



# Effect of L-Arginine on Maintaining Storage Quality of the White Button Mushroom (*Agaricus bisporus*)

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## Abstract

Decreased postharvest quality is one of the main reasons for the short shelf life of the white button mushroom. The effects of L-arginine on color, weight loss, firmness, electrolyte leakage rate, malondialdehyde (MDA) content, PPO activity, PAL activity, SOD activity, POD activity, total phenolic levels, flavonoid amounts, total sugar, and soluble protein amounts in white button mushrooms were assessed during storage at 4 °C for 8 days. The results showed that treatment with 10 mM L-arginine maintained tissue firmness, reduced electrolyte leakage, and delayed browning compared with the control treatment. In addition, 10 mM L-arginine treatment inhibited PPO and PAL activities, while inducing SOD and POD activities. Furthermore, L-arginine treatment increased the accumulation of phenolic substances and flavonoids, while total sugar and soluble protein contents were maintained at high levels throughout the storage period. These findings suggested that 10 mM L-arginine treatment may maintain the quality of the button mushrooms and extend their shelf life.

**Keywords** White button mushroom · L-Arginine · Postharvest quality · Browning

## Introduction

The white button mushroom (*Agaricus bisporus*) is highly popular among consumers and widely recognized for its organoleptic, medicinal, and nutritional properties (Nasiri et al. 2017). Indeed, this fungus offers various nutrients, and its regular consumption can prevent scurvy, cancer, and atherosclerosis, while improving neurological function (Wu et al. 2017; Cui 2010). However, due to early veil breaking, and accelerated physiological, morphological, and microbial

changes that lead to browning and sliminess, white button mushrooms can only be stored for 3–4 days at room temperature (Jiang 2012). Button mushroom postharvest browning is mainly due to oxidation of phenols into quinones through the activities of enzymes such as polyphenol oxidase (PPO), with subsequent polymerization and browning (Ding et al. 2016). PPO and peroxidase (POD) are two important enzymes that cause postharvest browning of white button mushrooms (Wu et al. 2017; Sun et al. 2013). Electrolyte leakage and cellular decompartmentalization cause cell defects and reduce the repairing capacity of the cell membrane system, and may disrupt the cell membrane structure, subsequently causing browning and reducing the quality of white button mushrooms (Li et al. 2017).

Mushrooms have a short shelf life, about 3–4 days at room temperature, which hampers their distribution over long distances; therefore, prolonged postharvest storage would benefit the mushroom industry and consumers (Meng et al. 2012). At present, in the field of edible fungi, biophysical, chemical, and physical methods are mainly used to inhibit PPO activity to achieve the purpose of controlling the browning and preservation of white button mushrooms (Lu 2016). In the study by Wills and Li (2016), arginine treatment was used to inhibit the browning of fresh cut Granny Smith apple and iceberg lettuce,

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and they believed that the postharvest life was doubled by dipping in 100 mM arginine for 5 min. In Li et al. (2015), L-arginine was used to inhibit enzymatic browning of mandarin orange, and the results showed that at optimal concentration (200  $\mu\text{mol/L}$ ), L-arginine effectively inhibited the browning of mandarin fruits. Studies assessing preservation techniques for the white button mushroom are mainly focused on chemical coating, electron beam irradiation, modified atmosphere packaging, and frozen storage (Zhang et al. 2017). Compared with the hydroxypropyl methylcellulose coating and the control group, Perez-Gago et al. (2005) found that the whey protein-coated apple slices had a better effect and they believed that whey protein acts against browning. Sarengaowa et al. (2017) studied the effects of alginate-based edible coatings (EC) containing different concentrations of thyme oil on fresh-cut “red Fuji” apples. The results showed that the combination of 0.5  $\mu\text{L/mL}$  thyme oil and alginate-based EC significantly inhibited the microbial growth, respiration, weight loss, hardness, and browning of fresh-cut “red Fuji” apples. However, these technologies have drawbacks, including odors, residual toxins, expensive equipment, and improvement challenges (Wu et al. 2017; Zhang et al. 2013). Therefore, safe and effective preservation methods for white button mushrooms are urgently required.

