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Effects of high CO_2 in-package treatment on flavor, quality and antioxidant activity of button mushroom (*Agaricus bisporus*) during postharvest storage

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ABSTRACT

Button mushroom (*Agaricus bisporus*) is marketed for its good flavor and health benefits. However, the shelf life of fresh button mushroom is limited and quality is lost rapidly during storage. In this study, button mushrooms were treated with high CO_2 (95%–100%) at the time of sealing of the packages and the packages were ventilated after 0, 12, 24 and 48 h by puncturing the film at four corners. Results showed that 12 h high CO_2 treatment had a significant effect in reducing browning index (BI) and maintaining flavor of button mushroom during storage. In addition, the malonaldehyde (MDA) content was significantly inhibited while catalase (CAT) and peroxidase (POD) activities were significantly promoted by high CO_2 treatment. High CO_2 treatment increased antioxidant ability of button mushroom, which in turn maintained the flavor, quality and consumer acceptance of button mushroom during postharvest storage.

1. Introduction

Button mushroom (*Agaricus bisporus*) is a popular edible mushroom, which is considered not only as nutritional vegetable but also as functional food due to the free radical scavenging and antioxidant activities (Guan et al., 2013; Wu et al., 2016). Mushrooms accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids. The smell of button mushroom is primarily ascribed to an abundance of flavor volatiles, particularly 8-carbon compounds, with 1-octen-3-ol being reported to be one of the major 8-carbon components responsible for the typical mushroom smell (Dong et al., 2012). The secondary compounds in mushrooms are of great interest to consumers and are possible protective agents for human health.

However, mushrooms lose their quality rapidly during postharvest storage at ambient temperature because of their high moisture content and overall structure (Oliveira et al., 2012). Loss of qualities for mushrooms include browning, softening, cap development, off-flavor and secondary mold growth (Kim et al., 2006). Different treatment have been reported to extend the shelf life of mushrooms such as modified atmosphere packaging, washing with hydrogen peroxide and ozone treatment (Kim et al., 2006; Yuk et al., 2006).

Modified atmospheres are created by altering normal air composition, in order to provide an appropriate atmosphere surrounding the product for decreasing its deterioration rate and increasing its shelf life (Ares et al., 2007). It has been reported that modified atmospheres rich in CO_2 can modify respiration rate, energy metabolism, ethylene reaction and physiological changes in postharvest storage or package of many fresh products (Blanch et al., 2015; Lumpkin et al., 2015; Yi et al., 2016). However, excessive accumulation of CO_2 in modified atmosphere packages can damage the cell membrane and cause physiological injuries to the product, such as enzymatic browning and loss of firmness (Briones et al., 1992; Burton et al., 1987). Thus, the exact concentration and exposure time should be determined for specific fresh produce during modified atmosphere packaging or storage.

In this study, button mushrooms were treated with high CO_2 (95%–100%) at the time of sealing of the packages and then the packages were ventilated at 0, 12, 24 and 48 h using a new packaging method. The sensory evaluation, browning index (BI), flavor compounds, total phenolics, total antioxidant activity and

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antioxidant enzyme activities in treated and untreated mushrooms were measured. The objective is to select appropriate high CO_2 in-package treatment for button mushroom during postharvest storage.

2. Materials and methods

2.1. Plant material and CO_2 package

Button mushrooms (*Agaricus bisporus*) were harvested from a local edible fungus cultivation base in Beijing, China. Intact, closed and uniform mushrooms with a fresh white color were selected and pre-cooled for 12 h at 4 °C in a cold room. Afterwards, three mushrooms were placed in a 28 cm × 20 cm × 4 cm box and were sealed with high CO_2 (95%–100%) using low density polyethylene (PE) of 0.04 mm thickness (He Yuan Hua Feng Plastic Co., Ltd., China). The packages were stored at 4 °C with 85% relative humidity (RH) in refrigerators, and were punched with 4 holes (r = 0.3 cm) at each corner of the package after 0, 12, 24 and 48 h storage. Analyses were carried out on the first day and subsequently at 4 d intervals until 16 d. Mushroom caps were frozen by liquid nitrogen and stored at −80 °C for analysis.

