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## Atmospheric Composition and Respiration of Fresh *shiitake* Mushroom in Modified Atmosphere Packages

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The respiration rates of fresh *shiitake* mushroom were measured in modified atmosphere packaging (MAP) with LDPE and PVC films at 0, 5, 15, 20 and 30 °C. The results indicated that respiration rates were suppressed by decreasing O<sub>2</sub> and increasing CO<sub>2</sub> concentrations at 5, 15, 20 and 30 °C, but it was not changed much at 0 °C inside both LDPE and PVC packages. The respiratory quotient (RQ) breakpoints were mainly controlled by O<sub>2</sub> concentration and little affected by CO<sub>2</sub> concentration. Moreover, there was temperature dependence of RQ breakpoint for fresh mushroom in MAP with high linear correlation coefficient ( $r=0.928$ ). In the aerobic respiration period, the respiration rates of fresh mushroom were also significantly affected by O<sub>2</sub> concentration. The effect of the storage time became greater factor next to O<sub>2</sub> concentration at 15 and 20 °C. These results suggest that better keeping quality for fresh *shiitake* mushroom could be obtained from low O<sub>2</sub> atmosphere conditions above RQ breakpoint at low temperature.

### INTRODUCTION

In recent years, fresh *shiitake* mushroom has been accepted as main food dishes in Japan, its production has been increased to very high levels. But there are two main problems due to its high respiration rate and rapid quality deterioration, namely low levels of freshness and short shelf-life. It has been long been demonstrated that MAP is an economical and effective way of extending the shelf life of fresh mushroom by creation and maintaining an optimum gaseous atmosphere surrounding the produce (Burton, *et al.*, 1987; Roy, *et al.*, 1995a, 1995b). Moreover, MAP has continuing beneficial effects until the package is opened.

Over several years, many studies on MAP of mushrooms focused on creating and maintaining optimal atmosphere conditions and on the suitability of the various films (Burton, *et al.*, 1987; Lopez-Briones, *et al.*, 1993; Exama, *et al.*, 1993; Roy, *et al.*, 1995a). However, the benefits from MAP were limited if the gas concentrations in the package are out of recommended level because the gaseous conditions were under unsteady state with the changes in respiration of produce and permeability of film. In especial, it is

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difficult to ensure constant temperature during storage and transportation, temperature variations will affect the respiration rates of produce and the permeability of MAP film. But the permeability of the package may not change to the same extent as respiration rate, and hence the gas concentrations inside the package will be altered (Exama, *et al.*, 1993). The excessive accumulation of CO<sub>2</sub> inside the package can cause physiological injuries to produce and results in severe browning (Nichols & Hammond, 1973; Lopez-Briones, *et al.*, 1992). The lack of O<sub>2</sub> leads to anaerobic respiration accompanied by off-odors due to the production of volatile substances, such as ethanol and acetaldehyde. Similar to other mushroom (*Agricus bisporus*), fresh *shiitake* mushroom has relatively short shelf life with a higher respiration rate and susceptibility to surface enzymatic browning that the reaction occurred during storage and marketing. It is not easy to get a desired atmosphere in package because the gas concentration in MAP is affected by many factors such as film properties, temperature and characteristics of product. The respiration rate of postharvest fresh *shiitake* mushroom is important physiological index for optimal MAP design under unsteady atmosphere conditions of storage and marketing. The objective of this study was to investigate the respiratory characteristics of fresh *shiitake* mushroom in MAP with different permeability of films. The effects of O<sub>2</sub> and CO<sub>2</sub> concentrations on the respiration rate in the aerobic respiration period were also investigated by multiple regression analysis.

## MATERIALS AND METHODS

### Mushrooms and storage conditions

Mushrooms (*Lentinus edodes* Sing.) cultivated at laboratory were hand-harvested and sorted by size, shape and appearance. Stems were hand trimmed to 10 mm and were precooled for 10 hours at experimental temperatures. And then mushrooms were selected at random and samples of 100 ± 5 g were placed in the pouches and heat sealed. The free volume of pouch was determined by subtracting the mushroom volume from the total volume, which was measured by displace water. The pouches were stored at 0, 5, 15, 20 and 30 °C.

