Global Advanced Research Journal of Agricultural Science (ISSN: 2315-5094) Vol. 7(11) pp. 333-339, November, 2018 Issue.
Available online http://garj.org/garjas/home

Full Length Research Paper

Effect of Perforation-mediated Modified Atmosphere Packaging (PM-MAP) on browning and enzymatic characteristics of shiitake mushrooms (*Lentinus edodes*)

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Accepted 14 November, 2018

Shiitake mushrooms (*Lentinus edodes*) were treated under different perforation-mediated MAP conditions. In-package O2 and CO2 concentrations, the surface color difference, browning degree, total phenolics, the enzymatic activity PPO, POD, and PAL were determined. Then the correlation analysis of the indexes was carried out. The results showed that O2 concentration decreased and CO2 concentration increased gradually, and reached the equilibrium on the day 6. All the treatments of PM-MAP could maintain the surface color and decrease the browning degree, and the P2 and P4 were the best. The total phenolics of shiitake mushroom decreased gradually, and were higher in MAP treatments than control till the end of the storage. All the MAP treatments had obvious influence on the PPO and PAL activity, and P2 and P4 treatments retarded the increase of POD activity to some extent.

Keywords: perforation-mediated MAP; browning degree; total phenolics; enzymatic activity; antioxidant activity

INTRODUCTION

Shiitake mushrooms are important edible and medicinal fungus. They are known as their rich protein, special taste and rich nutrition (Antmann, Ares et al. 2008). Shiitake mushrooms are one of the specialties in China and are also popular edible fungus in the world (Jiang, Luo et al. 2010). However, shiitake mushrooms are prone to get brown during storage, transportation and processing, which not only affects the appearance of processed products, but also changes the flavor and nutritional components (Jiang, Luo et al. 2015; Li, Ishikawa et al; Ye, Li et al. 2012). Therefore, browning is an urgent problem in shiitake mushrooms during the storage and transportation.

Modified Atmosphere Packaging (MAP) is a kind of packaging method which utilizes the balance between respiration and membrane permeability to change the composition of ambient gas in the packaging to inhibit the respiration and metabolism of the product (Zhuang, Barth, et al. 2014) and prolong its storage life (Hu, Uchino et al. 2001; Gholami, Ahmadi et al. 2017; Cliffe-Byrnes,and O'Beirne. 2008) . Compared with other preservation methods, modified atmosphere packaging (MAP) technology not only has lower cost, but also has the advantages of simple operation, convenient storage and transportation, and maintaining the original nutrients of

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shiitake mushrooms (Jafri, Jha et al. 2013). Modified atmosphere packaging technology can prevent browning, reduce deterioration, maintain original nutrition and flavor, and prolong shelf life of shiitake mushrooms (Wei, Lv et al. 2017).

In this study, the changes of gas concentration, cap color, browning degree and related enzymes (PPO, POD, PAL) under different modified atmosphere packaging conditions were determined.

MATERIALS AND METHODS

Experiment materials

The polyethylene packages were punched 2, 4 and 6 holes with 0.7 mm diameter and marked as P2, P4 and P6, respectively. The packages with four 6 mm diameter holes were marked as CK for control set. For each package, 70±5g mushrooms were packaged and stored at 5℃. Under each treatment, 3 replicates were set up. The in-packaging concentration of O2 and CO2, browning degree, enzymatic activities, total phenolics, antioxidant activity of shiitake mushrooms were determined.

In-packaging gas composition determination

O2 and CO2 concentrations were measured by gas analyzer (PBI densensor) during the storage. Gas concentration was measured every day till day 6, and measured every 3 days from 7 to 15 days.

Browning degree determination

Shiitake mushroom flesh were mixed with refrigerated distilled water at 1:10 (W/W) for 30 seconds, then centrifuged at 8000 rpm for 15 minutes. Then the supernatant was kept at 25℃ for 5 minutes. The absorbance at 410nm (A410) was measured. The browning degree was expressed as 10×A410.

