

## Extension of Postharvest Life of Oyster Mushroom under Ambient Conditions by Modified Atmosphere Packaging

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**ABSTRACT.** Suitability of polypropylene, low-density polyethylene, linear low-density polyethylene (LLDPE) as a packaging material and effectiveness of 0.5% calcium chloride and 0.5% citric acid (CA) as a washing treatment were tested based on off-colour and off-odour development in mushroom. A suitable package surface area to weight ratio was also established. Effectiveness of magnesium oxide in modifying the in-package gaseous atmosphere and thereby, extending the postharvest life was tested by monitoring the concentrations of oxygen, carbon dioxide, acetaldehyde, ethanol, weight loss and the 'L' value of oyster mushroom during storage for 6 days at  $27\pm 2^\circ\text{C}$  and  $82\pm 3\%$  RH. The developed modified atmosphere packaging system was compared with a commercial package.

Oxygen concentrations were 2.6, 3.8, 7.6 and 9.5% in the control and packages containing 1, 3 and 5 g of magnesium oxide, respectively, on day 4 in storage. Under similar conditions, carbon dioxide concentrations were 12.7, 7.9, 3.5 and 1.7%, respectively. Ethanol contents in the control and mushroom packaged with 1 and 3 g of magnesium oxide were 210, 126 and 103 ppm and acetaldehyde contents were 43, 19 and 10 ppm, respectively on day 4 in storage. Washing of oyster mushroom with 0.5% calcium chloride and 0.5% CA followed by packaging in 0.015 mm LLDPE in a 3:1 surface area to weight ratio with 3 g of magnesium oxide as a carbon dioxide scavenger was successful in extending the shelf life up to 5 days at  $27\pm 2^\circ\text{C}$  and  $82\pm 3\%$  RH. Shelf life of the commercial sample was 2 days.

### INTRODUCTION

Shelf life of commonly grown oyster mushrooms (*Pleurotus spp.*) in Sri Lanka is about two days under ambient conditions. The physiological disorders such as colour changes, shrivelling, wilting, liquefaction, textural and flavour changes shortens its shelf life (Bardon *et al.*, 1990). Storage of mushroom at  $0^\circ\text{C}$  and 95% RH has been reported to be the optimum condition to extend marketable life (Sea Land Services Inc., 1991). Modified atmospheric packaging (MAP) is reported to be the most economical and effective method of extending the shelf life of mushrooms (Roy *et al.*, 1995; Tano *et al.*, 1999). MAP can provide an economical and effective way of extending the shelf life of fresh mushroom during transportation and marketing. In MAP, low oxygen and high carbon dioxide environment resulting from respiration has been successful in slowing down deterioration and growth of microorganisms in fresh mushrooms (Kader *et al.*, 1989). However, excessive accumulation of carbon dioxide inside the MA package has caused loss

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of firmness and severe browning of mushroom due to cell membrane damage (Devece *et al.*, 1999). Moreover, an anaerobic respiration created inside the package has enhanced production of volatile compounds such as acetaldehyde and ethanol giving off-odours in mushroom (Tano *et al.*, 1999). Therefore, devising a MAP system of optimum gaseous composition for mushroom requires selecting a film with correct permeability properties and appropriate film surface area to product weight ratio. Furthermore, use of carbon dioxide absorbers such as magnesium oxide and potassium hydroxide are found to be beneficial in creating optimum atmospheric conditions (Cameron *et al.*, 1989; Henig and Gilbert, 1975).

Most researchers have concentrated on button mushroom (*Agaricus bisporus*), which is more popular than oyster mushroom in the western countries. This study describes development of a MAP system to extend the storage life of oyster mushroom under ambient conditions. The effectiveness of magnesium oxide as a carbon dioxide absorber in establishing an optimum modified atmosphere condition was also tested.

## MATERIALS AND METHODS

Fresh oyster mushrooms (*Pleurotus spp.*) were harvested from a commercial farm at Haragama in Kandy district and transported to the laboratory. Mushrooms were sorted by size and appearance. Diseased, damaged and extremely large or small mushrooms were discarded to minimize biological variability.

