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RESEARCH ARTICLE

RESPIRATION RATE OF OYSTER MUSHROOMS - EFFECT OF TIME, TEMPERATURE AND GAS CONCENTRATION AND ITS KINETIC MODELLING.

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Manuscript Info Abstract	
Manuscript History:	Experiments were conducted for the measurement of respiration rate of
Received: 15 April 2016 Final Accepted: 29 May 2016 Published Online: June 2016	oyster mushrooms at seven different storage temperatures (0, 5, 10, 15, 20, 25 ad 30 °C) using the closed system respirometer method. The effect of time, temperature and gas concentration on the respiration rate of oyster mushroom was studied and the models involving these parameters were
<i>Key words:</i> Oyster mushroom, respiration rate, enzyme kinetics, Peleg model, modeling	developed. The experimental data were fitted to two different mathematical models; Peleg model, and an uncompetitive inhibition based enzyme kinetics model, for the prediction of respiration rate within the given experimental range. The suitability of models to predict the respiration rate was examined
*Corresponding Author	with the help of relative deviation modulus by calculating the difference between the actual and the predicted respiration rates at 3 °C. The mean
Sudhanshu Billoria.	relative deviation moduli of predicted versus experimental respiration rates were found to be 10.3% and 11.2% for O_2 and CO_2 respiration expressions of Peleg model and 9.3% and 8.5% for O_2 and CO_2 for uncompetitive inhibition based enzyme kinetics model, respectively. The uncompetitive inhibition based enzyme kinetics model showed better agreement with the actual remainstring rate than the Peleg model

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a	Peleg rate constant, h % ⁻¹
b	Peleg capacity constant, % ⁻¹
Ε	mean relative deviation modulus, %
E_{a}	activation energy, kJ g^{-1} mol ⁻¹
$G_{\rm CO2}$	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 _{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
Т	storage temperature, °C
t	storage time, h

NOMENCLATURE

T _{abs}	absolute temperature, K
$T_{\rm ref}$	reference temperature, K
Δt	time difference between two gas measurements, h
$V_{ m fr}$	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

Introduction:-

There are considerable postharvest losses in the horticultural commodities and mushroom is one of those highly perishable commodities (Bano et. al., 1988; Villaescusa and Gill, 2003). The major factor that leads to shorter shelf life of mushrooms is its high respiration rate, which is 28.2-43.6 mg CO₂ per kg fresh weight per hour at 0 °C for button mushrooms (Hammond and Nichols, 1975; USDA, 1977).

Mushrooms undergo various other physiological processes also after harvesting, which include growth, maturation, senescence, weight loss, veil-opening, browning and wilting(Rai and Arumuganathan, 2008). These processes are also responsible for the short shelf life of the mushrooms. There are various types of mushrooms among which *Agaricus bisporus* (button mushroom), *Lentinus edodes* (shiitake mushroom), *Pleurotus spp* (oyster mushrooms), *Auricula spp* (wood ear), *Flamulina velutipes* (enokitake) and *Volvariella volvacea* (straw mushroom) are the commonly cultivated mushrooms (Aida *et al.*, 2009). Nearly all the mushrooms have poor shelf-life, whereas the oyster mushroom has even poorer (few hours at the ambient conditions).

The number of recognized mushroom species has been reported to be 14,000, which is just 10% of the total estimated mushroom species on the earth (Cheung, 2008; Koushki *et al.*, 2011). Total commercial mushroom production worldwide has increased from about 350,000 tons in 1965 to about 3.4 million metric tons (Koopman and Laney, 2010). However, references regarding the respiration rate of these varieties are scarce in the literature. Measurement of respiration rate of produce at a particular storage temperature and gas composition is time consuming and requires special equipment for gas analysis (Cameron *et al.*, 1989; Bhande and Goswami, 2008).

In order to prolong the availability in the market throughout the year, the perishable commodities need to be handled and stored with due care. Certain postharvest constraints, including short shelf life, moisture loss, ripening and susceptibility to diseases limit their long duration storage and transportation (Mangaraj and Goswami, 2011).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. These methods provide control of moisture loss, retardation of senescence and browning in cut produce, reduction in respiration rate and ultimately prolong the shelf life. Depending on the specific requirements, MA and CA can extend market availability of the produce up to the next harvest (Mangaraj and Goswami, 2011).

