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Kirandeep
Department of processing and Food Engineering, Punjab Agricultural University, Ludhiana, Punjab, India

Preetinder Kaur
Department of processing and Food Engineering, Punjab Agricultural University, Ludhiana, Punjab, India

Baljit Singh
Department of Food Science and Technology, Punjab Agricultural University, Ludhiana, Punjab, India

Nitin Kumar
Department of Processing and Food Engineering, Punjab Agricultural University, Ludhiana, Punjab, India.

Correspondence
Kirandeep
Department of processing and Food Engineering, Punjab Agricultural University, Ludhiana, Punjab, India

Effect of temperature and headspace O_2 and CO_2 concentration on the respiratory behaviour of fresh yellow bell-pepper (*Oribelli*)

Kirandeep, Preetinder Kaur, Baljit Singh and Nitin Kumar

Abstract
In this study, the influence of storage temperature on the respiratory parameters of fresh yellow bell pepper (*Oribelli*) was investigated. The rate of respiration was assessed using closed system technique at 5 °C, 10 °C, and 15 °C for 5 hours. The gaseous concentration of the closed container headspace was continuously measured at regular intervals using gas analyser. The rate of oxygen consumption and CO_2 evolution has been described using enzyme kinetics theory that proposes different types of inhibition caused by CO_2 at different temperatures. This inhibitory effect in the present study has been assessed using the inter-relationship between four inhibition constants which were determined using non-linear regression techniques. Analysis of the respiration data using different models (competitive, uncompetitive, non-competitive and mixed model) revealed that respiration of yellow bell-pepper could be described using mixed type of inhibition. Rate of respiration was found to increase significantly with increase in temperature. Enzyme kinetics parameters were also analysed using simple linear equations. Inhibition by CO_2 was found to be predominantly uncompetitive at 5 °C and predominantly competitive at 10 °C and 15 °C.

Keywords: Effect of temperature, headspace O_2, CO_2, respiratory behaviour

Introduction
Yellow bell-pepper are used as culinary ingredients for their colour, flavour and pungency. They are good source of vitamins A and C (Howard *et al* 1994). Vitamin C and carotenoids are basic antioxidants in human diet. Vitamin C is the most abundant among the water soluble antioxidants in human body (Frei *et al* 1989) [2]. Bell peppers are usually harvested when they are of marketable size and bright green. For coloured peppers (yellow, red) an additional harvesting criterion is to have a minimum 50% colouration (Phan & Weichmann, 1987) [3]. It is a hot-weather crop. A light frost will damage plants (28º F to 32º F), and temps below 55º F slow growth and cause leaves to look yellowish. They need well-drained, nutrient-rich soil. Amend soil with 3 to 5 inches of compost or other organic matter prior to planting. Soil pH should be 6.2 to 7.0. The bright yellow or orange varieties of the bell pepper bring all the same range of nutrients in other peppers such as healthy amounts of vitamins A and C, B6, and folate, along with important minerals, all in a high-fiber, low-calorie package. The yellow bell pepper has an enormous amount of vitamin C due to the concentration of carotenoids and so offers a lot of antioxidants to fight cancer and other diseases. One study has shown that foods with deep yellow to orange colors contain a phytonutrients found to reduce the risk of some kinds of arthritis.

Quality retention and decreasing loss in the postharvest quality of fresh fruits and vegetables are the primary targets of postharvest engineering. The modified atmosphere conception for foodstuff comprises altering the atmosphere around a food product by vacuum, gas flushing or controlled permeableness of the pack hence curbing the biochemical, enzymatic and microbial activities so as to avoid or diminish the main degradations that may happen. Respiration is a crucial factor in planning modified atmosphere packaging (MAP) for fresh produce. The reaction of fresh produce respiration rate to modifications in oxygen and carbon dioxide levels and temperature should be known so as to get information about package design parameters (Hagger *et al.* 1992) [5]. Thus, respiration rate modelling is important to the design of MAP for fresh fruits and vegetables.

