducted at 12°C storage temperature using the closed system respirometer method as reported above. The experimental respiration rate of the tomatoes in terms of CO_2 and O_2 was calculated using [Eq-1 and 2] respectively. Moduli below 10% are indicative of reasonably close fit, 10-20% fairly close fit and 20-30% not satisfactory fit for all practical purposes.

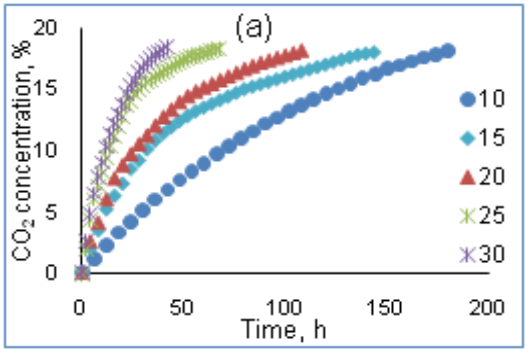
$$E = \frac{100}{N} \sum_{1}^{N} \frac{\left|(R_{\exp} - R_{pre})\right|}{R_{\exp}} \quad [15]$$

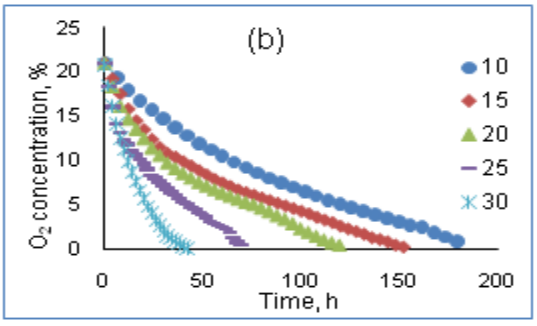
Where, E is the average relative deviation modulus, %; N is the number of respiration data points; R_{exp} is the experimental respiration rate, mL kg^{-1} h^{-1} and R_{pre} is the predicted respiration rate, mL kg^{-1} h^{-1}. In general, lower modulus shows the closer agreement between predicted and experimental values. The free volume and weight of the tomatoes, taken for the validation were 1235 mL and 0.511 kg, respectively.

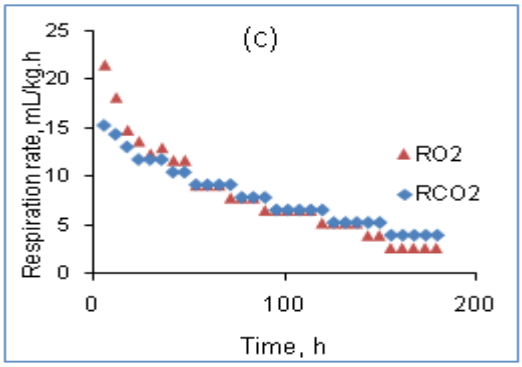
Results and Discussion

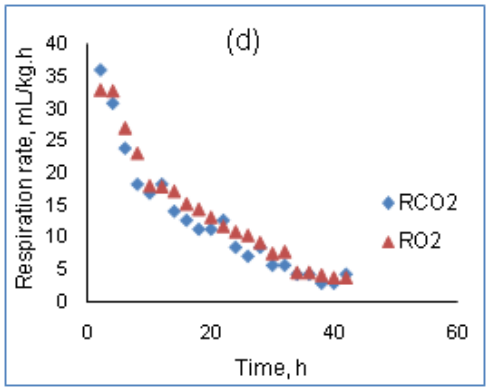
The respiration rate increased considerably with the rise in temperature and it was recorded as 15.21 ± 0.26 and 18.98 ± 0.14 at 10°C and 48.5 ± 0.65 and 32.12 ± 0.06 at 30°C in terms of CO_2 evolution (mL of CO_2 kg^{-1} h^{-1}) and O_2 consumption (mL of O_2 kg^{-1} h^{-1}), respectively. Moreover, the gas concentrations reached their upper limits CO_2>18% and O_2 ~ 0% in 186 h at 10°C and in 42 h at 30°C [Fig-1a and 1b], similar trend was reported by Saltveit [20].