### Package atmosphere

Throughout storage, three packages for each treatment were taken out and 1.0 ml gas samples were withdrawn by gas-tight 1.0 ml syringe to measure gas composition inside the package by gas chromatography (GC-390, GL Sciences Inc.) equipped with thermal conductivity detector (TCD) and integrator D2000 (Hitachi, Ltd.). Helium was used as carrier gas and the flow rate was 30 ml min<sup>-1</sup>. The injector and column temperatures were 80 and 50 °C, respectively. The column was WG100 with molecular sieve 5 A and Porapak Q 80/100 mesh. Finally, the packages were opened to evaluate the quality attributes of fresh mushrooms after storage. The measurements were carried out in triplicates.

### Film permeability

The two kinds of film used for mushroom packaging were polyvinyl chloride (PVC) and low-density polyethylene (LDPE) (Sumitomo Bakelite Co., Ltd.), whose properties were given in Table 1. The permeability coefficient of the films were measured at 0, 5, 10,

**Table 1.** Gas permeability coefficient of films used for packaging mushroom ( $\times 10^{-5} \text{ ml} \cdot \text{m m}^{-2} \text{ h}^{-1} \text{ atm}^{-1}$ )

Film	Thickness (mm)	0°C		5°C		15°C		20°C		30°C	
		O <sub>2</sub>	CO <sub>2</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>	O <sub>2</sub>	CO <sub>2</sub>
LDPE	0.035	284.1	1277.0	494.1	1766.1	620.0	2727.8	682.0	3137.5	1314.1	5496.8
PVC	0.095	208.5	210.6	355.5	386.8	409.9	664.3	490.9	619.5	750.0	1148.5

15, 20 and 30 °C. The gas of high CO<sub>2</sub> concentration (99.9%) was injected into the pouch (10 cm  $\times$  15 cm, 300 cm<sup>2</sup> in surface area) with 200 ml syringe and the pouch was placed in incubator at experimental temperatures for measuring O<sub>2</sub> and CO<sub>2</sub> concentrations. The changes in gas concentrations inside the pouches were determined by gas chromatography. The permeability coefficient was calculated from the changes in gas concentrations.

### Mathematical model for MAP

To determine the respiration rate of mushroom in MAP, the basic volume balance given in equations can be used for flexible polymeric film (Akimoto & Maezawa, 1997). The changes in the free volume of gas within package with time were shown in following equations.

$$\frac{dV_s}{dt} = \frac{dV_c}{dt} + \frac{dV_o}{dt} + \frac{dV_N}{dt} \quad (1)$$

$$\frac{dV_c}{dt} = \frac{A}{L} K_c (P_{c_a} - P_c) + R_c W \quad (2)$$

$$\frac{dV_o}{dt} = \frac{A}{L} K_o (P_{o_a} - P_o) + R_o W \quad (3)$$

$$\frac{dV_N}{dt} = \frac{A}{L} K_N (P_{N_a} - P_N) \quad (4)$$

where  $V$  is the volume of gas in pouch (ml),  $A$  is surface area of pouch (m<sup>2</sup>),  $L$  is thickness of film (m),  $K_c$ ,  $K_o$  and  $K_N$  are the permeability coefficient of film for CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> (ml  $\cdot$  m m<sup>-2</sup> atm<sup>-1</sup> h<sup>-1</sup>),  $R$  is respiration rate (ml kg<sup>-1</sup> h<sup>-1</sup>),  $W$  is weight of produce (kg),  $P_{c_a}$ ,  $P_{c_a}$  and  $P_{N_a}$  are partial pressure of CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> outside pouch (atm),  $P_c$ ,  $P_o$  and  $P_N$  are partial pressure of CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> inside pouch (atm). These equations showed that gas volume of package was determined by permeability of film and respiration of produce. According to gas partial pressure between internal and external package, CO<sub>2</sub> evolution and O<sub>2</sub> consumption rates were determined simultaneously at different temperatures.