As respiration rate is an important factor (Hammond and Nichols, 1975), its decrease or careful alteration and manipulation can help in achieving the extended shelf life of the commodity. Product type and variety, production conditions, maturity stage at harvest, ageing (time), temperature, gas composition, and cutting (mechanical damage) are important internal and external factors influencing the respiration rate of the fresh produce (Menon and Goswami, 2008;Iqbal *et al.*, 2009; Li *et al.*, 2009). In addition to this, manipulation of storage conditions also results in the lowered enzymatic activities leading to extended storage life and reduction in the utilization of the respiratory substrates.

Peleg model and uncompetitive inhibition based enzyme kinetics model were the commonly used models (Hagger *et al.*, 1992; Mahajan and Goswami, 2001; Bhande and Goswami, 2008; Menon and Goswami, 2008; Mangaraj and Goswami, 2011). Although a number of other models were also proposed by the researchers for the prediction of respiration rate of various fruits and vegetables, 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 (Yang & Chinnan, 1988; Cameron *et al.*, 1989; Talasila *et al.*, 1992; Lee *et al.*, 1991; Hagger *et al.*, 1992; Makino *et al.*, 1996). Among the models, the most theoretically based model is the Michaelis–Menten equation

which is based on the principle of enzyme kinetics but simplifies the complex pathway of respiration assuming it to be based on one limiting enzymatic reaction where the substrate is O_2 (Mangaraj and Goswami, 2011).

Nevertheless, studies regarding respiration modeling and postharvest preservation techniques for *Agaricus bisporus* (Byrnes and O'Beirne, 2007; Iqbal *et al.*, 2009; Li *et al.*, 2009; Oliveira *et al.*, 2012) were abundantly found in the literature, information on the respiration rate of other species like oyster mushrooms (*Pleurotus ostreatus*) is scarce. Few studies on MAP of some of *Pleurotus sps.* such as *P. ostreatus*, *P. sajor-caju* and *P. eryngii*(Sapata *et al.*, 2009; Villaescusa and Gill, 2009) were found, but sufficient amount of respiration rate modelling of oyster mushrooms is still lacking. Azevedo *et al.*(2014) studied and modelled the effect of temperature and time on respiration rate of fresh oyster mushroom. However, the gas concentration along with the storage time and the temperature is an important factor that influences the respiration rate of a commodity (Menon and Goswami, 2008; Peppelenbos, 1996). Therefore, the need was realized to develop models that include the effect of gas concentration, storage time and temperature on the respiration rate of oyster mushrooms. The models were suitably modified for temperature dependence based on the linear model fitting and using an Arrhenius type of relationship (incorporating reference temperature) in Arrhenius equation (Lakakul *et al.*, 1999; Hertog *et al.*, 1998).

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 $V_{m(CO2)}$ or V_m and K_{mO2} and K_{mCO2} for K_m and K_{iO2} , K_{iCO2ox} and $K_{m(O2)f}$ until mentioned otherwise.

Materials and methods:-

Measurement of Respiration Rate:-

Fresh mushrooms (*Pleurotus ostreatus*) were obtained from the experimental farm of Agricultural & Food Engineering Department, Indian Institute of Technology Kharagpur, West Bengal, India. These were washed to remove adhering dirt before using for the experiments. It was ensured that the mushrooms were of uniform size, mass and maturity (depending on color and size of the mushrooms). The sample used for study varied from 0.044 m to 0.060 m in circumference of the veil.

The respiration rate was measured using closed system respirometer (Cameron *et al.*, 1989; Hagger *et al.*, 1992; Morales-Castro *et al.*, 1994; Lee and Lee, 1996; Hong and Kim, 2011; Mahajan and Goswami, 2001; Fonseca *et al.*, 2002; Song *et al.*, 2002; Menon and Goswami, 2008; Bhande *et al.*, 2008; Iqbal *et al.*, 2009; Mangaraj and Goswami, 2011), as this method is more convenient and simpler than continuous flow method which is technically difficult because of the need of highly accurate analytical equipment (Cameron *et al.*, 1989). The sample was kept in airtight respirometer chambers made up of acrylic sheets having dimensions $0.2 \times 0.2 \times 0.18$ m and volume 7200 mLthat were previously equilibrated with the experimental temperature.