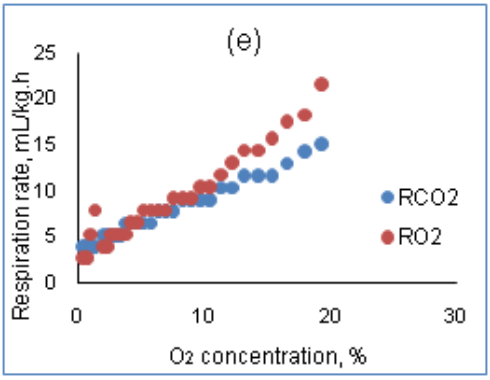
Though the respiration rate increased with the increase in temperature, it followed a decreasing trend with the progress in time due to the accumulation of CO_2 concentration and the decrease in O_2 concentration inside the respirometer chamber [2, 25]. At the outset of the experiment, the respiration rate was very high (15.21 and 21.58) and reduced as the time progressed [Fig-1c and 1d] to 3.9 and 2.6 (mL kg^{-1} h^{-1}) in terms of CO_2 evolution and O_2 consumption, respectively, after 186 h because of the accumulation of CO_2(18.5%) and reduction of O_2 to 0.35% at 10°C. This can be attributed to the inhibitory effect of accumulated CO_2 on the rate of respiration as evident from [Fig-1(g) and 1(h)]. Similar results were reported in earlier studies [2, 16].

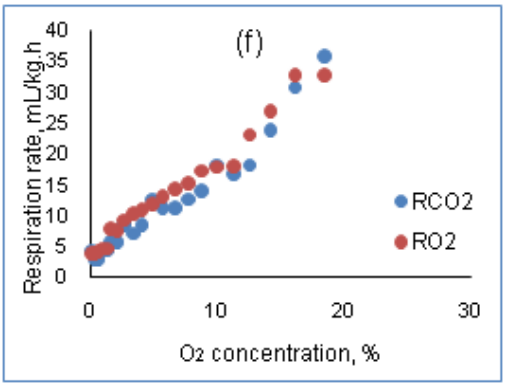


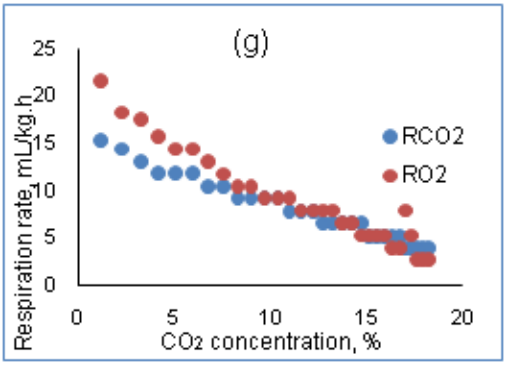












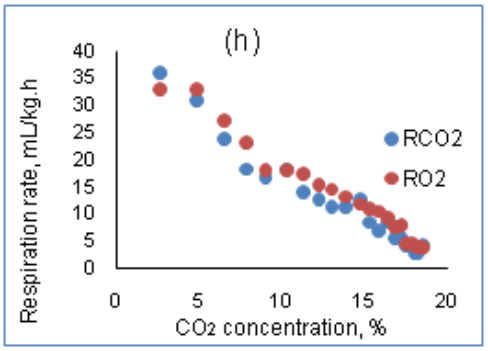


Fig-1 Change in gas concentration inside the respirometer chamber with change in temperature (a) evolution of CO_2 and (b) consumption of O_2. Change in respiration rate of tomatoes with time (c) at 10°C, (d) 20°C. Change in respiration rate of tomatoes with change in O_2 concentration at (e) 10°C and (f) 30°C and with change in CO_2 concentration at (g) 10°C and (h) 30°C. Data corresponds to the average of the independent triplicates for all the figures.