Respiration is a dynamic process that supplies energy for biochemical processes of fresh produce. Respiration comprises of oxidative break up of organic substrates like carbohydrates, lipids and organic acids to less complicated molecules, including carbon dioxide and water, with release of energy (Fonseca *et al.* 2002) [18]. The respiration rate of fresh produce can be expressed as O_2 consumption rate and/or CO_2 production rate. The respiration rates are calculated from the absolute differences in gas concentrations between the outlet and the inlet when the system reaches steady state. The steady-state concentrations of O2 and CO2 are determined and a mass balance is performed on the system in order to estimate the respiration rates.

Material and Methods

Sample Preparation

Fresh yellow bell pepper (*Oribelli*) was procured from farms of Punjab Agricultural University, Ludhiana grown under greenhouse conditions. Samples were washed and were kept at desired temperature for 10 minutes for equilibration.

Experimental set up

Respiration rate experiment for yellow bell-pepper was conducted in closed at different temperatures (5, 10, and 15 ºC) with RH of 75%. Approximately 200g of sample was kept in glass jar of volume 750ml. The containers were tightly covered with plastics caps with the help of vacuum grease. Re-sealable septums were attached to the cap of the containers in order to measure the gaseous concentration inside the container. Gas concentration was measured at regular interval of 30 mins with the help of gas analyzer (Make: PBI DANSENSOR).

Void volume and true density

For each experiment the volume of the sample crop kept in closed container was determined.

$$V_s = \frac{W_S}{\rho_S} \qquad (1)$$

The void volume (V_V) of the glass container used for the respiration experiment was measured. The density of fresh yellow bell pepper was determined by water displacement method (Ishikawa *et al* 1992) [12] through the evaluation of true volume (V_T) of a known mass of freshly harvested bitter gourd. The void volume (V_V) for each experiment was determined using the relationship.

$$V_V = V_T - V_S \qquad (2)$$

Respiration rates and respiration quotient

Respiration rates were determined using following equations:

$$R_{O_2} = \frac{(p^{in}_{CO_2} - p^{f}_{CO_2}) \times V_v}{100 \times W \times (t^f - t^i)} \qquad (3)$$

$$R_{CO_2} = \frac{(p^{f}_{CO_2} - p^{in}_{CO_2}) \times V_v}{100 \times W \times (t^f - t^i)} \qquad (4)$$

Respiration quotient is the ratio of rate of carbon dioxide evolution to the rate of oxygen consumption.