Alternatively, another set of experiments was conducted at the same conditions except that after the measurement of gas concentration, the respirometer container was kept open for 10 min so that it gets replenished with new air. This was done in order to study the effect of time on the respiration rate of mushrooms as the time progressed without changing the gas concentration.

The respirometer containers were kept in thermostatically controlled test chambers which were maintained at the desired temperatures with a tolerance limit of \pm 0.5 °C. Gas composition of respirometer was analyzed at regular intervals depending on the storage temperature of mushrooms. The sampling interval was varied depending on the rate of respiration at different temperatures, as at higher temperature the rate of evolution of CO₂ and consumption of O₂ was faster, therefore, the sampling was done at shorter intervals and for lower temperatures long sampling interval was chosen because of the lowered respiration rates. In the headspace analyzer, a zirconium sensor is used for O₂ determination and an infrared detector is used to detect CO₂. The change in gas concentration was measured till the aerobic respiration persisted(i.e. O₂ concentrations reached 0%). The responses studied were R_{CO2} , R_{O2} and RQ with respect to changes in temperature and time. All measurements were taken in triplicate.

Data Analysis:-

The respiration rates in terms of CO_2 and O_2 at a given temperature were calculated using the following Eqs. (1-3) as given by Kays (1991):

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

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(3)

30

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

(3) $RQ = \frac{R_{CO2}}{R_{O2}}$

Where, R_{O2} is the respiration rate, mL O₂ kg⁻¹h⁻¹; R_{CO2} is the respiration rate, mL CO₂ kg⁻¹h⁻¹; G_{O2} and G_{CO2} are the gas concentrations (%) of O₂ and CO₂, respectively (Bhande and Goswami, 2008; Menon and Goswami. 2008; Iqbal et al., 2009; Mangaraj and Goswami, 2011); t is the storage time in hours; Δt is the time difference between two gas measurements; $V_{\rm fr}$ is the free volume of the respiration chamber in mL and W is the mass of the fruit in kg. Mass of mushrooms and free volume of respirometer taken during the experiment is presented in Table 1. The volume of mushrooms was measured by water displacement method and free volume was calculated by subtracting the volume of mushrooms from the total volume of respirometer.

Storage temperature (°C)	Weight of mushrooms, $W(kg)$	Free volume of respirometer, $V_{\rm fr}$ (mL)
0	0.579 ± 0.03	6410 ± 84.81
5	0.498 ± 0.08	6508 ± 111.87
10	0.510 ± 0.11	6523 ± 95.27
15	0.500 ± 0.09	6536 ± 126.65
20	0.532 ± 0.12	6493 ± 94.83
25	0.515 ± 0.07	6516 + 88 12

 6460 ± 103.9

Table 1:- Free volume of respirometer and weight of mushrooms taken for generating the respiration data^a

^a Values are average of three replications and values after \pm are standard deviation.

 0.556 ± 0.12

Respiration Rate Modeling:-

Model 1 (Peleg Model)

Experimental respiration data in which the container was kept open for 10 min to replenish the air was used to perform nonlinear regression analysis (OriginPro 8.5) to fit O₂ and CO₂ concentration at different storage time periods. The respiration rate at a given time is determined from the first derivative of the regression function (Cameron et al., 1989; Hagger et al., 1992; Mahajan and Goswami, 2001; Bhande and Goswami, 2008; Menon and Goswami, 2008; Mangaraj and Goswami, 2011). Respiration rates can be theoretically obtained by measuring the slope of the graph of gas concentration versus time. However, this method is not recommended since data sets have large experimental variations (Mangaraj and Goswami, 2011). Instead, a regression function (Eqs. 4 and 5) is often 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 Eqs. 6 and 7.

$$G_{CO_2} = \frac{t}{(at+b)}$$

$$G_{O_2} = 0.21 - \left[\frac{t}{at+b}\right]$$
(4)
(5)

