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## Research on energy metabolism, lignification and veil opening in postharvest white mushroom (*Agaricus bisporus*) under high O<sub>2</sub>/CO<sub>2</sub> controlled atmospheres (HOC-CA)

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### Summary

White mushroom is an abundant nutritional but perishable product; veil opening and stipe lignification are two of the obvious characteristics of senescence. In order to study the relationship between the energy charge, veil opening and lignification of the white mushroom, high O<sub>2</sub>/CO<sub>2</sub> controlled atmospheres (HOC-CA) were utilized at 2±1 °C. The treatments comprised the following 100% O<sub>2</sub>, 80% O<sub>2</sub>+20% CO<sub>2</sub>, 60% O<sub>2</sub>+40% CO<sub>2</sub>, 40% O<sub>2</sub>+60% CO<sub>2</sub> respectively, with air as the control. In this study, sensorial and physiological qualities, adenosine triphosphate (ATP) content, energy charge level, lignin content, phenylalanine ammonia-lyase (PAL), cinnamyl alcohol dehydrogenase (CAD) and peroxidase (POD) activity were studied. The optimal condition 80% O<sub>2</sub>+20% CO<sub>2</sub> treatment inhibited both respiration rate and veil opening of the mushroom; suppressed PAL, POD and CAD activity. It also retarded the reduction of ATP level, energy charge level, and growth of lignin content. Exogenous ATP had an effect on inhibiting veil opening and lignification. These results indicated that there was a negative correlation between the ATP content, cap opening and stipe lignification. Appropriate HOC-CA maintained high energy, preserved the white mushroom well and extended the storage time to 24 days.

**Key words:** *Agaricus bisporus*, high O<sub>2</sub>, controlled atmosphere, energy, lignification, veil opening

### Introduction

The white mushroom is widely consumed in lots of places in the world due to its nutritional, healthy and medical benefits (LAGNIKA et al., 2011). It also contains high-quality protein and is superior to other vegetables and cereal proteins (JAWORSKA et al., 2011; LINDEQUIST et al., 2005). However, white mushroom is highly perishable, and its shelf life is just 2-3 days at ambient temperature. Thus, preserving good quality and prolonging the storage period would benefit the mushroom industry as well as consumers. Veil opening and lignification are two of the obvious characteristics of senescence besides browning and losing water.

Veil opening as well as growth of gills and production of spores, is the normal maturation process of white mushroom, but involves negative quality factors (BRAAKSMA et al., 2001). It is the first and most important factor that determines consumer acceptability and marketability. Veil opening was related to temperature, gas concentration of storage (QUE et al., 2015) and harvest time (BRAAKSMA et al., 1999). Lignification, as a biochemical process of sealing a plant cell wall through lignin deposition, usually occurs in the process of tissue development (BUBNA et al., 2011). It easily occurs in post-harvest bamboo shoots (ZENG et al., 2015), green asparagus (LIU and JIANG, 2005) and loquat fruit (CAI et al., 2006), and also in white mushroom. Actually, lignification is another factor that affects the

taste, nutritive value and quality of the postharvest mushroom (JIANG et al., 2010). Phenylalanine ammonia-lyase (PAL; 4.3.1.24), cinnamyl alcohol dehydrogenase (CAD; 1.1.1.195) and peroxidase (POD; 1.11.1.7) are the key enzymes in forming lignin (CAI et al., 2006). Notwithstanding lignification strongly influences the decisions of consumers, it is usually neglected by researchers. Therefore, how to control the lignification should provide an effective way to preserve white mushroom.

As the “energy currency” for the metabolic processes in vast majority of organisms, adenylate triphosphate (ATP) also plays an important part in the preservation of postharvest fruit and vegetables. Recent research has indicated that energy supply in cell is a critical factor in controlling ripening and senescence of postharvest plants. There was a negative correlation between the extent of ATP and the senescence of broccoli and bamboo shoots (LI et al., 2016; LIU et al., 2016).

In recent years, high O<sub>2</sub> controlled atmosphere (CA) has been utilized increasingly in fruit and vegetables storage. In white mushroom storage, high O<sub>2</sub>+N<sub>2</sub> CA (LIU and WANG, 2012) and 100% O<sub>2</sub>+2% alginate coating (JIANG, 2013) preserves them relatively well, especially surface colour. However, 100% O<sub>2</sub> significantly stimulated and increased respiration rate and ethylene production of the broccoli (LI et al., 2013) and fresh-cut bell peppers (CONESA et al., 2007), and further lead to a rapid yellowing of broccoli. Moreover, a combination of high O<sub>2</sub> and elevated CO<sub>2</sub> CA keeps the texture of products, decreases the incidence and severity of decay due to bacteriostatic action, reduces some physiological disorders and retards the senescence at last (GUO et al., 2013; LI et al., 2016; RUIZ-CAPILLAS and MORAL, 2002). In previous study, high O<sub>2</sub>/CO<sub>2</sub> CA could preserve broccoli well, however, different products might have different optimum concentrations of O<sub>2</sub>/CO<sub>2</sub>, depending on the metabolic characteristics of the specific product (CONESA et al., 2007; LI et al., 2014). There are little published data on the effects of high O<sub>2</sub>/CO<sub>2</sub> CA (HOC-CA) on cap opening, lignification or energy status of white mushrooms. In this study, we utilized HOC-CA to investigate the sensorial and physiological qualities, ATP content, respiration rate, ATP content, energy charge, veil opening, firmness, moisture content, lignin content and its related enzyme activity at 2±1 °C. The objective of this work is to investigate the effects of HOC-CA treatment on cap opening, lignification and energy metabolism under a low temperature and to clarify the relationship between them. The optimum HOC-CA for white mushrooms is established through these parameters and their storage time.

### Materials and methods

#### Mushroom materials

The white mushrooms (*Agaricus bisporus*, As2796) were freshly harvested from Dezhou in Shandong Province on 1 April 2016. All samples were harvested at the “close cap” stage with pileus dia of 40±5 mm and were cut stipes of 1.5 cm below the caps. The white mushrooms were delivered to the laboratory in Shandong University of Technology in a refrigerated truck at 5±2 °C. The mushrooms

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without bruise, damage, or blemishes were selected for uniformity of size and shape. Thereafter, they were stored at 2±1 °C and 90-95% relative humidity (RH).

### Postharvest treatments

The mushrooms were randomly divided into 5 sets with 5 kg mushrooms per set. Three replicates were used for each of the following treatments: 100% O<sub>2</sub>, 80% O<sub>2</sub> + 20% CO<sub>2</sub>, 60% O<sub>2</sub> + 40% CO<sub>2</sub> and 40% O<sub>2</sub> + 60% CO<sub>2</sub>. The mushrooms exposed to air at 2±1 °C were used as the control. Each treatment was respectively put into a 0.5 m<sup>3</sup> sealable container. The containers were linked with continuous mixed gas flows (0.80 L s<sup>-1</sup>) according to each treatment. Gases were checked regularly with FBI-Dansensor CheckPoint O<sub>2</sub>/CO<sub>2</sub> (MR-07825-00, FBI-Dansensor America Inc.) during storage. The mushrooms from each group were taken out to determine parameters such as sensory evaluation, respiration rate, ATP content, energy charge, cap opening, firmness, moisture content, lignin content and phenylalanine ammonia-lyase (PAL; 4.3.1.24), cinnamyl alcohol dehydrogenase (CAD; 1.1.1.195) and peroxidase (POD; 1.11.1.7) activity every 4 days. The fresh weight of mushrooms was used for the measuring parameters.

