

## EFFECT OF TEMPERATURE ON THE RESPIRATION OF MILKY MUSHROOM

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**Abstract:** A study was conducted to determine the influences of storage temperature (5, 15 and 25°C) on respiration rate (RR) of milky mushroom. The RR of milky mushroom was measured as 1.05, 1.10 and 1.14 for 5, 15 and 25°C respectively for oxygen and 0.67, 0.91 and 0.94 for 5, 15 and 25°C respectively for carbon dioxide. The influence of higher storage temperature in increasing the RR of milky mushroom was more pronounced towards the end of the storage period. The effects of temperature on rates of O<sub>2</sub> consumption and CO<sub>2</sub> production of mushrooms were adequately described by an Arrhenius type model. The model was validated for mushroom stored at 10°C, and a good agreement was found between experimental and predicted data.

**Keywords:** Milky mushroom, Respiration.

### INTRODUCTION

*Calocybe indica* commonly known as milky mushroom is a well recognized tropical edible mushroom and promising for cultivation in India (Purkayastha and Chandra, 1976). This is the first indigenous mushroom to be commercialized in India. It contains highest protein (17.2%) and has 12 essential amino acids. Cropping requires an optimum temperature of 32-35°C, humidity of 85-90%, diffused light and ventilation. Biological efficiency potential for this mushroom is 50 – 100%. Shelf life of milky mushroom is 2-3 days at 25- 30°C and 10-15 days at 4°C if microbial spoilage is taken care of (ICAR- IIHR, 2016). Accurate measurement of respiration rates and modeling is pivotal to the design of MA for agricultural commodity (Mangaraj and Goswami, 2011).

Babu and Rao (2011) studied on the antioxidant properties and electrochemical behavior of milky mushroom. Their research shows that milky mushroom has more total flavonoid content along with DPPH scavenging, FRAP and reducing power abilities. Talasila *et al.* (1992) developed a non-linear empirical model for predicting the respiration rate of strawberry as a function of temperature, O<sub>2</sub> and CO<sub>2</sub> concentration. Most of the model have

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not incorporated one or other dependent factors such as O<sub>2</sub>, CO<sub>2</sub>, temperature and time, and hence, are not flexible enough to predict the respiration rate at various storage conditions. The recent approach for modeling respiration rates by using Michaelis-Menten type equation is based on enzyme kinetics. This provides a simple description of respiration, based on the assumption that diffusion and solubility of O<sub>2</sub> and CO<sub>2</sub> in plant tissue regulates reactions catalyzed by enzymes (Lee *et al.*, 1991).

Respiration study is having a great influence on the shelf life of mushroom. The respiration of milky mushroom has not been reported in previous literatures. Keeping this in view, the present study was undertaken to find out the effect of temperature on respiration of milky mushroom.

## **MATERIALS AND METHODS**

Fresh milky mushroom was harvested from local farm and packed in polyethylene bags for transportation. On arrival, mushroom were cleaned thoroughly to remove any foreign materials and wiped with clean dry cloth and stored in 5°C. Thereafter, the mushroom was used for the experiments.

Closed system method was used for generating the respiration data for mushrooms at different temperatures (Amir *et al.* 2010). Air tight glass bottles of capacity 3L was used for the study. Mushrooms were kept in bottles and were closed with a lid and made it hermetically sealed using cilliputty. A septum was glued to the bottle for taking the gas sample. The setup was kept in humidity control chamber (Technico, Chennai, India), which was maintained at desired temperatures with a variation of  $\pm 0.5^{\circ}\text{C}$  and relative humidity of  $90\pm 2\%$ . Experiments were conducted at 5, 15 and  $25 \pm 0.5^{\circ}\text{C}$  temperatures.

### ***Gas analysis***

The head space oxygen and carbon dioxide concentrations within the glass bottles were monitored periodically using a portable O<sub>2</sub>/CO<sub>2</sub> gas analyzer (PBI Dansensor), after calibrated with standard gases. Using the O<sub>2</sub> and CO<sub>2</sub> concentrations obtained at regular intervals, the respiration rates of sample were found out at different temperatures.

### ***Modeling and data analysis***

The respiration study has been conducted in IICPT laboratory UNDER various storage temperatures viz. 5, 15 and 25°C for milky mushroom. The data on oxygen consumption and carbon dioxide release was studied with time till their values got stabilize. The respiration was modeled using regression analysis and enzyme kinetic models as explained by Salu *et al.* (2016). The temperature dependence of the model parameters of the Michaelis- Menten

equations were quantified using an Arrhenius type relationship as given by equation (1) following Caleb *et al.*, 2012.