L-Arginine, a conditionally essential amino acid, simultaneously acts as a precursor for nitric oxide (NO) and polyamines (PAs) (Mirmiran et al. 2017). Both NO and PA are important messenger molecules in plants, which participate in almost all physiological and biochemical processes including growth development and stress resistance. Arginine decarboxylase (ADC), arginase, and nitric oxide synthase (NOS) are key enzymes in L-arginine catabolism. Arginine can regulate plant growth and development through its metabolites and related enzymes; NOS catalyzed L-arginine to produce NO and regulate root growth and differentiation, flower formation, and break dormancy (Yamasaki 2005; Grün et al. 2006; Bethke et al. 2006); ADC catalyzes L-arginine to produce PA and regulate lateral root formation fruit development, promote flower bud differentiation, and pollen germination (Kalač and Krausová 2005). In addition, PA synthesis was increased in the plants infected by pathogenic bacteria (Walters 2003). Exogenous spermine can activate plant defense genes and hypersensitive response (HR) through reactive oxygen species (ROS) to achieve systemic acquired resistance (SAR), while NO can synergize with ROS (Takahashi et al. 2003). Some studies indicate that decreased arginine levels in nitrate reductase-deficient *Arabidopsis thaliana* plants impaired nitric oxide synthesis and inhibited the hypersensitive response to *Pseudomonas syringae* (Modolo et al. 2006; Zheng et al. 2011). Exogenous arginine improved salt resistance of rice via modification of the put/spd + spm ratio (Kakkar et al. 2000). L-Arginine derived from animal-based food or plant extracts are natural preservatives (Wang et al.

2017). Numerous reports have demonstrated that postharvest dipping in a NO donor or fumigation with NO extends postharvest life by senescence in non-climacteric produce and slows down the ripening of climacteric fruits (Wang et al. 2017). Wills and Li (2016) reported that an ambient dip of arginine inhibits development of browning in fresh-cut apple and lettuce and does not affect the taste. The potential for the addition of L-arginine to extend postharvest life of vegetables would be worth to investigate (Wang et al. 2017). This study reports the effects of L-arginine on postharvest quality of white button mushrooms and related enzyme activities that provide a basis for their preservation.

## Materials and Methods

### Chemical Reagent

All chemicals were of analytical reagent-grade purity and purchased from Beijing Solarbio Science & Technology Co., Beijing, China.

### Sample Preparation

White button mushrooms as the experimental material from the first flush that were harvested at commercial maturity stage (approximately 3–4 cm) were obtained from Kuancheng Jiarun Edible Mushroom Co., Ltd., Hebei, China. The mushrooms were transported to the laboratory within 3 h and stored in darkness at 4 °C and 80–90% relative humidity for 24 h. The mushrooms were selected based on color uniformity and absence of mechanical damage and then randomly divided into four lots of 160 sporophores each. One lot was dipped in distilled water used as a control. Another three lots were respectively dipped in aqueous solutions of 5 mM L-arginine, 10 mM L-arginine, and 25 mM L-arginine. Twenty white button mushrooms were dipped into 1 L of solution for 10 min. Treatment was replicated three times. The mushrooms were allowed to completely dry at 25 °C for 1 h and packed in low-density polyethylene bags with a thickness of 0.03 mm (20 intact sporophores for each bag). The samples were stored in darkness at 4 °C and 80–90% relative humidity for 8 days. Twenty-six sporophores per replicate of each treatment were taken immediately following treatment (time 0) and after 2, 4, 6, and 8 days. Sixteen mushroom caps per replicate of each treatment were selected to assess surface color, firmness, weight loss, and electrolyte leakage rate measurement. Another ten mushroom caps per replicate of each treatment were diced, frozen in liquid nitrogen, and stored at -80 °C to assess MDA, total phenolic, flavonoid, total sugar, total soluble protein content, and PPO, POD, SOD, and PAL enzyme activities.

## Color Measurement

Three mushrooms were randomly selected from control, 5 mM L-arginine, 10 mM L-arginine, and 25 mM L-arginine treatment groups. The change in colorimetric value was determined by measuring three fixed positions on the fungus, respectively, on a CR-400 colorimeter (Konica Minolta Co., Ltd., Japan) in the reflectance mode. The  $L^*$  value (lightness),  $a^*$  (red/green), and  $b^*$  (yellow/blue) were determined after 0, 2, 4, 6, and 8 days of storage at 4 °C, respectively, according to a previous report (Meng et al. 2012). Total color difference ( $\Delta E$ ) indicates the magnitude of total color difference and is expressed by the following equation (1), where  $L_0^*$ ,  $a_0^*$ , and  $b_0^*$  are the values of control samples at day 0. The browning index (BI) represents the purity of brown color and was calculated according to the following equations (2) (Borchert et al. 2014; Wu et al. 2017):

$$\Delta E = \left[ (L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2 \right]^{1/2} \quad (1)$$

$$BI = [100 (x - 0.31)] / 0.172 \quad (2)$$

where  $x = (a^* + 1.75L^*) / (5.645L^* + a^* - 3.012b^*)$ .

## Scanning Electron Microscope

White mushrooms in the control group and the 10 mM L-arginine-treated group were selected for observation on day 8. They were cut into 4 mm × 3 mm pieces and placed in 1.5-mL Eppendorf tubes and fixed at 4 °C for 24 h. The samples were dried in 40 °C for 1 h by a Leica EM CPD 300 automatic critical point dryer (Leica, Germany). Then, they were then placed on a metal stage and observed at 300, 500, and × 1000 magnifications, respectively.