2.2. Sensory evaluation

Sensory evaluation was conducted according to the method from Huang et al. (2008). Five key attributes, color, off-odour, cap shape, texture and consumer acceptance, were selected for evaluation. A scale of 0 to 10 was used in sensory evaluation: Color (White: 10-8, Slight browning: 8-6, Mild browning: 6-4, Heavy browning <4); Off-odour (No: 10-8, Slight: 8-6, Obvious: 6-4, Severe <4); Cap shape (Closed: 10-8, Slightly open: 8-6, Half open: 6-4, Totally open <4); Texture (Stretchy: 10-8, Slight soft: 8-6, Mild soft: 6-4, Severe soft <4); Consumer acceptance: (Intense: 10-8, Acceptable: 8-6, Discount: 6-4, Unacceptable <4). The sensory evaluations were carried out by ten panelists. The sensory score for each sample was calculated as a mean value. Fresh mushrooms were used as the control each time (score = 10).

2.3. Color analysis

The surface color of mushroom caps was measured with a WSC-S Colorimeter (Shanghai precision instrument Co. Ltd., China). 'L^*' (light/dark), 'a^*' (red/green) and 'b^*' (yellow/blue) values were used to calculate the browning index (BI) according to the following equation (Gao et al., 2014):

$$BI = [100(x - 0.31)]/0.172, \quad \text{where} \quad x = (a^* + 1.75L^*)/(5.645L^* + a^* - 3.012b^*).$$

2.4. Flavor compounds analysis

Flavor volatiles were measured according to the method from Costa et al. (2013) with modifications. Exactly 1 g of each sample was placed in a 10 mL vial and homogenized with 5 mL buffer solution, containing 20% $CaCl_2$ and 200 mM ethylene diamine tetraacetic acid (EDTA). Solid-phase micro-extraction (SPME) was carried out in the headspace mode by means of an AOC-5000 autosampler (Shimadzu, Kyoto, Japan) connected with the GC–MS-QP2010 Plus system (Shimadzu, Kyoto, Japan). The polydimethylsiloxane/divinylbenzene (65 μm, 1 cm) fiber was held in the headspace for 30 min at 50 °C under agitation for extraction.

Analytes were then desorbed for 2 min at 250 °C in the GC injector with a splitless mode fitted with DB-WAX column (30 m × 0.25 mm × 0.25 μm). The column temperature was held at 40 °C for 2 min, and then increased to 120 °C with a temperature gradient of 3 °C min^{-1}, before being increased to 200 °C at 5 °C min^{-1} and held for 5 min.

Identification of the metabolites was conducted using the NIST/EPA/NIH Mass Spectral Library (NIST-11) of the GC–MS data system. Relative contents of the identified compounds were normalized by an internal standard method.

2.5. Malondialdehyde (MDA) content analysis

MDA content was measured according to the method described by Heath and Packer (1968) with modifications. Absorbencies of the aqueous phase at 450 nm, 532 nm and 600 nm were measured. The MDA content in the aqueous phase was calculated according to the following formula:

$$MDA\ (mol\ L^{-1}) = [6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}] \times 10^{-6}.$$

2.6. Total phenolics content analysis

The total phenolics content analysis was carried out using the method described by Gao et al. (2014).

2.7. Antioxidant assay

The 2,2-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and ferric reducing antioxidant power (FRAP) assay were used as described by Lu et al. (2016).

2.8. Enzyme activity analysis

One gram mushroom powder was homogenized with 8 mL of 0.05 mol L^{-1} PBS at pH 6.8, then centrifuged at 8000*g* at 4 °C for 10 min. The supernatant was used to measure the activity of polyphenol oxidase (PPO) and peroxidase (POD). The PPO and POD activity was measured according to the method described by Hu et al. (2015).

Catalase (CAT) activity was analyzed according to the method described by Kan et al. (2010) with modifications. One gram of mushroom powder was homogenized with 5 mL 0.1 mol L^{-1} PBS (pH 7.0) and then centrifuged for 20 min at 8000*g* at 4 °C. The supernatant was used as the crude extract. The reaction mixture contained 0.02 mol L^{-1} H_2O_2 and crude enzyme. Catalase activity was determined as the amount of enzyme that caused an absorbance decrease of 0.01 at 240 nm in 1 min. The protein concentrations for all the enzyme assays were determined with Bradford and Williams (1977) method.