The O_2 concentration inside the chamber also affected the respiration rate of tomatoes, both in terms of CO_2 evolution as well as O_2 consumption [Fig-1e and 1f]. In the beginning when the O_2 concentration inside the chamber was 15%, the respiration rate was 11.7 mL kg^{-1} h^{-1} and later when O_2 concentration declined to 0.4% the respiration rate became 3.9 mL kg^{-1} h^{-1}, in terms of CO_2 evolution, respectively at 10°C storage temperature. Similarly, at the same O_2 concentration respiration rate was found to be 15.6 and 2.9 mL kg^{-1} h^{-1} in terms O_2 consumption, respectively.

The analysis of variance (ANOVA) test was conducted on the means of the average respiration rate (average of respiration rates from time, $t = 0$ to $t = t$) of all the temperatures. From the result of overall ANOVA, it can be inferred that at least two of the five temperatures were found to have significantly different means of respiration rate, since the p-value was found to be 0.001 i.e. >0.05. To evaluate further, means of all the levels were compared with the Tukey's test and respiration rate means of all the temperatures were found to differ with each other, significantly ($p < 0.05$). Therefore, it can be concluded that the respiration rate of the tomatoes increased significantly ($p < 0.05$) with the increase in temperature. In order to determine whether all the five temperatures showed equal variation, Levene' test for homogeneity of variance was performed. One-way ANOVA result showed that the degree of variance among all the five groups was not equal (the experimental p-value 0.3474 > 0.05), therefore, increase in respiration rate was not regular with increase in temperature and vice versa.

Peleg model

The values of model parameters *a* and *b* of the regression equation indicated the temperature dependence of both the parameters, whereas temperature had a more pronounced influence on *b* than *a*. The model parameter *a*, corresponds to rate constant that explains the influence of consumption and evolution rate of gases in the initial stages of respiration. Whereas, *b*, is the capacity constant that relates to the attainable gas concentrations by the system when the time approaches to infinity and at that moment the above equations establish the relation between the equilibrium gas content and *b*. The values of *a* and *b* obtained in this study, were found to have similar trends of the parameters reported for respiration of banana [2] and apples [14]. The values of regression coefficients *a* and *b* of [Eqs-4 and 5], and correlation coefficients (R^2) at different storage temperatures are presented in [Table-1]. Since the R^2> 0.992 (Adjusted R^2), the regression functions suitably fitted the experimental data for tomatoes.

The values of model parameters *a* and *b* of different storage temperatures were subjected to regression analysis and were found to follow the linear model for parameter *a*(R^2 = 0.97 and 0.99 for CO_2 and O_2 expressions, respectively) and second order polynomial model for parameter *b*, (R^2 = 0.99 and 0.98 for CO_2 and O_2 expressions, respectively). The regression equations found can be used for the prediction of model parameters of Peleg equation at any temperature. The [Eq-6 and 7] can be used to estimate dGO_2/dt and $dGCO_2/dt$, respectively, by

substituting the corresponding values of *a* and *b*, that can further be substituted in the [Eq-8 and 9] for the prediction of respiration rate.

Enzyme kinetic model

The multiple linear regression analysis was used to estimate the model parameters [Table-2]. The model parameters showed temperature dependence which was established by fitting the Arrhenius model on the values of model parameters against the inverse of absolute temperature. All the model parameters (R_m) such as V_m, K_m, and K_i for O_2 and CO_2 (both oxidative as well as fermentative) were plotted as a natural logarithm against the difference of inverse of absolute temperature and the reference temperature in the linear form of [Eq-14]. The values of slope (E_a/R) and *y*-axis intercept (ln R_p) were used to calculate the values of the activation energy and respiration pre-exponential factor [Table-3].

In case of competitive inhibition, the inhibition by CO_2 can be counteracted by increasing O_2 because both the gases (O_2 and CO_2) are competing for the same active site. But in case of uncompetitive inhibition, the CO_2 reacts with enzyme substrate complex, so by increasing the concentration of O_2 will not be able to counteract CO_2 as there is always some amount enzyme-substrate complex available for CO_2 to bind, therefore, the maximum respiration rate (V_m) would never be achieved until the presence of CO_2 or at the beginning of the experiment when CO_2 is very low. Temperature dependence of the model parameters was established by the increase in their values with the temperature and the results were in agreement with the existing literature [14, 16, 26].