### Multiple regression analysis

To determine effects of O<sub>2</sub> concentration ( $C_o$ ), CO<sub>2</sub> concentration ( $C_c$ ) and storage time ( $T$ ) on the respiration rate of mushroom in the aerobic respiration period, CO<sub>2</sub> evolution rate ( $R_o$ ) and O<sub>2</sub> consumption rate ( $R_c$ ) were described in following regression equations.

$$R_o \text{ or } R_c = a_0 + a_1 C_o + a_2 C_c + a_3 T + a_4 C_o^2 + a_5 C_c^2 + a_6 T^2 + a_7 C_o C_c + a_8 C_o T + a_9 C_c T \quad (5)$$

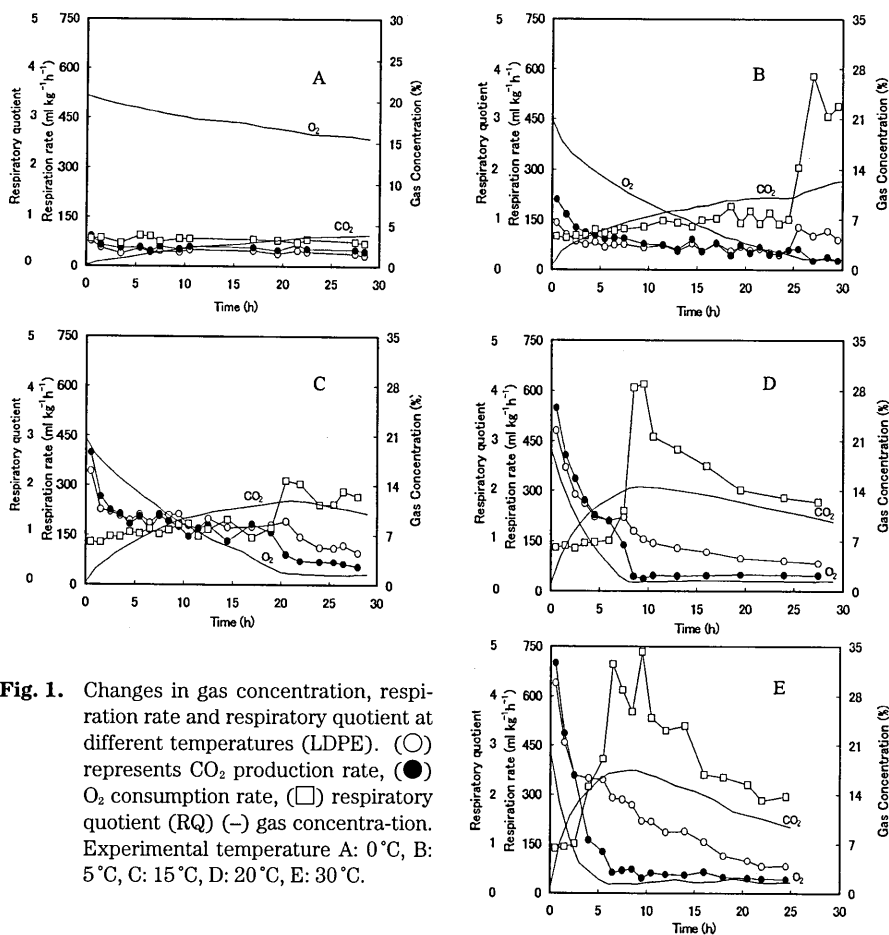
Where  $R_o$  and  $R_c$  were represented as functions of O<sub>2</sub> concentration ( $C_o$ ), CO<sub>2</sub>

concentration ( $C_c$ ) and storage time ( $T$ ),  $C_o$  and  $C_c$  are in fraction and  $T$  is in hour. Further,  $R_o$  and  $R_c$  were considered to be quadratic functions of  $C_o$ ,  $C_c$  and  $T$ .  $\alpha_i$  ( $i=0$  to 9) are the fitted constants.