### Evaluation of sensorial qualities of HOC-CA

Sensory parameters such as firmness, opening caps, color of surface and flesh, off-flavor and overall acceptability were evaluated by a panel of 8 trained judges, employing the method of SHI et al. (2005). The standards of sensory properties of mushrooms are shown in Tab. 1. For scoring, 0-10 was utilized, where 0 represented for strong dislike and 10 for excellent. The storage period of mushrooms was determined by the score of sensory evaluation. The score of 5 was considered as limit of marketability (MOHAPATRA et al., 2011).

### Determination of respiration rate of HOC-CA

A sealed system was the method chosen to measure the respiration rates of the white mushrooms (WANG et al., 2008). Approximately 100 g of tissue from 18 mushrooms of each set was weighed, and then put into a sealed container for 4 hours under 2±1 °C. The CO<sub>2</sub> content released through respiration was measured by FBI-Dansensor CheckPoint O<sub>2</sub>/CO<sub>2</sub>. The measurement was replicated 3 times. Respiration rate (mL CO<sub>2</sub>·kg<sup>-1</sup>·h<sup>-1</sup> FW) was expressed as CO<sub>2</sub> production rate and calculated using following formula:

$$r_{CO_2} = \frac{(y_{CO_2}^{t_0} - y_{CO_2}^t)V}{100W(t - t_0)}$$

Here,  $V$  is the free volume of the container (mL);  $W$  is the weight of each sample (kg);  $t_0$  and  $t$  are initial and final time respectively (h).  $y_{CO_2}^{t_0}$  and  $y_{CO_2}^t$  represent initial and final volume fraction of CO<sub>2</sub> respectively (%).

### Determination of moisture content of HOC-CA

Weighing method was used. Moisture content was determined by weighing of all slices of 18 mushrooms contained in a petri dish before and after drying in thermostatic drier box with 95 °C for 5 hours every 4 days during the storage period.

Moisture content (%) =  $(W_o - W_d) / W_o \times 100\%$

Where,  $W_o$  and  $W_d$  were the original and drying weight during storage, respectively.

### Determination of veil opening rate of HOC-CA

Veil opening, the detachment of the head from the stem of the mushroom, was calculated manually by visual observation.

Veil opening rate (%) =  $C_o / C_t \times 100$

Where,  $C_o$  and  $C_t$  were the number of mushrooms with cap opening and the total number of mushrooms during storage, respectively.

### Determination of ATP and energy charge of HOC-CA

Extractions and measurements of ATP, ADP and AMP contents were determined according to OZOGUL et al. (2000) method, with slight modification. In brief, 2.4 g tissue from 18 mushrooms was ground in liquid nitrogen, thereafter it was homogenized in an ice bath for 6 min with 9 mL of 0.6 mol L<sup>-1</sup> perchloric acid. The homogenate was centrifuged at 16,000 × g for 12 min at 4 °C. The filtered supernatant was adjusted to pH 6.5-6.8 with 1 mol L<sup>-1</sup> KOH, after which, it was placed in ice for 30 min to precipitate the perchloric acid. The homogenate was centrifuged at 6,000 × g for 7-8 min at 4 °C. The filtered supernatant was filtered through a 0.45 μm filter for subsequent determination of ATP, ADP and AMP. A high performance liquid chromatography (Waters 600, Waters Corporation, USA) equipped with an UV detector and a Megres<sup>TM</sup> C18 column (4.6 mm × 250 mm) was used at 254 nm. The HPLC separation was achieved using the LIU et al. (2006) method of continuous gradient elution. The ATP, ADP and AMP in mushroom samples were identified by comparison with retention times of standards. The ATP, ADP and AMP contents were calculated in accordance with the external standard program. The measurement was performed 3 times. Energy charge was calculated by  $[ATP + 1/2ADP] / [ATP + ADP + AMP]$ .

### Determination of pileus and stipe lignin content of HOC-CA

The lignin content was measured according to the JIANG et al. (2010) method with some modifications. Two grams of pileus and stipe tissue from 18 mushrooms was ground with 5 mL of 95% ethanol, respectively, then centrifuged at 12,000 × g for 7 min at 4 °C. The supernatant was discarded, and the precipitate was washed in ethanol 3 times and then by ethanol and n-butane (the volume ratio was 1:2) for 3 times. The above precipitate was dried, and then dissolved in 2 mL of 25% bromoacetyl glacial acetic acid at 70 °C for 30 min. It was centrifuged at 12,000 × g for 15 min at 20 °C, then the supernatant was discarded and the pellet was washed with distilled water. The pellet was resuspended in 3 mL of 1.0 mol L<sup>-1</sup> NaOH along with agitating at 20 °C for 12 h. After centrifugation at 12,000 × g for 15 min, the supernatant was collected and then mixed with 5 mL acetic acid and lignin thioglycolic acid. The mixture was precipitated for 2 h and centrifuged at 8,000 × g for 7 min. The pellet was suspended in 0.9 mL of 2 mol L<sup>-1</sup> NaOH for measuring. Lignin content was defined as the absorbance value at 280 nm g<sup>-1</sup> fresh weight.

### Determination of phenylalanine ammonia-lyase (PAL) activity of HOC-CA

The PAL activity was measured according to the KIM et al. (2006) method, with some modifications. Two grams of pileus and stipe tissues from 18 mushrooms was ground with 5 mL of 0.2 mol L<sup>-1</sup> sodium borate buffers in an ice bath, respectively, and then centrifuged at 10,000 × g for 20 min at 4 °C. The supernatant (enzyme solution) was collected to determine enzyme activity. PAL activity was measured by incubating 0.5 mL supernatant with 2 mL of 0.2 mol L<sup>-1</sup> boric acid-borax buffer solution (pH 8.8), 1 mL of 20 mmol L<sup>-1</sup> 1-phenylalanine and 1 mL enzyme solution for 1 h at 37 °C. PAL activity was measured by the change in absorbance at 290 nm. One unit was defined as a 0.01 change in absorbance at 290 nm h<sup>-1</sup> and the PAL activity was expressed as U mg<sup>-1</sup> protein.

