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Estimation of Respiratory Dynamics of Fresh Green Chilli (*Capsicum annuum* L.)

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ABSTRACT

The respiratory behavior of fresh green chillies under different temperatures were evaluated to study its respiratory dynamics based upon enzyme kinetics. The dependence of respiration rate on the O_2 and CO_2 concentrations was described assuming the enzyme kinetics model for combined type of inhibition caused by CO_2. Various respiratory parameters viz. maximal O_2 consumption rate Michaelis-Menten constants, inhibition constants under the specified environmental conditions of 5, 10, 15°C and 85% relative humidity (RH) were estimated using non-linear regression technique. It was found that storage temperature had substantial affect on the partial pressures within the closed containers and subsequently the respiration rates. The concentration of O_2 inside the container and surrounding temperature had synergistic effect on the rate of respiration and respiratory parameters. Though the inhibition by evolved CO_2 was observed to be of combined type, it was predominantly competitive at all temperatures. Thus, this study can be utilized for design of modified atmosphere packages for storage of fresh green chillies. It was observed that the respiration rate (oxygen consumption and carbon dioxide evolution rate) increased with increase in temperature.

Key words: *Respiration rate, Enzyme kinetics, Mixed inhibition, Green chillies, Modified atmosphere packaging*

INTRODUCTION

Chilli ("*Capsicum annuum* L") belongs to the family *Solanaceae*, are herbaceous or semi-woody annuals or perennials. Fresh chilli is good source of vitamin A, vitamin B and vitamin C[1]. The high content of carotenoids is the reason for chilli's nutritional value because it acts as pro vitamin A which after digestion is converted into vitamin A and it also imparts red colour to the ripe fruit[2]. Chillies are perishable in nature having limited shelf life and high susceptibility to postharvest problems like shriveling, wilting and are also susceptible to fungal infections caused by Botrytis cinerea and Alternaria alternate[3]. Because of their large surface to weight ratio, chillies are also prone to water loss and shriveling. The most effective method of maintaining quality and controlling decay of peppers is by rapidly cooling post-harvest followed by storage at low temperature with high relative humidity.

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However, chillies are very sensitive to chilling injury which limits storage temperature (i.e. above 10°C) [4]. On the other hand, without refrigeration, chillies turn colour and deteriorate in a few days as a result of rapid aging and parasitic infections. High moisture content of chillies creates high vapour pressure gradient across the skin of chillies leading to an increase in rate of transpiration and degradation in the physical quality of chillies in terms of physiological loss in weight, shriveling and firmness thus reducing its market value. The quality degradation can be controlled to a great extent by reducing its respiration rate since there exist an inverse relationship between respiration rate and shelf life of the commodity. Temperature control along with atmospheric modification is the key to maintain the quality of fresh produce and enhance its shelf life. Use of low O_2 and high CO_2 concentration and low temperature maintain sensorial as well as microbial quality of the crop[5].

Packaging of peppers in polymeric films has been reported to inhibit fruit respiration, delay ripening, decrease ethylene production, reduce chilling injury, retard softening, slow down compositional changes associated with ripening, maintain color and extend shelf-life[6]. These beneficial effects can be explained by the modified atmosphere created inside the package as well as the reduction in water loss. Modified atmosphere packaging (MAP) is a technique, where fresh food products are enclosed in gas-barrier materials using different gas combinations as per the requirement of the respective food products, to prolong their shelf-life[7]. Instead of air, a gas combination of reduced O_2, elevated CO_2, and a balance of N_2 is used to reduce respiration rate, ethylene production, decay and the physiological changes in the packaged produce in MAP. The design and generation of an optimum MA requires thorough understanding of the interaction between the various factors such as film characteristics (surface area, gas and water vapour permeability), temperature, free volume inside the package, weight and respiration rate of the produce and initial gaseous composition[8]. The MAP preserves the quality of food, and also improves overall cost effectiveness. It is a well established technology which along with low temperature storage helps in extending the shelf life and maintenance of quality of perishable produce by the way of creation of appropriate gaseous atmosphere around the surrounding of the produce packaged in plastic films. In recent years, a rapid growth of MAP for preservation of fresh-cut products has occurred[9].