### Preliminary experiments

A preliminary experiment was carried out to select a suitable washing treatment and a packaging material. The experimental treatment structure was a two-factor factorial with packaging materials and washing treatments as the two factors laid out in a completely randomised design. Six packaging materials, 0.05 mm polypropylene (PP), 0.0375 mm PP, 0.075 mm low density polyethylene (LDPE), 0.05 mm LDPE, 0.0375 mm LDPE, 0.015 mm linear low density polyethylene (LLDPE) and four washing treatments, water, 0.5% citric acid, 0.5% calcium chloride and 0.5% citric acid with 0.5% calcium chloride, were used as the levels of the factors. Mushrooms were dipped in the washing treatment solutions for 1 min, air-dried and kept in packages of 2:1 surface area to weight ratio ( $\text{cm}^2 \text{g}^{-1}$ ). Twenty packages each of six packaging materials were used for the study. The heat sealed packages were stored at  $27 \pm 2^\circ\text{C}$  and  $82 \pm 3\%$  RH for three days. Packages were opened on day 2 and subjective measurements on off-colour and off-odour developments were taken as described below. The data were analysed by Kruskal-Wallis test using the MINITAB statistical package. A suitable packaging material and a washing treatment were selected by comparing the means at  $p < 0.05$  according to the multiple comparison procedure.

In the second stage of the preliminary study, the following experiment was carried out to find out the optimum surface area to weight ratio for packaging of mushroom. Three surface areas to weight ratios, 2:1, 3:1 and 4:1, were used in a completely randomised design, and the in-package concentrations of carbon dioxide and oxygen and the colour of mushroom were measured daily in triplicate for 6 days. Data of this experiment were subjected to variance analysis using the SAS package. Treatment means were compared

at  $p < 0.05$  according to the LSD mean separation procedure and the best surface area to weight ratio was selected.

### Main experiment

Fresh oyster mushrooms were sorted, washed in the washing solution containing 0.5% calcium chloride and 0.5% citric acid, air-dried and packaged (200 g per package) in 0.015 mm LLDPE in a 3:1 surface area to weight ratio with carbon dioxide scavengers. Magnesium oxide 1, 3 or 5 g wrapped with muslin cloth was used as carbon dioxide scavengers in a completely randomised design. Self-sealing septa were fixed onto the packages to facilitate gas measurements. The packages were stored at  $27 \pm 2^\circ\text{C}$  and  $82 \pm 3\%$  RH for six days. In-package concentrations of carbon dioxide and oxygen, acetaldehyde and ethanol contents, the colour and the weight loss were measured daily in triplicate as described below for 6 days. Data of this experiment were subjected to variance analysis using the SAS package. Treatment means were compared at  $p < 0.05$  according to the LSD mean separation procedure.

Three commercial mushroom packages were analysed for in-package concentrations of carbon dioxide and oxygen, acetaldehyde and ethanol contents, weight loss and colour on day 2 in storage at  $27 \pm 2^\circ\text{C}$  and  $82 \pm 3\%$  RH. Results of this experiment were compared with those obtained for mushroom in the MAP system developed in this study and stored for 4 days at  $27 \pm 2^\circ\text{C}$  and  $82 \pm 3\%$  RH. Mushroom packaged in perforated LLDPE was used as a control.

### Gaseous composition

In-package concentrations of oxygen and carbon dioxide were measured using a gas chromatograph (Shimadzu, model GC-14B). For oxygen measurement, a molecular sieve column, a thermal conductivity detector, helium carrier gas at a flow rate of 40 ml/min. and column, injector and detector temperatures of 50, 90 and  $110^\circ\text{C}$ , respectively, were used. Carbon dioxide was measured using a Poropak Q column, and the conditions used were the same as for oxygen.

### Acetaldehyde and ethanol contents

Mushroom (5 g) was homogenised with five ml of distilled water using a mortar and pestle. The homogenate was centrifuged at  $6000 \times g$  for 10 min, and 2  $\mu\text{l}$  of the supernatant was injected to the gas chromatograph (Shimadzu, model GC-14B). Acetaldehyde and ethanol contents were analysed according to the methods of AOAC (1990) using a Poropak Q column, a flame ionization detector, nitrogen carrier gas at a flow rate of 40 ml/min, and column, injector and detector temperatures of 190, 200 and  $200^\circ\text{C}$ .