### Determination of cinnamyl alcohol dehydrogenase (CAD) activity of HOC-CA

The CAD activity was determined using the method described by

JIANG et al. (2010) with several modifications. Two grams of pileus and stipe tissue from 18 mushrooms was ground with 6 mL extracting buffer (5.5 mL of 0.1 mmol L<sup>-1</sup> phosphate buffer and 0.5 mL PVP), respectively. After centrifugation at 12,000 × g for 20 min at 4 °C, the supernatants were used as crude enzyme solutions for enzyme activity determination. Thereafter, the enzyme extract was incubated with an assay medium containing 1 mL of 0.5 mol L<sup>-1</sup> phosphate buffer (pH 6.25), 1 mL of 2 mmol L<sup>-1</sup> NADP<sup>+</sup> and 1 mL of 1 mmol L<sup>-1</sup> trans-cinnamic acid as substrate at 37 °C for 30 min. PAL activity was measured by incubating 0.5 mL supernatant with 2 mL of 0.2 mol L<sup>-1</sup> boric acid-borax buffer solution (pH 8.8), 1 mL of 20 mmol L<sup>-1</sup> 1-phenylalanine and 1 mL enzyme solution for 1 h at 37 °C. CAD activity was measured by the change in absorbance at 340 nm. One unit was defined as a 0.01 change in absorbance at 340 nm min<sup>-1</sup> mg<sup>-1</sup> fresh weight and the CAD activity was expressed as U mg<sup>-1</sup> protein.

#### Determination of peroxidase (POD) activity of HOC-CA

The activity of POD was measured according to Li's (2006) method with slight modifications. Three grams of pileus and stipe tissue from 18 mushrooms was respectively ground in an ice bath with 6 mL of 0.05 mol L<sup>-1</sup> phosphate buffer solutions (5% polyvinyl pyrrolidone, 0.01% Triton-X100). After centrifuging at 15,000 × g for 15 min at 4 °C, the supernatant (enzyme solution) was collected in order to measure enzymatic activity.

Phosphate buffer (4.4 mL, pH 6.8) and 0.4 mL guaiacol (2%) were added to tubes and kept at 25 °C for 15 min. Thereafter, 0.2 mL H<sub>2</sub>O<sub>2</sub> (0.46%) and 1 mL the above enzyme solution were added, the absorbances of 0, 30, 60 s were measured at 470 nm. One unit of POD activity was defined as the increase of absorbance 0.01 min<sup>-1</sup> and the POD activity was expressed as U mg<sup>-1</sup> protein.

#### Determination of veil opening, pileus and stipe lignin of exogenous ATP

A total of 720 mushrooms were applied for the experiment. The mushrooms were divided into 2 groups (360 mushrooms group<sup>-1</sup>), and each group contained 3 subgroups for replication. One group was infiltrated in a desiccator which contained 1.1 mM ATP (150 mushrooms L<sup>-1</sup>) under vacuum (72 kPa for 4.5 min), and another group was infiltrated in distilled water as the control. After air-drying, both groups of mushrooms were stored at 22±1 °C and 90-93% relative humidity (RH) for 7 days. The determination of veil opening and lignin contents of the pileus and stipe tissues was the same as the above.

#### Statistical analyses

Experiments were performed randomly and data expressed as mean standard deviation. Data were analyzed by analysis of variance (ANOVA) using SPSS 16.0 statistical software (IBM SPSS, Inc., Chicago, IL, USA). Then, Tukey's test and t-test were carried out for multiple and pair-wise comparison. Differences between treatments at *P* < 0.05 were considered as significant.

## Results

#### Sensorial qualities of HOC-CA

The sensorial qualities of mushrooms with all treatments decreased during the entire period (Fig. 1). In specific, the 80% O<sub>2</sub> + 20% CO<sub>2</sub> treatment maintained the mushroom white color of surface and flesh, high firmness, without sliminess and off-flavor, and with a high overall acceptability score of 9.1 on the 8<sup>th</sup> day during storage (Fig. 1D). Nevertheless, the control had a high rate of opening caps, serious browning and sliminess, low firmness, and only with 4.3 of overall

acceptability score on the 8<sup>th</sup> day (Fig. 1A). Although the 100% O<sub>2</sub> treatment maintained the white color of surface, it didn't retain the firmness or white color of flesh (Fig. 1E). In the meantime, the mushroom under 40% O<sub>2</sub> + 60% CO<sub>2</sub> had serious off-flavor especially during the later storage period (Fig. 1B). On the whole, 60% O<sub>2</sub> + 40% CO<sub>2</sub> also kept the white mushrooms relatively well besides the 80% O<sub>2</sub> + 20% CO<sub>2</sub> treatment. Therefore, the storage period of 100% O<sub>2</sub>, 80% O<sub>2</sub> + 20% CO<sub>2</sub>, 60% O<sub>2</sub> + 40% CO<sub>2</sub>, 40% O<sub>2</sub> + 60% CO<sub>2</sub> and the control were respectively 12, 24, 16, 12 and 8 days according to the overall acceptability score.

#### Storage period

The 80% O<sub>2</sub> + 20% CO<sub>2</sub> treatment prolonged the storage period of mushroom to 24 days, while 100% O<sub>2</sub>, 60% O<sub>2</sub> + 40% CO<sub>2</sub>, 40% O<sub>2</sub> + 60% CO<sub>2</sub> and control were only 12, 16, 12 and 8 days, respectively (Tab. 1).

#### Effects of HOC-CA on respiration rate, moisture content and veil opening rate

Basically, the respiration rate of mushrooms undergoing all treatments increased at the previous stage of storage, and declined after reaching a peak during the later stage of storage (Fig. 2A). The respiration peak of 80% O<sub>2</sub> + 20% CO<sub>2</sub> and 60% O<sub>2</sub> + 40% CO<sub>2</sub> treatments appeared on the 16<sup>th</sup>, 12<sup>th</sup> day with 83.0 and 90.6 mL CO<sub>2</sub>·kg<sup>-1</sup>·h<sup>-1</sup> FW, while that of the control appeared on the 4<sup>th</sup> day with 122.3 mL CO<sub>2</sub>·kg<sup>-1</sup>·h<sup>-1</sup> FW. The respiration rate of 80% O<sub>2</sub> + 20% CO<sub>2</sub> treatment showed the lowest and relatively stable level during the whole storage period, while that of the control exhibited the highest level.

Moisture content of all samples decreased progressively during the whole storage period (Fig. 2B). Compared with the control, 80% O<sub>2</sub> + 20% CO<sub>2</sub> treatment significantly (*P* < 0.01) retarded the decrease of moisture content with 89.7 % on the 8<sup>th</sup> day, whilst those of 100% O<sub>2</sub>, 60% O<sub>2</sub> + 40% CO<sub>2</sub>, 40% O<sub>2</sub> + 60% CO<sub>2</sub> and the control were respectively 81.4%, 86.0%, 83.5% and 78.4%.

As shown in the Fig. 2C, the veil opening rate of the control showed a higher level than others, whereas that of 80% O<sub>2</sub> + 20% CO<sub>2</sub> treatment exhibited a lower level. Veil opening of both 100% O<sub>2</sub> and the control started on the 6<sup>th</sup> day of storage, while those of 80% O<sub>2</sub> + 20% CO<sub>2</sub>, 60% O<sub>2</sub> + 40% CO<sub>2</sub> and 40% O<sub>2</sub> + 60% CO<sub>2</sub> treatments started on the 20<sup>th</sup>, 16<sup>th</sup> and 8<sup>th</sup> day, respectively.