2.9. Statistical analysis

The figures were drawn using Origin 8.6 software (Microcal Software Inc., Northampton, MA, USA). Least significant difference (LSD) or Duncan's test at the 0.05 level were analyzed by SPSS Statistics 22 Software.

3. Results and discussion

3.1. Sensory evaluation

The assessment of produce quality is one of the core aspects of applied postharvest biology and the sensory evaluation of both the control and high CO_2 treated button mushrooms was conducted using a sensory score. The sensory scores all declined in button mushroom in all the treatments during storage, but the decline was reduced by the CO_2 packaging treatment. Compared with high CO_2

in-package treatment, the sensory score in control mushrooms declined more rapidly, losing commercial acceptability after 8 d storage (a sensory score below 6 was determined to be the limit of commercial acceptability), with a sensory score of 5.18 at 12 d. Compared with the control, the 12 h CO_2 treatment gave 8 additional days of shelf life, while the 24 and 48 h CO_2 treatment has given 4 additional days of shelf life. Thus, high CO_2 in-package treatment was effective in maintaining sensory quality of button mushroom during storage, especially for the 12 h package treatment (Table 1).

3.2. Browning index (BI) variation

The browning index is one of the main quality features for measuring the extent of deterioration on the surface of white mushrooms. As shown in Fig. 1, the degree of mushroom browning increased with storage time. During the first four days, the high CO_2 treated mushrooms had higher BI values than the control, and the BI was proportional to treatment time, indicating damage to mushroom quality from high CO_2 treatment. By 12 d of storage, the 12 and 24 h CO_2 treated mushrooms showed lower BI values than control. At 16 d storage, the BI of the control sample reached 35.17, while BI of 12 and 24 h treated mushrooms were 30.21 and 32.85, respectively. However, the BI of 48 h CO_2 treated mushrooms was higher than that of the control during the whole storage period. Thus, it was concluded that high CO_2 could cause damage to the mushroom cap surface tissue, but it obviously also suppressed browning of button mushrooms during cold storage, which was similar to effect of UV-C treatment on button mushroom (Guan et al., 2005).

3.3. Flavor quality

Mushrooms accumulate a variety of secondary metabolites and 13 identified metabolites (1-octen-3-ol, (E)-2-octen-1-ol, 1-octanol, 1-octen-3-one, (E)-2-octenal, 3-octanone, benzyl alcohol, benzaldehyde, benzeneacetaldehyde, azulene, 2,4-bis(1,1-dimethylethyl)-phenol, (E,E)-2,4-decadienal and (E,E)-2,4-nonadienal) and 2 unknown metabolites were observed using SPME-GC–MS. The levels of these compounds, relative to those detected at 0 d, were measured and clustered into two classes according to the change in content during storage (Fig. 2A).

Previous research has reported that the volatile organic compounds produced by button mushroom are predominantly 8-carbon molecules (Noble et al., 2009). From our results, six kinds of 8-carbon compound were investigated and used in principal component analysis (PCA), which showed that PC1 and PC2 accounted for 93% of the total variability for 8-carbon compounds in button mushroom during storage. Mushrooms with a high sensory evaluation score after cold storage were clustered (green circle) with fresh control treatment (CK-0d), while mushrooms with low score were clustered (red circle) with stored control

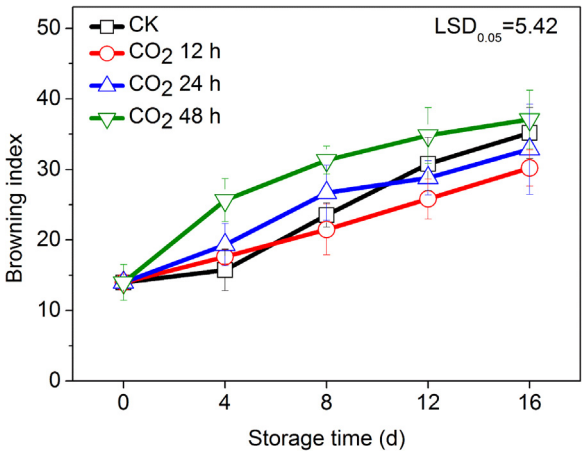

Fig. 1. Effects of CO_2 treatments on browning index of button mushroom during storage. The error bars represent the standard errors. LSDs represent least significant differences at the 0.05 level. 'CK' represents the control. Twelve mushrooms in each treatment were used for analysis on each sampling day.

treatment mushrooms (CK-16d) (Fig. 2B), thus, the contents of 8-carbon molecules be characteristic indicators of mushroom quality during postharvest storage.