Respiration rate is the measure of metabolic activity of fruit or vegetable tissue and is affected by crop physiology and its surrounding environment. Thus to monitor the respiration rate, it is necessary to monitor either the crop physiology or the surrounding environment which mainly comprises of O_2 and CO_2 concentrations, temperature and relative humidity. Respiration rate is dependent on multiple factors including storage temperature, gas composition, variety and maturity of the commodity.

All the factors that affect respiration are rather complex. The important parameters that can be studied experimentally are gaseous concentrations, temperature, time, etc.[10]. Linear relationships to describe the complex phenomenon of respiration in sharp contrast to the actual non-linearity of the enzyme kinetics reaction and its temperature dependence have been described by Michaelis-Menten type equation to demonstrate the relation between respiration and gas concentration. As respiration is strongly affected by temperature, Arrhenius relationship has been incorporated in the Michaelis-Menten type equation. Michaelis-Menten equation is the most general form of the enzyme kinetics relation that assumes no inhibition of O_2 consumption by the evolved CO_2. But actually, the evolved CO_2 may or may not inhibit the respiration reaction in some way or the other depending upon the storage conditions and produce characteristics.

In this study, the respiratory behavior of fresh chilli as affected by temperature has been evaluated and discussed in the following sections.

MATERIAL AND METHODS

Theoretical concept

Respiration can be defined as the metabolic process that provides energy for plant biochemical process. It is the process by which the stored organic materials (carbohydrates, proteins and fats) are broken down into simple end-products with the release of energy. Respiration is accompanied by release of heat according to the chemical reaction[11].

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 2816KJ$

Dependence of respiration rate on temperature

The respiratory behavior of fresh produce is found to have been influenced by the extrinsic factor temperature. It has been observed that metabolic reactions increase two or three fold for every 10^0C rise in storage temperature[12,13].

Respiration studies of fresh green chillies

Fresh green chillies of variety CH-1 was procured from local farmers of Ludhiana and brought to fruits and vegetables pilot plant, department of processing and food engineering, PAU, Ludhiana. The closed or static system was used for measuring respiration rate of freshly harvested chillies. Respiration rate experiment for the crop was conducted at different temperatures (5, 10, and 15^{o}C) with RH of 85%. For each experiment the volume of the sample crop kept in closed container was determined.

$$V_S = \frac{W_s}{\rho_s}$$

The void volume (V_V) of the impermeable glass container used for the respiration experiment was measured. The water displacement method was used for the determination of density of chillies[14] through the estimate of true volume of a known mass. The void volume (V_V) for each experiment was determined using the relationship.

$$V_V = V_T - V_S$$

The container neck was air-sealed by applying vacuum grease. Crop sample of 200 g was taken in each of the three containers. The impermeable glass containers were then kept in environmental chambers. Respiration experiment was conducted at 5, 10, and 15^{o}C ($\pm 1^o$C) with 85% RH ($\pm$5%).

The gaseous concentration of the container headspace was continuously measured at regular intervals using gas analyzer (Make: SYSTECH NSTRUMENTS, UK; Model: GASPACE Advance). The gaseous concentrations obtained in percent are converted into partial pressures. 1 atmospheric pressure (101.325 kPa) is equivalent to 100% gaseous composition in the atmosphere. Gas analyses were done till the difference between two consecutive concentrations in the headspace became almost constant.

$$R_{O_2} = \frac{(p^{in}_{CO_2} - p^{f}_{CO_2}) \times V_v}{100 \times W \times (t^f - t^i)}$$

$$R_{CO_2} = \frac{(p^{f}_{CO_2} - p^{in}_{CO_2}) \times V_v}{100 \times W \times (t^f - t^i)}$$

The headspace partial pressure and respiration rates were studied at selected storage temperature. The enzyme kinetics model parameters of determined respiration rates were determined by non-liner analysis (Graph Pad Prism).