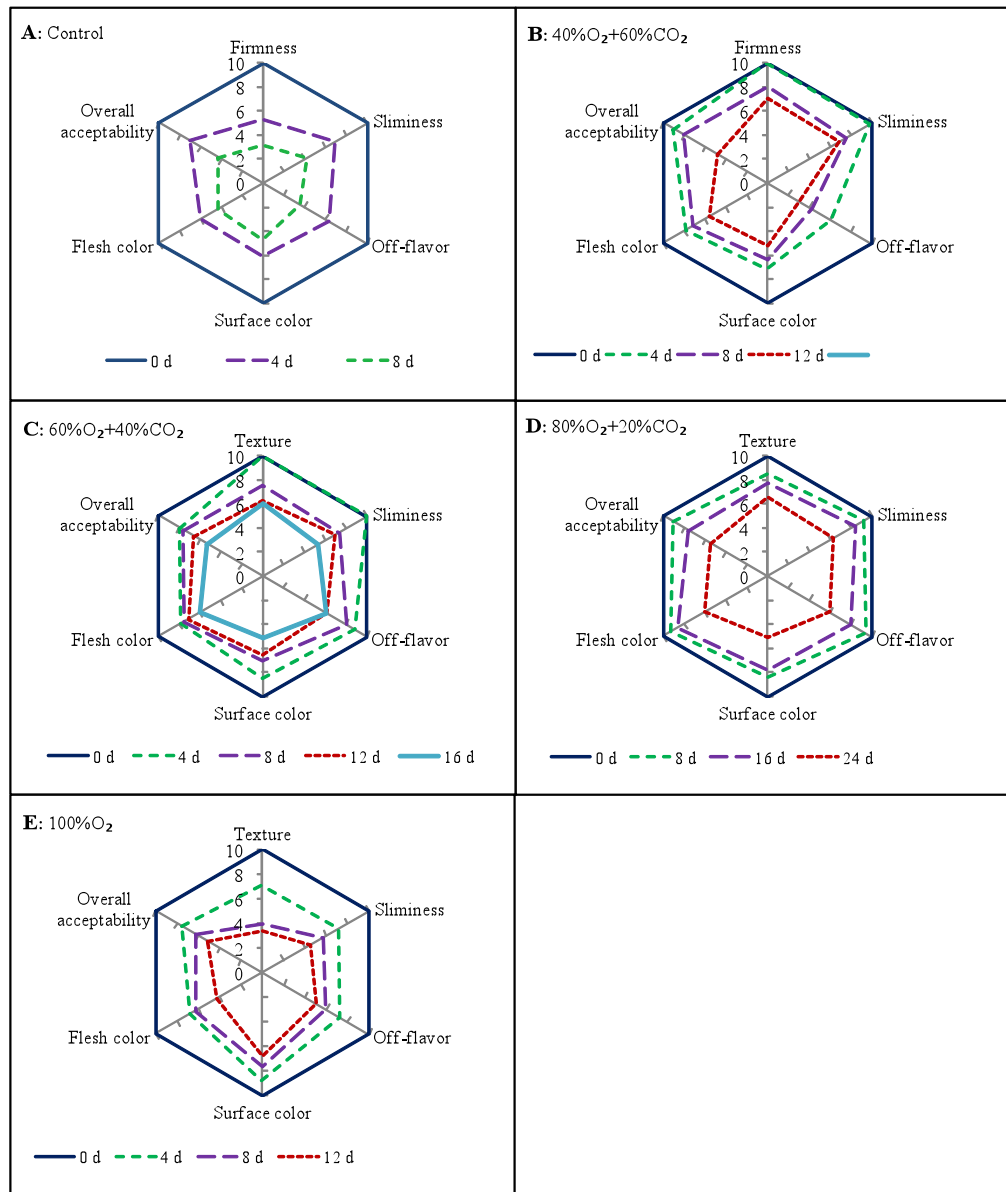
#### Effects of HOC-CA on ATP, energy charge

Overall, the ATP content and energy charge levels of all treatments showed a downward trend during storage (Fig. 3). The ATP content and energy charge level of 80% O<sub>2</sub> + 20% CO<sub>2</sub> treatment decreased significantly with a 16.7% reduction of ATP between 0-8 days' storage, whilst that of the control was obviously faster with 69.2%. Treatment of 80% O<sub>2</sub> + 20% CO<sub>2</sub> inhibited the decrease in the ATP and energy charge level.

As shown in the Fig. 3B, the texture of mushrooms undergoing all treatments exhibited a downward tendency during storage. Compared with others, the firmness of mushrooms treated with 80% O<sub>2</sub> + 20% CO<sub>2</sub> kept a relatively high level and declined slowly from 8.6 of initial level to 8.2 between 0-8 days. Meanwhile, the firmness of 60% O<sub>2</sub> + 40% CO<sub>2</sub> was also relatively high, whereas those of the control and 100% O<sub>2</sub> declined quickly and were only 6.4 and 5.1 on the 8<sup>th</sup> day of storage.

#### Effects of HOC-CA on lignin contents

The changes of lignin content in the pileus (Fig. 4A) and stipe (Fig. 4B) parts of mushrooms undergoing all treatments increased during storage. Lignin content of stipe was higher than that of the



**Fig. 1:** Sensory quality changes of the control (A), 40% O<sub>2</sub>+60% CO<sub>2</sub> (B), 60% O<sub>2</sub>+40% CO<sub>2</sub> (C), 80% O<sub>2</sub>+20% CO<sub>2</sub> (D) and 100% O<sub>2</sub> (E) during the whole storage at 2±1 °C. Air treatment was used as the control. Data are the mean of 3 replications.

**Tab. 1:** Storage periods of white mushrooms treated with HOC-CA at 2±1 °C.

Treatments	Storage period (d)
80% O <sub>2</sub> + 20% CO <sub>2</sub>	24 a
60% O <sub>2</sub> + 40% CO <sub>2</sub>	16 b
40% O <sub>2</sub> + 60% CO <sub>2</sub>	12 c
100% O <sub>2</sub>	12 c
Control	8 d

