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Research Article

The Mechanism of Micro-vacuum Storage Delaying *Pleurotus ostreatus* Senescence

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Abstract: In order to study the mechanism of micro-vacuum storage delaying *Pleurotus ostreatus* senescence, the modified atmosphere package storage was used as a control group to explore the delaying senescence mechanism of *Pleurotus ostreatus* under micro-vacuum storage. The activity of endogenesis antioxidative enzymes (SOD, CAT, POD), PPO, the regression relationship of superoxide anion (O_2^-•), H_2O_2, MDA content and relative leakage rate were studied. The results showed that micro-vacuum storage could significantly improve the activities of SOD, CAT and POD, decrease the accumulation of O_2^-• and H_2O_2, delay the increasing of MDA content and relative leakage rate, decline the activities of PPO ($p<0.05$). It showed that the micro vacuum storage can reduce the accumulation of free radicals, keep cell membrane structure and function, inhibit browning and change of quality through improving the endogenous antioxidant enzymes activity to delay senescence process of *Pleurotus ostreatus*.

Keywords: Micro-vacuum storage, *Pleurotus ostreatus*, quality, senescence mechanism

INTRODUCTION

Pleurotus ostreatus also called *Pinggu* mushroom in China, is widely cultivated in China, Japan and Thailand (Xiao *et al*., 2011). It is a delicate variety of mushroom, requiring a growing temperature range between 5 and 22°C, depending on the cultivar, as well as good ventilation and high relative humidity (Villaescusa and Gil, 2003). Now days the *Pleurotus ostreatus* have a very successful and great consumer's demand, due to their high nutritive content, peculiar taste and texture, unique flavour and medicinal properties. Lowering the temperature of mushrooms reduces respiration and transpiration, delaying senescence, preventing wilting and shriveling and thus extending shelf life (Burton and Twyning, 1989; Beit-Halachmy and Mannheim, 1992). It is a highly perishable mushroom with a normal shelf life of 1 to 3 days at ambient temperature during marketing (Xiao *et al*., 2011). During this period the *Pleurotus ostreatus* presents increased respiration, metabolic rate and several biochemical reactions. The commercial value of the *Pleurotus ostreatus* also decreases drastically in this period (Ju *et al*., 2000). Hence, effective control of physiological processes to prolong the post-harvest storage time of the *Pleurotus ostreatus* is an important area of research.

Modified Atmosphere Packaging (MAP) was demonstrated in some reports to be an effective method of extending the shelf-life of this mushroom and other mushrooms' varieties (such as *Agaricus bisporus*) (Gabriela *et al*., 2008; Borton *et al*., 1987). Modified atmosphere packaging alters the atmosphere *Pleurotus ostreatus* surrounding that is created by respiration and reduces water loss to preserve the quality of mushroom (Mir and Beaudry, 2004). However, exposure of mushroom to a level above their CO_2 tolerance limit can increase physiological damage; exposure below their O_2 tolerance limit may create an anaerobic condition and accumulation of acetaldehyde leading to change in flavor. In spite of the benefits, film gas permeability must match the requirement of mushroom and the storage temperature to prevent creating undesirable conditions (e.g., anaerobic condition, CO_2 damage, condensation within the package) (Lange, 2000).

Hypobaric storage is an effective storage measure which can rapidly remove the respiration heat, reduce the oxygen concentration and the accumulation of detrimental volatile metabolic products such as ethylene and α-farnesene. This storage system can significantly overcome the disadvantages of refrigeration and MAP storage (Dilley, 2003; Li *et al*., 2006). Nevertheless, based on the design principle, the construction of the storeroom should bear greater atmospheric pressure than MAP storage. The possible solution is to increase the rigidity of storeroom wall material or equip with upholders inside the facility. This results in heavy containers and increased cost. In addition, it has been reported that maintenance of low vacuum in hypobaric storage facility results in high moisture loss (Li *et al*., 2006). These difficulties make the hypobaric storage