RESULTS AND DISCUSSION

The respiratory dynamics of fresh chillies was studied in terms of headspace partial pressures of $p^{in}_{o_2}$ and $p^{in}_{co_2}$ inside the impermeable glass containers containing coriander samples and maintained at different temperatures (T_1=5°C, T_2=10°C, T_3=15°C). At all the temperatures, values of $p^{in}_{o_2}$ and $p^{in}_{co_2}$ decreased with the progress of time and came to steady state in 4-4.5 hours of storage. The rate of decrease of $p^{in}_{o_2}$ and increase of $p^{in}_{co_2}$, increased with the increase in environmental temperature as shown in figure 1. The rates of respiration were higher at the start of the experiment and gradually decreased as the storage period increased before becoming almost constant. There was no significant difference in O_2 consumption rate and CO_2 evolution rate for all the temperatures. The partial pressure concentration of both O_2 and CO_2 remained within aerobic respiration range and no fermentation was observed. For fresh chillies the initial rate of oxygen consumption was 85.11, 112.13 and 125.64 ml kg^{-1} h^{-1} at start of the experiment and became constant at 23.04, 39.56 and 47.81 ml kg^{-1} h^{-1} for temperature from 5 to 15 ^{0}C, respectively as represented in figure 2. Whereas CO_2 evolution rate at start of experiment was 38.51, 58.77 and 65.53 ml kg^{-1} h^{-1} which stabilized at 23.04, 39.56 and 47.81 ml kg^{-1} h^{-1}. The difference in O_2 consumption and CO_2 evolution rate as observed from the plotted graph increased gradually with increase in temperature which indicates an increase in water vapor production. It can be put forward that moisture condensation may take place at higher temperature leading to spoilage.

The steady state O_2 and CO_2 evolution rate increased from by 50.33% and 71.66 % respectively when temperature increased from 5 to 10 ^{0}C whereas increase of 73.82% and 107.49 % was observed when temperature increased from 5 to 15 ^{0}C. The difference in O_2 consumption and CO_2 evolution rate as observed from the plotted graph (figure 2) increased gradually with increase in temperature, which indicates an increase in water vapour production. It can be put forward that moisture condensation may take place at higher temperatures leading to spoilage. The increase in temperature increases the metabolic activity in the commodity, which results in enhanced rate of respiration[15].

Respiration quotient

The respiration quotients (RQ) of chilli at experimental temperatures were determined from the calculated steady-state respiration rates of fresh chilli. The steady state respiration quotient values were found to be 0.52, 0.59, and 0.61 at 5, 10 and 15°C, respectively. As the time lapsed, RQ increased constantly and became stable as shown in figure 3.

Evaluation of respiration and inhibition using enzyme kinetics theory

The enzyme kinetics parameters, viz. $V_{m_{O_2}}$, $K_{m_{O_2}}$, $K_{mc_{CO_2}}$ and $K_{mu_{CO_2}}$ are determined by non-linear analyses of respiration data on the basis of enzyme kinetics equation for mixed or combined inhibition (15). The value of inhibition constants is a measure of the extent to which respiration can be inhibited by CO_2. The inhibition constants obtained by non-linear analyses of the respiration data shows that respiration of chilli was prone to combined inhibition ($K_{mc_{CO_2}}$ and $K_{mu_{CO_2}}$ are finite and unequal). The variability in inhibition of oxygen consumption rate of fresh green chillies by carbon dioxide with increase in temperature is shown in figure 4. At 5, 10 and 15°C, predominantly competitive type ($K_{mc_{CO_2}} < K_{mu_{CO_2}}$) of combined inhibition was observed. The Table 1 also shows the temperature dependence of all the parameters. Although, $V_{m_{O_2}}$ increased from 105 to 144.1 with an increase in temperature from 5°C to 15°C. Both $V_{m_{O_2}}$ and $K_{mu_{CO_2}}$ was observed to be less temperature-dependent than the other constants i.e. $K_{m_{O_2}}$, $K_{mc_{CO_2}}$, as expressed by its lower activation energy. $K_{mc_{CO_2}}$ was observed to be highly temperature dependent as compared to the inhibition constants. Other enzyme kinetics parameters have been assumed to be temperature independent. It is found that all the parameters related to chilli were temperature dependent and also the nature of dependence was different for all the parameters as given in table 2. The values of enzyme kinetic parameters are presented in table 1.