Enzymatic activities determination

Enzymatic activities determination was according to some references with some modifications (Liu and Wang 2012).

PPO extraction and enzyme activity determination

(1) PPO extraction: 2g shiitake mushroom flesh was mixed with 6 mL phosphate buffer (PBS: pH 7.8, containing 5% (w/v) PVP) and grinded in ice bath, and then the mixture was centrifuged at 10 000 rpm and 4℃ for 15 min. The supernatant was as the PPO extract.
(2) Determination of PPO activity: Acetic acid buffer of pH 4.75 was added to 2.0 mL catechol solution of 0.1 mol/L and 0.5 mL PPO extract. The absorbance at 410nm (A410) was recorded every 30 seconds, and continuously recorded for 3 minutes. A unit of activity (U) was defined as the change of A410 of 0.01 per minute. PPO activity was expressed as U/g/min.

POD extraction and enzyme activity determination

(2) POD extraction: 2g shiitake mushroom flesh was added with 6 mL phosphate mixed buffer (PBS: pH 7.8, containing 5% (w / v) PVP) and grinded in an ice bath, and centrifuged at low temperature (10 000 rpm, 4℃) for 15 minutes. The supernatant was taken as enzyme extract.
(3) Determination of POD activity: 100 ml enzyme solution and 3 mL reaction solution were added sequentially, and PBS (pH 6.0) was used as control, then A470 was determined. The absorbance value was recorded every 30 seconds for 3 minutes. A unit of activity (U) was defined as the change of A470 of 0.01 per minute. POD activity was also expressed as U/g/min.

PAL extraction and enzyme activity determination

(1) PAL extraction: 2g shiitake mushroom flesh was added with pre-cooled 4 mL of 0.2 mol/L sodium borate buffer (pH 8.8) and then grinded in ice bath. Then the mixture was centrifuged at 8000rpm and 4℃, and the supernatant was taken as PAL extract.
(2) Determination of PAL activity: 0.8 ml of PAL extract was added with 2.2 mL of 0.05 mol/L l-phenylalanine. A290 was measured as the initial value. and then the mixture was incubated in 37℃ water bath for 90 min. After the incubation, the mixture was added 0.2 mL of 6 mol/l HCl to terminate the reaction, and then immediately re-determine A290. A unit of activity (U) was defined as the change of A290 of 0.01 per minute. PAL activity was expressed as U/g/min.

Total phenolic content determination

1g Shiitake mushroom flesh was added with 20 mL 95% methanol, and then extracted for 30 minutes in 60℃ water bath. After centrifugation (10000rpm, 4℃) for 10min, the supernatant was taken. And the extraction was taken twice. Then the supernatant was collected and diluted with 95% methanol to a final volume of 50mL. 0.4 mL extract was added with 2 mL Folin-Ciocalteu reagent (FCR) and 1.8 mL of 75% sodium carbonate solution. The absorbance at 765 nm was measured after the mixture was placed in the the dark for 1 hour. .

Antioxidant activity determination

Antioxidant activity determination method was according to some references with some modifications (Lin, Lu et al. 2017; Kaewnarin, Suwannarach et al. 2016). 400 µL total phenolic extract was added with 3.5 mL of 0. 14 mmol/L