## Firmness Measurement

A penetration test was performed on the white button mushrooms. Each group of three was paralleled by a TA-XT2i-plus textural analyzer (Stable Micro System, USA), equipped with a flat-end cylindrical probe of 2 mm diameter. The test mode was compression. The sample was penetrated with a probe speed of 1 mm/s during the testing penetration and 2 mm/s during the pretest and the posttest. Firmness was defined as the average force when the probe penetrated samples during 3–4 s.

## Determination of Weight Loss

The weight loss ( $X$ ) was determined by weight difference between the initial weight of the mushrooms  $W_0$  and that of mushrooms during storage at time  $t$ ,  $W_t$ , and was calculated by the following expression (3):

$$X = (W_0 - W_t) / W_0 \times 100\% \quad (3)$$

(where  $X$  is the weight loss rate in %,  $W_0$  is mushroom weight during storage in g, and  $W_t$  is mushroom weight during measurement).

## Electrolyte Leakage Rate

The electrolyte leakage rate was evaluated according to Zhao et al. (2009), with minor modifications. Mushrooms were sliced into small blocks of 1 cm and 0.3 cm in diameter and depth, respectively, by a puncher. Surface contamination was removed from each of the six pieces collected by washing with distilled water. Then, the samples were placed into a 100-mL beaker containing 40 mL of deionized water. The conductivity of each suspension ( $P_0$ ) was determined immediately on a DDS307 conductivity meter (Precision Scientific Instruments Ltd., Shanghai, China).  $P_1$  was measured after shaking the suspension at 100 cycles/min for 2 h. The suspension including the sample was subsequently boiled for 10 min and cooled to room temperature, after which deionized water was added to achieve a volume of 40 mL. A final conductivity measurement was performed ( $P_2$ ). The relative electrolyte leakage rate was derived by the following equation (4):

$$\text{Electrolyte leakage (\%)} = 100\% \times (P_1 - P_0) / (P_2 - P_0) \quad (4)$$

## MDA Content

The MDA content was determined with reference to the method described by Ding et al. (2007) with slight modifications. White button mushroom samples (1 g) were homogenized in 7 mL of 10% (w/v) trichloroacetic acid and then centrifuged at 12,000×g for 10 min at 4 °C. Two milliliters of 0.67% (w/v) thiobarbituric acid was mixed with 2 mL of the supernatant. The obtained mixture was incubated in a water bath at 95 °C for 20 min and then centrifuged at 12,000×g for 10 min at 4 °C after cooling to room temperature. The absorbance of the supernatant was measured at 450, 523, and 600 nm, respectively. The MDA content is expressed in micrograms per gram of fresh weight (FW).

## PPO and POD Activities

PPO activity was measured with a polyphenol oxidase activity assay kit (Beijing Solarbio Science & Technology Co., Ltd., Beijing). The measurement method is as follows. First, 40 μL of 50 mmol/L of acetic acid-sodium acetate buffer at pH 5.5 and 40 μL of 50 mmol/L catechol solution were added to the control tubes and assay tubes, respectively. Then, 10 μL of boiling sample has been boiled into the control tube, mixed thoroughly, and centrifuged at

5000g at room temperature for 10 min. The collected 200  $\mu\text{L}$  of the supernatant was added to a 96-well plate, and the absorbance was measured at 410 nm for the measurement and control tubes. PPO activity was calculated based on the fresh weight of the tissue sample in the reaction system (210  $\mu\text{L}$ ); a change in absorbance at 410 nm of 0.005 was defined as unit enzyme activity. PPO activity was expressed in U/g FW.

POD was measured with a peroxidase activity test kit (Beijing Solarbio Science & Technology Co., Ltd., Beijing). First, 50 mmol/L acetate buffer (pH 5.5), 0.5 mol/L  $\text{H}_2\text{O}_2$  solution, and 25 mol/L guaiacol solution were placed at 25 °C for more than 10 min. For the test, 120  $\mu\text{L}$  of 50 mmol/L acetate buffer, 30  $\mu\text{L}$  of 0.5 mol/L  $\text{H}_2\text{O}_2$  solution, 30  $\mu\text{L}$  of 25 mol/L guaiacol solution, 60  $\mu\text{L}$  of distilled water, and 5  $\mu\text{L}$  of sample were added successively into EP tubes and mixed. Then, 200  $\mu\text{L}$  of the reaction was immediately transferred to 96-well plates, and absorbance at 30 s was read at 470 nm (A1). Absorbance A2 was obtained after 1 min 30 s with the activity defined as POD activity per gram of fresh tissue. A change of 0.005 in POD was defined as unit enzyme activity.