## RESULTS AND DISCUSSION

### Respiration rate of fresh mushroom packaged with LDPE film

The changes in gas concentration, respiration rate and RQ at 0, 5, 15, 20 and 30°C in the LDPE film packages were given in Fig. 1.  $O_2$  concentrations were decreased and  $CO_2$  concentrations were increased throughout storage period at 0 and 5°C. Changes in gas concentrations were quicker at 5°C than that at 0°C. But  $O_2$  levels decreased and  $CO_2$  levels increased quickly at an initial 18 h, and then  $CO_2$  levels decreased slowly and  $O_2$  levels fluctuated at about 2% at 15°C. For 20 and 30°C,  $O_2$  levels decreased and  $CO_2$  levels increased markedly at an initial 8 h, and then  $CO_2$  levels decreased slowly and  $O_2$



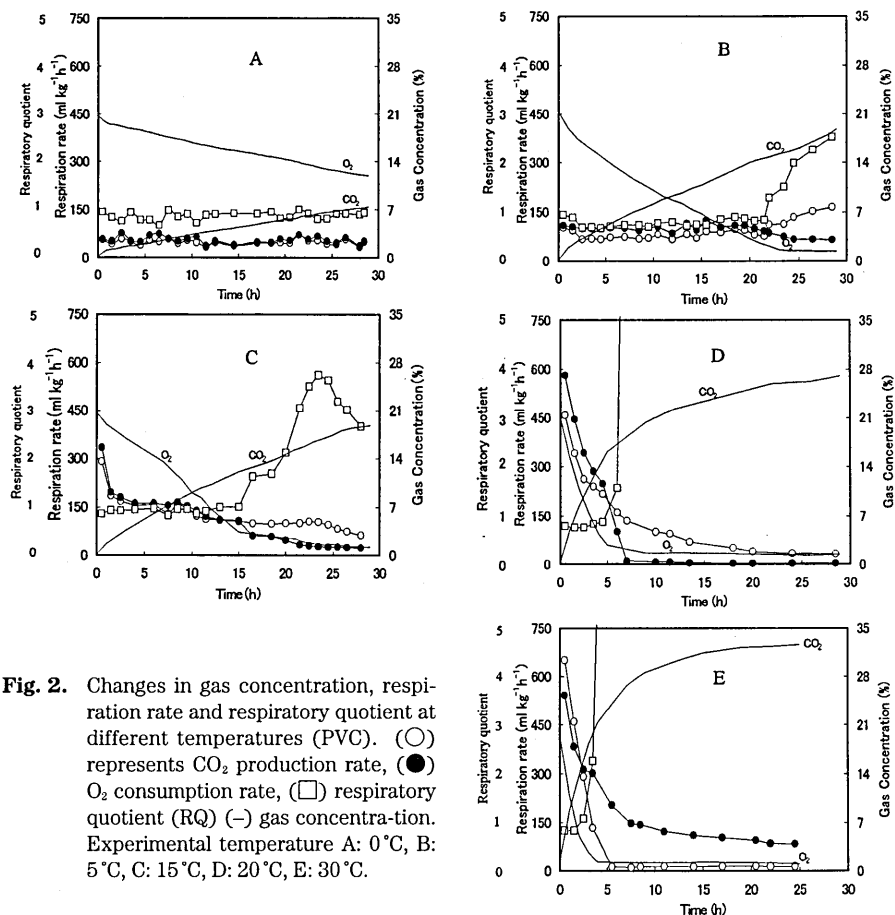
**Fig. 1.** Changes in gas concentration, respiration rate and respiratory quotient at different temperatures (LDPE). (○) represents CO<sub>2</sub> production rate, (●) represents O<sub>2</sub> consumption rate, (□) represents respiratory quotient (RQ) (—) gas concentration. Experimental temperature A: 0°C, B: 5°C, C: 15°C, D: 20°C, E: 30°C.