Temperature dependence of respiratory parameters

Temperature dependence of respiration rate (RO_2 and RCO_2) and RQ was evaluated by Arrhenius relationship for calculating activation energies for respiration rate and RQ. It was found that all parameters are highly temperature dependent as expressed by their higher activation energies as RO_2 (E_a= 22.02*10^7J/mol), RCO_2 (E_a = 16.51*10^7J/mol) and RQ (E_a = 6.6*10^6J/mol).

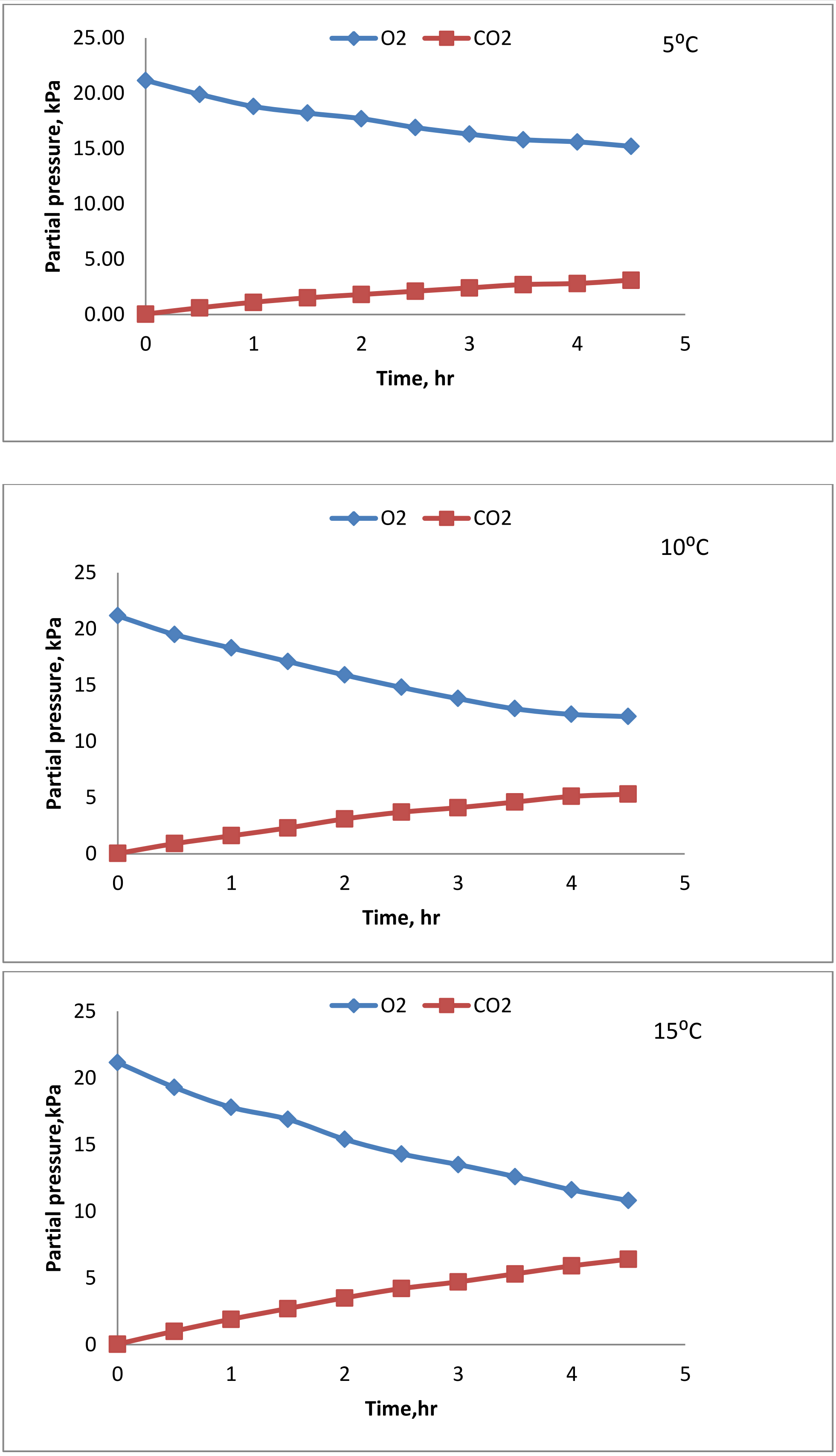

Fig. 1: Partial pressures of oxygen and carbon dioxide in the container head space during the closed system experiment for the measurements of the rates of oxygen consumption (RO_2) and carbon dioxide evolution (RCO_2) for fresh green chillies