### SOD and PAL Activities

SOD activity was measured with a superoxide dismutase activity test kit (Beijing Solarbio Science & Technology Co., Ltd., Beijing). First, crude enzyme extract was performed. Weigh 0.1 g tissue, add 1 mL 0.5 mol/L phosphate buffer (pH 7.8), and mix, 8000g 4 °C centrifugation 10 min; the supernatant is the crude enzyme solution. Then, 45  $\mu\text{L}$  of 100  $\mu\text{mol/L}$  EDTA- $\text{Na}_2$  solution, 100  $\mu\text{L}$  of 750  $\mu\text{mol/L}$  nitroblue tetrazolium solution, 3  $\mu\text{L}$  of xanthine oxidase, 18  $\mu\text{L}$  of the sample, and 35  $\mu\text{L}$  of 130 mmol/L methionine solution were added to 96-well plates. The control tube included 18  $\mu\text{L}$  of double-distilled water instead of the sample. After mixing, the samples were incubated for 30 min at room temperature, and absorbance was read at 560 nm. SOD enzyme activity was calculated based on fresh weight. At a percentage inhibition in the above xanthine oxidase conjugate reaction system of 50%, SOD enzyme activity in the reaction system was defined as unit enzyme activity. SOD activity was expressed in U/g FW.

PAL activity was measured with a phenylalanine ammonia-lyase activity assay kit (Beijing Solarbio Science & Technology Co., Ltd., Beijing) as follows. In a 96-well plate, 5  $\mu\text{L}$  of crude enzyme solution, 145  $\mu\text{L}$  of 50 mmol/L boric acid buffer (pH 8.8), and 40  $\mu\text{L}$  of 20 mmol/L L-phenylalanine solution were added per well. Then, 150  $\mu\text{L}$  of reagent I and 40  $\mu\text{L}$  of 20 mmol/L L-phenylalanine solution were added to the control tube. After mixing, the samples were incubated at 30 °C for 30 min; then, 10  $\mu\text{L}$  of 6 mol/L hydrochloric

acid solution was added to the assay and control tubes, respectively, mixed, and incubated for 10 min. Absorbance was read at 290 nm. PAL activity was calculated based on the fresh weight of the tissue sample in the reaction system; a change in absorbance at 290 nm of 0.05/min was defined as unit enzyme activity. PAL activity was expressed in U/g FW.

### Total Phenolic and Flavonoid Content Assessment

Total phenol and flavonoid content was determined according to Pirie and Mullins (1976) method with modifications. To 1.0 g of fruiting body, 4.0 mL of pre-chilled 1% hydrochloric acid-methanol solution was added, followed by the mixing of the sample with a vortex mixer in an ice bath and transferred to a 20-mL scaled test tube. Then, the mortar was rinsed with 1% hydrochloric acid-methanol solution and transferred to the test tube; after adjustment the volume to 20 mL, the sample was gently mixed, incubated with shaking at 4 °C for 2 h, and centrifuged at 10,000 rpm (4 °C) for 15 min. The supernatant was used for testing. The blank reference calibrator was a 1% hydrochloric acid-methanol solution, and optical densities were measured at 280 nm (total phenolic content) and 325 nm (flavonoid content), respectively, with three repetitions using a TU-1901 spectrophotometer (Beijing Purkinje General Instrument Co. Ltd., Beijing, China). Gallic acid was used as the calibration standard, and the data were expressed as gallic acid equivalents in mg/g FW.

### Total Sugar and Total Soluble Protein Content Assessment

A plant soluble sugar content test kit (Beijing Solarbio Science & Technology Co., Ltd., Beijing) was used for the determination of sugar content.

The total soluble protein was determined with BCA protein assay kit (Beijing Solarbio Science & Technology Co., Ltd., Beijing). 2.5 g of frozen white button mushrooms was homogenized with 5 mL PBS (50 mM, pH 7.0). The obtained mixture was centrifuged at 12,000 $\times$ g for 15 min at 4 °C. Then, the total soluble protein in the supernatant was detected using the above kit.