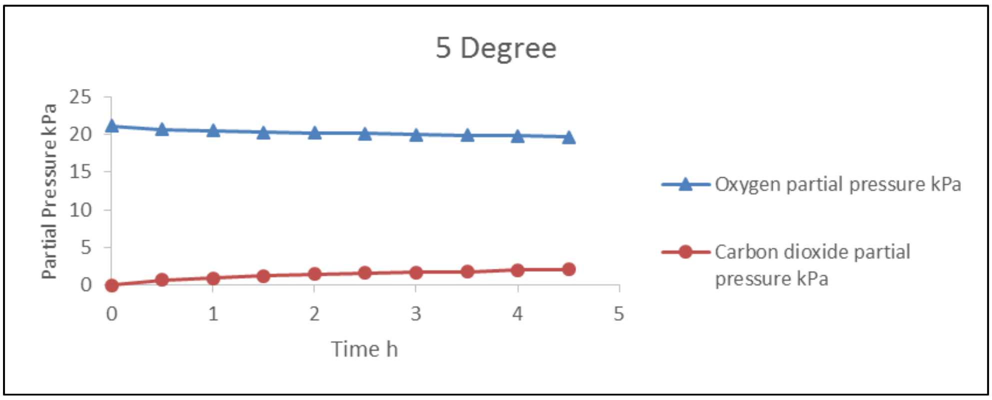
Statistical Analysis

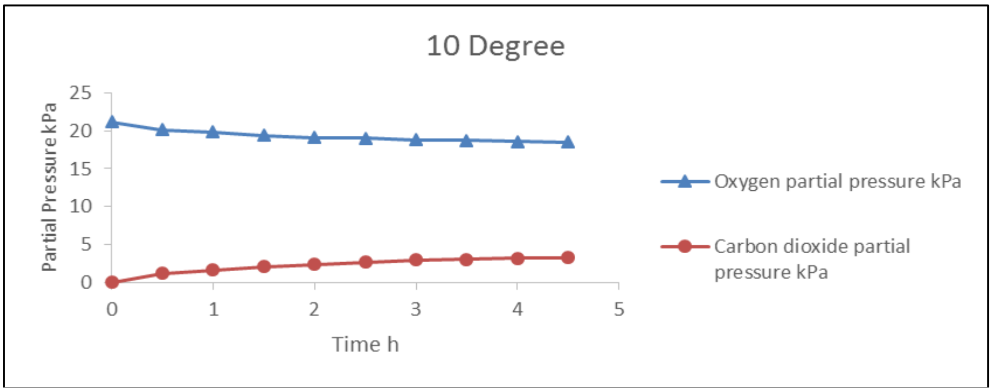
The analysis of respiration data was done using non-linear regression technique (GraphPad PRISM Version 5.00.288 software USA).

Results and Discussion

Respiration Rate

Gas analysis was done till the steady state was reached. Steady state was achieved within 4-4.5 h. Partial pressure of oxygen decreased whereas partial pressure of carbon dioxide increased gradually with time (Fig 1). Initially the respiration rate was high and it decreased with the increase in time till the steady state was achieved where respiration rate became nearly constant. Oxygen consumption rate and carbon dioxide evolution rate declined gradually with time (Fig 2). The rate of oxygen consumption and carbon dioxide evolution at steady state are given in table 1. The steady state oxygen consumption and carbon dioxide evolution rate increased by 45.11% and 36.69% respectively when the temperature was increased from 5 to 10 ºC whereas an increase of 69.96% and 56.60% was observed when temperature increased from 5 to 15 ºC. It can be clearly seen that with increase in temperature, difference between oxygen consumption rate and carbon dioxide evolution rate increased significantly indicating increase in production of water vapour. At higher temperatures moisture condensation may take place leading to spoilage of produce (P Kaur *et al.* 2009).





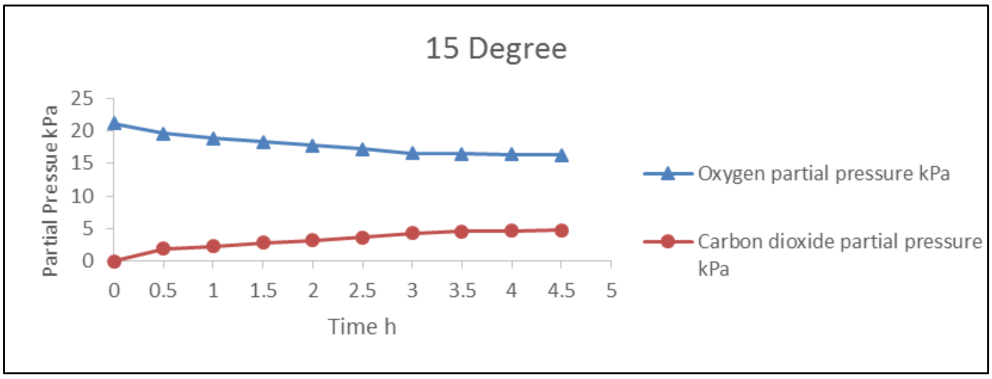
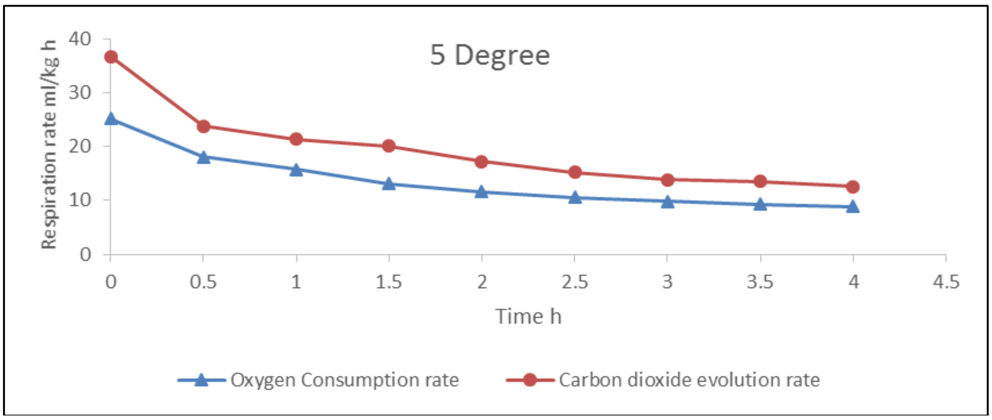