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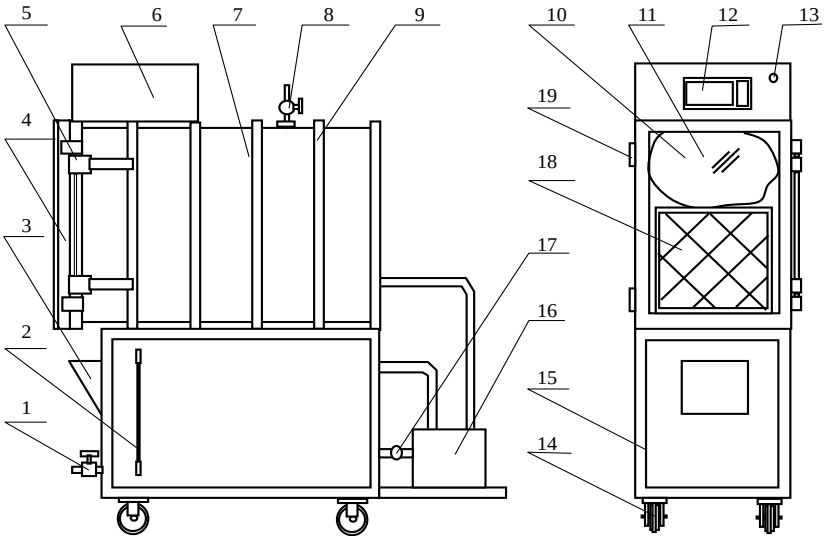


Fig. 1: Structure schematic diagram of micro-vacuum storage experimental equipment; 1 Blow-down valve, 2 Water Level Gauge, 3 Filling pipe end, 4 Air-tight door, 5 Hinge, 6 Control box, 7 Storage chamber, 8 Inlet or outlet air valve, 9 Stiffener, 10 Air bag, 11glass, 12 PLC display screen, 13 Indicator lamp, 14 Return pulley, 15 Water tank, 16 Vacuum pump, 17 Electromagnetic Valve, 18 Turnover box, 19 Fastening handle

technology less acceptable despite its advantages over MAP storage. In an effort to overcome the limitations of hypobaric storage, a "three-stage hypobaric storage" technology was established (Li and Zhang, 2006). But this technique was suitable only for products with high surface area such as soft-textured leafy vegetable. The applicability of this system on the storage of *Pleurotus ostreatus* (Li *et al*., 2008) is not well defined.

This study used equipment, Micro-Vacuum (MV) storage experimental equipment (Fig. 1), designed and manufactured by the Food Science and Engineering College of Qingdao Agriculture University. This device is a modified hypobaric storage equipment based on the invention patent CN1530290A (Wang, 2004). The most characteristic part of this experimental equipment is the soft air bag inside the storage chamber which is connected to the surrounding atmosphere. The air bag expands automatically when the internal air is excluded from the storage chamber. The expanding air bag formed by the decline of air pressure inside the storage chamber occupies the little chamber space. It buffers the change of internal air pressure and creates a "micro-vacuum" inside the chamber. In this MV condition, the air constituents in the chamber, including the oxygen and harmful gases, are decreased dramatically. The buffer action caused by the soft air bag lowers the vacuum of storage chamber, due to which MV storage equipment bears lesser atmospheric pressure. This reduces the material and manufacturing costs. In addition, the expanding air bag in the MV storage chamber inhibits the water loss of the products and plays an important role in improved preservation for a longer time.

The objective of this study was to investigate the effect of MV storage and MAP storage on delaying senescence of *Pleurotus ostreatus* in post-harvest period.

MATERIALS AND METHODS

Plant materials: *Pleurotus ostreatus* were harvested from plantations in Xiazhuang village of Qingdao and transported to the laboratory of Qingdao Agricultural University, within 3 h of harvest. They were then sorted and mushroom of uniform size, free of scars and insects were selected and precooled at 3°C for 12 h. The *Pleurotus ostreatus* were packaged using a plastic bag (low-density polyethylene LDPE).

