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RESPIRATION RATE MODELS OF FRESH CHICKPEA SPROUTS (*Cicer arietinum* L.) UNDER MODIFIED ATMOSPHERE PACKAGING

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Abstract: The closed system glass container headspace gaseous composition, in terms of O_2 and CO_2 concentrations, in modified atmosphere packaging (MAP) of Chickpea Sprouts (*Cicer arietinum* L.) at 5, 10, 15, 20 and 25^{o}C temperature were respectively monitored within 5h by a O_2/CO_2 gas analyzer. The respiration rate of Chickpea sprouts under modified atmosphere packaging at any instant of time were calculated by a C^+ program based on closed system. The dependence of respiration rate on gas composition was well described by a Michaelis-Menten type enzyme kinetic model equation with uncompetitive type of mixed CO_2 inhibition. The model parameters were estimated by fitting the model to the experimental data by non-linear regression using GraphPad Prism 5. The relationship between enzyme kinematics model parameters and temperature was well described by the Arrhenius equation. The results suggest that, the respiration rate of Chickpea sprouts as well as kinematic parameters increased with temperature. Results of the study can be applied to the MAP design for Chickpea sprouts.

Keywords: *Chickpea (Cicer arietinum) sprouts, MAP, respiration rate, modeling*

INTRODUCTION

An effective way to extend the shelf life of fresh produce is to use a modified atmosphere package (MAP) [9]. The package should maintain an optimal atmosphere that will reduce respiration and slow physiological and microbiological changes that decrease shelf life. Modified atmosphere packaging (MAP) is a well-established

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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 [1]. Chickpea sprouts are the major seed sprouts in most Asian countries. Chickpea seeds can be stored for long periods of time and sprouts can be easily obtained by germinating the seeds in the dark for up to four days. This process has been used by the Asian countries for centuries. Large amounts of sprouts can be obtained in a relatively short time. Sprouts are a cheap source of certain vitamins in the diet and some vitamins are synthesized in the germinating seeds. The sprouting process results in an improvement in the vitamin content [5].

Sprouts are highly perishable in nature and get spoiled if not stored properly. Due to a short shelf life, sprouts are susceptible to get spoiled during distribution or in supermarket. However, postharvest of chickpea sprouts is prone to deteriorate; therefore it is crucial to use the MAP system to extend its shelf life. The gas constituents inside the package is an important factor affects the shelf life of the product. 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 [8]. The enzyme kinetics approach proposed by [3] 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 [4, 6, 2, 7, 11, 12]. The purpose of this work were: (i) to study the respiration rate of sprouts under different storage conditions and develop a modified atmosphere packaging (MAP) system for sprouts in order to increase shelf life; (ii) to develop a predictable model relating respiration rate to O_2 and CO_2 concentrations and temperature that maybe used in the design of MAP for Chickpea sprouts.

MATERIALS AND METHODS

Plant Materials

Chickpea seeds (PBG-5) cultivar was procured from Krishi Vigyan Kendra, Gurdaspur under aegis of Punjab Agricultural University, Ludhiana for experimentation and study. The seeds were washed and rinsed with Ca $(OCl)_2$ @ 20,000 ppm for 15 min and soaked in clean water (1: 3, w/v) for 12 hours overnight at ambient room temperature. The soaked seeds were shifted to clean sterile muslin cloth and placed in dark at ambient temperature for sprout growth. After 36 hours the sprouts were ready to harvest for experimentation.

Experiment Methods

A closed system is used to measure the respiration rate of the fresh chickpea sprouts [9]. A known weight (100g) of chickpea sprout was filled into air tight glass container. After packing, container was kept at 5°C, 10°C, 15°C , 20°C , 25°C temperature and

75 % RH in a cold room (Frig India Ltd, India). The closed system container headspace analysis for O_2 and CO_2 concentrations was carried out by a portable headspace gas analyzer (Model 902 D DualTrak O_2/CO_2 Analyzer, USA) at every 30 min interval.