Fig 1: Partial pressure of oxygen and carbon dioxide in the headspace during closed system experiment for the measurements of the rates of oxygen consumption (RO_2) and carbon dioxide evolution (RCO_2) for fresh yellow bell pepper at 5, 10 and 15°C respectively.

Table 1: The rate of O_2 consumption and CO_2 evolution at steady state

Temperature	Oxygen Consumption rate (ml $kg^{-1}h^{-1}$)	Carbon dioxide evolution rate (ml $kg^{-1}h^{-1}$)	Respiration Quotient
5°C	8.889	12.604	1.418
10 °C	16.196	19.910	1.229
15 °C	29.591	29.043	0.981



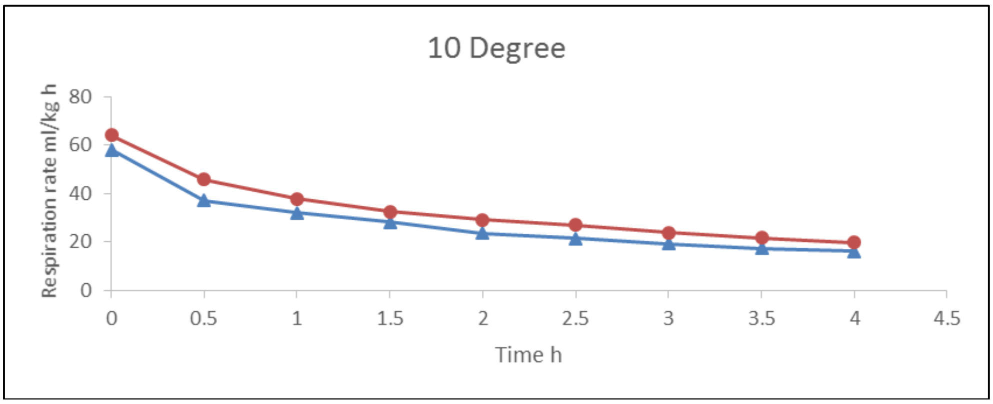


Fig 1: Oxygen consumption rate and carbon dioxide evolution rate for fresh yellow bell pepper

Respiration Quotient
From steady state respiration rates, the respiration quotients were determined. Fig 3 shows RQ at different temperatures. With the increase in time RQ values decreased constantly and became stable. Steady state RQ is given in table 1.

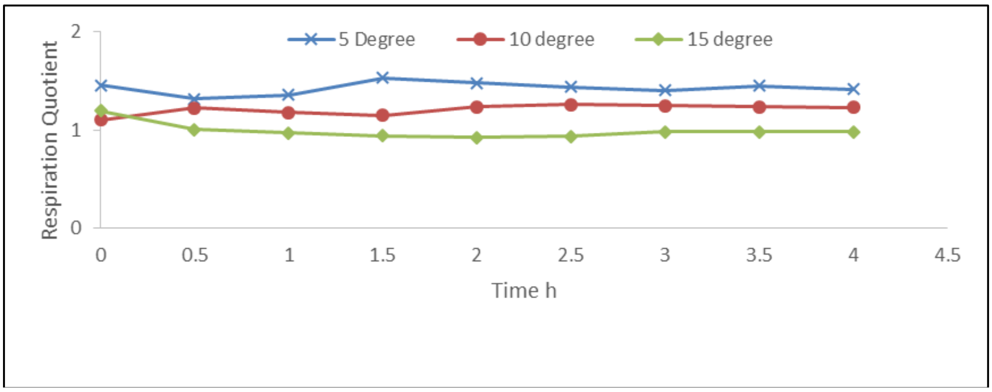


Fig 3: Respiratory quotient of fresh bell pepper at different temperature

Enzyme Kinetics
Enzyme kinetics parameters for respiration data obtained by fitting different inhibition models have been presented in the table 2 and table 3.