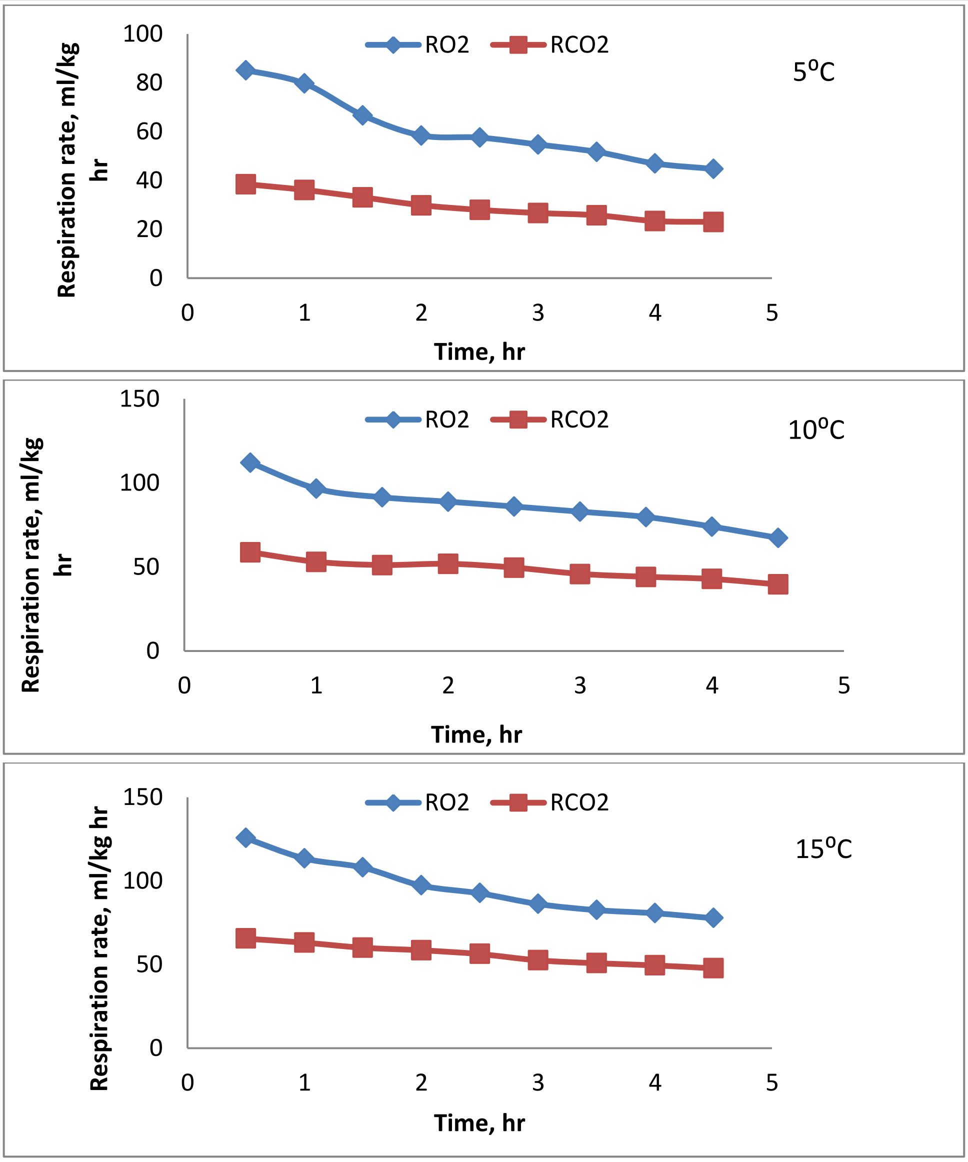

Fig. 2: Oxygen consumption rate and carbon dioxide evolution rate in the container headspace during the closed system experiment for the measurement of rates of oxygen consumption (RO_2) and carbon dioxide evolution (RCO_2) for fresh green chillies