### Off-odour and off-colour

Off-odour and off-colour were determined as described by Burton *et al.* (1987) using a scale of 1-4. 1-none, 2-slight, 3-moderate and 4-high with 10 trained panellists.

### Weight loss and colour

Weight loss was determined during storage by monitoring the weight of the contents of the package before and after the storage period. Weight loss was expressed as the percentage loss of weight with respect to the initial weight. Mushroom colour was measured using a Colour Difference Metre (ZE 2000 Nippon Denshuku). The lightness value (L) was used to evaluate the colour. The measurements were made directly on the cap surface three times on each mushroom.

## RESULTS

### Preliminary study

The effect of packaging material  $\times$  washing treatment interaction was significant ( $p < 0.05$ ) on both off-odour and off-colour. Regardless of the washing treatment, off-colour and off-odour development of mushroom packaged in PP was more pronounced than that packaged in other materials (Tables 1 and 2). Mushroom packaged in LDPE also developed off-odour and off-colour under all washing treatments. Washing with water or 0.5% citric acid followed by packaging was not effective in preventing off-odour and off-colour development in fresh mushrooms (Tables 1 and 2). Though washing with 0.5% calcium chloride followed by packaging in 0.015 mm LLDPE was effective in preventing off-odour development, it was not successful in preventing off-colour development. Packaging in 0.015 LLDPE after washing with a solution containing 0.5% citric acid and 0.5% calcium chloride was found to be the best treatment to prevent off-odour and off-colour development.

Table 1. Estimated means of off-odour development in mushrooms on day 2 on storage at  $27 \pm 2^\circ\text{C}$  and  $82 \pm 3\%$  RH.

Packaging material	Washing treatment			
	Water	0.5% citric acid	0.5% CaCl <sub>2</sub>	0.5% citric acid + 0.5% CaCl <sub>2</sub>
PP, 0.05 mm	4.0	3.6	3.8	2.2
PP, 0.0375 mm	4.0	3.7	3.9	2.6
LDPE, 0.075 mm	3.3	2.5	2.4	2.7
LDPE, 0.05 mm	3.4	2.7	2.6	2.0
LDPE, 0.0375 mm	2.5	1.7	2.0	1.4
LLDPE, 0.015 mm	2.0	1.6	1.0	1.0

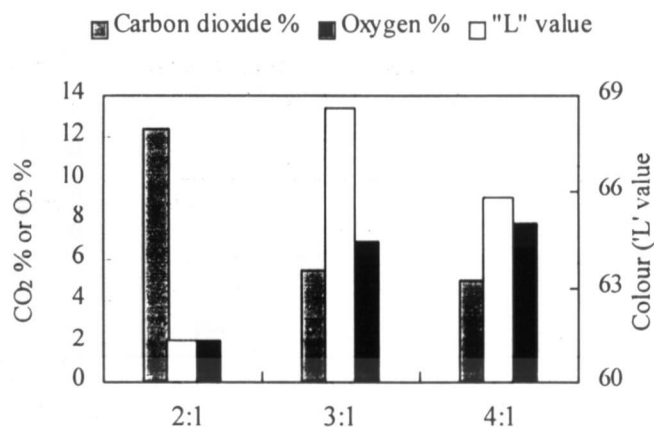
Each value represents the mean of ten replicates. Scale: 1-none, 2-slight, 3-moderate and 4-high.

**Table 2. Estimated means of off-colour development in mushrooms on day 2 at 27±2°C and 82±3% RH.**

Packaging material	Washing treatment			
	Water	0.5% citric acid	0.5% CaCl <sub>2</sub>	0.5% citric acid + 0.5% CaCl <sub>2</sub>
PP, 0.05 mm	3.8	3.3	2.2	1.7
PP, 0.0375 mm	3.7	2.6	2.3	2.0
LDPE, 0.075 mm	3.3	2.7	2.3	1.3
LDPE, 0.05 mm	3.0	2.5	2.1	1.0
LDPE, 0.0375 mm	2.7	2.3	1.8	2.0
LLDPE, 0.015 mm	2.5	1.7	1.3	1.0

Each value represents the mean of ten replicates. Scale: 1-none, 2-slight, 3-moderate and 4-high.