Table 2: Enzyme kinetics parameters of different models

5 °C	Competitive	Non-competitive	Uncompetitive	Mixed Model
K_m	4503	1.304e+011	297.4	59.52
I	2.10	2.10	2.10	2.10
Ki	0.1885	0.189	0.008833	0.1411
V_{max}	25259	7.251e+011	2323	444.3
R^2	0.952	0.951	0.9502	0.952
10°C	Competitive	Non-competitive	Uncompetitive	Mixed Model
K_m	19.58	17023	-21.53	1.243e-010
I	3.30	3.30	3.30	3.30
Ki	4.982e-005	0.0001585	7.427	0.6668
V_{max}	1.321e+006	3.614e+008	5.284	405.4
R^2	0.9839	-	0.980	0.942
15°C	Competitive	Non-competitive	Uncompetitive	Mixed Model
K_m	1.641e+006	-1.805	-38.94	5.818e-011
I	4.80	4.80	4.80	4.80
Ki	0.7107	0.0007003	0.01004	1.305
V_{max}	4.658e+007	373456	28774	714.5
R^2	0.9458	0.954	0.954	0.933

Table 3: Enzyme kinetics parameters and type of inhibition

Temperature (K)	Km_{O_2}	Kmc_{CO_2}	Kmu_{CO_2}	Type of inhibition
278	4503	0.1885	0.008833	Predominantly uncompetitive
283	19.58	4.982e-005	7.427	Predominantly competitive
288	-1.805	0.0007003	14.22	Predominantly competitive

Effect of CO_2 on respiration
The value of inhibition constants is a measure of extent to which respiration can be inhibited by CO_2. A high, value of inhibition constants implies that the backward reaction of inhibition is much faster than the forward reaction and hence inhibition by CO_2 is not possible. The inhibition constants shows that respiration of fresh yellow bell pepper was prone to combined inhibition. At 10 and 15 °C predominantly competitive type inhibition was observed whereas at 5 °C predominantly uncompetitive type of combined inhibition was observed (Fig 4). The inhibitor can bind to the enzyme at the same time as the enzyme's substrate. However, the binding of the inhibitor affects the binding of the substrate, and vice versa. If both competitive and uncompetitive components are present in the inhibition it is said to be mixed. The O_2 versus RO_2 (Fig 4) curve at 5 and 10°C is shifted to the left but not down. Inhibition is said to be predominantly uncompetitive if the inhibitor (CO_2) binds with equal affinity to the enzyme, and the enzyme-O_2 complex. The inhibition is not surmountable by increasing substrate concentration. This suggests that when inhibition becomes uncompetitive at 15 °C and beyond, even at low O_2 concentrations that are attained within a few hours, the value of half maximum activity is attained. The O_2 versus RO_2 curve is shifted to the left and also down. The downward shift became more prominent, indicating that inhibition changed from competitive to uncompetitive, probably due to denaturization of enzyme (protein) at high temperature (Manpreet *et al* 2012).