RESULTS AND DISCUSSION

Rates of Respiration

The calculated value of RO_2 and RCO_2 of Chickpea sprouts at 5°C, 10°C, 15°C, 20°C and 25°C temperatures are plotted in Fig 1. The rate of respiration is gradually increases and higher at the start of the experiment and gradually declined as the storage period prolonged, before becoming almost constant. The steady-state respiration rate for O_2 consumption were observed to be 38.56, 89.17, 103.63, 140.98, 222.92 and 356.68 ml/kg-h at 5 °C, 10 °C, 15 °C, 20 °C, 25 °C and 35°C (control sample), respectively. The steady-state respiration rates for CO_2 evolution were 36.41, 57.84, 71.09, 89.17, 162.67 and 202.26 ml/kg-h at 5 °C, 10 °C, 15 °C, 20 °C, 25 °C and 35°C (control sample), respectively. The respiration rates of in steady-state conditions steady-state conditions increased with temperature. This can be attributed to the fact that the higher rate of gaseous diffusion across the closed system and the higher respiration rate of chickpea sprouts at a higher temperature when they reached the equilibrium state [9].

Respiration Rate Models

Michaelis-Menten type Enzyme Kinetic Model for Respiration Rate

Among the several reported models in the literature [1, 3, 9, 10, 11, 12] describing the respiration rate of perishable produces, the most used is that based on Michaelis-Menten type enzyme kinetic equation with uncompetitive CO_2 inhibition. The model can be written as following [3].

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

Where,

R	[ml/kg-h]	- respiration rate of the produce,
V_m	[ml/kg-h]	- maximum rates of O_2 consumption for the produce,
K_m	[kPa]	- Michaelis-Menten constant in CO_2 evolution,
K_i	[kPa]	- inhibition constant in CO_2 evolution,
y^0_{CO2}	[%]	- volumetric concentrations of CO_2,
y^0_{O2}	[%]	- volumetric concentrations of O_2.

By reciprocating and separating the terms of Eq. (1), we get:

$$\frac{1}{R} = \frac{1}{V_m} + \frac{K_m}{V_m\, y^0_{O_2}} + \frac{y\, co_2}{K_i\, V_m} \qquad (2)$$

Multiple regression analysis was carried out using the measured values of RO_2, RCO_2, $y^0{}_{CO2}$ and $y^0{}_{O2}$ to estimate the values of model parameters for O_2 consumption and CO_2 evolution of the chickpea sprouts. Temperature has been identified as the most important external factor Influencing respiration and the effect of gas composition increased with temperature [8, 10].

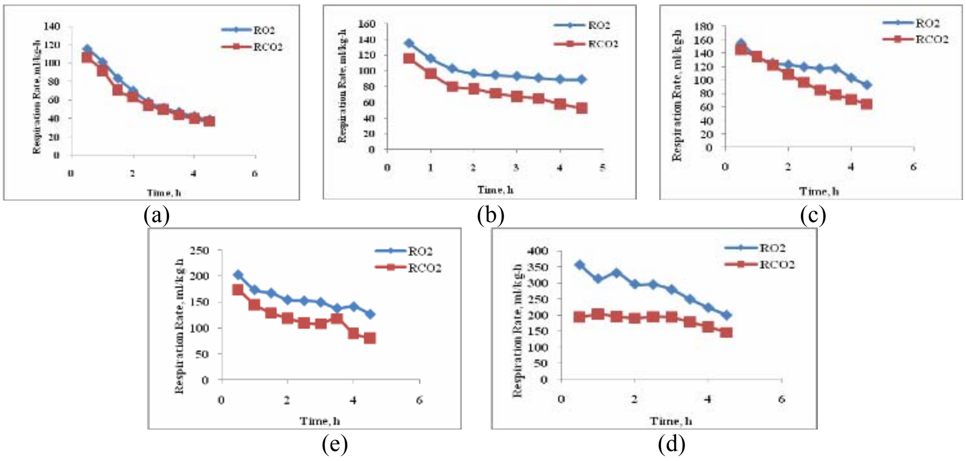

Figure 1. Oxygen consumption (RO_2) and carbon dioxide evaluation (RCO_2) rate in the container headspace during the closed system experiment for chickpea sprouts at different temperatures: (a) 5°C, (b) 10°C, (c) 15°C, (d) 20°C, (e) 25°C