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

Where:  $R_m$  is the model parameter of Michaelis- Menten equation;  $R_p$  is the respiration pre-exponential factor;  $E_a$  is the activation energy in  $\text{kJ g}^{-1} \text{mol}^{-1}$ ;  $T$  is the storage temperature in K and  $R$  is the universal gas constant in  $\text{kJ g}^{-1} \text{mol}^{-1} \text{K}^{-1}$  (  $8.314 \text{ kJ g}^{-1} \text{mol}^{-1} \text{K}^{-1}$ ). The above equation can be expressed in a linearised form as follows:

$$\ln R_m = -\frac{-E_a}{R} \left[ \frac{1}{T} \right] + \ln R_p \quad (2)$$

### **Verification of the model**

Respiration rates of mushroom predicted by models were verified with experimental respiration rates at  $10^\circ\text{C}$  storage temperature. The goodness of fit between experimental and predicted respiration rates was obtained by using the mean relative deviation modulus (E) as given in equation (3). According to McLaughlin and O'Beirne, 1999 moduli below 10% are representing a reasonable good fit, between 10 and 20% are fairly good fit and between 20 and 30% are of not satisfactory fit for all practical purposes.

$$E = \left[ \frac{100}{N} \sum_{i=1}^n \frac{(R_{\text{exp}} - R_{\text{pre}})}{R_{\text{exp}}} \right] \quad (3)$$

Where: E is the mean relative deviation modulus in %; N is the number of respiration data points;  $R_{\text{exp}}$  is the experimental respiration rate in  $\text{ml kg}^{-1} \text{h}^{-1}$  and  $R_{\text{pre}}$  is the predicted respiration rate in  $\text{ml kg}^{-1} \text{h}^{-1}$ .

## **RESULTS AND DISCUSSIONS**

### **Free volume and the weight of mushroom**

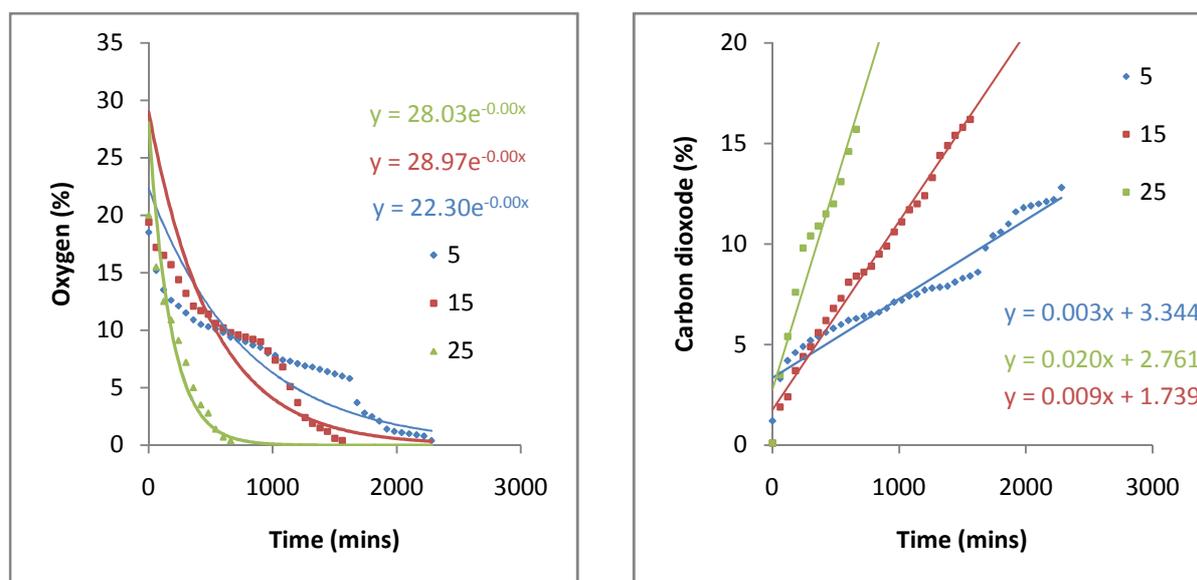
Free volume of the mushroom holding glass bottles and the weight of the mushroom kept in the respective glass bottles are shown in table 1.

**Table 1: Free volume and the weight of the mushroom kept for respiration rate study**

| Storage temperature, °C | Weight (W), g | Volume (V), ml |
|-------------------------|---------------|----------------|
| 5                       | 675           | 2320           |
| 15                      | 635           | 2220           |
| 25                      | 610           | 2150           |

### *Oxygen and Carbon dioxide concentration during respiration*

Figure 1 shows the actual gas composition in terms of oxygen and carbon di oxide inside the glass bottles kept in different temperatures. From the obtained graphs it is observed that as the temperature increased, the respiration also increased in a faster rate. This is due to the change in the respiration rate which is influenced by the storage temperature. The maximum time taken for reaching the least oxygen content of 0.5% is 2280 minutes, in which the sample was kept in 5°C. Minimum time taken (660 minutes) is by the mushrooms kept in 25°C.