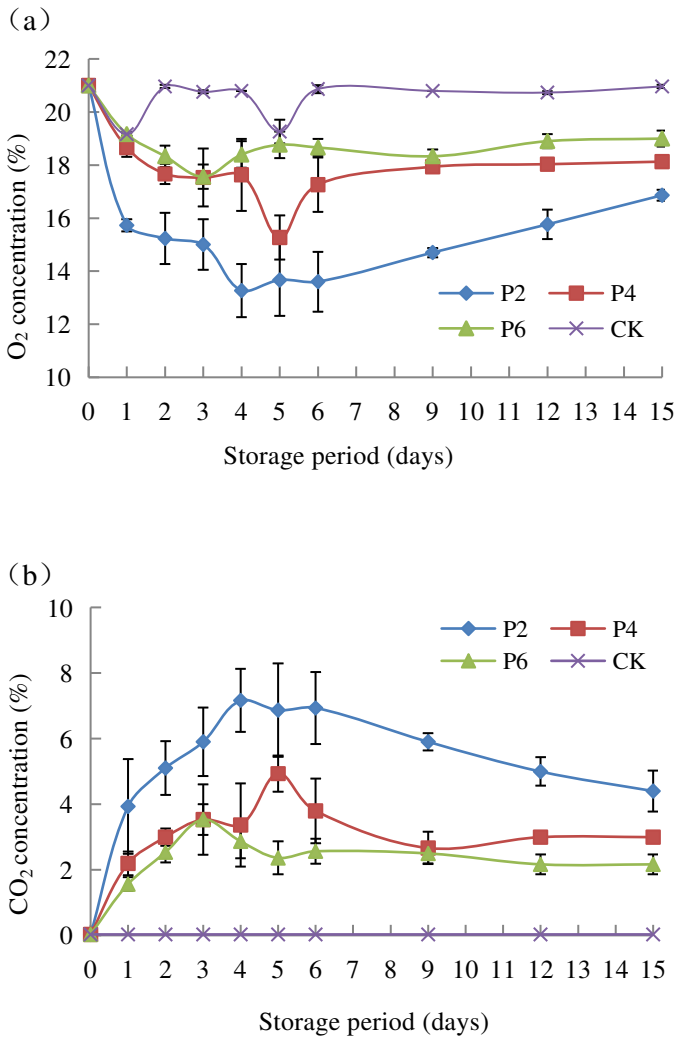

Figure1: gas concentrations in different packages during the storage (a. O_2 concentration; b. CO_2 concentration)

DPPH. After mixing uniformly, it was placed in the room temperature for 30 minutes to determine its absorbance at 517 nm. 80% methanol solution was used as blank, 400 µL of 80% methanol was added to 3.5 mL 0.14 mmol/L DPPH solution as control. Each sample should be measured three times.

RESULT AND DISCUSSION

In-packaging gas composition

From Fig.1, it could be indicated that the oxygen concentration in the P2, P4 and P6 decreased during the 0-5 days of storage, and the oxygen concentration in P2 decreased fastest, while that in P6 decreased slowly. By the 6th day, O2 and equilibrium concentration of oxygen was 13%, 17%, 18% and 21% respectively, and the O2 and CO2 concentrations changed slightly and tended to be stable. CO2 concentration in P2, P4 and P6 increased gradually during the 0-5 days of storage. CO2 concentration increased the fastest in P2 while increased the slowest in P6. O2 and CO2 concentrations in control group kept the same as that in the air.

The decrease of O2 concentration and the increase of CO2 concentration during storage were related to high respiration rate of fresh shiitake mushrooms (Park, Sangwanangkul, et al. 2018; Sanguinetti, Del Caro, et al.