Where, GCO_2 and GO_2 are the gas concentrations of CO₂ (%) and O₂ (%), 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 Eqs. (6) and (7) and then substituted in Eqs. (8) and (9) to calculate the respiration rate of mushrooms at any given conditions within the experimental range.

$$\frac{dG_{co_2}}{dt} = -at(at+b)^{-2} + (at+b)^{-1}$$
(6)

(1)

$$\frac{dG_{o_2}}{dt} = at(at+b)^{-2} - (at+b)^{-1}$$
(7)

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

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

A similar model was applied to the respiration data of apples by Mahajan and Goswami (2001), for banana by Bhande and Goswami (2008) and other researchers (Cameron *et al.*, 1989; Hagger *et al.*, 1992; Menon and Goswami, 2008; Mangaraj and Goswami, 2011). According to the previous studies, the values of model parameters at given temperature could hitherto be predicted by linear interpolation of the values. Therefore, the linear regression analysis was performed on the values of model parameters at different temperatures. 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 (Uncompetitive Inhibition based Enzyme Kinetics Model)

Uncompetitive inhibition based enzyme kinetics model was developed to predict the respiration rate on the basis of consideration that CO_2 acts as a respiratory inhibitor (McLaughlin and O'Beirne, 1999). 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 (Peppelenbos*et al.*, 1996; Hertog*et al.*, 1998; Lammertyn*et al.*, 2003; Ho *et al.*, 2008; Ho *et al.*, 2010). The first part in equations 11 and 13 is CO_2 production by oxidative respiration and the second part is by the fermentation. The linearized forms (Eqs. 12 and 13) of the equations (10 and 11) were fitted to the gas concentrations at different storage time using multiple linear regression analysis (OriginPro 8.5).

However, at high levels of CO₂concentration (18%) and reduced levels of O₂ concentration (0%) the respiration mechanism changes from aerobic to anaerobic pathway (Mahajan and Goswami,2001; Bhande and Goswami, 2008). The model parameters (Eqs. 10 and 11), $V_{\rm m}$, $K_{\rm m}$, and $K_{\rm i}$ for O₂ consumption and CO₂evolution were determined using the experimental respiration data. The Eqs. 10 and 11 were converted to their linearized forms (Eqs. 12 and 13) to estimate the model parameters by multiple linear regression analysis using OriginPro 8.5.

$$R_{CO_{2}} = \left[\frac{V_{m(CO_{2})}G_{O_{2}}}{K_{m(CO_{2})} + \left(1 + \frac{G_{CO_{2}}}{K_{i(CO_{2})}}\right)G_{O_{2}}}\right] + \left[\frac{V_{m(CO_{2})f}}{\left(1 + \frac{G_{CO_{2}}}{K_{m(O_{2})f}}\right)}\right]$$
 Or

$$R_{CO_{2}} = RQ \times RO_{2} + \frac{V_{m(CO_{2})f}}{\left(1 + \frac{G_{CO_{2}}}{K_{m(O_{2})f}}\right)}$$
(10)

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

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

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

Where, $V_{m(O2)}$ is the maximum O₂ consumption rate; $V_{m(CO2)}$ and $V_{m(CO2)/3}$ are the maximum CO₂ production rates by oxidative respiration and fermentation (mL.kg⁻¹.h⁻¹) respectively; K_{mO2} and K_{mCO2} are the Michaelis-Menten constants for O₂ consumption and CO₂ evolution (%O₂), respectively; and K_{iO2} and K_{iCO2} are the inhibition constants for O₂ consumption and CO₂ evolution (%CO₂), respectively, $K_{m(O2)/3}$ is the inhibition constant of O₂ on fermentative CO₂ production rate (%O₂); G_{CO2} and G_{O2} are gaseous concentrations of CO₂ and O₂ (%).