levels fluctuated at about 2%. The CO<sub>2</sub> evolution and O<sub>2</sub> consumption rates were approximately stable about 38.8 ml kg<sup>-1</sup> h<sup>-1</sup> under the conditions of slowly increasing CO<sub>2</sub> and decreasing O<sub>2</sub> concentrations during 30 hours at 0 °C (Fig. 1A). RQ was fluctuated around 1.0, indicating that anaerobic respiration did not occur at 0 °C. But both CO<sub>2</sub> evolution and O<sub>2</sub> consumption rates decreased remarkably with increasing CO<sub>2</sub> and decreasing O<sub>2</sub> concentrations in MAP at 5, 15, 20 and 30 °C (Fig. 1B-E). These decreases were more significant with the increase in temperature from 5 to 30 °C. The O<sub>2</sub> concentration continued to decrease and reached certain point, O<sub>2</sub> consumption rates were remarkably decreased, while CO<sub>2</sub> evolution rates were increased conversely and then decreased gradually (Fig. 1B-1D). At 30 °C, O<sub>2</sub> consumption rate were decreased quickly at over storage of 7h, after that it fluctuated around 40.5 ml kg<sup>-1</sup> h<sup>-1</sup> while CO<sub>2</sub> evolution rate was decreased much slower than that of O<sub>2</sub> consumption rate, indicating that a shift from aerobic to anaerobic respiration confirmed by high RQ values. This shift was also demonstrated by off-flavor of fermentation when the package was opened after storage. The lowest O<sub>2</sub> concentration surrounding the product that dose not induce fermentation was called RQ breakpoint or lower O<sub>2</sub> limit. In this experiment, the RQ breakpoint was increased with rise in temperature, indicating higher O<sub>2</sub> level was needed for the higher respiration rate at high temperature. The RQ continued to increase to the peak and then decreased slowly, indicating that the anaerobic behavior was not reversed.

#### Respiration rate of fresh mushroom packaged with PVC film

The changes in gas concentration, respiration rate and RQ at 0, 5, 15, 20, 30 °C in PVC film packages can be seen in Fig. 2. The trends of changes in respiration rates were similar as the results of packaged with LDPE film (Fig. 2A-E). But the changing rates of gas concentrations were quicker than that of LDPE film due to lower permeance of PVC film. The results also indicated that less permeable film provided much higher CO<sub>2</sub> and lower O<sub>2</sub> levels in MAP. After 20 hours, CO<sub>2</sub> and O<sub>2</sub> levels in LDPE package were 12.3% and 3%, while in PVC package CO<sub>2</sub> and O<sub>2</sub> levels reached 25.5% and 1.3% at 20 °C, respectively. It was suggested that higher permeability coefficient of film (LDPE) was appropriate for the higher respiration rate of mushroom due to supply much more oxygen to a certain extent through film from outside package.

The storage life of horticultural products has well been related to the respiration rate of produce, the suppression of respiratory metabolism will extend the storage life of fresh produce (Lopez-Briones, *et al.*, 1992; Kader, *et al.*, 1986). Burton, *et al.* (1989) reported that CO<sub>2</sub> level higher than 5% enhance discoloration during storage and CO<sub>2</sub> also exhibits a marked effect on the mushroom development, possibly due to repression of aerobic metabolism and O<sub>2</sub> concentration is less important than CO<sub>2</sub> concentration, so the main condition in case of MAP should be maintain the CO<sub>2</sub> concentration below 5% (*Agaricus bisporus*) (Burton, *et al.*, 1989). Kader, *et al.* (1986) also proved that 5% O<sub>2</sub> concentration seems to be phytotoxic for plant tissues and CO<sub>2</sub> inhibits specific enzymes of the Krebs's cycle resulting in an uncoupling effect on transitory metabolism and enzymatic activities (Kader, 1986). The phytotoxic effect of CO<sub>2</sub> could account for internal and external browning of mushroom and the sharp increase in their potential respiration rate when stored in controlled atmospheres containing more than 5% CO<sub>2</sub>. But for the fresh *shiitake* mushroom, it was more sensitive to low O<sub>2</sub> than high CO<sub>2</sub> concentration



**Fig. 2.** Changes in gas concentration, respiration rate and respiratory quotient at different temperatures (PVC). (○) represents CO<sub>2</sub> production rate, (●) O<sub>2</sub> consumption rate, (□) respiratory quotient (RQ) (—) gas concentration. Experimental temperature A: 0 °C, B: 5 °C, C: 15 °C, D: 20 °C, E: 30 °C.