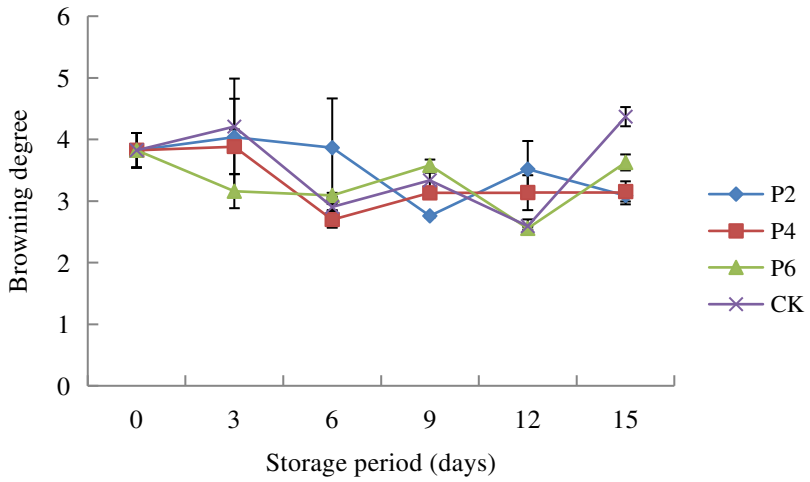

Figure 2: Browning degree of shiitake mushrooms during the storage

2016; Deza-Durand, and Petersen 2014). The fast decrease of O2 concentration in P2 was due to high respiration rate and low gas permeability of the package. High O2 concentration in P6 was not effective to inhibit the respiration, even may accelerate the metabolism and get perished. Extremely low O2 concentration and high CO2 concentration could induce the anaerobic metabolism and CO2 injury to plant tissue (Techavuthiporn, C. and P. Boonyaritthongchai 2016; Donglu, Wenjian, et al. 2016). In all the treatments, O2 and CO2 concentrations in P4 maintained the appropriate range for shiitake mushrooms during the storage.

Browning degree

Browning degree was related to the sensory quality and commodity value of shiitake mushrooms. Fig. 2 showed that there is no obvious change of browning degree of shiitake mushrooms before 12 days of storage, but the change is obvious on 15th day. At the end of the storage, browning degree in CK showed the highest and lower in P2 and P4. It could be indicated that P2 and P4 treatments could inhibit discoloration of shiitake mushrooms during the storage.

Enzymatic activities

PPO activity analysis

Fig. 3 showed that the PPO activity of shiitake mushrooms fluctuates up and down during the first 12 days of storage. After 12 days, all the three treatments showed a downward trend, and the control group had the fastest and lowest decline rate, while P2 treatment had a small change during the storage. Browning of shiitake mushrooms was correlated with PPO activity. From 12 to 15 days, PPO activity decreased while browning degree increased, indicating that browning reaction began to occur obviously.

POD activity analysis

The change of POD was also very unstable. POD activity in P2 showed an overall upward trend, and increased first and began to decline on the 9th day of storage in P4. The change trend of POD in P6 was similar to that in CK. The main reason is that the POD activity increases with the high concentration of oxygen in P6 and CK. With the prolongation of storage period, POD and substrates are gradually consumed, and the activity decreases gradually. However, the oxygen concentration in P2 and P4 was lower, which slowed down the increase of POD activity to a certain extent.

PAL activity analysis

From day 3, PAL activities in P2 and P4 were higher than P6 and CK. PAL activity of shiitake mushrooms in P2 and P4 increased sharply at the beginning, and began to decline from day 6 to day 12. After that, PAL activity in P4 kept decreasing , but slightly increase in P2 on the 15th day. PAL activity of P6 and CK declined slowly till the end of the storage.

Total phenolic content analysis

Fig. 6 indicated that total phenolic content in P2 and CK of shiitake mushrooms increased till day 3, and then decreased till the end of the storage. Total phenolic content