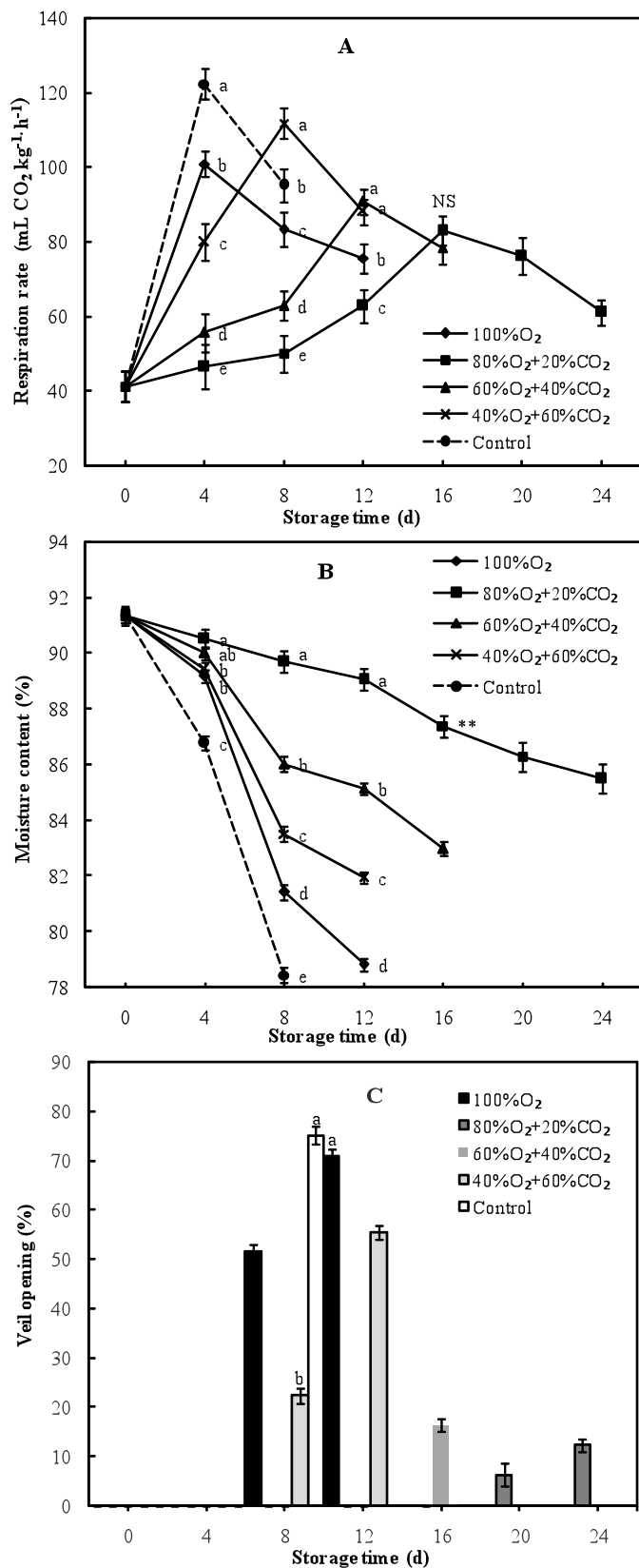
Different little letters represent significant differences by Tukey's test at  $P < 0.05$  ( $n = 3$ ).

pileus during storage, especially during later storage. Compared with others, the lignin content at 80% O<sub>2</sub> + 20% CO<sub>2</sub> treatment increased more slowly and exhibited lower values than those of the control which showed the highest levels during postharvest storage.

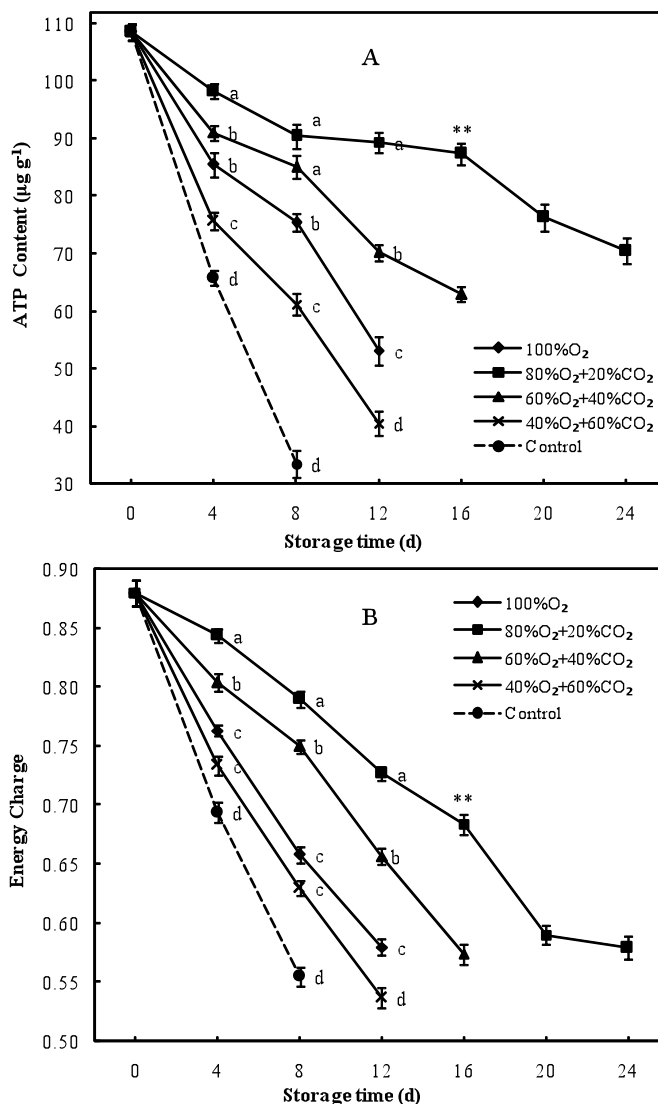
#### Effects of HOC-CA on PAL, CAD and POD activity

PAL activity of the pileus (Fig. 5A) and stipe (Fig. 5B) parts of the white mushrooms increased at early storage and then decreased except that of the control. On the whole, the PAL activity of the stipe was higher than that of the pileus during the whole storage period. Treatment of 80% O<sub>2</sub> + 20% CO<sub>2</sub> maintained PAL activity at a lower level than others. The maximal level of PAL activity of stipe parts at 80% O<sub>2</sub> + 20% CO<sub>2</sub> was 6.7 U mg<sup>-1</sup> on the 16<sup>th</sup> day, while that of the control was 12.1 U mg<sup>-1</sup> on the 8<sup>th</sup> day. However, the maximum PAL activity at 100% O<sub>2</sub>, 60% O<sub>2</sub> + 40% CO<sub>2</sub> and 40% O<sub>2</sub> + 60% CO<sub>2</sub> treatments were recorded at 9.5, 8.0 and 8.8 U mg<sup>-1</sup>, respectively. CAD activity of the pileus (Fig. 5C) and the stipe (Fig. 5D) parts of white mushrooms increased, while that of the pileus was higher than the stipe during the entire storage period. There were significant differences in CAD activity between 80% O<sub>2</sub> + 20% CO<sub>2</sub> treatment and the control. The CAD activity of pileus parts at 80% O<sub>2</sub> + 20% CO<sub>2</sub> increased 2.3% from 0 to 8 days, whereas those at 100% O<sub>2</sub> and the control increased 52.4% and 100.1%.





**Fig. 2:** Effect of HOC-CA on the respiration rate (A), moisture content (B) and veil opening (C) of the white mushrooms at 2±1 °C. Air was used as the control. The vertical bars indicate standard deviation (n = 3). Means followed by same letters are not significantly different among all treatments from 0 to 12<sup>th</sup> day during storage by Tukey's test (*P* < 0.05). Means followed by \*\* are significantly different at *P* < 0.01 by t-test on the 16<sup>th</sup> day, and NS = not significant.