Enzyme Kinematics Model Parameters for Respiration of Chickpea Sprouts

The enzyme kinematics model parameters for chickpea sprout samples were determined by fitting the experimental data at each specified temperature from 5 °C to 25 °C using the method of non-linear regression analysis. The model parameter (Vmo$_2$, Kmo$_2$, Kmc$_{CO2,}$ and Kmu$_{CO2}$) is temperature dependent and proceed accordingly as the temperature increased (Table 2). Temperature dependence (by Arrhenius equation) was estimated by plotting the log values of the model parameters against the inverse of corresponding temperature values in absolute units. It is observed that, as each enzyme may be affected differently by sudden change in temperature, a change in the rate of limiting enzymatic action was attributed Fig 2 . The Michaelis-Menten constant Kmo$_2$, is a measure of saturation of respiration with headspace gas O_2. It depicts the oxygen concentration at which half of the maximum respiration rate is reached, assuming no inhabitation by CO_2. A steep inclination in respiration rate with respect to all experimental temperatures was observed due to availability of large surface area of chickpea sprouts for respiration [9].

Inhibition of Chickpea Sprouts Respiration by CO_2

A high value for inhabitation constants implies that the backward reaction of the inhabitation is much faster than the forward reaction and hence inhibition by CO_2 is not occurring. The inhabitation constant obtained by non-linear analysis of the respiration data shows that respiration of chickpea sprouts was prone to mixed inhibition ($K\text{mc}_{CO2,}$ and $K\text{mu}_{CO2}$ are finite and unequal). At all defined temperatures (5°C, 10°C, 15°C, 20°C and 25°C) mixed type of uncompetitive inhibition ($K\text{mc}_{CO2,}$ and $K\text{mu}_{CO2}$ are finite and unequal; ($K\text{mc}_{CO2}$ > $K\text{mu}_{CO2}$) was observed. In the present study, the inhibition is uncompetitive inhibition and the inhibitor (CO_2) binds with equal affinity to the sprouts enzyme and the enzyme O_2 complex.

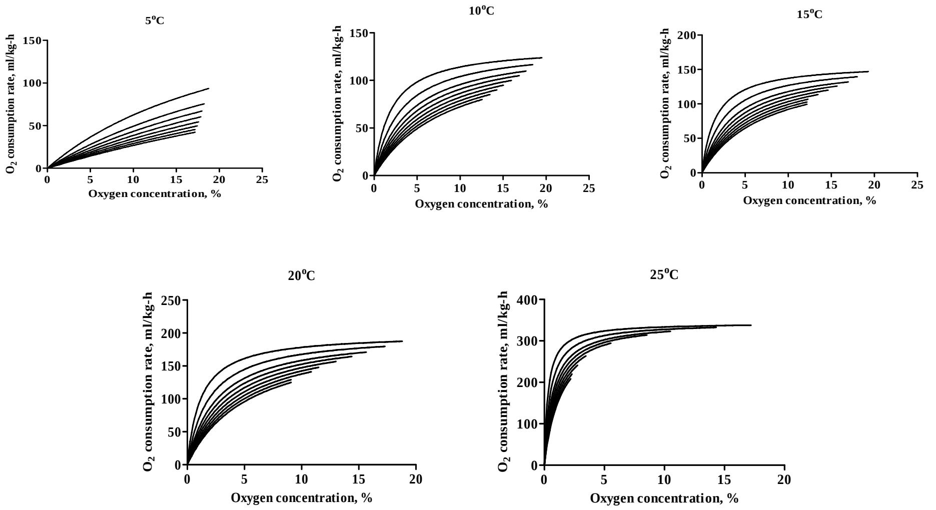

Figure 2. Graphical representation of variability in inhibition of oxygen consumption rate by CO_2 with increase in temperature with uncompetitive type of combined inhibition