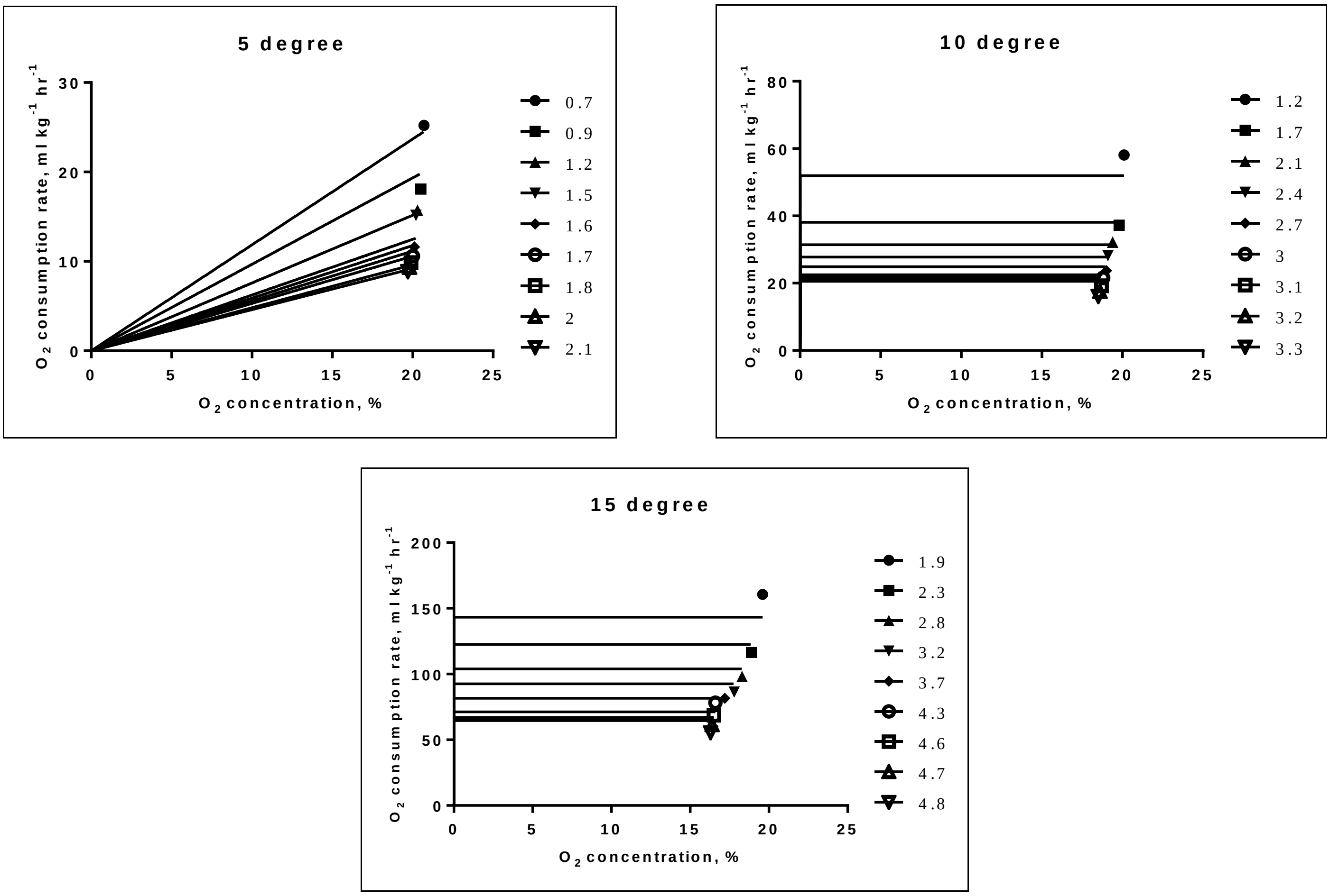
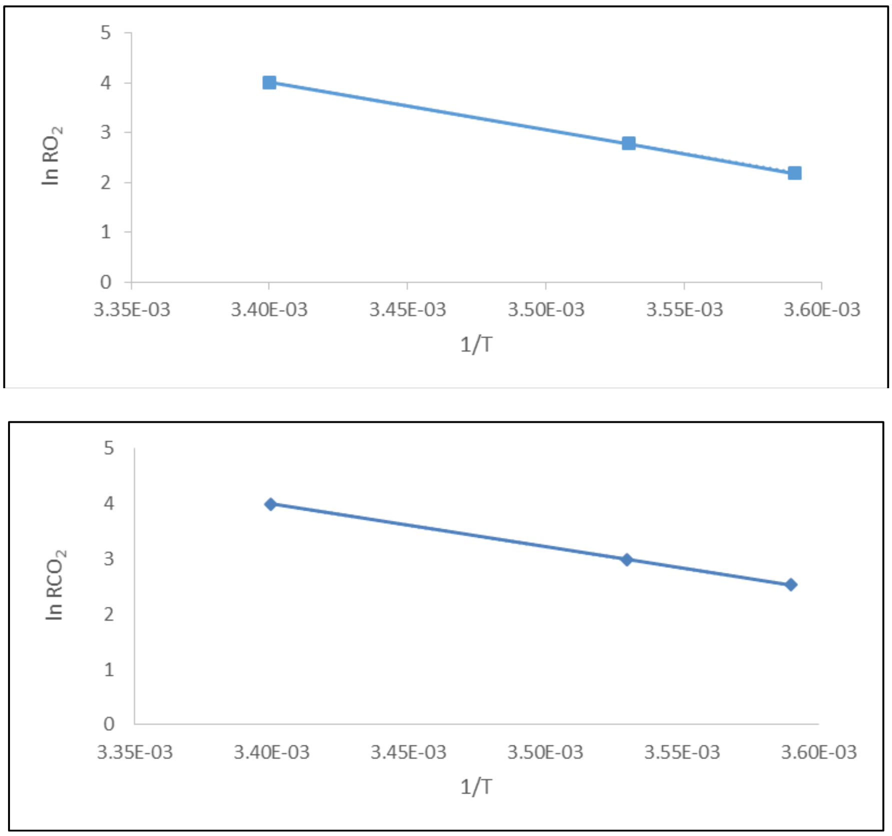