One of the major 8-carbon components is 1-octen-3-ol, which causes the typical mushroom odor (Dong et al., 2012). The content of 1-octen-3-ol declined in button mushroom during storage treatments. Compared with the control, high CO_2 in-package treatment was effective in maintaining 1-octen-3-ol content of button mushroom during storage, especially the 12 h in-package treatment (Fig. 2C). Previous reports have illustrated that high CO_2 treatment maintains the flavor quality in many plants, such as strawberry (Pelayo et al., 2003), table grape (Howe and Lee, 2015) and blueberry (Lange and Beaudry, 1991). Thus, there is a conclusion that the 12 h high CO_2 package treatment was effective in maintaining the appearance and flavor quality of button mushroom during postharvest storage.

3.4. MDA content

MDA is the main product of membrane lipid peroxidation (Gürbüz and Heinonen, 2015). Throughout the eight days of storage, MDA content increased in treated and untreated samples. Compared with the control, the MDA content increased more rapidly in untreated samples. Significant differences were observed between the CO_2 treatment and the control samples at 4 d and 8 d storage, while no significant difference in MDA content were observed at 12 and 16 d storage between the treated and untreated mushrooms (Fig. 3). The results indicate that CO_2 treatment could alleviate oxidative injury during the early stage of storage.

Table 1
Sensory evaluation of button mushroom during postharvest storage under different CO_2 treatments.

Treatment	Storage time (d)				
	0	4	8	12	16
CK	$9.57 \pm 0.08^{a,*}$	8.93 ± 0.21^{a}	7.03 ± 0.14^{b}	5.18 ± 0.17^{c}	3.15 ± 0.10^{c}
CO_2 12 h	9.54 ± 0.11^{a}	8.83 ± 0.18^{a}	8.07 ± 0.18^{a}	7.07 ± 0.10^{a}	6.02 ± 0.04^{a}
CO_2 24 h	9.61 ± 0.06^{a}	8.19 ± 0.35^{a}	7.07 ± 0.17^{b}	6.10 ± 0.13^{b}	4.35 ± 0.23^{b}
CO_2 48 h	9.69 ± 0.05^{a}	8.04 ± 0.21^{a}	7.17 ± 0.16^{b}	6.17 ± 0.10^{b}	5.03 ± 0.05^{b}

*Five key attributes, color, off-odour, cap shape, texture and consumer acceptance, were selected for evaluation. A scale of 0 to 10 was used in evaluation of each attribute. The sensory score was calculated as a mean value. A sensory score below 6 was determined to be the limit of commercial acceptability. The error margins represent standard errors. The letters of 'a', 'b' and 'c' represent significant differences at the 0.05 level. 'CK' represents the control. Twelve mushrooms in each treatment were used for analysis on each sampling day.