Table-1 *Regression coefficients a and b of Peleg model at different storage temperatures for consumption of O_2 and evolution of CO_2 respectively*

Storage temperature, °C	Respiration expression in terms of	Regression coefficients		Coefficient of determination (Adj. R^2)
		a $h\%^{-1}$	*b* $\%^{-1}$	
10	CO_2 evolution O_2 consumption	2.651 (0.13) 3.256 (0.16)	114.58 (2.39) 117.93 (3.18)	0.999 0.999
15	CO_2 evolution O_2 consumption	3.472 (0.03) 3.783 (0.11)	97.24 (3.21) 98.43 (4.38)	0.999 0.999
20	CO_2 evolution O_2 consumption	4.194 (0.07) 4.452 (0.19)	77.23 (3.03) 81.44 (2.66)	0.999 0.999
25	CO_2 evolution O_2 consumption	4.373 (0.12) 4.774 (0.09)	64.54 (5.84) 65.49 (6.28)	0.997 0.991
30	CO_2 evolution O_2 consumption	4.668 (0.07) 5.481 (0.11)	46.17 (2.36) 42.13 (1.89)	0.985 0.997

Values are average of triplicate measures and the value in parentheses is the standard deviation

Table-2 *Model parameters for uncompetitive inhibition enzyme kinetics based model at different storage temperatures*

Parameters	Storage Temperature (°C)				
	10°C	15°C	20°C	25°C	30°C
Enzyme kinetics coefficients for RO_2					
$V_{m(O_2)}$ (mL kg^{-1} h^{-1})	34.52 (2.22)	39.52 (2.67)	58.63 (3.95)	68.72 (4.32)	88.55 (4.28)
$K_{m(O_2)}$ (% O_2)	9.83 (0.26)	10.44 (0.48)	13.51 (0.27)	14.82 (0.35)	15.89 (0.21)
$K_{i(O_2)}$ (% CO_2)	8.29 (0.32)	6.16 (0.36)	5.85 (0.35)	4.83 (0.28)	4.61(0.15)
Adj. R^2	0.96	0.95	0.95	0.98	0.98
Enzyme kinetics coefficients for oxidative part of RCO_2					
$V_{m(CO_2)}$ (mL kg^{-1} h^{-1})	23.59 (1.23)	36.23 (3.14)	51.39 (4.31)	64.29 (5.01)	80.59 (3.59)
$K_{m(CO_2)}$ (%)	7.09 (0.34)	9.94 (0.55)	11.28 (0.34)	13.58 (0.26)	15.61 (0.45)
$K_{i(CO_2)}$ (% CO_2)	9.50 (0.24)	7.26 (0.41)	6.97 (0.29)	5.27 (0.26)	4.94 (0.18)
Adj. R^2	0.96	0.94	0.96	0.92	0.95
Enzyme kinetics coefficients for fermentative part of RCO_2					
$V_{m(CO_2)f}$ (mL kg^{-1} h^{-1})	19.37 (1.06)	27.83 (4.23)	48.46 (6.78)	72.27 (14.25)	102.67 (21.23)
$K_{i(O_2)f}$ (% CO_2)	6.79 (0.73)	5.43 (0.86)	5.24 (1.21)	3.98 (0.67)	3.39 (0.64)
Adj. R^2	0.91	0.85	0.86	0.96	0.86

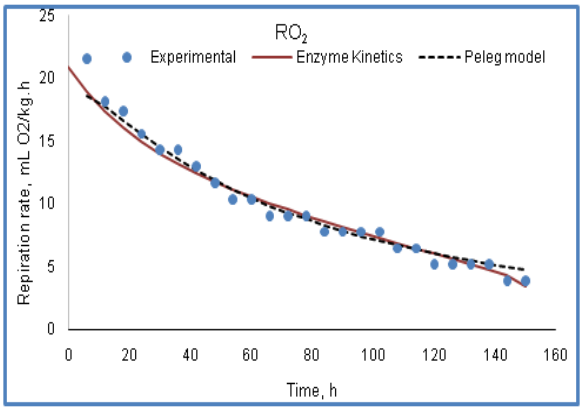
Values are average of triplicate measures and the value in parentheses is the standard deviation