In-package carbon dioxide increased and oxygen decreased with decrease in surface area to weight ratio (Fig. 1). Carbon dioxide concentration was 12.4, 5.5 and 4.3% in ratios of 2:1, 3:1 and 4:1, respectively, on day 4 in storage (Fig. 1). The 'L' value reduced with decrease in surface area:weight and was 61.5, 68.8 and 65.8 in 2:1, 3:1 and 4:1, respectively, on day 4 in storage (Fig. 1). However, the difference in in-package carbon dioxide concentration between 3:1 and 4:1 packages was not significant at  $p < 0.05$ .



**Fig. 1. Effect of package surface area:weight on mushroom colour ('L' value) in in-package oxygen and carbon dioxide concentrations on day 4 in storage.**

[Note:  $LSD_{0.05}$  for CO<sub>2</sub>%, O<sub>2</sub>% and 'L' Value was 1.5, 2.1 and 5.3, respectively].

## Main experiment

### Gaseous composition

In-package concentrations of oxygen and carbon dioxide between the control and packages containing 1, 3 and 5 g of magnesium oxide were significantly different at  $p < 0.05$  (Fig. 2). Oxygen concentrations were 2.6, 3.8, 7.6 and 9.5% in the control and the packages containing 1, 3 and 5 g of magnesium oxide, respectively, on day 4 in storage. Under similar conditions, carbon dioxide concentrations were 12.7, 7.9, 3.5 and 1.7%, respectively (Fig. 2).

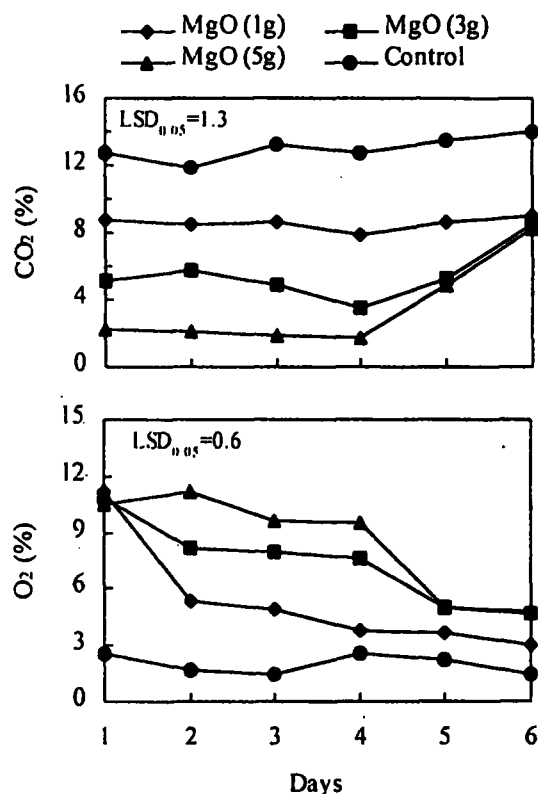


Fig. 2. Effect of magnesium oxide as a carbon dioxide absorber on in-package carbon dioxide and oxygen concentrations of mushroom stored in LLDPE at  $27 \pm 2^\circ\text{C}$  and  $82 \pm 3\%$  RH.

### Weight loss and colour

Weight loss was 2.1, 2.0 and 2.4% for mushroom in the control and in the packages containing 1 and 3 or 5 g of magnesium oxide, respectively, on day 4 in storage (Table 3). However, these differences in weight loss were not significant at  $p < 0.05$ .

Brightness or lightness of mushroom as indicated by the 'L' value was 80 initially and was 62.2, 71.6, 72.9 and 70.1 in the control and in the packages containing 1, 3 and 5 g of magnesium oxide, respectively, on day 4 in storage (Table 3).