The model parameters obtained were correlated at different temperatures using Arrhenius equation (Bhande and Goswami, 2008; Mangaraj and Goswami, 2011). However, the mathematical structure of the Arrhenius equation as proposed by Bhande and Goswami (2008); Menon and Goswami (2008) involving the exponentiation of the reciprocal of the absolute temperature, introduces a high correlation between two parameter estimates making parameter estimation difficult. To overcome this complication, the Arrhenius equation was represented in its linear form by Mangaraj and Goswami (2011). In order to further minimize the high correlation among the parameters of the Arrhenius equation, reparameterization was suggested by Schwaab and Pinto (2007). The reparameterization can be attained by involving a new temperature term T_{ref} into the Arrhenius equation which is the reference temperature (Eq. 14) allows the estimation of uncorrelated parameters and simultaneous improvement of the precision of the parameter estimates in problems involving a single kinetic constant (Schwaab and Pinto, 2007).

$$R_{m} = R_{p} \exp\left[\frac{-E_{a}}{R} \times \left(\frac{1}{T_{abs}} - \frac{1}{T_{ref}}\right)\right]$$

$$\ln R_{m} = \left[\frac{-E_{a}}{R} \times \left(\frac{1}{T_{abs}} - \frac{1}{T_{ref}}\right)\right] + \ln R_{p}$$
(14)
(14)

 $R_{\rm m}$ is the generalized form of model parameters (i.e. $V_{\rm m}$, $K_{\rm m}$, and $K_{\rm i}$), $R_{\rm p}$ is respiration pre-exponential factor, $E_{\rm a}$ is the activation energy(kJ g⁻¹ mol⁻¹), R is the universal gas constant(kJ g⁻¹ mol⁻¹ K) and $T_{\rm abs}$ is the absolute temperature (K) and $T_{\rm ref}$ is the reference temperature (K).

Validation of the models:-

The suitability of models to predict the respiration rate was examined by using the relative deviation modulus (Eq. 16) by calculating the difference between the actual and the predicted respiration rates at 3 °C. The experimental respiration rate of the mushrooms in terms of CO_2 evolution and O_2 consumption was calculated using Eqs. 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.

$$E = \frac{100}{N} \sum_{1}^{N} \frac{|(R_{\exp} - R_{pre})|}{R_{\exp}}$$

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⁻¹h⁻¹ and R_{pre} is the predicted respiration rate mL kg⁻¹h⁻¹. In general, lower modulus shows the close agreement between the predicted and the experimental values. The free volume and weight of the mushrooms taken for the validation were 6518 mL and 0.511 kg, respectively. The initial gas concentration of the respiration models.

Results and discussion:-

Effect of temperature and gas concentration on the respiration rate:-

Expectedly, the respiration rate increased significantly (P<0.05) with the rise in temperature and it was recorded as 29.52 ± 0.33 and 40.59 ± 0.45 at 0 °C and 360.18 ± 0.43 and 395.06 ± 0.38 at 30 °C in terms of CO₂ evolution (mL of CO₂ kg⁻¹ h⁻¹) and O₂ consumption (mL of O₂ kg⁻¹ h⁻¹), respectively. Moreover, the gas concentrations reached their upper limits (CO₂>18% and 0% O₂) in 80 h at 5 °C and in 28 h at 15 °C as can be seen from Figure 1 (Salveit, 2004).

(16)



Figure 1:- Gas concentrations of CO₂ and O₂ at (a) 5 °C and (b) 15 °C. Data correspond to the mean \pm SE of the independent triplicates.

Though the respiration rate increased with the increase in temperature, it followed a decreasing trend with the progress in time due to the increase in CO_2 concentration and the decrease in O_2 concentration (Bhande and Goswami, 2008; Menon and Goswami, 2008). At the outset of the experiment, the initial respiration rate was higher, i.e. 29.52 and 40.59 (mL kg⁻¹ h⁻¹) but it reduced to 6.80 and 6.29 in terms of CO_2 evolution and O_2 consumption, respectively, after the accumulation of 18.3% of CO_2 and reduction of O_2 to 0.75% at 0 °C. This can be attributed to the inhibitory effect of CO_2 on the rate of respiration. A similar trend is clearly reflected in Figure 2 by the gradual reduction in the slope of the data points with the time.



Figure 2:- Change in respiration rate with increase in concentration of CO_2 and decrease in O_2 concentration at 5 °C.Data correspond to the mean \pm SE of the independent triplicates.