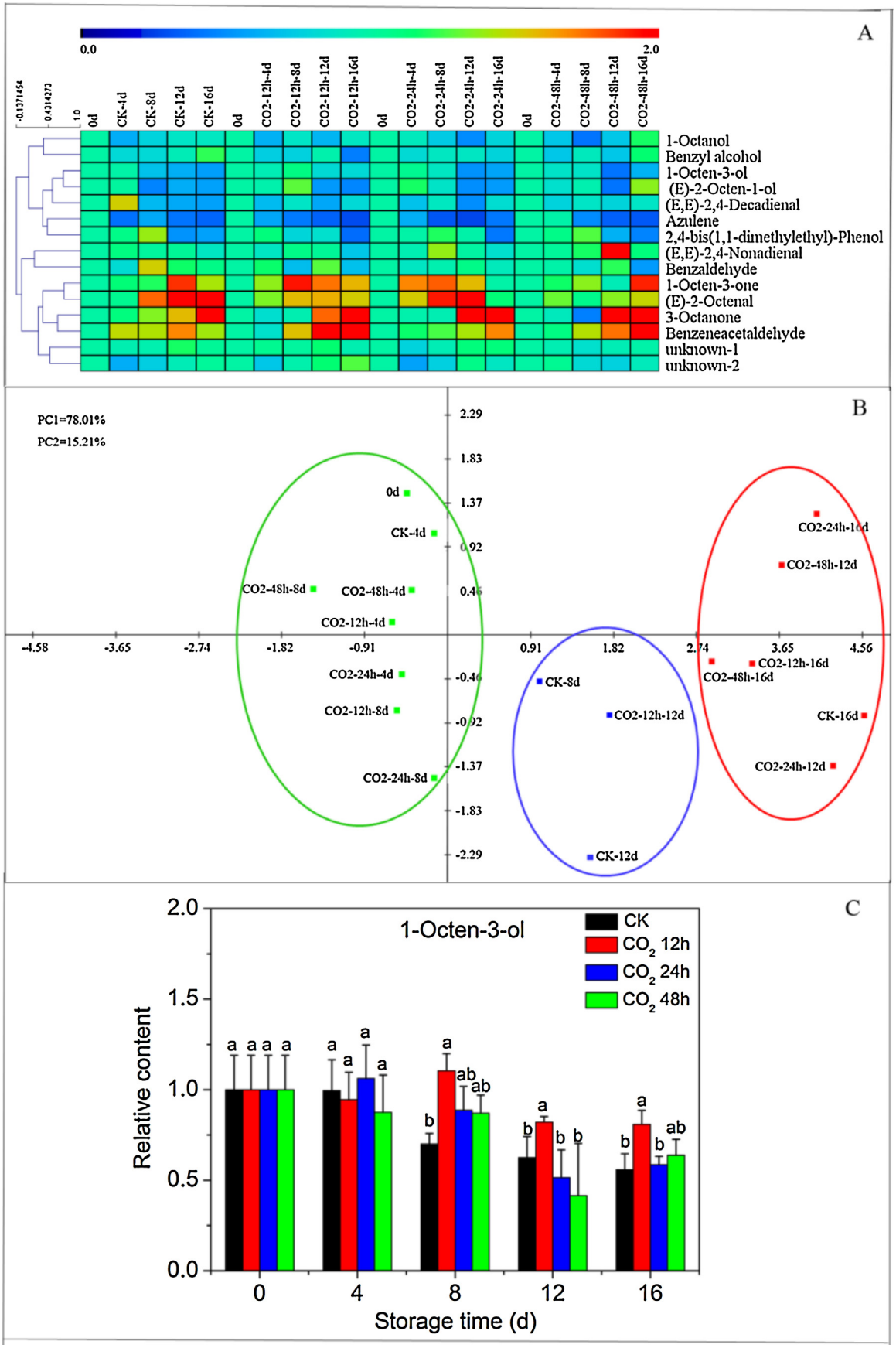

Fig. 2. Effects of CO_2 treatment on variation in contents of flavor compounds of button mushroom during storage. (A) Cluster and (B) PCA analysis of flavor compounds were conducted using MeV 4.8.1 software. The graph shows the relative level of each metabolite relative to its amount at 0 d. Normalized values are shown on a color scale (shown on the top of the figure), which is proportional to the content of each identified metabolite. (C) Effects of CO_2 treatment on 1-octen-3-ol content variation in button mushroom during storage. Different superscripts between columns represent significant differences between samples at the 0.05 level. 'CK' represents the control. Twelve mushrooms in each treatment were used for analysis on each sampling day.

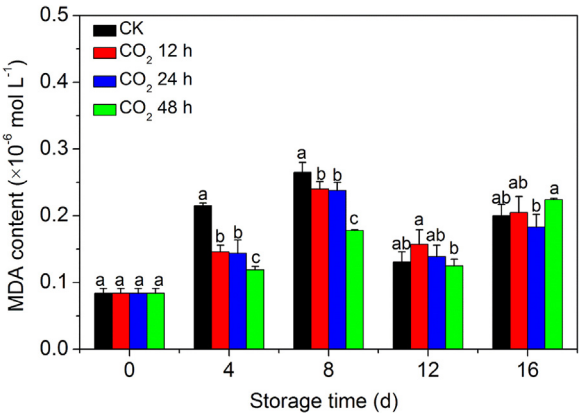

Fig. 3. Effects of CO_2 treatments on variation of MDA contents in button mushroom during storage. Different superscripts between columns represent significant differences between samples at the 0.05 level. 'CK' represents the control. Twelve mushrooms in each treatment were used for analysis on each sampling day.