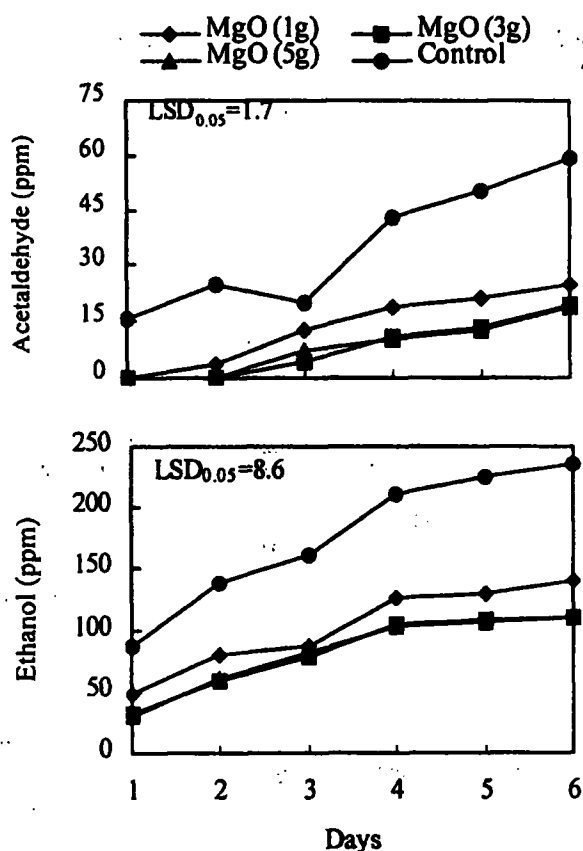
**Table 3. Weight loss and the 'L' value of mushroom as affected by the quantity of magnesium oxide during storage at  $27\pm 2^{\circ}\text{C}$  and  $82\pm 3\%$  RH.**

Storage period (days)	MgO (g)	Weight loss %	Colour ('L' value)
1	0	0.7 a	79.2 a
	1	0.5 a	80.4 a
	3	0.6 a	79.9 a
	5	0.8 a	77.9 a
	Duncan critical value	0.3	2.8
2	0	1.3 a	66.5 b
	1	1.5 a	78.6 a
	3	1.4 a	78.9 a
	5	1.3 a	78.5 a
	Duncan critical value	0.8	3.4
3	0	1.8 a	64.4 b
	1	1.9 a	70.0 a
	3	2.1 a	74.1 a
	5	1.9 a	70.6 a
	Duncan critical value	0.7	4.8
4	0	2.1 a	62.2 b
	1	2.0 a	71.6 a
	3	2.4 a	72.9 a
	5	2.4 a	70.1 a
	Duncan critical value	0.9	5.4
5	0	2.6 a	60.1 b
	1	2.5 a	65.5 a
	3	2.7 a	64.3 a
	5	2.7 a	64.0 ab
	Duncan critical value	0.3	3.9
6	0	3.1 a	50.0 a
	1	3.0 a	55.0 a
	3	3.2 a	54.0 a
	5	3.2 a	52.0 a
	Duncan critical value	0.3	5.1

Each value represents the mean of triplicate and when followed by the same letter within the same column was not significantly different (Duncan,  $p < 0.05$ ).

**Ethanol and acetaldehyde contents**

Concentrations of ethanol and acetaldehyde increased during storage and were significantly higher ( $p < 0.05$ ) in the control samples than those packaged with magnesium oxide (Fig. 3). Ethanol concentration in samples packaged with 1 g of magnesium oxide was 126 ppm on day 4 in storage and was significantly higher ( $p < 0.05$ ) than those packaged with 3 or 5 g of magnesium oxide. A similar pattern for acetaldehyde contents was also observed where its concentration was 19 ppm in samples packaged with 1 g of magnesium oxide on day 4 in storage and was significantly higher ( $p < 0.05$ ) than those packaged with 3 or 5 g of magnesium oxide (Fig. 3). Samples packaged with 3 or 5 g of magnesium oxide contained about 103 ppm of ethanol and 10 ppm of acetaldehyde (Fig. 3).



**Fig. 3.** Effect of carbon dioxide absorber on acetaldehyde and ethanol contents of mushroom stored in LLDPE at  $27 \pm 2^\circ\text{C}$  and  $82 \pm 3\%$  RH.



### Comparison of the experimental sample and commercial sample

In-package oxygen concentration was significantly higher ( $p < 0.05$ ) and carbon dioxide concentration was significantly lower ( $p < 0.05$ ) in the samples packaged in 0.015 mm LLDPE than those packaged in 0.0375 mm PP, the commercial samples, on day 4 and day 2, respectively, in storage (Table 4).