The respiratory quotient (RQ), estimated as the ratio of CO_2 evolved to the O_2 consumed, was found to vary with time for all the temperature levels studied. Similar results of variation with time and temperature were reported by earlier researchers (Bhande and Goswami, 2008; Mangaraj and Goswami, 2011). Nakamura *et al.*, (2004)also reported similar temperature dependence of RQ for mangoes. The RQ above 1.3 indicates that the respiration has turned into anaerobic respiration, the process being termed as anaerobiasis (Kader, 1986;Iqbal *et al.*, 2009). This was evident by off-odor produced inside the chamber after the onset of anaerobiasis. In the present study, the tolerance limits for oyster mushrooms were found to be 17.2% of CO_2 (maximum) and 1.55% of O_2 (minimum), i.e. the gas concentration after which the anaerobiasis sets in and the value of RQ exceeds 1.3.This combination of gases can, therefore, be used as the basis of selection of range for optimization of gas concentration in MAP for oyster mushrooms.

Estimation of model parameters:-

Model1 (Peleg model)

The values of model parameters a and b of the regression equation indicated the temperature dependence of both the parameters, whereas, the temperature had more a pronounced influence on b than a. The model parameters 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 slightly higher, but similar trend of the parameters reported for the respiration of apples by Mahajan and Goswami (2001) and banana by Bhande and Goswami(2008). This increase in the values of the parameters *a* and*b* as compared to the literature values of banana and apple could be attributed to the higher respiration rate of mushrooms. 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 2. Since the $R^2 > 0.992$, the regression functions suitably fitted the experimental data for mushrooms.

Table 2:- Regression co efficients *a* and *b* of Peleg model at different storage temperatures for consumption of O_2 and evolution of CO_2 , respectively

Temperature		CO_2			O_2	
	$a(h \%^{-1})$	$b (\%^{-1})$	Adj. R^2	$a (h \%^{-1})$	$b (\%^{-1})$	$\operatorname{Adj} R^2$
0	3.17±0.02	378.49±5.03	0.999	3.11±0.53	211.44±9.21	0.998
5	3.26±0.13	254.76±6.04	0.998	3.24±0.41	178.83±11.33	0.998
10	3.46±0.09	174.61±4.22	0.997	3.49±0.75	112.92±6.16	0.999
15	3.61±0.19	121.34±6.54	0.998	3.92±0.51	88.33±7.27	0.994
20	3.89±0.01	78.32±7.21	0.997	4.35±0.82	62.33±5.52	0.994
25	4.28±0.42	52.31±5.15	0.994	4.53±0.32	57.76±6.64	0.998
30	4.92±0.45	22.94±5.03	0.999	4.81±0.23	24.99±3.08	0.998

Values in the table are average of three replications and values after \pm are standard deviation.

The values of model parameters *a* and *b* of different storage temperatures were found to follow zero order kinetics (Figure 3) and the model equations found can be used for the prediction of model parameters for a given combination of gases (i.e. CO_2 and O_2). This method yields more precise values, otherwise, the value of *a* and *b* can also be determined by linear interpolation of the known values. The Eqs. 6 and 7 can be used to estimate dGO_2/dt and $dGCO_2/dt$ by substituting the corresponding values of *a* and *b*, respectively, that can further be substituted in the Eqs. 1 and 2 for the prediction of respiration rate by model 1.



Figure 3:-Fitting of linear model on (a) parameter *a* and (b) parameter*b* of Peleg equation.

Model 2 (uncompetitive inhibition based enzyme kinetics model)

The multiple linear regression analysis was used to estimate the model parameters (Table 3 and 4). The model parameters showed temperature dependence, which was established by fitting the Arrhenius model on the model parameters against the inverse of absolute temperature. All the model parameters (R_m) such as V_m , K_m , and K_i for O₂ and CO₂ (both oxidative as well as fermentative) were plotted as a natural logarithm against the difference of inverse of absolute temperature in the linear form (Eq. 15).

In case of competitive inhibition, the inhibition by CO_2 can be counteracted by increasing O_2 , because both the gases $(O_2 \text{ and } CO_2)$ are competing for the same active site. But in case of uncompetitive inhibition, the CO_2 reacts with enzyme substrate complex, therefore, increase in the concentration of O_2 will not be able to counteract CO_2 as there is always some amount of enzyme-substrate complex available for CO_2 to bind. Hence, 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. The high values of V_m (maximum respiration rate), indicate that the respiration rate was strongly affected by increase in temperature and were found to be in agreement with those reported in the literature(Mahajan and Goswami, 2001; Mangaraj and Goswami, 2011).