Experimental apparatus: The MV storage experimental equipment consisted of a vacuum pump, compressor, moistener, soft air bag, storage chamber (100×75c×65 cm) and a control box (Fig. 1). This equipment was placed in a refrigeration house (3±1°C, 85-95% relative humidity [RH]).

Treatments: *Pleurotus ostreatus* were divided into two sets of 3 kg each. One set of mushroom was placed in MAP experimental equipment storage chamber and stored for 12 days at 101 kPa (3±1°C; 85-95% RH). The other set of mushroom was stored in MV store room (105×55×100 cm) for 14 days. This was monitored by an atmosphere analyzer (FC-701, Italy) kept at the same temperature and RH. *Pleurotus ostreatus* were taken randomly at an interval of 1 day to determine the characteristics. Each treatment was repeated thrice.

Determination of physiological and physicochemical characteristics:

SOD, CAT and POD activity: SOD was extracted from 5g of mushroom tissue ground in 5 mL of 0.1 mol/L phosphate buffer, pH 7.8, containing 1% PVPP at 4°C and then centrifuged at 12,000g for 30 min at 4°C. The supernatant was used to determine SOD activity. SOD activity was detected in mushroom extracts using the method given by Crosti *et al*. (1987). One unit of enzyme activity is defined as 50% inhibition of NBT autooxidation under assay conditions.

CAT was extracted from 5 g fresh weight of mushroom tissue ground in 5 ml of 0.1mol/L phosphate buffer, pH 7.5 at 4°C and then centrifuged at 12,000g for 30 min at 4°C. The supernatant was used to determine CAT activity by the method of Candan and Tarhan (2003). CAT activity was assayed in reaction mixture containing 0.1mol/L phosphate buffer (pH 7.5) 20mmol/L H_2O_2 and enzyme. The unit of CAT activity was defined as the amount of enzyme, which decomposes 1 lmol H_2O_2 perminute at 25°C.

POD was extracted from 5g fresh weight of mushroom tissue ground in 5 ml of 0.1mol/L phosphate buffer (pH 7.0) and then centrifuged at 12,000g for 30 min at 4°C. The supernatant was used to determine POD activity. For the measurement of guaiacol-dependent peroxidase activity, the reaction mixture contained 0.1mol/L phosphate buffer (pH 7.0), 25 mmol/L guaiacol, 0.5 mol/L H_2O_2 and enzyme. Activity was determined by the increase in absorbance at 470 nm due to guaiacol oxidation (Nakano and Asada, 1981).

O_2^-• and H_2O_2 activity: The rate of superoxide anion generation was measured following the method of Chaitanya and Naithani (1994). About 1g fresh mushroom samples were homogenized in 50 mmol/L sodium phosphate buffer (pH 7.8) containing 1 mmol/L diethyl dithiocarbamate to inhibit SOD activity. After centrifugation at 10,500g for 20 min, superoxide anion (O_2^-•) in the supernatant, was measured by its capacity of reducing Nitroblue Tetrazolium (NBT). The assay mixture in total volume of 3 ml contained 50 mmol/L sodium phosphate buffer (pH 7.8) containing 1 mmol/L diethyl thiocarbamate, 0.25 mmol/L NBT and the supernatant. The absorbance of the end product was measured at 540 nm in a Bausch and Lomb spectronic-20 spectrophotometer and (O_2^-•) formation was expressed as ΔA540 min/mg/protein.

The H_2O_2 activity was measured according to the methods of Sa-Ouk *et al*. (1993). H_2O_2 was extracted from 5 g fresh weight of mushroom tissue ground in 5 mL of acetone pre-cooling, then centrifuged at 12, 000g for 30 min at 4°C. The supernatant was used to determine H_2O_2 activity.