3.5. Total phenolics and antioxidant ability

Phenolic compounds have been reported as the major naturally occurring antioxidant components from commercial mushrooms (Dubost et al., 2007) and other plants (Christopoulos and Tsantili, 2015; Selcuk and Erkan, 2015). The biological properties of phenolic compounds may be due to their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals, terminating the radical-stimulated chain reactions that occur during the oxidation of unsaturated fats (Cheung et al., 2003). From our results, total phenolic content in the control samples decreased continuously during storage, while in the CO_2 treated mushrooms it increased immediately after the start of treatment and decreased afterwards. Compared with control, total phenolic contents were significantly different under CO_2 treatments, particularly 24 and 48 h, during the early 8 d storage, however, no significant difference were investigated between the treated and untreated mushrooms during later storage (Fig. 4A).

FRAP and ABTS methods are commonly applied to determine the antioxidant activity. The FRAP method measures the capacity of the sample to reduce ferric complex to the ferrous form, while the ABTS method is based on the capacity to scavenge the radical cation ABTS+· (Cheng et al., 2015). Compared with the control, the total phenolics content of button mushrrom was significantly different in 24 and 48 h CO_2 treatment before 12 days storage, while significant difference was found in the 12 h CO_2 treated mushrooms at 8 d storage (Fig. 4A). The total antioxidant activity measured by the FRAP method decreased during the storage period both in control and in CO_2 treated samples and the trend was closely associated with the total phenolics content. The total antioxidant activity in the control sample decreased continuously during storage, while in CO_2 treated mushrooms it increased immediately after treatment onset and decreased thereafter (Fig. 4B). However, no significant difference was found between the control and the treated mushrooms in total antioxidant activity measured by the ABTS method, although it increased as expected during the whole storage time in all the treatments (Fig. 4C).

3.6. Antioxidant enzyme activities

PPO plays an important role in enzymatic browning of mushrooms. Decreasing the PPO activity has been shown to be efficient in preventing the synthesis of melanin in the process of

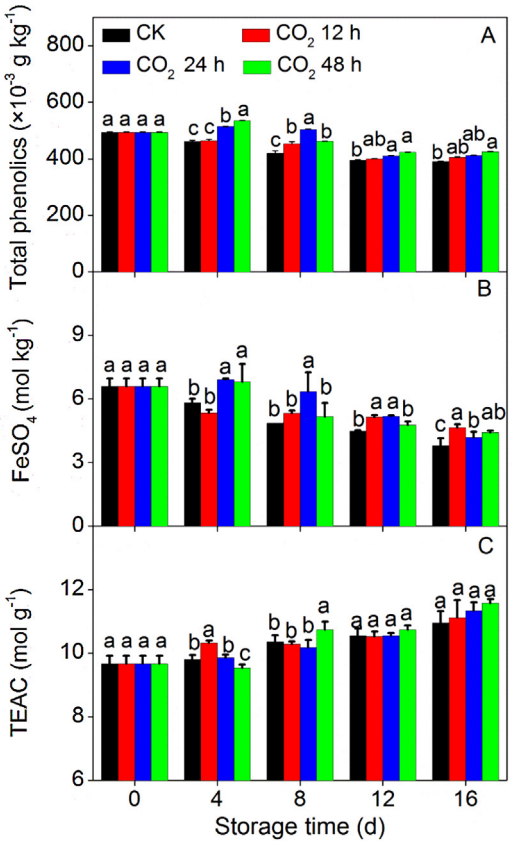

Fig. 4. Effects of CO_2 treatments on (A) total phenolic contents and antioxidant activity measured by (B) FRAP and (C) ABTS in button mushroom during storage. Different superscripts between columns represent significant differences between samples at the 0.05 level. 'CK' represents the control. Twelve mushrooms in each treatment were used for analysis on each sampling day.