Effect of Temperature Dependence on Respiration

The respiration rate of chickpea sprouts was influenced by the temperature. The activation energies for kinematic parameters, namely $V\text{mo}_2$, $K\text{mo}_2$, $K\text{mc}_{CO2,}$ and $K\text{mu}_{CO2}$ were determined and the effect was expressed as Arrhenius-type relations in the defined temperature range for modified atmosphere packages of chickpea sprouts. The values of $K\text{mu}_{CO2}$ varied from 0.482 to 11.732 with an increase of temperature from 5 to 25°C (Table 1). An increasing trend in $K\text{mu}_{CO2}$ value was noticed between temperature ranges from 278 to 288 °K. After slight decline in $K\text{mu}_{CO2}$ value (2.905), the value finally settled (11.732) at 298 °K. The higher value of $K\text{mu}_{CO2}$ indicates the stronger influence of temperature dependence on respiration kinetics [3, 9].

The mixed enzyme kinematic parameter $K\text{mu}_{CO2}$ seen to be more temperature dependent than $V\text{mo}_2$ and $K\text{mo}_2$ as expressed by its higher activation energy (E_a=74.38 kJ/mol). Enzyme kinematic parameter $V\text{mo}_2$ was observed to be highly temperature dependent (E_a=53.92 kJ/mol) as compared to inhibition constant [E_a (Kmo_2) = 38.25 kJ/mol] and [E_a ($Kmcco_2$) = 43.82 kJ/mol] (Fig 3).

Table 1. Model parameters for mixed enzyme kinematic at different temperature

Temperature (K)	Vmo_2	Kmo_2	$Kmcco_2$	$Kmuco_2$
278	247.3	9.313E-010	0.833	0.482
283	175.4	2.911E-011	2.179	2.795
288	151.5	7.266E-012	2.131	3.021
293	230.4	1.391E-011	2.143	2.905
298	366.8	2.775E-004	5.382E-004	11.732

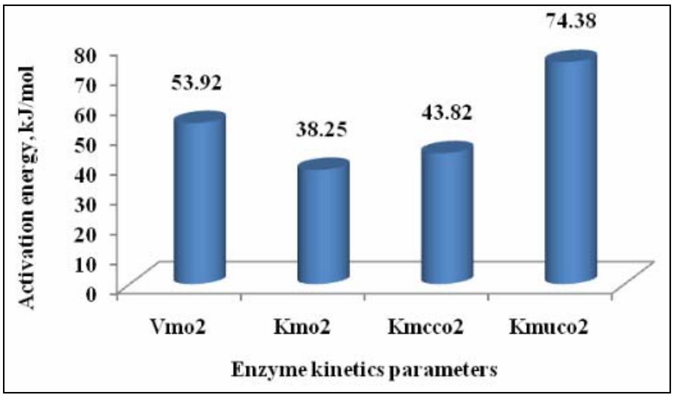

Figure 3. Activation energy of chickpea sprouts at various temperatures

The mathematical equations and temperature dependence relationship for mixed inhibition enzyme kinematic model parameters at different temperature was given in Table 2. It was observed that, all the studied enzyme kinematic parameters are temperature dependence and the dependence was different for all the parameters in varied degrees.