**Table 4. Gaseous composition, chemical and physical parameters of mushroom subjected to different packaging conditions and stored at  $27 \pm 2^\circ\text{C}$  and  $82 \pm 3\%$  RH.**

Condition of packaging	Storage period (days)	CO <sub>2</sub> %	O <sub>2</sub> %	CH <sub>3</sub> CHO (ppm)	C <sub>2</sub> H <sub>5</sub> OH (ppm)	'L' value	Weight loss (%)
Experimental sample*	4	3.50 b	7.6 b	11.0 b	105 b	73 a	2.4 b
Commercial sample**	2	14.90 a	2.7 c	31.7 a	277 a	65 b	1.3 c
Control***	1	0.09 c	20.9 a	nd	nd	60 b	4.2 a
LSD <sub>0.05</sub>		2.21	0.8	2.6	18	8	0.7

\*washed with 0.5% CA+ 0.5% CaCl<sub>2</sub> and packaged in 0.015 mm LLDPE with carbon dioxide scavenger (3g MgO).

\*\*washed with water and packaged in 0.05 mm PP.

\*\*\*washed with 0.5% CA+ 0.5% CaCl<sub>2</sub> and packaged in perforated (0.378 cm<sup>2</sup>/100 cm<sup>2</sup>) LLDPE with carbon dioxide scavenger (3 g MgO).

Each value represents mean of triplicate and when followed by the same letter within the same column was not significantly different at  $p < 0.05$

nd - not detectable

Mushroom packaged in LLDPE contained significantly lower ( $p < 0.05$ ) concentrations of acetaldehyde and ethanol and significantly higher ( $p < 0.05$ ) 'L' value than the commercial mushroom samples after storage for 4 and 2 days, respectively (Table 4).

### DISCUSSION

Mushroom colour is one of the most important factors that contribute to postharvest quality. Off-odour development caused by anaerobic respiration is a common problem in packaging of fresh mushroom (Tano *et al.*, 1999). Therefore, selection of a suitable packaging material is a must to establish an atmosphere within packages, which does not cause anaerobic respiration. Mushrooms are also washed before packaging to make them more suitable for use as ingredients in salads, pizza toppings and other foods, particularly by improving colour (Sapers *et al.*, 1994). Bardon *et al.* (1990) and Kukura *et*

*al.* (1998) reported the effectiveness of calcium chloride in improving mushroom colour when included in irrigated water probably due to reduced tyrosinase activity and bacterial growth. Moreover, washing of mushroom with hard water containing 151 ppm calcium carbonate and 0.016% calcium chloride has been more effective in delaying browning of mushroom than washing with soft water (Bardon *et al.*, 1990). Sapers *et al.*, 1994 reported that washing with 1.0% citric acid had no effect on preventing off-colour development but functioned as an anti-microbial. A combination of 0.5% citric acid and 0.5% calcium chloride was found to be effective in preventing off-colour and off-odour development in mushroom for up to 2 days in storage at  $27\pm 2^\circ\text{C}$  and  $82\pm 3\%$  RH when packaged in LLDPE. Moreover, LDPE (0.075 mm, 0.05 mm and 0.0375 mm) and PP (0.05 mm and 0.0375 mm) were found to be unsuitable for packaging of mushroom due to off-colour and off-odour development.

Decrease in oxygen concentration lower than the tolerable level and increase in carbon dioxide concentration higher than the tolerable level are the major problems of developing MAP systems for mushroom (Tano *et al.*, 1999). Lopez-Briones *et al.* (1993) reported a minimum tolerable oxygen concentration of 1 to 2 while Burton *et al.* (1987) suggested a higher oxygen concentration of 3-4%. Use of correct ratio between package surface area and the product weight, and carbon dioxide scavengers inside packages were tried out to establish and maintain an optimum gaseous composition. Mushrooms (200 g) are sold in packages of 2:1 surface area to weight ratio ( $\text{cm}^2/\text{g}^{-1}$ ). However, packaging of mushroom in 3:1 or 4:1 surface area to weight ratio was found to be better than a 2:1 ratio as reflected by higher 'L' value for the former than for the latter. This decrease in the 'L' value with decrease in surface area to weight ratio could be due to increase in carbon dioxide and decrease in oxygen with decrease in surface area to weight ratio. Increase in in-package carbon dioxide concentration may have damaged the tissues as suggested by Lopez-Briones *et al.* (1993) and reduced the 'L' value. Based on these results, a surface area to weight ratio of 3:1 could be recommended for mushroom packaging.