**Figure 1: Changes in the concentration for oxygen and carbon dioxide kept in different storage temperatures (5°C, 15°C, 25°C)**

The regression coefficients a and b and their corresponding  $r^2$  (coefficients of determination) values at different storage temperatures are shown in table 2. The experimental data fitted very well with the regression equation having  $r^2$  values  $> 0.877$  indicates that the regression functions fit the data very well. From the values of the regression coefficients a and b as shown in table 2, it can be inferred that respiration was influenced by the storage temperature (Mangaraj and Goswami, 2011). However, coefficient b was more influenced than coefficient a.

The enzyme kinetic model parameters were calculated and are given in table 3 along with their corresponding coefficients of determination ( $r^2$ ) values. The value of table 3 shows that, Michaelis- Menten type equation parameters were also dependent on the storage temperature. Coefficient of determinants of this model is indicating that the relationship between respiration rate and oxygen and carbon dioxide concentrations is fitted well.

**Table 2: Regression coefficients a and b for oxygen consumption and carbon dioxide evolution respectively, for treated and untreated samples stored in different temperatures**

| Storage Temperature | Respiration expression in terms of | Regression Coefficients |         | r <sup>2</sup> |
|---------------------|------------------------------------|-------------------------|---------|----------------|
|                     |                                    | a                       | b       |                |
| 5                   | O <sub>2</sub> Consumption         | 4.31                    | 2554.66 | .964           |
|                     | CO <sub>2</sub> evolution          | 6.27                    | 6222.96 | .913           |
| 15                  | O <sub>2</sub> Consumption         | 2.47                    | 4082.78 | .965           |
|                     | CO <sub>2</sub> evolution          | 2.41                    | 6269.61 | .877           |
| 25                  | O <sub>2</sub> Consumption         | 3.04                    | 1165.70 | .952           |
|                     | CO <sub>2</sub> evolution          | 4.33                    | 1632.20 | .953           |

**Table 3: Model parameters of uncompetitive inhibition enzyme kinetics for different storage temperatures and ozone treatments**

| Storage temperature, °C | Respiration expression in terms of | Maximum respiration rate (V <sub>m</sub> ), ml/kg h | Michaelis-Menten constant (K <sub>m</sub> ), % O <sub>2</sub> | Inhibition constant (K <sub>i</sub> ), % CO <sub>2</sub> | r <sup>2</sup> |
|-------------------------|------------------------------------|---|---|--|----------------|
| 5                       | O <sub>2</sub> Consumption         | 7.45  | 9.69  | 0.02   | 0.965          |
|                         | CO <sub>2</sub> evolution          | 3.33  | 3.24  | 0.02   | 0.877          |
| 15                      | O <sub>2</sub> Consumption         | 3.60  | 10.67   | 0.04   | 0.878          |
|                         | CO <sub>2</sub> evolution          | 0.99  | 2.48  | 0.25   | 0.891          |
| 25                      | O <sub>2</sub> Consumption         | 3.6   | 10.48   | 0.85   | 0.863          |
|                         | CO <sub>2</sub> evolution          | 3.0   | 2.91  | 0.17   | 0.964          |

**Table 4: Activation energy and pre-exponential factor of Arrhenius type equation for different model parameters of uncompetitive enzyme kinetics**

| Parameters for Arrhenius equation | V <sub>m</sub>        |                       | K <sub>m</sub>        |                       | K <sub>i</sub>        |                       |
|-----------------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
|                                   | O <sub>2</sub>        | CO <sub>2</sub>       | O <sub>2</sub>        | CO <sub>2</sub>       | O <sub>2</sub>        | CO <sub>2</sub>       |
| E <sub>a</sub> (kJ/g-mole)        | 24.76                 | 41.55                 | 3.29                  | 9.24                  | -12.82                | -8.78                 |
| R <sub>p</sub>                    | 1.4 x 10 <sup>6</sup> | 7.4 x 10 <sup>8</sup> | 2.5 x 10 <sup>8</sup> | 1.3 x 10 <sup>2</sup> | 1.5 x 10 <sup>6</sup> | 7.9 x 10 <sup>4</sup> |