(Minamida, *et al.*, 1980a; Pujantoro, *et al.*, 1995). Moreover, Minamida, *et al.* (1980a) reported that 40% CO<sub>2</sub> and 1–2% O<sub>2</sub> concentrations were optimal atmosphere conditions for keeping quality of mushroom in controlled atmosphere. In this study, CO<sub>2</sub> concentration has little effect on the respiration rate of *shiitake* mushroom, while O<sub>2</sub> level was more important than CO<sub>2</sub> level in control respiration of mushroom. On the other hand, O<sub>2</sub> levels beyond its tolerance limits can induce anaerobic respiration to result in browning and development of off-flavors.

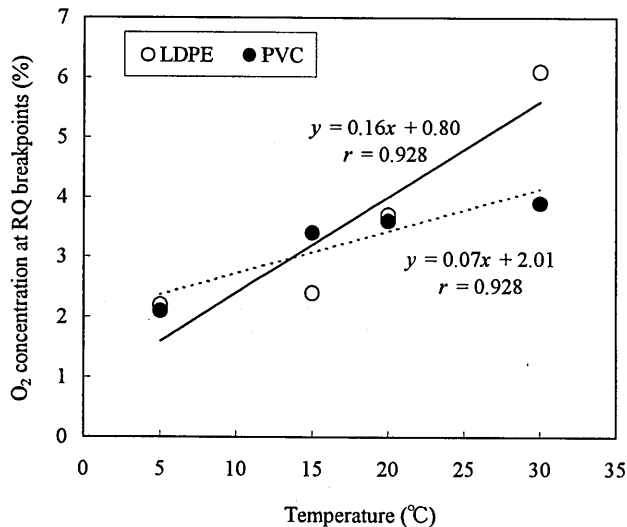
### Gas concentration and respiration rate at RQ breakpoint

Gas concentration, CO<sub>2</sub> evolution and O<sub>2</sub> consumption rates of fresh mushroom at RQ breakpoints were shown in Table 2. Beadry, *et al.* (1992) reported that O<sub>2</sub> concentration at RQ breakpoint increased with temperature for blueberries. Others also reported that there was increase in the O<sub>2</sub> tolerance limit with increasing temperature (Joles, *et al.*, 1994; Lakakul, *et al.*, 1999). In this experiment, similar results of both CO<sub>2</sub> evolution and

**Table 2.** Gas concentrations, CO<sub>2</sub> production and O<sub>2</sub> consumption rates of fresh shiitake mushroom at RQ breakpoints under different temperatures

Film	Temperature (°C)	CO <sub>2</sub> concentration (%)	O <sub>2</sub> concentration (%)	CO <sub>2</sub> evolution rate (ml kg <sup>-1</sup> h <sup>-1</sup> )	O <sub>2</sub> consumption rate (ml kg <sup>-1</sup> h <sup>-1</sup> )	RQ
LDPE	5	10.0	2.2	59.4	59.4	1.0
	15	11.5	2.4	180.0	158.2	1.1
	20	12.9	3.7	211.6	209.4	1.0
	30	11.2	6.1	359.4	357.5	1.0
PVC	5	14.6	2.1	75.9	91.4	0.8
	15	12.1	3.4	108.2	107.0	1.0
	20	15.2	3.6	217.8	248.2	0.9
	30	16.0	3.9	314.1	291.3	1.1