PPO activity: PPO was extracted from 5 g mushroom homogenized in 5 ml of 0.1 mmol/Lof phosphate buffer (pH 7.0) containing 0.1 mmol/L ascorbic acid and polyethylene glycol (1% of mushroom in weight) using a DS-1 (Shanghai, China). After filtration of the homogenate through muslin, the filtered material was centrifuged at 12,000g for 30 min at 4°C and the supernatant was collected. The supernatant was used to determine PPO activity. PPO activity was assayed with catechol as a substrate by a spectrophotometric procedure (Jiang, 1999). The assay was performed using 0.5 ml 0.01 mmol/L catechol, 5.0 ml of 0.1 mmol/L sodium phosphate buffers (pH 7.0) and 0.5 ml of crude enzyme. The increase in absorbance at 420 nm was recorded for 5 min. One unite of enzyme activity was defined as the amount of the enzyme which caused a change of 0.001 in absorbance per minute.

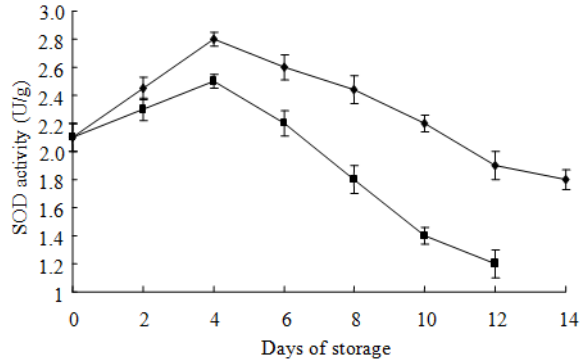
MDA and relative leakage rate: The cell-membrane permeability was measured according to the methods of Liu *et al*. (2013). From each treatment, 10 g of flesh was collected from 8 fruits and placed into 50 mL of distilled water. The sample was then incubated for 3 h. The initial electric conductivity was measured with a conductivity meter (DDS-11C, Shanghai, China). Disks were boiled for 30 min, cooled in room temperature and then the total conductivity was measured. Membrane permeability was expressed as relative electric conductivity percentage of the initial electric conductivity.

Malondialdehyde (MDA) content was assayed using the Thiobarbituric Acid (TBA) reaction (Li *et al*., 2013). Approximately 1 g of flesh from the fruits was homogenized with 10 mL of 5% Trichloroacetic Acid (TCA) and then centrifuged at 10000g for 10 min at 4°C. The supernatant (2 mL) was incubated with 2 mL of 6.7 g/L TBA for 30 min at 95°C and the mixture was then cooled rapidly in an ice bath. It was centrifuged again at 4000g for 10 min. The absorbance of the supernatant was recorded at 532, 450 and 600 nm using a UV-2000 spectrophotometer (Unico Instrument Co., Shanghai, China) and the results were expressed as μmol/g using the formula: MDA content (μmol/gFW) = $6.45\times (OD_{532}-OD_{600}) -0.56\times OD_{450}$. All measurements were repeated at least three-times.

Statistical analysis: The experimental design was completely randomized with three replications. Statistical evaluation of the different storage conditions were performed using analysis of variance (ANOVA). The treatment means were separated using the Least Significant Difference (LSD) method. Mean values were considered significantly different at $p<0.05$.

RESULTS AND DISCUSSION

Effects of MV and MAP storage on SOD, CAT and POD of mushroom: Figure 2A showed the effect of MV and MAP storage on SOD activity. Dismutation of



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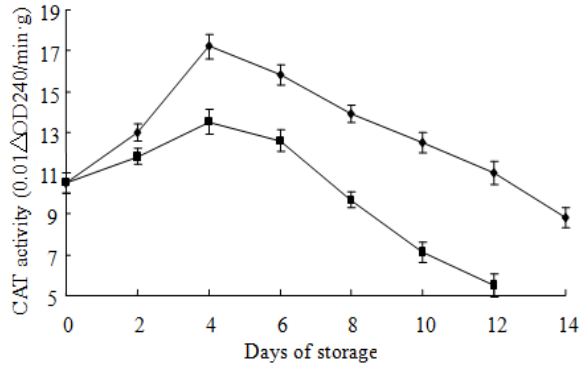