browning in mushroom (Yang and Mou, 2009). The PPO activity increased gradually during early 8 d storage in both the treated and untreated samples, however, a more rapid increase was observed in the untreated mushrooms compared to the treated samples (Fig. 5A). CAT is an iron containing enzyme, which plays a crucial role in antioxidant defense during the fruit ripening process (Duan et al., 2011) that catalyzes H_2O_2 accumulated by the plant into O_2 and H_2O to reduce the oxidative damage caused by H_2O_2 in plant tissues. Compared with the control sample, the CAT activity increased and peaked at 4 d storage in 24 h treated mushrooms, while the peak occurred at 8 d storage in 12 and 48 h treated mushrooms (Fig. 5B). Previous research has shown POD is closely related to both enzymatic browning and antioxidant defense (Jiang et al., 2004). The results showed that the POD activity increased sharply during the whole storage period, and significant difference were found between the control and CO_2 treated mushrooms during the whole storage (Fig. 5C).

High CO_2 treatment significantly increased antioxidant enzymes of button mushroom during postharvest storage, as has been found with other plants in modified atmosphere storage. For examples, short-term CO_2 treatment has been shown to extend the shelf life of fresh-cut burdock by decreasing the PPO activity and enhancing the activity of CAT, POD and superoxide dismutase (SOD); Application of pure oxygen significantly induced the activities of antioxidant enzymes, including SOD, ascorbate peroxidase (APX) and CAT, in harvested litchi fruit and was beneficial in scavenging of H_2O_2 and superoxide and alleviating

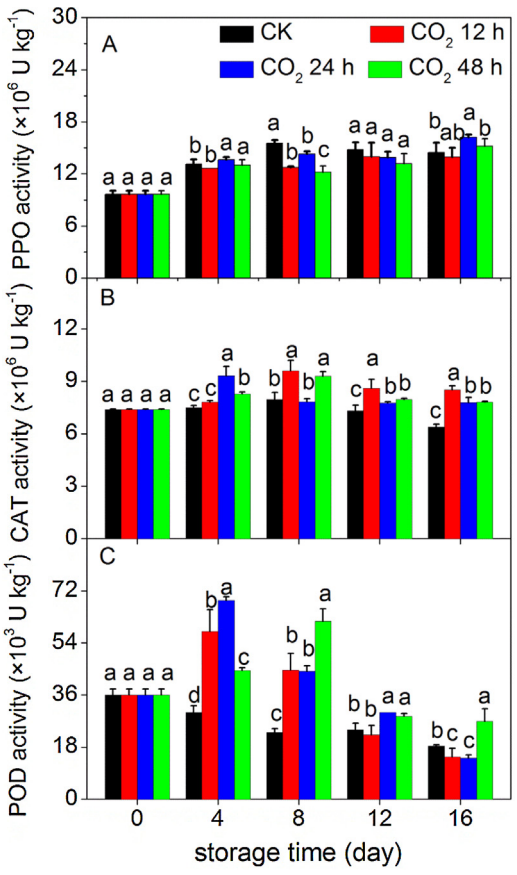

Fig. 5. Effects of CO_2 treatments on variation of (A) PPO, (B) CAT and (C) POD activities in button mushroom during storage. Different superscripts between columns represent significant differences between samples at the 0.05 level. 'CK' represents the control. Twelve mushrooms in each treatment were used for analysis on each sampling day.

lipid peroxidation; High oxygen treatment was effective in increasing the PPO activity and maintaining the quality of mushroom during postharvest storage (Dong et al., 2015; Duan et al., 2011; Liu et al., 2010). Thus, it can be concluded that CO_2 treatment was effective in increasing antioxidant ability by inducing the POD and CAT activities, and maintaining flavor and consumer acceptance of mushroom during postharvest storage.

4. Conclusion

The findings of the present study have shown that a 12 h high CO_2 in-package treatment has a positive effect on reducing browning and maintaining flavor quality in button mushroom. Treated mushrooms maintained higher levels of total phenolics, total antioxidant activity, POD and CAT activities, and lower levels of MDA content and PPO activity during postharvest storage, suggesting a significant effect on activation of antioxidant activity by high CO_2 treatment. These results indicated that high CO_2 in-package treatment for 12 h could be used as favorable treatment to extend shelf life of button mushroom and represent a promising alternative as an environment-friendly application to be used in the complementation of low temperature storage in mushrooms.

Conflict of interest

The authors declare no competing financial interest.

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