### Statistical Analysis

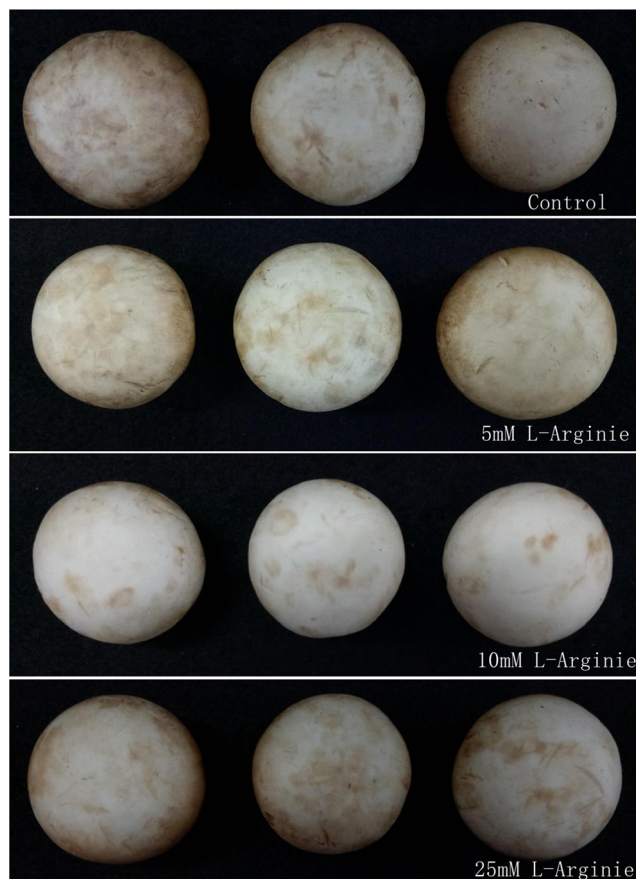
All experiments were performed in triplicate. Data are mean  $\pm$  SD. Origin 8.6 was used for graphs, and the SPSS 9.4 statistical analysis software for one-way analysis of variance and Duncan's multiple comparison test.  $P < 0.05$  indicated statistical significance.

## Results and Discussion

### Effect of L-Arginine Dose on $L^*$ Value and Browning Appearance

Color is an important factor that determines the quality of fresh button mushrooms and also a key indicator affecting sales. The 10 mM L-arginine treatment exhibited a pronounced effect with respect to delaying button mushroom browning, followed by 5 mM L-arginine treatment and 25 mM L-arginine, compared to the control. The visual inspection on day 8 directly reflected these results (Fig. 1).

When the lightness ( $L^*$ ) value of button mushroom is less than 80 and 69, its whiteness is unacceptable from wholesale and consumer perspectives (Meng et al. 2012; Wu et al. 2017). Browning of button mushroom usually occurs at the callus during harvesting. As shown in Table 1, the  $L^*$  value in all mushrooms gradually decreased during storage, with the browning reaction phenomenon continuously increasing.  $L^*$  values in the control and 25 mM L-arginine groups decreased rapidly at 2–8 days, indicating increased degree of browning after 2 days in these groups as well as decreased quality and commercial value. Meanwhile, the  $L^*$  values in the 5 mM and



**Fig. 1** Browning appearance of white button mushrooms treated with control, 5 mM L-arginine, 10 mM L-arginine, and 25 mM L-arginine and stored at 4 °C on the 8th day

10 mM L-arginine groups were significantly higher at 2 and 4 days compared with those of the control and 25 mM L-arginine groups ( $P < 0.05$ ). The 10 mM L-arginine group showed significantly higher  $L^*$  values compared with the 5 mM L-arginine group at days 4, 6, and 8 ( $P < 0.05$ ). Although the  $L^*$  values of the 25 mM L-arginine-treated mushroom were not significantly different from those of control group on days 0, 2, and 4 ( $P > 0.05$ ), they were 4.4% and 4.5% higher than those of the control group on days 6 and 8, respectively ( $P < 0.05$ ). As shown in Table 1, the browning of the control samples developed quickly during the storage, and the  $\Delta E$  value dramatically increased from 0 at day 0 to  $32.17 \pm 1.42$  at day 8. However, the  $\Delta E$  values of the mushrooms treated with 5, 10, and 25 mM L-arginine were still 22.1%, 47.9%, and 3.6% lower than that of the control mushrooms at day 8, respectively. The BI values of the control samples also increased sharply from  $14.91 \pm 1.85$  on day 0 to  $55.45 \pm 0.14$  on day 8. However, the BI of the mushrooms treated with 5, 10, and 25 mM L-arginine were 3.6%, 32%, and 3.4% lower than that of the control mushrooms at day 8, respectively. L-Arginine is the immediate precursor of NO (Gao et al. 2009). Numerous studies have shown that the browning of fresh-cut apple and lettuce and decays caused by *Botrytis cinerea* in tomato fruit were effectively controlled by L-arginine treatment (Wills and Li 2016; Zhang et al. 2017). Zheng et al. (2011) and Zhang et al. (2013) pointed out that pretreatment with L-arginine could elevate NOS activity and NO content in tomato fruit under fungal infection and chilling stress. Castellano et al. (2001) studied the effects of different doses of arginine on NO formation in rat substantia nigra. A persistent and high increase in NO levels was observed when a low dose (8 nM) of L-arginine was co-infused with glutamate. The co-infusion of glutamate with a moderate dose (32 nM) of L-arginine induced drastic and persistent NO production, and this effect was significantly higher than that caused by 8 nM L-arginine. However, very high doses (128 nM) of either L-arginine or D-arginine inhibit NO production. In our experiments, we considered that low concentration of L-arginine (5 mM) induces a slight increase of NO level, which can delay the development of surface browning of white button mushrooms. A suitable concentration of L-arginine (10 mM) induces a higher concentration of NO, which can achieve the best effect of preservation. However, higher concentration (25 mM) of L-arginine might decrease NOS activity and NO level. In addition, Todoroki et al. (1998) found that high concentration of L-arginine suppresses NOS activity and produces ROS in NB9 human neuroblastoma cells. Bayliak et al. (2017) also indicated that the concentration of L-arginine in the diet affects the oxidative stress intensity of adult fruit flies, where high concentrations of L-arginine cause more intensive oxidative stress. Thus, 25 mM L-arginine treatment might cause oxidative stress and increase browning of white button mushrooms, compared with 10 mM L-arginine