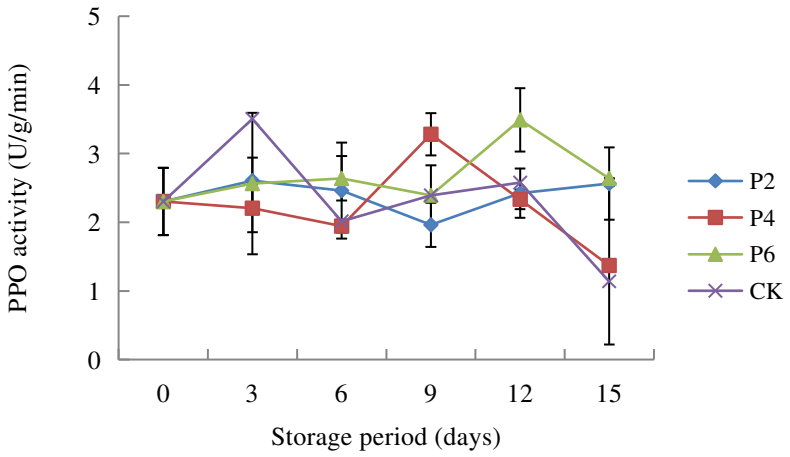

Figure 3: Changes of PPO activity of shiitake mushrooms during the storage

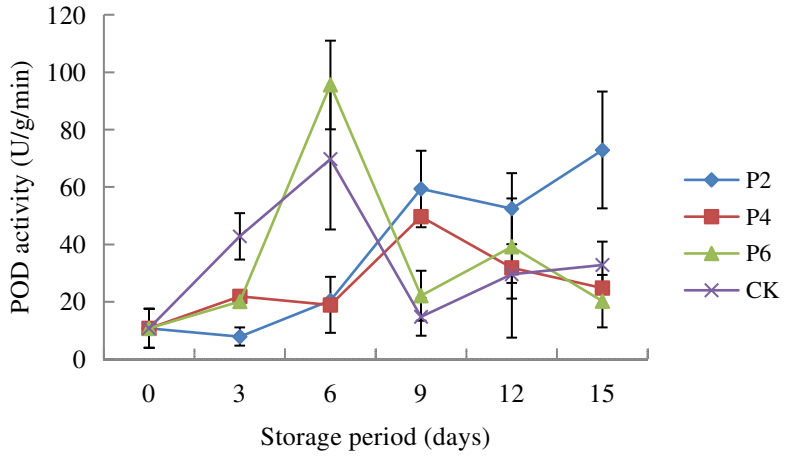

Figure 4: Changes of POD activity of shiitake mushrooms during the storage

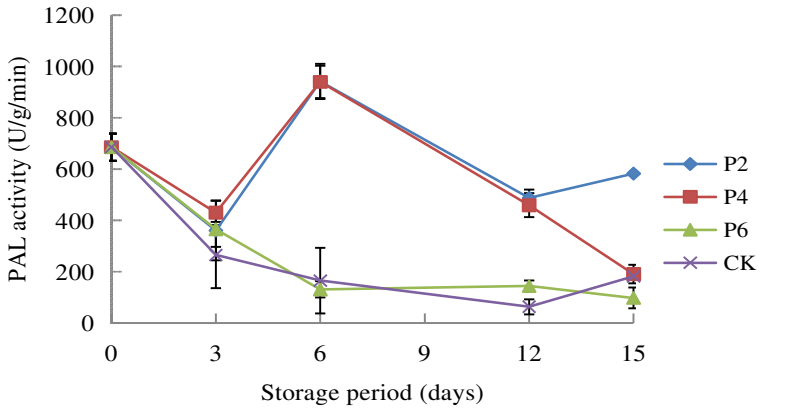

Figure 5: Changes of PAL activity of shiitake mushrooms during the storage

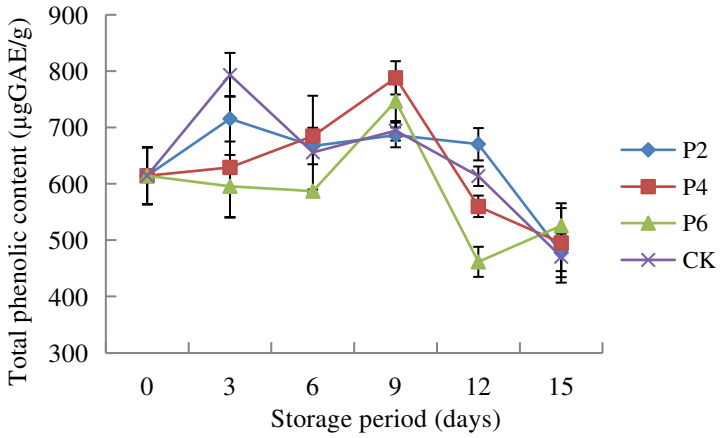

Figure 6: Changes of total phenolic content of shiitake mushrooms during the storage