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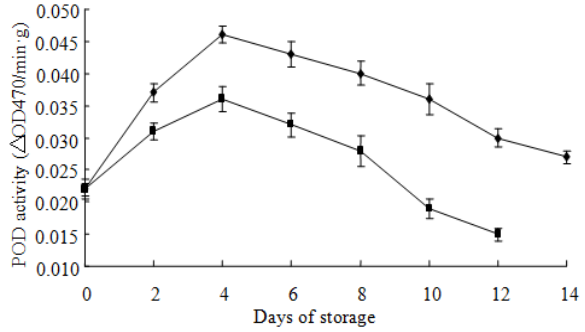


(c)

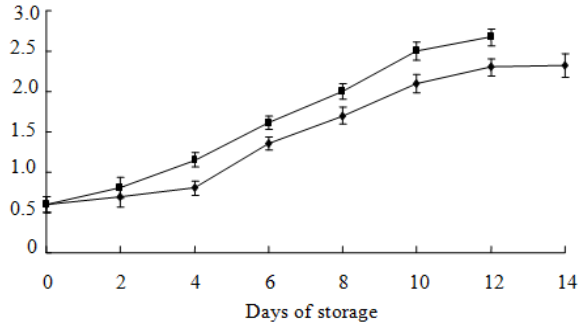
Fig. 2: Changes of SOD (A), CAT (B) and POD (C) in MV and MAP storage (◆, stored under MV; ■, stored under MAP) *Pleurotus ostreatus* during storage at 3±1°C. Each value is the average of three replicates± standard deviation. Values with different letters are significantly different according to Duncan's multiple range test at $p<0.05$

O_2^-• by SOD might be the primary step for the defense against chilling injury (Saruyama and Tanida, 1995). In this study, results showed that MV storage had a significant effect on SOD activity of the mushrooms ($p<0.05$) after the 4 days. Total SOD activity increased to its maximum on 4th in MV storage, 2.84 U/g, in MAP 2.49 U/g. The mushrooms stored under MV storage maintained remarkably higher SOD activity during the storage and we found the SOD activity of the mushrooms had significant difference throughout storage time ($p<0.05$). This result indicated that MV storages could efficiently extend the shelf life of the mushrooms.

The CAT is a kind of endogenous active oxygen scavenger, decomposing organism H_2O_2, balancing the H_2O_2 metabolism of free radicals and reducing the damage of membrane structure .The increase in CAT activity was found after 4 days of storage of the mushroom stored under MV and MAP storage, which was followed by its decrease (Fig. 2B). In the whole storage, the mushrooms stored under MV storage maintained remarkably higher CAT activity, total CAT activity increased to its maximum on 4th in MV storage, 16.75 (0.01△OD240/min•g), in MAP 14.28 (0.01ΔOD240/min•g). The maximum CAT activity in MAP storage is 85% of in the MV storage. The initial enhancement of CAT activity might be an adaptive response to cold storage and Micro-vacuum storage treatment. More rapid increase in H_2O_2 content at later senescence phase might cause the CAT activity following decline. The results showed that MV storage had a significant effect on CAT activity of the mushrooms ($p<0.05$).

The significant increase of peroxidase activity was found in the mushrooms after MV storage treatment ($p<0.05$) (Fig. 2C). The lower enzyme activity was detected in the mushroom stored under MAP storage treatment. However, no significant difference of peroxidase activity was observed before 4 days of storage in two stoeages ($p<0.05$). Total POD activity increased to its maximum on 4th in MV storage, 0.046 (△OD470/min•g), maximum POD activity in MAP storage is 85% of in MV storage. The enzyme activity measured in the mushrooms stored under MV storage treatment was much higher than under WAP storage. The results indicated that MV storage could induce higher SOD activity that had positive effect on prolong the shelf-life of the mushroom.