Fig 4: Variability in the inhibition of O_2 consumption rate by CO_2 with increase in temperature

Effect of temperature on respiratory parameters

Temperature dependence of respiration rate (R_{O2} and R_{CO2}) and RQ was evaluated by calculating activation energies for respiration rate and RQ with the help of Arrhenius relationship as shown in Fig. 5. It was found that all the terms were highly temperature dependent as expressed by their higher activation energies of 8.31 x 10^8 J/mol for RO_2, 6.34 x 10^8 J/mol for RCO_2 and -1.98 x 10^8 J/mol for RQ (Table 4).



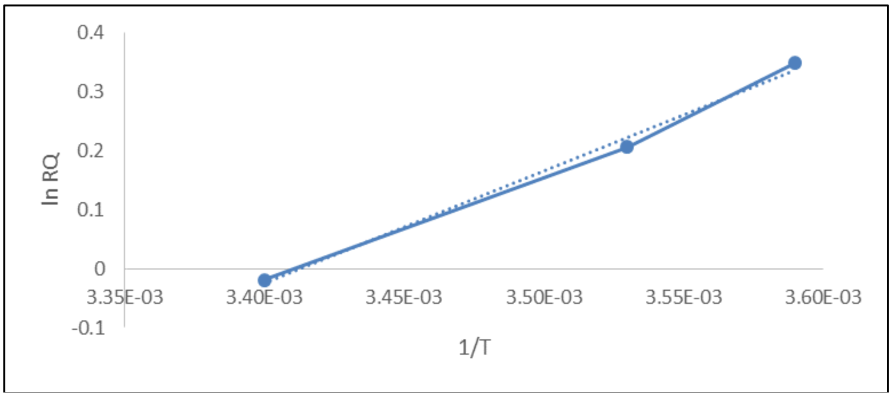


Fig 5: Effect of temperature on respiratory parameter

Table 4: Effect of temperature on respiratory parameters

Temperature (K)	R_{O2}	R_{CO2}	RQ
278	39.53	58.81	1.48
283	56.98	62.10	1.10
288	75.78	108.23	1.42
E_a (J/mol)	8.31 x 10^8	6.34 x 10^8	-1.98 x 10^8

Conclusion

This research was focused on respiratory behaviour of fresh yellow bell pepper for temperature ranging from 5 to 10 ºC. Rate of respiration was found to be dependent upon temperature, it increased with increase in temperature. With the increase in temperature difference between partial pressure of O_2 and CO_2 increased significantly whereas difference between CO_2 consumption rate and O_2 evolution rate increased with increased in temperature. The steady state oxygen consumption and carbon dioxide evolution rate increased by 45.11% and 36.69% respectively when the temperature was increased from 5 to 10 ºC whereas an increase of 69.96% and 56.60% was observed when temperature increased from 5 to 15 ºC. Non-linear regression analysis for enzyme kinetics of the data shows that mixed inhibition was found to be the best fit for all temperatures. This study helped to develop an integrated model on the basis of enzyme kinetics theory and temperature dependence relationships for different parameters encompassing the effect of headspace gas concentrations as well as surrounding temperature.

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