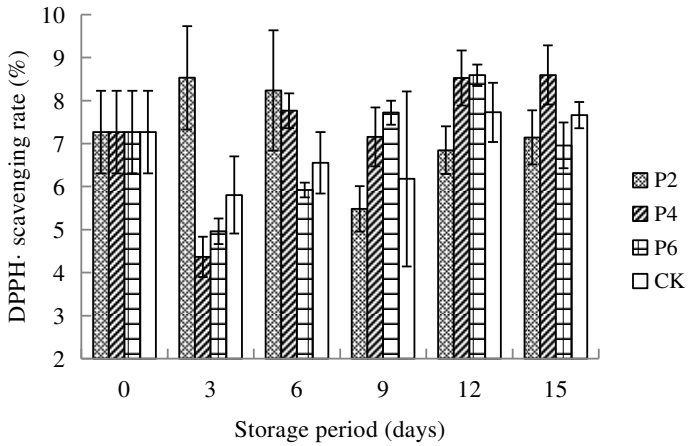

Figure 7: Changes of DPPH· scavenging rate of shiitake mushrooms during the storage

in P4 and P6 increased till day 9 and decreased in P4 till the end of the storage. After that, total phenolic content in P6 decreased till day 12, and then slightly increased at the end of the storage. Total phenolic content in all the treatments kept increasing at the beginning of the storage mainly due to the physiological response to injuries or infections, which plants usually repair wound damage or defend against infection by increasing phenolic compounds. However, total phenolic content decreased then for the reason of phenolic compounds was consumed gradually as substrate of some enzymes with the storage prolonged. P4 and P6 treatments could retard the release of phenolic compounds although at the end of the storage, there were not obvious differences of total phenolic contents in all the treatments.

Antioxidant activity analysis

Fig. 7 showed that DPPH• scavenging rate of P4, P6 and CK declined dramatically on day 3 , and then gradually increased till the end of the storage. DPPH• scavenging rate of P2 increased on day 3, and then decreased till the day 9 followed by a gradual increase till the end of the storage. DPPH• scavenging rate reflected the antioxidant ability of shiitake mushrooms during the storage. At the beginning of the storage, O2 concentration decreased to a low level in P2, which could retard the oxidation reaction. Therefore, DPPH• scavenging rate of P2 kept a high level at the beginning. However, with the storage prolonged,

antioxidant substances were consumed gradually, and the antioxidant ability began to decrease. From day 6 to day 15, antioxidant ability of P4 could kept higher than other treatments.

CONCLUSION

Shiitake mushrooms were packaged in micro perforation-mediated modified atmosphere packages of P2, P4 and P6. Macro perforation-mediated packaging was as the control (CK). All the shiitake mushrooms were stored for 15 days at 5℃.

During the storage, O2 concentration was lowest in P2 but highest in CK, and CO2 concentration was highest in P2 but lowest in CK. P4 and P6 could keep a relative appropriate O2 and CO2 concentrations during the storage. Browning degree in P4 kept lower than other treatments during the storage. Total phenol content and DPPH• scavenging rate will also change in some extent. Through this experiment, it can be concluded that modified atmosphere packaging can slow down the decline of hardness after harvest of shiitake mushrooms; modified atmosphere packaging also can protect the sensory quality of postharvest mushrooms. It also can slow down the oxidation rate of total phenols, and improve the DPPH• scavenging capacity of shiitake mushrooms. In this experiment, total phenol content and DPPH• content of the modified atmosphere packaging with 4 holes (P4) were higher than those of other treatments and the control group, and the physiological characteristics and quality were also better maintained.

ACKNOWLEDGEMENT

This work was supported by National Natural Science Foundation of China (grant number: 31601394).

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