Table 2. Temperature dependence relationship for mixed enzyme kinematic at different temperature

Parameter	Model	Equation	R^2 value	Temperature Range (K)
Vmo_2	Mix-inhibition	$y = 0.0051\,T^3 - 2.9173\,T^2 + 418.2\,T$	0.99	$278 \le T \le 298$
Kmo_2	Mix-inhibition	$y = 2E\text{-}07\,T^3 - 0.0002\,T^2 + 0.0451\,T - 4.2864$	0.98	$278 \le T \le 298$
$Kmcco_2$	Mix-inhibition	$y = 0.2692\,T - 74.005$ and $y = -0.0431\,T^2 + 25.037T - 3634.6$	1.00 1.00	$278 \le T \le 283$ to $283 \le T \le 298$
$Kmuco_2$	Mix-inhibition	$y = 0.0074\,T^3 - 6.317\,T^2 + 1808.7\,T - 172597$	0.98	$278 \le T \le 298$

Influence of Respiratory Quotient (RQ)

The respiratory quotient exhibits slight minor fluctuations during the initial stage of respiration rate experiments (Fig 4). At a given temperature condition, RQ was found varying insignificantly with the time under aerobic condition. With respect to temperature series investigated, a gradual increase in RQ value was observed with rising temperature. This phenomenon is corresponding to some other fresh produce [4]. RQ is less than unity; the O_2 consumption was always higher than the oxidative CO_2 production.

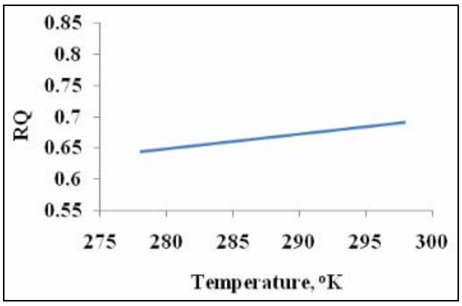

Figure 4. Change in the respiratory quotient at different temperature

CONCLUSIONS

The respiration characteristics of chickpea sprouts under MAP within 5h were measured. The glass container headspace compositions, in terms of oxygen and carbon dioxide concentrations of chickpea sprouts were monitored based on closed system. The Michaelis Menten enzyme kinetics equation, assuming the effect of CO_2 as that of uncompetitive inhibition, has been found to describe well the relationship between the respiration rate and the in-pack gas composition at a constant temperature. The model parameters of models at 5 ºC, 10 ºC, 15 ºC and 20 ºC were evaluated and it has been found to have a linear as well as polynomial relationship. All the studied enzyme kinematic parameters are temperature dependent and the dependence was different for all the parameters in varied degrees. the transpiration coefficient of chickpea sprouts varied according to Arrhenius relationship. This approach can be easily used to design a MAP system for storage of chickpea sprouts in polymeric film packages under viable temperature systems.

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MODELI STOPE RESPIRACIJE SVEŽIH KLICA LEBLEBIJA (*Cicer arietinum* L.) U MODIFIKOVANOM ATMOSVERSKOM PAKOVANJU

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Sažetak: Sastav gasova na vrhu staklenog kontejnera u zatvorenom sistemu, uključujući koncentracije O_2 i CO_2, u modifikovanom atmosverskom pakovanju (MAP) klica leblebija (*Cicer arietinum* L.) pri temperaturama od 5, 10, 15, 20 i 25^oC je praćen pojedinačno u periodima od po 5 časova O_2/CO_2 gas analizatorom. Stopa respiracije svežih klica leblebija u modifikovanom atmosverskom pakovanju proračunavana je u realnom vremenu C^+ programom zasnovanim na zatvorenom sistemu. Zavisnost stope respiracije od sastava gasova je dobro opisana jednačinom Michaelis-Menten tipa enzimskog kinetičkog modela sa nekonkurentnim tipom inhibicije izmešanog CO_2. Parametri modela su procenjeni prilagođavanjem modela eksperimentalnim podacima primenom nelinearne regresije u GraphPad Prism 5. Odnos između parametara modela kinematike enzima i temperature bila je dobro pisana Arrhenius jednačinom. Rezultati ukazuju na porast stope respiracije klica leblebija i kinematičkih parametara sa porastom temperature. Rezultati studije mogu se primeniti na proračun MAP za klice leblebija.

Ključne reči: *Klice leblebija (Cicer arietinum), MAP, stopa respiracije, modeliranje*

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