O<sub>2</sub> consumption rates were increased with increase in temperature at RQ breakpoint. But no correlation between CO<sub>2</sub> concentration and temperature was found, while O<sub>2</sub> concentration was increased from 2.2% to 6.1% for LDPE, 2.1% to 3.9% for PVC with rise in temperature from 5°C to 30°C, indicating that there may be a higher O<sub>2</sub> requirement for aerobic respiration at RQ breakpoint under higher temperature. The strong correlations between O<sub>2</sub> concentration and temperature with high linear correlation coefficient ( $r=0.928$ ) were found in both LDPE and PVC packages (Fig. 3). It was demonstrated that there was temperature dependence of RQ breakpoint for fresh *shiitake* mushroom in MAP. It could be possibly deduced that skin's permeability to O<sub>2</sub> dose not rise as rapidly as the increase of O<sub>2</sub> consumption rate with temperature rise, resulting in increase of RQ

**Fig. 3.** Relationship between temperature and O<sub>2</sub> concentration at breakpoints.



breakpoint. It also showed that the shift from aerobic to anaerobic respiration was controlled by O<sub>2</sub> concentration at RQ breakpoint. Lopez-Brionez, *et al.* (1992) reported that a minimal O<sub>2</sub> concentration of 1–2% has been suggested for preventing fermentation in mushrooms, while Burton, *et al.* (1987) suggested that the O<sub>2</sub> level must not fall below 3–4%. Once anaerobic respiration has been initiated, the O<sub>2</sub> level remained constant during subsequent fluctuation cycles regardless of the change in temperature. In this experiment, similar results were found that O<sub>2</sub> level and the O<sub>2</sub> consumption rate of fresh mushroom were almost constant under 2–4% O<sub>2</sub> levels. Which CO<sub>2</sub> levels were increased inside PVC packages, and then decreased slowly inside LDPE packages. It was considered that anaerobic respiration became main path of respiratory metabolism in *shiitake* mushroom below RQ breakpoint.

### Effect of gas concentration on respiration rate in aerobic respiratory period

As mentioned above, CO<sub>2</sub> evolution and O<sub>2</sub> consumption rates were greatly suppressed with decreasing O<sub>2</sub> and increasing CO<sub>2</sub> concentrations in the aerobic respiratory period. In order to clarify the effects of O<sub>2</sub> and CO<sub>2</sub> levels on respiration of mushroom in MAP, multiple regression analysis was carried out. The results of statistical analysis showed that there was higher multiple correlation coefficients (0.83–0.99) at different temperatures. The proportion of the total absolute values to standard partial regression coefficients of O<sub>2</sub> and CO<sub>2</sub> concentrations and storage time were presented in Table 3. The degree of effect of some factors can be determined by the absolute value of standard partial regression coefficient of each factor. Among the three factors of storage time, O<sub>2</sub> and CO<sub>2</sub> concentrations, the proportion of the standard partial regression coefficients of O<sub>2</sub> concentrations was accounted for 45.6–61.8% of the total absolute values to standard partial regression coefficients for three factors as 100%. The proportion of standard partial regression coefficients of storage time was 29.5 and 38.4% at 15 and 20 °C, respectively. The standard partial regression coefficients of CO<sub>2</sub> concentration was 37.4–32.4% at 0 and 10 °C, and 24.5–16.0% at 15 and 20 °C. It indicated that the respiration rate of fresh mushroom was also mainly affected by O<sub>2</sub> concentration in the aerobic respiration period. CO<sub>2</sub> concentration was secondary factor to O<sub>2</sub> concentration at 0 and 10 °C. The storage time became more significant factor next to that of O<sub>2</sub> concentration at 15 and 20 °C. It was possible explained that this inhibition of respiration rate was due to short of respiratory substrate at high temperature during storage.