Table-3 *Slope (E_a/R) and Y-axis intercept (ln R_p) of equation and coefficient of determination (R^2), Activation energy and pre-exponential factor for Arrhenius equation for different model parameters for uncompetitive type enzyme kinetics model*

Slope and Y-axis intercept of equation	Maximum respiration rate (V_m), mL.kg^{-1}.h^{-1}			Michaelis-Menten constant (K_m), % O_2		Inhibition constant (K_i), % CO_2		
	O_2	CO_2.ox[a]	CO_2.f[b]	O_2	CO_2	O_2	CO_2.ox	CO_2.f
Slope	-3996	-5217.5	-7368	-3255	-2254	2442.6	2799.7	2914
Y-axis intercept	3.987	3.859	3.8311	2.413	2.544	-1.756	-1.881	-1.567
R^2	0.956	0.983	0.995	0.971	0.948	0.931	0.952	0.965
Activation energy, E_a, kJ.g^{-1}.mol^{-1}	33.22	43.38	61.25	27.06	18.74	-20.31	-23.28	-24.22
Pre-exponential factor, R_p	53.89	47.41	46.11	11.17	12.73	0.173	0.15	0.208

CO_2.ox is the CO_2 production from the oxidative part and CO_2. f is the CO_2 production from fermentative part

K_m is the measure of saturation of respiration by O_2 at which V_m becomes half (1/2 V_m) when there is no inhibition by CO_2. The value for K_i is a measure of the degree to which respiration (both in terms of CO_2 production and O_2 consumption) can be inhibited by CO_2. The higher values of K_i show the backward reaction is faster than the forward reaction and hence, there is no inhibition in respiration due to CO_2 [6].The values of $K_{i(CO2)}$ and $K_{i(O2)}$ for tomatoes indicated that the respiration was inhibited by CO_2 and the values were similar to the literature [2, 16]. The high values of parameter K_{icO2f}, which signifies the extent to which fermentation can be inhibited by O_2 indicate that the tomatoes show fermentative CO_2 production at even higher levels of O_2. The negative value of activation energy for parameter K_i indicated that the temperature had a negative effect on the inhibition by CO_2 that means with the increase in temperature the inhibitory effect of CO_2 was less pronounced [2]. These constants can be used to predict the values of model parameters of [Eq-8 and 9] at any temperature, and which in turn can be used to predict the respiration rate for any given combination of gaseous concentration i.e. CO_2 and O_2.



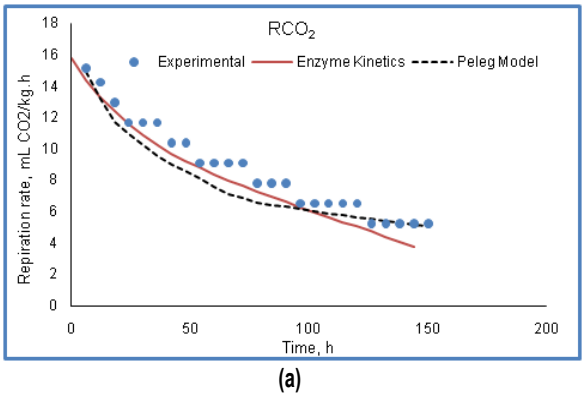
(b)

Fig-2 Predicted and experimental respiration rate of tomatoes at 10°C in terms of (a) CO_2 and (b) O_2.

Verification of the model

The suitability of models to predict the respiration rate was examined by using the relative deviation modulus [Eq-15] by calculating the difference between the actual and the predicted respiration rates at 12°C storage temperature using the closed system respirometer method as reported above (free volume of the chamber – 1235 ± 43 mL and weight of tomatoes – 0.511 ± 16 kg). The actual respiration rates of tomatoes in terms of O_2 consumption and CO_2 evolution were estimated by putting experimental data into [Eqs-1 and 2]. The predicted respiration rate was calculated by using [Eq-4-9] for Peleg model and [Eq-10-11] for enzyme kinetics model.