**Fitting Arrhenius equation**

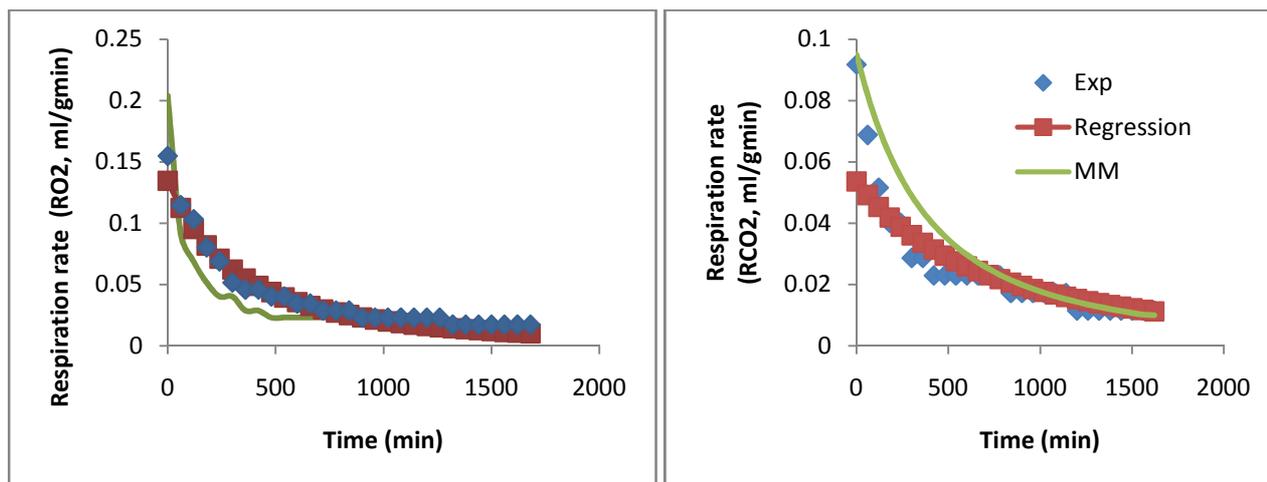
The model parameters such as v<sub>m</sub>, k<sub>m</sub> and k<sub>i</sub> for O<sub>2</sub> and CO<sub>2</sub> concentrations, were found to vary with the temperature hence Arrhenius equation was used to co- relate the model

parameters at different storage temperatures. As per the equation 1 the model parameters were plotted at different storage temperatures; by plotting the log values of the model parameters against the inverse of corresponding temperature in absolute temperature. The activation energy was calculated from the slope of the straight line and the pre-exponential factor was calculated from the Y-axis intercept. Table 4 shows the activation energy and pre-exponential factor for different model parameters of enzyme kinetics. Activation energy was found to be in negative side for  $k_i$  ( $O_2$ ) and  $k_i$  ( $CO_2$ ). The negative values of activation energies for  $k_i$  could be attributed to the inhibitory effect of  $CO_2$  concentration on respiration rate. By using these constants, the model parameters at any temperatures can be predicted by using equation 2 and then, the respiration rate at the given temperature can be estimated for respiration rates in terms of  $O_2$  consumption and  $CO_2$  evolution, respectively.

#### ***Verification of respiration rate models***

The respiration study was done at temperatures of 5, 15 and 25°C for milky mushrooms. However, the developed models were verified to assess the capability of its predictability of the respiration rates at any temperature between 5 and 25°C. The respiration rate models were verified at 10°C storage temperature for mushrooms. For generating the experimental data for  $O_2$  and  $CO_2$ , the free volume of the chamber and the weight of the mushrooms were as 2320 ml and 675g, respectively. Figure 2 shows the change in  $O_2$  and  $CO_2$  concentrations with storage time at 10°C. The regression coefficients a and b at 10°C storage temperature was found out using the regression model. The respiration rates of mushroom in terms of  $O_2$  consumption and  $CO_2$  evolution were estimated by using equations 9 and 10.

. The mean relative deviation moduli (equation 13) between respiration rates of mushroom at 10°C predicted by regression analysis and that obtained through experiments were 10.6% and 7.24% for  $O_2$  consumption and  $CO_2$  evolution, respectively. Similarly the mean relative deviation moduli between respiration rates of mushroom at 10°C predicted by enzyme kinetics and that obtained through experiments were found to be 2.9% and 9.48% for  $O_2$  consumption and  $CO_2$  evolution, respectively. The predicted and experimental respiration rates are given in figure 2. This suggested that the predicted respiration rates for ozone treated and non- treated mushroom were close agreement with the experimental respiration rates.



**Figure 2: Predicted and experimental respiration rates of mushroom at 10°C storage temperature**

## CONCLUSION

Respiration rates for milky mushrooms at different temperatures from 5 to 25°C in step of 10°C were estimated using a closed system method. The respiration data generated by this method can be used to model the respiration rate. The respiration rates predicted by the regression model and enzyme kinetic model were found to be in close agreement with those obtained experimentally. Predicted respiration rates by the models were found to be in good agreement with the experimental respiration rates. The activation energy and respiration pre-exponential factor could be used to predict the model parameter of enzyme kinetics at any storage temperature. There was a good agreement between experimental and predicted respiration rate at 10°C storage temperature.

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