Effects of MV and MAP storage on O_2^-• and H_2O_2 activity of mushroom: The effect of MV and MAP storage on superoxide anion generation was shown in Fig. 3. The superoxide anion generation increased with the duration of storage. As it was evident, a higher level of superoxide anion production was noticed in the mushrooms stored under MV storage treatment throughout the storage time, as compared to MAP treatment. At the first 4 days, the effect of MV and MAP storage on superoxide anion generation had no significant differences ($p>0.05$). At the end of the storage, the superoxide anion generation of the mushrooms stored under two conditions had significant differences ($p<0.05$). The results showed that MV storage treatment had a significant effect on superoxide anion generation of the mushrooms.



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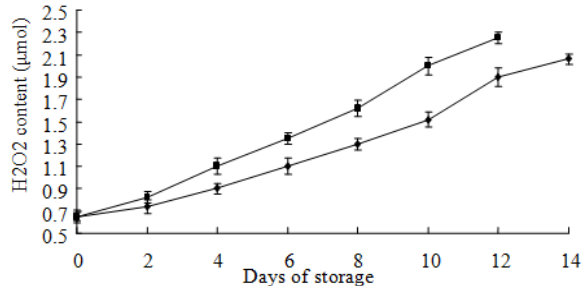

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Fig. 3: Changes of O_2^{-}• and H_2O_2 in MV and MAP storage (◆, stored under MV; ■, stored under MAP) *Pleurotus ostreatus* during storage at 3±1°C. Each value is the average of three replicates±standard deviation. Values with different letters are significantly different according to Duncan's multiple range test at $p<0.05$

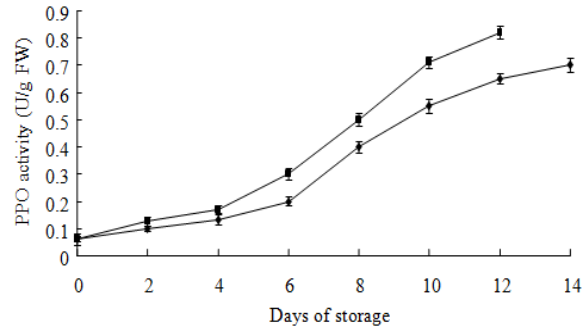


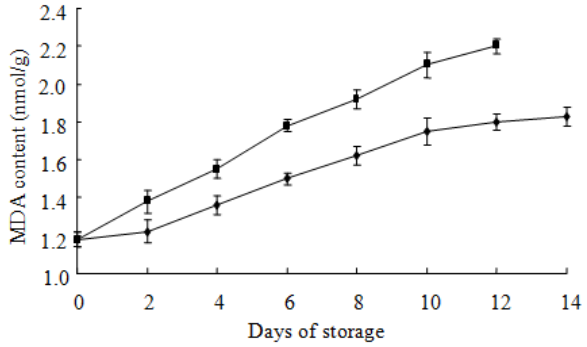
Fig. 4: Changes of PPO in MV and MAP storage (◆, stored under MV; ■, stored under MAP) Pleurotus ostreatus during storage at 3±1°C. Each value is the average of three replicates±standard deviation. Values with different letters are significantly different according to Duncan's multiple range test at $p<0.05$

The effect of MV and MAP storage on H_2O_2 generation was shown in Fig. 3B. Throughout the storage of 14 days, H_2O_2 activity increased from two storages. Significant differences ($p<0.05$) in H_2O_2 activity of mushrooms were observed between MV storage and MAP storage. The results indicated that MV storage treatment could prevent the existence of the mushroom oxidative injury to some degree. The effect of MV storage treatment was comparable to MPA treatment.

Effects of MV and MAP storage on PPO of mushroom: The influence of MV and MAP storage *Pleurotus ostreatus* on the activity of polyphenoloxidase was shown in Fig. 4. The PPO activity increased with the duration of storage. Until 12th, the PPO activity of the mushroom under MAP storage is 0.81 U/g·FW but PPO activity of the mushroom under MV storage is 0.66 U/g·FW. This was probably due to water evaporation from mushroom surface during MV storage process. The significant difference of PPO activity was found after 4 days storage ($p<0.05$). The lower PPO activity was detected in the mushroom stored under MV storage. This indicated that MV treatment storage showed comparable reduction of polyphenoloxidase activity.