The predicted and actual respiration rates of tomatoes in terms of CO_2 evolution and O_2 consumption are shown in [Fig-2 (a and b)], respectively. The difference (relative deviation modulus) between the predicted and the experimental respiration rates was found to be 8.3% and 9.2% for oxygen (O_2) and carbon dioxide (CO_2) respiration expressions for regression model and 7.5% and 8.4% for O_2 and CO_2 for uncompetitive inhibition based enzyme kinetics model, respectively, which suggested both the models fit fairly well. Although the predicted respiration rates of tomatoes for both the models used in the study were in close agreement with the experimental respiration rates.



(a)

The study suggested that the uncompetitive inhibition based enzyme kinetics model showed more uniformity in the prediction of respiration rate in terms of closeness to the actual respiration rate over the entire experimental temperature range. Also the uncompetitive inhibition based enzyme kinetics model is more generic and complete model and could be fully predictive and extrapolated out of current boundary conditions (i.e. different levels of O_2 and CO_2 concentrations). Although the respiration is affected more by gas concentration and temperature, but the time is also an important factor that affects the respiration rate [1] and, therefore, the Peleg model was also used for modeling the respiration rate.

Conclusion

The respiration rate of the tomatoes increased with temperature, but it decreased with the storage time as compared to the initial respiration rate at time zero. This trend could be attributed to the diminishing amounts of O_2 and accumulation of CO_2. The models developed are specific to the cultivar used for tomatoes at light red stage and are, therefore, valid only within the temperature range where experiments were conducted i.e. from 10°C to 30°C storage temperatures. At a given storage temperature range of the study (10°C to 30°C)the pre-exponential factor and activation energy of Arrhenius equation were found to be useful for predicting model parameters. There was a good agreement between experimental and predicted respiration rate at 12°C storage temperature. The information obtained regarding the respiration rate and the developed models would be of immense help in designing suitable postharvest storage and handling techniques like MAP and AP system for the tomatoes at light red stage.

Abbreviations

a	Peleg rate constant, h %$^{-1}$
b	Peleg capacity constant, %$^{-1}$
E	mean relative deviation modulus, %
E_a	activation energy, kJ g^{-1} mol^{-1}
G_{CO2}	carbon dioxide concentration, %
G_{O2}	oxygen concentration, %

$K_{i(CO2)}$	inhibition constant for CO_2 evolution, % CO_2
$K_{i(O2)}$	inhibition constant for O_2 consumption, % CO_2
$K_{m(O2)f}$	inhibition constant of O_2 on fermentative CO_2 production rate, % CO_2
$K_{m(CO2)}$	Michaelis–Menten constant for CO_2 evolution, % O_2
$K_{m(O2)}$	Michaelis–Menten constant for O_2 consumption, % O_2
N	number of respiration data points
R	universal gas constant, 8.314, kJ g^{-1} mol^{-1} K
R	universal gas constant, 8.314, kJ g^{-1} mol^{-1} K
R_{CO2}	respiration rate, mL [CO_2] kg^{-1} h^{-1}
R_m	model parameter for Michaelis–Menten equation
R_{O2}	respiration rate, mL [O_2] kg^{-1} h^{-1}
R_p	respiration pre-exponential constant factor
T	storage temperature, ºC
t	storage time, h
T_{abs}	absolute temperature, K
T_{ref}	reference temperature, K
Δt	time difference between two gas measurements, h
V_{fr}	free volume of the respiration chamber, mL
$V_{m(CO2)}$	maximum oxidative respiration rate for CO_2 evolution, mL kg^{-1} h^{-1}
$V_{m(O2)}$	maximum fermentation rate for O_2 consumption, mL kg^{-1} h^{-1}
$V_{m(CO2)f}$	maximum respiration rate for CO_2 evolution, mL kg^{-1} h^{-1}
W	mass of fruit, kg

Authors' Contributions

Billoria conceptualized and designed the work; collected, analyzed and interpreted data; Patel revised the drafted manuscript; Mishra gave technical support, conceptual advice and final approval of the manuscript to be published.

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Conflict of Interest: None declared

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