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Storage	Respiration	Enzyme kinetics c		Coefficient of			
tempera-	expression in terms				determination		
ture, °C	of	V _m	K _m	Ki	$(\mathrm{Adj}.R^2)$		
		$(mL kg^{-1} h^{-1})$	% O ₂	% CO ₂			
0	CO ₂ evolution	35.81±3.87	8.14±1.12	12.13±3.01	0.938		
	O ₂ consumption	28.98±4.14	7.87±1.36	9.76±2.15	0.992		
5	CO ₂ evolution	176.99±12.32	9.27±1.73	11.89±3.52	0.983		
	O ₂ consumption	173.41±12.18	8.32 ± 2.81	8.95±2.57	0.995		
10	CO ₂ evolution	188.55±19.41	10.33±1.01	10.15±2.23	0.998		
	O ₂ consumption	192.87±11.87	9.23±1.33	8.02±1.36	0.995		
15	CO_2 evolution	203.25±12.01	11.15±2.15	9.03±2.43	0.961		
	O ₂ consumption	213.45±13.36	10.45±0.91	7.42±1.95	0.976		
20	CO ₂ evolution	298.34±17.74	12.87±2.21	8.78±1.56	0.988		
	O ₂ consumption	308.78±12.14	11.98±1.32	6.57±0.93	0.935		
25	CO ₂ evolution	354.56±21.24	13.54±1.43	7.88±1.51	0.979		
	O ₂ consumption	369.43±24.92	13.14±3.32	6.34±1.42	0.984		
30	CO_2 evolution	436.68±25.34	14.06±1.72	6.74±1.81	0.985		
	O ₂ consumption	574.72±26.87	13.41±2.83	5.72±1.72	0.993		

Table 3:- Model	parameters for	uncompetitive	inhibition	enzyme	kinetics	based	model at	different	storage
			tommomotive						

Values in the table are average of three replications and values after \pm are standard deviation.

Table 4:- Model	parameters of	uncompetitive	inhibition	enzyme	kinetics	based	model	for ferr	nentative	eCO_2
		production at (different st	orage ter	nperatur	es.				

Storage temperature, °C	$V_{m(CO2)f}$	$K_{m(O2)f}$	Coefficient of
			determination (Adj. R^2)
0	62.02±9.82	1.94±0.43	0.86
5	137.06±19.59	2.95±0.63	0.91
10	187.13±32.23	3.28±0.81	0.91
15	204.39±41.9	5.07±2.66	0.90
20	289.38±32.56	8.36±1.13	0.91
25	360.74±28.45	9.31±2.41	0.93
30	454.13±40.19	11.71±2.41	0.89

Values are average of three replications and values after \pm are standard deviation.

 K_m is the measure of saturation of respiration by O₂ at which V_m becomes half (1/2 V_m) when there is no inhibition by CO₂ and the values of parameter K_m at different temperature were found similar to the literature (Bhande and Goswami, 2008; Menon and Goswami, 2008; Mangaraj and Goswami, 2011). The value for K_i is a measure of the degree to which the respiration (both in terms of CO₂ production and O₂ consumption) can be inhibited by CO₂. 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 CO₂ (Hertog*et al.*, 1998). In the present study, the values of $K_{i(CO2)}$ and $K_{i(O2)}$ for mushrooms indicated that the respiration was inhibited by CO₂ and the values were similar to the literature (Bhande and Goswami, 2008; Mangaraj and Goswami, 2011). The high values of parameter $K_{m(O2)f}$, which signifies the extent to which fermentation can be inhibited by O₂ indicate that the mushrooms show fermentative CO₂ production at even higher levels of O₂.