Effects of MV and MAP storage on MDA and relative leakage rate of mushroom: The changes in relative conductivity and MDA content under two different storage conditions are presented in Fig. 5. There was a continuous increase in relative conductivity during the storage period, showing a gradual loss of cell membrane integrity (Fig. 5B). However, MV storage was found to maintain the cell membrane integrity better than MAP treatment ($p<0.05$) (Fig. 5B). At the end of storage, the relative conductivity of mushroom under MV storage treatment was 70.5% of that under MAP storage. MDA is one of the intermediate products of lipid peroxidation. The content of MDA is often used as an indicator of lipid peroxidation resulting from oxidative stress (Dhindsa *et al*., 1981). Lower MDA content indicates less lipid peroxidation. In Fig. 5A, MDA content under two storage conditions increased with prolonged storage time. It was observed that MDA content of MAP storage treatment was higher than that of MV storage treatment ($p<0.05$). At the end of storage, MDA content of MV storage treatment was 80.2% of that under MAP storage.

The breakdown of cell wall component and membrane disruption is important physiological phenomena of mushroom senescence, resulting in increased membrane permeability (Paliyath and Droillard, 1992). Membrane permeability is expressed as increasing leakage of ions (Marangoni *et al*., 1996). Peroxidation of fatty acids with resulting free radical formation has been described as one of the major deteriorative processes of membranes. This process is accompanied by increased contents of MDA (Ye *et al*., 2000), higher production of Reactive Oxygen Species (ROS; Thompson *et al*., 1987) and gradual loss in the ability of scavenging enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), Peroxidase (POD) to neutralize the free radicals (Pastori and Del Rio, 1997). Our results suggest that MV storage condition



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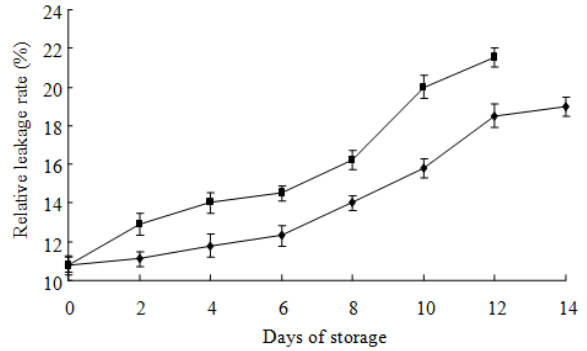

(b)

Fig. 5: Changes of MDA (A) and relative leakage rate (B) in MV and MAP storage (◆, stored under MV; ■, stored under MAP) Pleurotus ostreatus during storage at 3±1°C. Each value is the average of three replicates±standard deviation. Values with different letters are significantly different according to Duncan's multiple range test at $p<0.05$

could inhibit cell membrane deterioration and accumulation of the MDA. The effect of MV storage on the production of ROS and activity of the certain antioxidant enzymes needs to be studied further.

CONCLUSION

This study showed that by improving the activities of SOD, CAT and POD, decreasing the accumulation of superoxide anion (O_2^{-}•), delaying the increasing of MDA content and relative leakage rate, declining the activities of PPO, MV storage can delay *Pleurotus Ostreatus* ripening and senescence, when compared with MAP storage. During the MV storage period, the SOD, CAT and POD activity content were at higher level than that during the MAP storage. MV storage was more effective in inhibiting the increase in relative conductivity and MDA accumulation. The ability of MV storage in delaying the senescence of *Pleurotus Ostreatus* could be associated with the inhibition of ROS accumulation and maintenance of the activity of certain antioxidant enzymes. However, the mechanisms of inhibiting *Pleurotus ostreatus* senescence need further study.

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