**Table 1** The values of lightness ( $L^*$ ), the total color difference ( $\Delta E$ ), and browning index (BI) from button mushrooms treated with control, 5 mM L-arginine, 10 mM L-arginine, and 25 mM L-arginine and stored at 4 °C for 8 days

Days at 4 °C	Control	5 mM L-arginine	10 mM L-arginine	25 mM L-arginine
$L^*$				
0	92.93 ± 0.85Aa	92.80 ± 0.11Aa	92.84 ± 0.66Aa	92.78 ± 0.70Aa
2	88.88 ± 0.21Bb	91.39 ± 0.48Ba	91.52 ± 0.17Ba	89.09 ± 0.36Bb
4	79.29 ± 0.16Cc	84.60 ± 0.27Cb	88.08 ± 0.67Ca	80.94 ± 0.19Cc
6	73.40 ± 0.46Dc	77.23 ± 0.58Db	84.74 ± 0.45 Da	76.64 ± 0.32Db
8	65.42 ± 0.46Ed	74.64 ± 0.37Eb	80.79 ± 0.43Ea	68.39 ± 0.52Ec
$\Delta E$				
0	0	1.27 ± 0.52Dab	1.98 ± 0.66 Da	1.32 ± 0.08Eab
2	7.52 ± 0.44 Da	1.46 ± 0.04Dd	2.81 ± 1.08Dc	5.64 ± 0.60Db
4	16.85 ± 1.40Ca	10.57 ± 0.10Cb	8.58 ± 1.55Cb	15.45 ± 0.70Ca
6	25.26 ± 0.27Baa	17.32 ± 0.82Bb	13.86 ± 0.92Bc	19.58 ± 3.05Bab
8	32.17 ± 1.42Aa	25.09 ± 0.78Ab	16.76 ± 1.13Ac	31.04 ± 1.39Aa
BI				
0	14.91 ± 1.85 Da	14.51 ± 2.01Ca	13.41 ± 1.07 Da	15.03 ± 0.14Ca
2	25.34 ± 0.73Ca	15.84 ± 0.62Cd	18.57 ± 1.91Cc	21.89 ± 5.86Cb
4	37.15 ± 3.75Ba	27.10 ± 1.73Bb	26.88 ± 2.56Bb	34.28 ± 3.57Ba
6	51.65 ± 0.21Aa	30.85 ± 1.62Bb	35.23 ± 1.64Ab	47.61 ± 7.84Bb
8	55.45 ± 0.14Aab	53.42 ± 5.86Ab	37.71 ± 3.57Ac	53.59 ± 4.60Aa

Different capital letters (A–E) within the same column represent significant difference ( $P < 0.05$ ); different small letters (a–d) within the same line represent significant difference ( $P < 0.05$ ). Data are presented as means ± standard deviation,  $n = 3$

treatment. Based on our experimental results, we found that white button mushrooms treated with 10 mM L-arginine showed significantly lower browning index than the other groups, so 10 mM L-arginine was selected for further analysis.