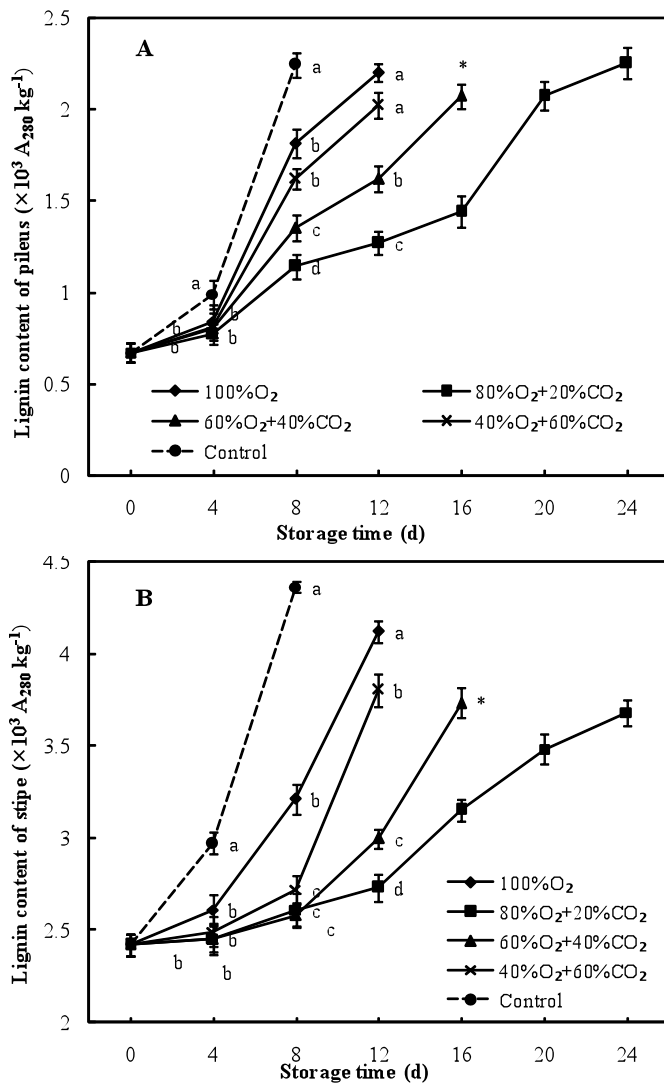


**Fig. 3:** Effect of HOC-CA on the ATP content and energy charge level of white mushrooms under 2±1 °C (Fig. 3). Air was used as the control. Energy charge was calculated by [ATP + 1/2ADP] / [ATP + ADP + AMP]. The vertical bars indicate standard deviation (n = 3). Means followed by same letters are not significantly different among all treatments from 0 to 12<sup>th</sup> day during storage by Tukey's test (*P* < 0.05). Means followed by \*\* are significantly different at *P* < 0.01 by t-test on the 16<sup>th</sup> day.

The change trends of POD activity with all treatments except that of the control were similar during storage; all increased at early storage and then decreased (Fig. 5E, F). On the whole, the POD activity of the stipe was higher than that of the pileus during the entire storage period. The POD activity of the mushrooms treated with 80% O<sub>2</sub> + 20% CO<sub>2</sub> maintained a moderate level (*P* < 0.05), while that of the control retained a higher level and a lower level at 100% O<sub>2</sub>.

**Effects of exogenous ATP on veil opening rate and lignin contents**

Veil opening of white mushrooms treated with exogenous ATP was lower than that of the control (Fig. 6A). The time of veil opening of white mushrooms was delayed by 1 day during storage. Lignin content of pileus (Fig. 6B) and stipe (Fig. 6C) parts of white mushrooms treated with exogenous ATP were lower than those of



**Fig. 4:** Effect of HOC-CA on the lignin content of pileus (A) and stipe (B) of the white mushrooms at  $2\pm 1$  °C. Air was used as the control. The vertical bars indicate standard deviation ( $n = 3$ ). Means followed by same letters are not significantly different among all treatments from 0 to 12<sup>th</sup> day during storage by Tukey's test ( $P < 0.05$ ). Means followed by \* are significantly different at  $P < 0.05$  by t-test at 16<sup>th</sup> day.

the control. In the meantime, the effect of exogenous ATP on the inhibition of stipe lignification was more significant than of the pileus.

### Discussion

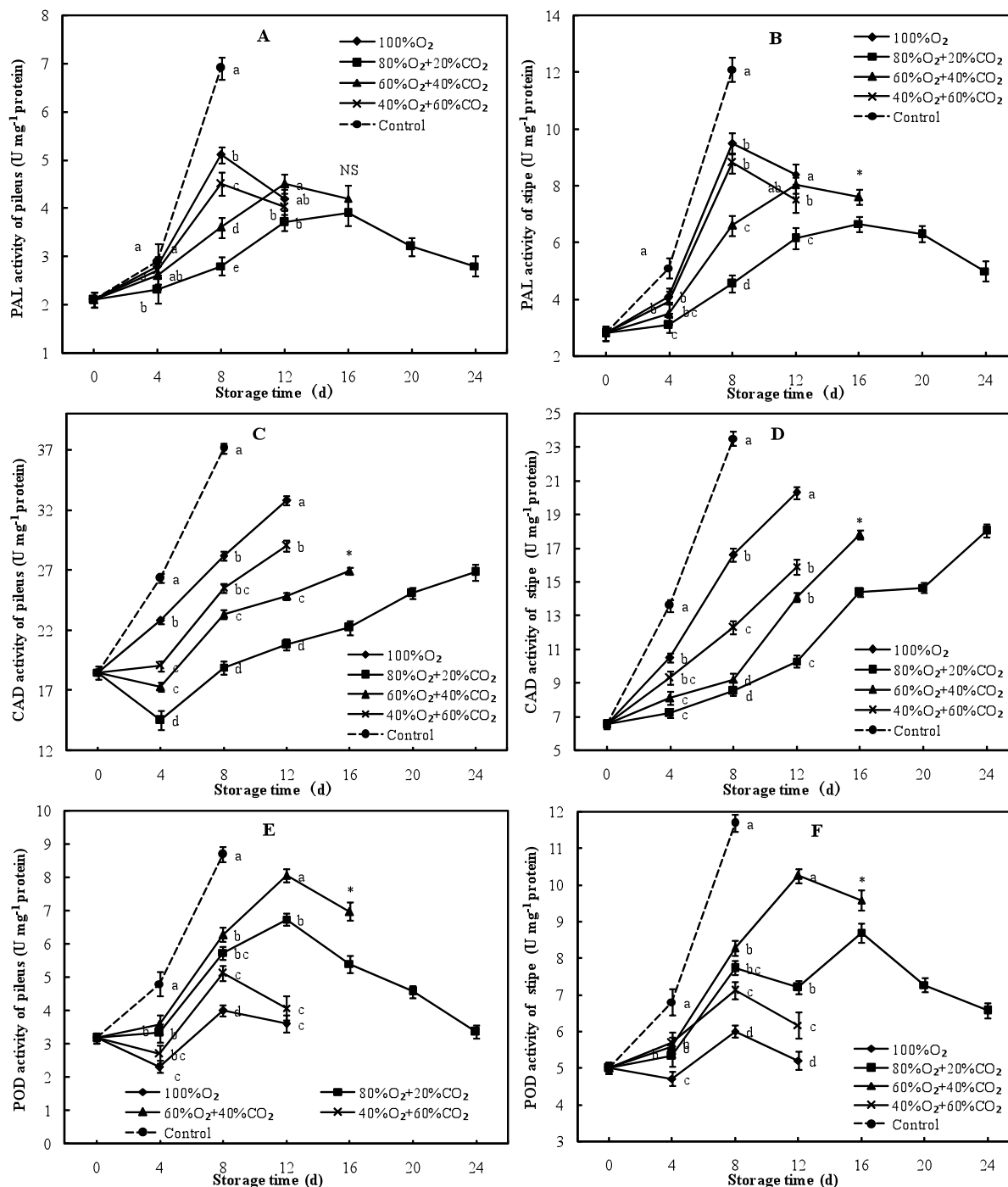
High respiration rate easily consumed metabolic substrates, accelerated senescence and shortened storage time of products (SINGH et al., 2010). The white mushroom is a kind of climacteric product that exhibits an initial high respiration rate (MENG, 2014). In our study, respiration rates at 100% O<sub>2</sub> and the control (about 21% O<sub>2</sub> + 78% N<sub>2</sub>) were similar and high. In contrast, the 80% O<sub>2</sub> + 20% CO<sub>2</sub> treatment suppressed the respiration rate significantly ( $P < 0.05$ ) and delayed the appearance of respiratory peak, which indicates that it is a potential strategy in reducing the respiration rate of mushrooms. MENG et al. (2010) pointed out that high CO<sub>2</sub> inhibited respiration rate, microorganism action, veil opening and stipe elongation, and delayed senescence of mushroom. Thus, it indicated that CO<sub>2</sub> could affect the respiration rates of white mushroom greatly. We also found