Lopez-Briones *et al.* (1993) identified a need to establish in-package oxygen and carbon dioxide concentrations within 5-10% and 2.5-5%, respectively, to optimise marketing conditions for mushroom. In this study, in-package concentrations of carbon dioxide and oxygen were within these limits when packaged with 3 or 5 g of magnesium oxide. Generation of an anaerobic atmosphere surrounding the mushroom was reported to be hazardous (Burton *et al.*, 1987) and accompanied by off-odours due to production of volatile substances such as ethanol and acetaldehyde (Tano *et al.*, 1999). Thus the quantity of acetaldehyde and ethanol would indicate the level of induction of anaerobic respiration in the mushroom tissues. A requirement for a minimal oxygen concentration of 1-2% (Lopez-Briones *et al.*, 1993) and a maximum carbon dioxide concentration of 3-5% (Burton *et al.*, 1987) has been identified to prevent anaerobic respiration of mushroom. Both acetaldehyde and ethanol concentrations increased during storage. However, the rate of increase of these metabolites was lower in mushroom packaged with magnesium oxide than that in the control. This indicates that magnesium oxide is effective in reducing the level of induction of anaerobic respiration by maintaining the carbon dioxide and oxygen concentrations within the required levels identified by Lopez-Briones *et al.* (1993) and (Burton *et al.*, 1987). The effectiveness of magnesium oxide increased with increase in the quantity.

Kader *et al.* (1989) reported that high carbon dioxide levels are toxic to plant tissues. However, the carbon dioxide concentration required to produce toxic effects vary

with the type of plant tissue. In-package carbon dioxide concentration of the samples packaged without magnesium oxide increased to 12.7% within 4 days, which is beyond the maximum tolerance limit of 12% as described by Devece *et al.* (1999). They mentioned that carbon dioxide at 12.7% caused physiological injuries resulting in severe browning of mushroom tissues, which was in agreement with the finding of this study where the 'L' value of mushroom packaged without magnesium oxide was significantly lower than those packaged with magnesium oxide.

Weight loss of mushrooms is mainly due to moisture loss by diffusion through the film and loss of carbon reserves due to respiration (Roy *et al.*, 1995). Moisture loss by transpiration is also a problem as mushrooms lack a protective epidermal structure (Roy *et al.*, 1995). Therefore, selection of a suitable packaging film to reduce the rate of moisture diffusion and the rate of respiration is important. Weight loss increased during storage and there was no significant difference between the samples packaged with and without magnesium oxide. However, weight loss of the commercial samples packaged in 0.05 mm PP was lower than that packaged with 3 g of magnesium oxide in 0.015 mm LLDPE, on day 2 and 3, respectively, in storage. However, 0.015 mm LLDPE is a more suitable material than 0.05 mm PP for mushroom as indicated by lower carbon dioxide, acetaldehyde and ethanol concentrations, higher oxygen concentration and 'L' value, and longer shelf life when packaged in the former than in the latter. Based on the concentrations of acetaldehyde and ethanol and the 'L' value, the quality of mushroom packaged in LLDPE with 3 g of magnesium oxide for 4 days was better than the quality of commercial sample stored for 2 days. The quality of the samples packaged in LLDPE with 3 g of magnesium oxide for 5 day was the same as the commercial samples stored for 2 days. Additional cost of fifty cents per package (200 g) over the commercial sample was rather economical considering the postharvest losses. Quality of mushroom packaged in perforated LLDPE was not acceptable due to high weight loss and dark colour.

## CONCLUSIONS

Washing of oyster mushroom with a solution of 0.5% citric acid and 0.5% calcium chloride followed by packaging in 0.015 mm LLDPE with 3 g of magnesium oxide as a carbon dioxide scrubber was effective in extending the postharvest life up to 5 days under ambient conditions.

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