### Effects of 10 mM L-Arginine on Ultrastructure

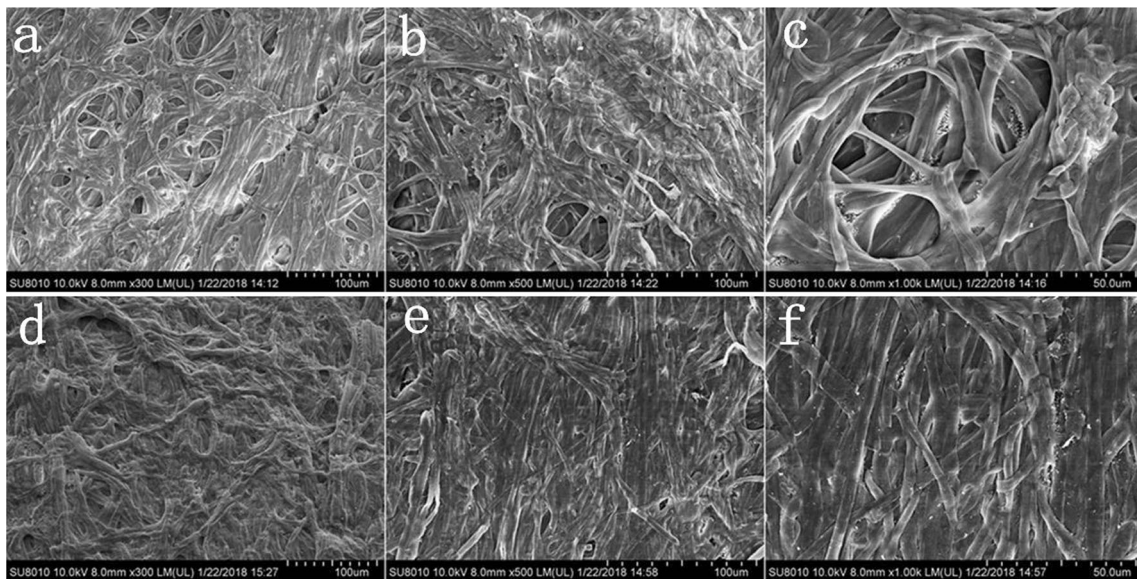
Scanning electron microscopy was carried out to investigate the effects of L-arginine on microstructure of white button mushrooms. It was used to observe the cell structure changes of white button mushrooms in the control group and 10 mM L-arginine treatment group on day 8. As shown in Fig. 2a, b, the 300 and 500 magnification views showed that the internal network structure of the white mushroom in the control group was severely damaged, and the cells were severely damaged and disordered. As shown in Fig. 2c, the 1000 magnification views showed that the surface of the white button mushroom cells in the control group was damaged and cracked. As shown in Fig. 2d–f, the internal cells of the white button mushroom in the 10 mM L-arginine treatment group were closely connected to form a dense and firm network structure. At 1000 magnification, the cells of the white button mushroom in the 10 mM L-arginine treatment group had intact appearance without any damage and no debris. Compared with the 10 mM L-arginine-treated group, the control group of mushrooms had severe cell shrinkage and was no longer full and smooth. The cell wall is degraded and the integrity of

the cell is destroyed. Results obtained in the present study were in agreement with those of Quiles et al. (2010) who studied the effect of calcium propionate on the microstructure of fresh-cut apples using SEM. After 1 week of storage, they observed that the cell walls of samples treated with calcium propionate maintained the integrity and uniformity as compared to untreated fresh-cut apples.

### Effects of 10 mM L-Arginine on Weight Loss, Firmness, Electrolyte Leakage, and MDA Content

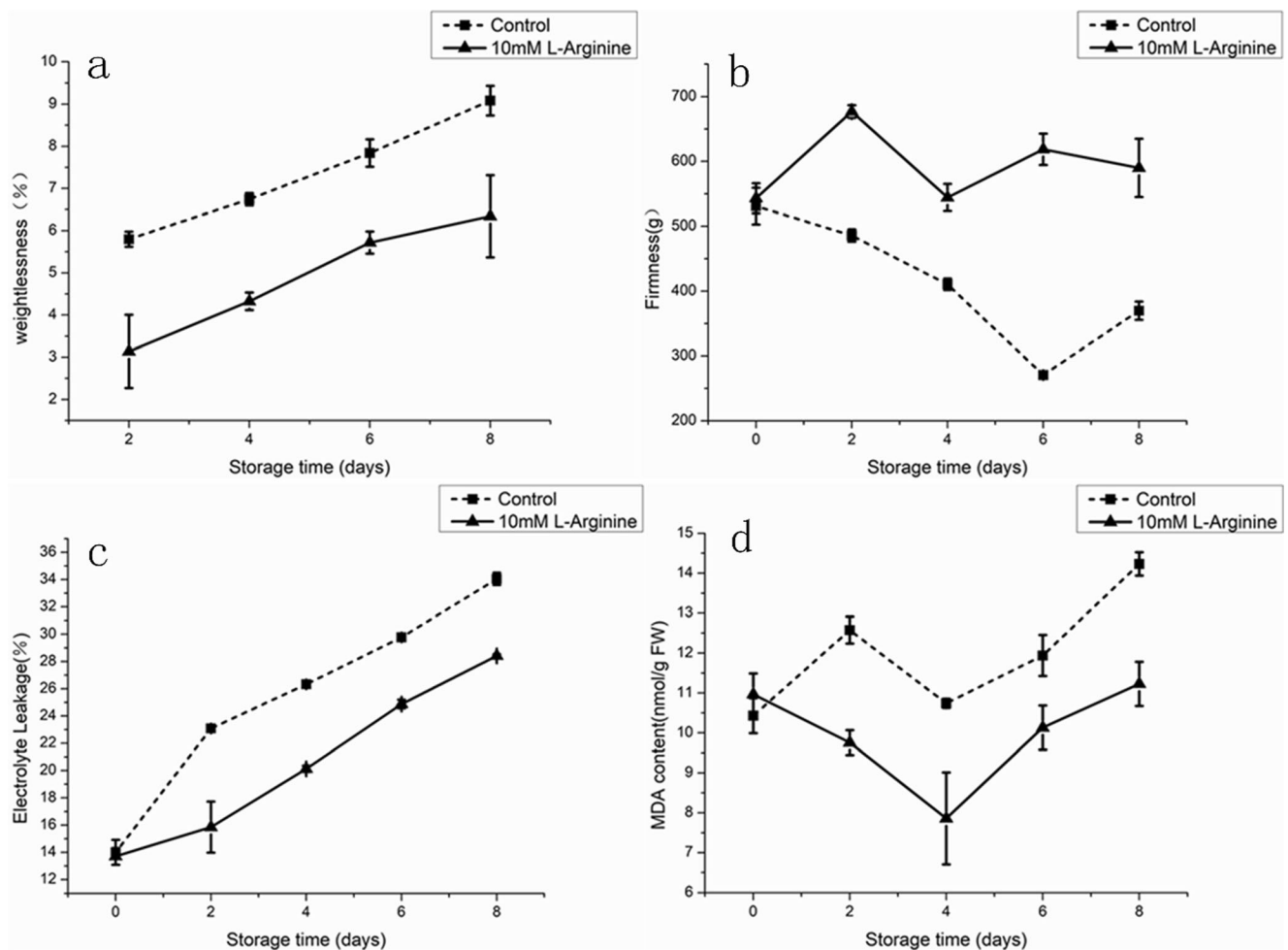
As shown in Fig. 3a, weightlessness rate increased with storage time in both treatments, suggesting that dehydration is an important process in mushroom quality loss during postharvest storage. During the storage period, weightlessness rate of the 10 mM L-arginine treatment group was significantly lower than that of the control group. The reason for low weightlessness rate in 10 mM L-arginine-treated button mushroom is that L-arginine treatment protects the button mushroom epidermis and prevents rapid dehydration of the surface. Our results are consistent with findings in L-arginine-treated green asparagus (Gong et al. 2017).