that mushrooms treated with 40% O<sub>2</sub> + 60% CO<sub>2</sub> have off-flavor during the late period storage. Improper high proportion CO<sub>2</sub> may stimulate the occurrence of fermentation pathway, which produces ethanol (LI et al., 2014; WANG et al., 2008). On the contrary, it was found that a combination of appropriate high O<sub>2</sub> and elevated CO<sub>2</sub> CA reduced off-flavor, maintained the texture, decreased the incidence of decay due to bacteriostatic action of postharvest mushrooms. The results are consistent with research of RUIZ-CAPILLAS and MORAL (2002), KE and KADER (1990) and our previous study about broccoli (LI et al., 2014). It might be because appropriate high O<sub>2</sub> and elevated CO<sub>2</sub> CA could inhibit the embden-meyerhof-parnas (EMP) of respiration pathway to reduce metabolic substrates consumption, and enhance the tricarboxylic acid cycle (TCA) and cytochrome oxidase pathway (CCP) to increase ATP production (LI et al., 2016). On the other hand, improper high O<sub>2</sub> such as 100% O<sub>2</sub> didn't maintain the texture or, effectively, inhibit the veil opening of white mushroom, and it also kept an obvious high respiration rate, which was similar with broccoli (LI et al., 2013) and fresh-cut bell peppers (CONESA et al., 2007). Therefore, the 80% O<sub>2</sub> + 20% CO<sub>2</sub> treatment, as the optimum HOC-CA preserved the white mushroom well, kept it good quality and prolonged the storage time to 24 days, although storage time of 8.0% O<sub>2</sub> + 8.5% CO<sub>2</sub> at 1 °C (WANG, 2004) and at 4 °C in 100% O<sub>2</sub> + 2% alginate coating (JIANG, 2013) were 15 and 16 days, respectively.

Moisture, involved in metabolism such as various physiological and biochemical reactions of the cell, is a critical parameter of fruit and vegetable storage. A higher moisture content maintained a higher hardness and elasticity of edible fungi (MANZI et al., 1999). However, fresh white mushrooms have a higher moisture content but no cuticle as the protection structure, so it is easier for them to lose water. In our study, the 80% O<sub>2</sub> + 20% CO<sub>2</sub> treatment maintained a higher moisture content than the others, resulting in greater turgor, crispness and tenderness. In general, firmness is very important for mushroom, and it can reflect metabolic changes in the cell wall, water content, and turgidity of cells and senescence of products (LIU et al., 2013; ZIVANOVIC, et al., 2000). The rapid loss of firmness results in a short storage time and rapid deterioration when the mushroom is senescent during storage. In our study, the 80% O<sub>2</sub> + 20% CO<sub>2</sub> treatment maintained the firmness of the mushroom very well. There was a significant difference ( $P < 0.05$ ) between 80% O<sub>2</sub> + 20% CO<sub>2</sub> treatment and 100% O<sub>2</sub> treatment and the control. The activity of enzymes about degradation cell wall might be suppressed when treated with appropriate storage and then cell wall degradation is inhibited, thus, the integrity of cell was protected and the firmness would be maintained.

Veil opening was chosen as one of the most appropriate parameters for sensory quality by consumers (QUE et al., 2015). Earlier harvest time (BRAAKSMA et al., 1999), lower temperature (QUE et al., 2015) and 100% O<sub>2</sub> + 2% alginate coating (JIANG, 2013) inhibited veil opening of the mushroom. In our study, exogenous ATP suppressed veil opening during storage. Meanwhile, 80% O<sub>2</sub> + 20% CO<sub>2</sub> treatment inhibited cap opening more than other treatments, especially at 100% O<sub>2</sub> and the control at low temperature. In our present study, HOC-CA regulated energy metabolism of the white mushroom, which gave a new insight into the mechanism of suppression of veil opening. It could be hypothesised that energy affecting veil opening through retarding the senescence of mushroom.