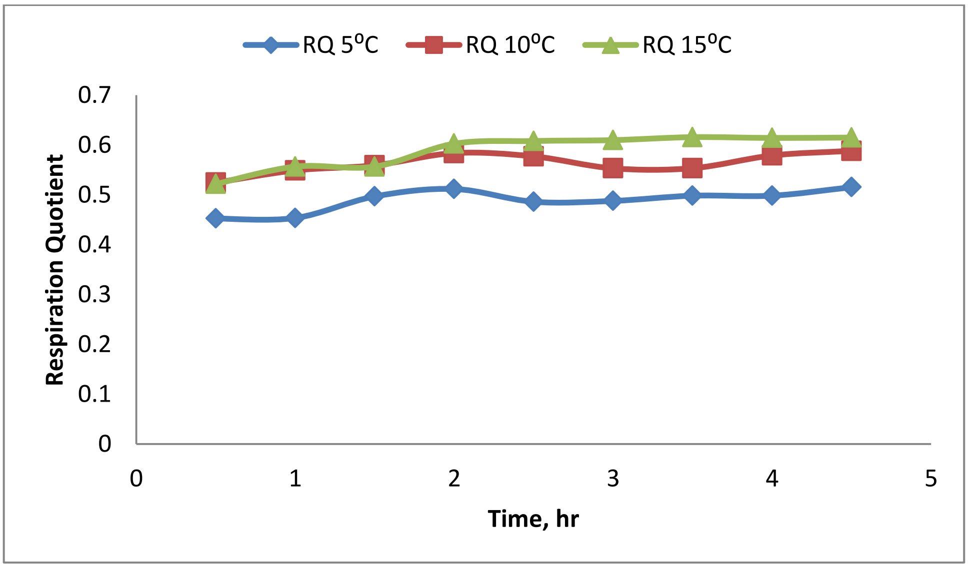

Fig. 3: Respiratory quotient of fresh green chillies at different temperatures

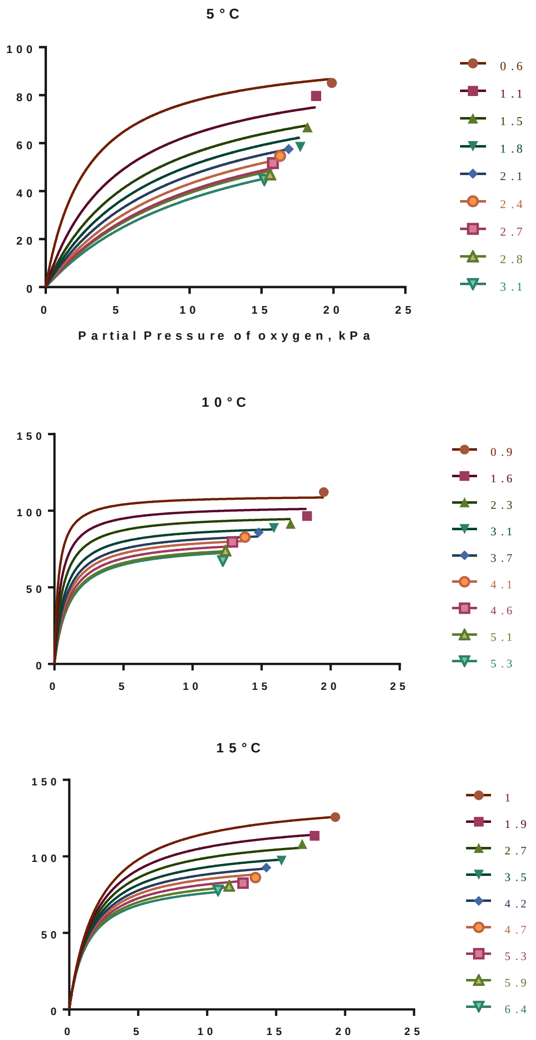

Fig. 4: Graphical representation of variability in inhibition of oxygen consumption rate of fresh green chillies by carbon dioxide with increase in temperature

Table 1: Enzyme kinetics model parameters

Temperature (K)	$V_{m_{O_2}}$		$K_{m_{O_2}}$		$K_{mc_{CO_2}}$		$K_{mu_{CO_2}}$	
	Actual	Predicted	Actual	Predicted	Actu	Predicte	Actual	Predicted
278.15	105	103.52	0.029	0.0215	0.005	0.006	10.746	10.78
283.15	120.1	121.59	0.055	0.100	0.18	0.127	10.16	9.96
288.15	144.1	142.02	0.622	0.440	2.31	2.41	9.2	9.93
E_a (kJ mol^{-1})	21.067		202.911		399.404		10.325	
Temperature dependence relationship	$V_{m_{O_2}}$ =936589 exp(-2534/T) R^2 = 0.990		$K_{m_{O_2}}$ = 2.737*10^{36}exp(-2663/T) R^2 = 0.889		$K_{mc_{CO_2}}$= 6.13 exp(-48040/T) R^2 = 0.994		$K_{mu_{CO_2}}$= 0.124exp(-1242/T) R^2 = 0.971	

Table 2: Temperature dependence of respiratory parameters

Temperature (K)	RO_2	RCO_2	RQ
278	44.7353	23.0431	0.5151
283	67.2531	39.5562	0.58817
288	77.7614	47.8127	0.61486
E_a (J/mol)	$22.02*10^7$	$16.51*10^7$	$6.6*10^6$

CONCLUSION