For some horticultural products, both O<sub>2</sub> and CO<sub>2</sub> concentrations have a significant influence on respiration and shelf-life (Kader, 1986). The respiration rate is good index of physiological metabolism and storability. Base on this experimental results, physiological metabolism of the mushroom was mainly controlled by O<sub>2</sub> concentration and little

**Table 3.** Standard partial regression coefficients of O<sub>2</sub> and CO<sub>2</sub> concentrations

Factor	Temperature (°C)				
	0	5	10	15	20
O <sub>2</sub> concentration	0.65	0.55	0.62	0.49	0.65
CO <sub>2</sub> concentratin	0.32	0.28	0.31	0.24	0.22

affected by CO<sub>2</sub> concentration. The lower O<sub>2</sub> concentration not only suppresses the respiration, but also inhibited the phenol oxidase activity, which resulted in enzymatic browning of mushroom surface (Minamid, *et al.*, 1980b; Murr, *et al.*, 1975a). The shelf life of the mushroom is mainly determined by its metabolic activity, which causes consumption of respiratory substrates. The mannitol as a main soluble carbohydrate in the common mushroom, a half of the total mannitol may be metabolized within 4 days of storage (Hammond, *et al.*, 1979; Exama, *et al.*, 1993). Therefore, to extend the shelf life of fresh mushroom, metabolic activities of fresh mushroom could be suppressed more effectively by using low O<sub>2</sub> atmosphere condition in the package over RQ breakpoint.

### Temperature coefficient

The creation and maintenance of an optimal atmosphere inside MAP depends on the respiration rate of product and on the permeability of the films to O<sub>2</sub> and CO<sub>2</sub>, both of them are affected by temperature. Temperature coefficient is the ratio of respiration reaction at a given temperature to the ratio of respiration reaction at a temperature 10°C lower, is denoted by  $Q_{10}$ . Biological reactions tend to increase by a factor of 2 to 3 for each increase in temperature by 10°C (Beaudry, *et al.*, 1992; Exama, *et al.*, 1993). Exama, *et al.* (1993) reported that  $Q_{10}$  for mushroom (*Agaricus bisporus*) is 3.0, while in this experiment,  $Q_{10}$  value of fresh *shiitake* mushroom was 3.39 and 4.36 at temperature range of 0–10°C for LDPE and PVC film packages (Table 4), respectively, indicating that there was higher metabolic activity and the respiration for mushroom. It is also shown that fresh *shiitake* mushroom was much more sensitive to temperature fluctuation, especially at lower temperature range. But the  $Q_{10}$  values for the permeability of the LDPE film were 1.84 and 1.96 for CO<sub>2</sub> and O<sub>2</sub>, and PVC film were 2.35 and 1.81 for CO<sub>2</sub> and O<sub>2</sub>, respectively. This disparity resulted in an accumulation of CO<sub>2</sub> and a decreased in O<sub>2</sub> inside the packages subjected to temperature variations. It was considered that temperature was the most important factor for controlling the respiration of mushroom during storage as well O<sub>2</sub> concentration.

**Table 4.** Temperature coefficient ( $Q_{10}$ )

Film	0–10°C	5–15°C	10–20°C	20–30°C
LDPE	3.39	2.44	1.80	1.33
PVC	4.36	2.88	1.89	1.18

### CONCLUSIONS

The CO<sub>2</sub> evolution and O<sub>2</sub> consumption rates were mainly affected by O<sub>2</sub> concentration and less CO<sub>2</sub> concentration in atmosphere conditions surrounding product. The shift from aerobic to anaerobic respiration was also significantly controlled by O<sub>2</sub> concentration and little affected by CO<sub>2</sub> concentration at RQ breakpoint in MAP. Moreover, there was temperature dependence of RQ breakpoint for fresh mushroom in MAP with high linear correlation coefficient ( $r=0.928$ ). In the aerobic respiration period, the respiration rate

of fresh mushroom was also affected by O<sub>2</sub> concentration markedly. The effect of the storage time became greater factor next to O<sub>2</sub> concentration at 15 and 20 °C. These results suggest that better keeping quality for fresh mushroom could be obtained from low O<sub>2</sub> atmosphere conditions above RQ breakpoint at low temperature.

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