Lignification is a problem that often occurs during postharvest mushroom storage, which decreases taste and market value (JIANG et al., 2010). However, mushroom lignification has not attracted great attention or importance. We found that stipe lignification was a more serious problem than pileus lignification during white the mushroom storage. In our study, the 80% O<sub>2</sub> + 20% CO<sub>2</sub> treatment significantly ( $P < 0.05$ ) reduced the lignin content and inhibited the lignification of the white mushroom. In the meantime, the higher degree of lig-

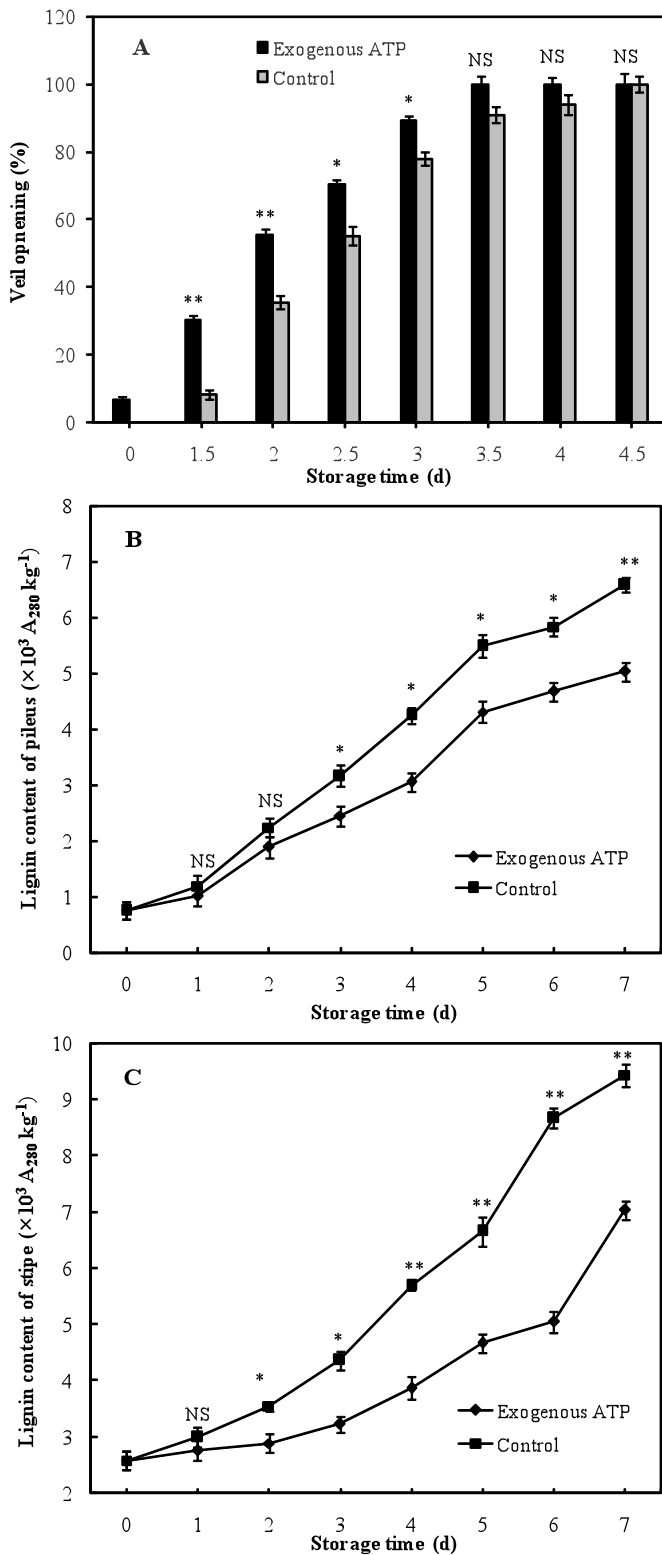


**Fig. 5:** Effect of HOC-CA on the PAL (A, B), CAD (C, D) and POD (E, F) activity of pileus and stipe of the white mushrooms at  $2\pm 1$  °C. Air was used as control. The vertical bars indicate standard deviation ( $n = 3$ ). Means followed by same letters are not significantly different among all treatments from 0 to 12<sup>th</sup> day during storage by Tukey's test ( $P < 0.05$ ). Means followed by \* are significantly different at  $P < 0.05$  by t-test at 16<sup>th</sup> day. NS = not significant.

nification, the greater degree of cap opening; the relationship and mechanism between them requires further study. In addition, exogenous ATP was found to suppress stipe lignification significantly in white mushroom storage.

PAL is the first enzyme in forming lignin, CAD is the enzyme responsible for catalyzing the conversion of coniferaldehyde into coniferyl alcohol, while POD is involved in the last step for the polymerization of lignin monomer in forming lignin (CAI et al., 2006). In our study, the mushrooms in the control group exhibited the highest degree of lignification, while 80% O<sub>2</sub> + 20% CO<sub>2</sub> treatment

showed a low level, which is consistent with the PAL and CAD activity changing of these treatments. A study of bamboo shoots also showed that there was involvement of PAL and CAD activity in lignin content increase during storage (ZENG et al., 2015). However, CAD activity of pileus was higher than that of the stipe, which indicated that CAD was not so important like PAL and POD in lignification of the mushroom. POD, relating to enzymatic browning, is commonly known as a crucial antioxidant defense enzyme in fruit and vegetables (LIU et al., 2013). In our study, in terms of the POD activity with HOC-CA exhibited a moderate and relatively stable level, while



**Fig. 6:** Effect of exogenous ATP on the veil opening (A), lignin content of pileus (B) and stipe (C) of the white mushrooms at 22±1 °C. Mushrooms infiltrated in distilled water were used as control. The vertical bars indicate standard deviation (n = 3). Differences between treatments were analyzed by t-test at \* $P < 0.05$  and \*\* $P < 0.01$ . NS = not significant.

that of the control group showed the highest level along with serious lignification. There is a significant positive correlation between lignin content and POD activity ( $P < 0.05$ ). The ability of HOC-CA

in decreasing lignin accumulation through reducing POD activity is in accordance with previous research (JIANG et al., 2010). What's more, moderate POD activity may not only scavenge reactive oxygen species (ROS), but also reduce browning and lignification of the mushroom. All these results demonstrated the effects of 80% O<sub>2</sub> + 20% CO<sub>2</sub> treatment in inhibiting lignification by suppressing PAL, CAD and POD activity, maintaining high energy availability and thus maintaining the mushroom's commercial value.

Limited energy availability is related to senescence and deterioration of horticultural products (JIN et al., 2013) CHEN et al. (2015) reported that exogenous ATP could retard senescence, maintain the quality and reduce the disease of harvested longan fruit. Under 80% O<sub>2</sub> + 20% CO<sub>2</sub> treatment storage conditions, the ATP content and energy charge of the white mushroom presented higher than in other treatments, and thus, the quality of these samples was higher and senescence was delayed. In addition, exogenous ATP suppressed veil opening and lignification of the white mushroom. These findings suggest that ATP level has a close relationship with veil opening and lignification of mushroom under HOC-CA.

## Conclusions

In conclusion, as the optimum HOC-CA for white mushroom, 80% O<sub>2</sub> + 20% CO<sub>2</sub> preserved the mushroom very well, and the effects were better than those of the other treatments, especially than the control during the whole storage process. It suppressed the respiration rate, cap opening and lignification of mushrooms, and inhibited PAL, CAD and POD activity. It also significantly retarded the reduction of ATP level, energy charge level, and growth of lignin content. Additionally, exogenous ATP suppressed lignification and veil opening in white mushroom storage. These findings demonstrate that there is a negative correlation between the ATP content and cap opening and stipe lignification. Accordingly, appropriate HOC-CA combination with low temperature might be an effective method to inhibit veil opening, lignification, preserve the white mushroom well, and prolong its long storage time to 24 days along with good sensorial and physiological qualities.

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