Respiration rates for chilli at differenct temperatures from 5 to 15 ˚C were estimated using closed system method. The respiration rates decreased with storage time due to decrease in O_2 concentrations and consequent increase in CO_2 concentrations. The difference in O_2 consumption rate and CO_2 evolution rate increased gradually with increase in temperature indicationg an increase in water vapour production. Non-linear regression analysis of the respiration data showed that enzymne kinetics relationship assuming mixed inhibition by CO_2 explained the effect of headspace gaseous concentrations. All enzyme kinetics parameters were found to be temperature dependent. It can be concluded that temperature plays a crucuial role in deciding the respiration of fresh chilli which affect the storage life of the produce.

NOMENCLATURE

$p_{CO_2}{}^{f}$: final concentration of CO_2, per cent,
$p_{CO_2}{}^{in}$: initial concentration of CO_2, per cent,
$p_{O_2}{}^{f}$:final concentration of O_2, per cent,
$p_{O_2}{}^{in}$:initial concentration of O_2, per cent,
Rco_2 :respiration rate (rate of CO_2 evolution, ml/kg-hr)
Ro_2 : respiration rate (rate of O_2 consumption, ml/kg-h)
V_s : volume of chilli sample, ml
V_t : total volume of container, ml
V_v : void volume of container, ml
t^f : final time, h
t^i : initial time, h
ρ_s : mean density of fresh chilli, kg l^{-1}
E_a : activation energy, J mol^{-1}
RH : relative humidity, %
W : product weight (kg)
Ws : weight of fresh chilli inside the impermeable container, kg

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