There was a change in the trend of the linear plot of Arrhenius equations and it appeared to have two different regions which can be attributed to the change in rate limiting enzymatic action due to the change in storage temperature (Song *et al.*, 1992). The values of activation energy and the respiration pre-exponential factor were calculated from the slope and *y*-axis intercept and are presented in Table 5. The negative value of the activation energy for parameter K_i indicated that the temperature had a negative effect on the inhibition by CO₂, which means with the increase in temperature the inhibitory effect of CO₂ was less pronounced (Bhande and Goswami, 2008). These constants can be used to predict the values of model parameters of equations (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. CO₂ and O₂.

Parameters of	Maximum resp	iration rate,	Michaelis-Menten equation,		Inhibition constant (K_i), % O ₂	
Arrhenius equation	$(V_{\rm m}), {\rm mL.}{\rm kg}^{-1}.$	h^1	$(K_{\rm m}), \% {\rm O}_2$			
	O ₂	CO ₂	O ₂	CO ₂	O ₂	CO ₂
Activation energy, E _a ,	43.2±1.39	32.71±1.16	37.18±1.25	40.59±1.42	-26.01±1.11	-38.15±1.31
$kJ.g^{-1}.mol^{-1}$						
Pre-exponential factor,	223.7±2.16	225.65±2.43	4.48±0.36	3.62±0.28	3.09±0.21	3.83±0.17
R _p						
\mathbb{R}^2	0.98	0.97	0.99	0.94	0.97	0.98
Parameters for fermentative CO ₂ production				$V_{m(CO2)f}$		$K_{m(O2)f}$
Activation energy, E_a , kJ.g ⁻¹ .mol ⁻¹				40.32		42.94
Pre-exponential factor, R_p				208		5.24
\mathbb{R}^2				0.92		0.97

Table 5:- Parameters of Arrhenius equation for different model parameters for un	ncompetitive inhibition based
enzyme kinetics model.	

Validation of the Models:-

In order to find the suitability of the developed models for the prediction of the respiration rate within the experimental domain, validation is necessary. Since, the experiments were conducted from 0 to 30 °C with the step of 5 °C, validation of the model was conducted at 3 °C storage temperature. The respiration rates of mushrooms in terms of O_2 consumption and CO_2 evolution were estimated by using Eqs. 1 and 2. The predicted and actual respiration rates of mushrooms as presented in Figures 4 and 5 in terms of CO_2 and O_2 , respectively, showed that the mean relative deviation moduli of predicted versus experimental respiration rates for model 1 (Peleg model) were found to be 10.3% and 11.2% for O_2 and CO_2 respiration expressions and 9.3% and 8.5% for O_2 and CO_2 for model 2(uncompetitive inhibition based enzyme kinetics model), respectively.

Although the predicted respiration rates of mushrooms for both the models used in the study were in close agreement with the experimental respiration rates. 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 mainly affected by gas concentration and temperature, the time also plays a crucial role in the respiration rate (Azevedo*et al.*, 2014) and, therefore, the Peleg model was also used for modeling the respiration rate.



Figure 4:- Predicted and experimental respiration rate of mushrooms at 3 °C in terms of CO₂ evolution.



Figure 5:- Predicted and experimental respiration rate of mushrooms at 3 °C in terms of O₂ consumption.

Conclusions:-

The respiration rate of the mushrooms 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 were found suitable for the prediction of respiration rate of mushrooms within the experimental range i.e. from 0 to 30 °C. The respiration rate predicted by the regression (Peleg) model and uncompetitive inhibition type enzyme kinetics model showed close agreement with the experimentally determined respiration rate of the mushrooms at 3 °C.

Based on the Arrhenius equation with a new term, the reference temperature (T_{ref}), a temperature dependent model was also developed which has shown a significant effect of temperature on the respiration rate of mushrooms. By the use of the term T_{ref} in the Arrhenius equation, the pre-exponential factor R_p becomes equal to the respiration rate of the mushrooms at the reference temperature. The models developed are specific to that cultivar of mushrooms and are, therefore, valid only within the temperature where experiments were conducted i.e. from 0 to 30 °C storage temperatures. Furthermore, the activation energy and pre-exponential factor of the Arrhenius equation were found to be useful for predicting the model parameters at given range of storage temperatures of 0 to 30 °C. The information obtained regarding the respiration rate and the developed models would be of immense help in designing MAP and AP system for the oyster mushrooms.

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Conflict of interest:-

The authors declare that there is no conflict of interest.

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