The texture of the button mushroom is often the first of many quality attributes assessed by the consumer and therefore extremely important for overall product acceptance (Jiang et al. 2011). The experimental results in Fig. 3b show that the firmness of mushroom in both treatments was reduced



**Fig. 2** Effects of 10 mM L-arginine on the ultrastructure of white button mushrooms during storage at 4 °C at 8 days. Control samples were magnified 300 (a), 500 (b), and 1000 (c) times, respectively, by electron

microscopy; samples treated with 10 mM L-arginine were magnified 300 (d), 500 (e), and 1000 (f) times, respectively, by electron



**Fig. 3** Effects of 10 mM L-arginine on weight loss rate (a), firmness (b), electrolyte leakage rate (c), and MDA content (d) in white button mushrooms during storage at 4 °C for 8 days. Data are mean ± SD, *n* = 3

throughout the storage period ( $P < 0.05$ ). However, the firmness diminution in 10 mM L-arginine treatment group was significantly lower than in the controls. Softening can occur due to cell wall degradation in postharvest mushrooms by bacterial enzymes and endogenous autolysins (Zivanovic et al. 2000). The experimental results in Fig. 3b show that the firmness of mushroom in both treatments was reduced throughout the storage period ( $P < 0.05$ ). However, the firmness diminution in 10 mM L-arginine treatment group was significantly lower than in the controls. This finding indicates that treatment with 10 mM L-arginine during storage can effectively control tissue softening in the white button mushroom and maintained acceptable texture.

Electrolyte leakage is closely related to tissue membrane integrity. When cell membrane integrity is impaired, the selective permeability function of the cell membrane is weakened, and intracellular materials can pass freely, with the relative tissue electrolyte leakage increasing accordingly (Wang et al. 2016). It is shown in Fig. 3c that the electrolyte leakage increased with storage time in the two groups, which indicated that membrane systems became more vulnerable to leakage. However, throughout storage, 10 mM L-arginine slowed the increase rate in electrolyte leakage compared with control group. Particularly, the electrolyte leakage in control group increased to 23.0% on day 2, while only 15.8% of that was observed in 10 mM L-arginine treatment group. At the end of storage, electrolyte leakages in control group and 10 mM L-arginine treatment group were 34.0% and 28.4%, respectively. L-Arginine treatment can maintain the structural integrity of the cell membrane and delay the button mushrooms' aging process. The results indicate that L-arginine treatment is an effective method to protect the cell membrane of button mushrooms to reduce the electrolyte leakage rate. Our results are consistent with findings in arginine-treated cherry tomato fruit (Zhang et al. 2010) and L-arginine-treated tangerine orange (Li et al. 2015).

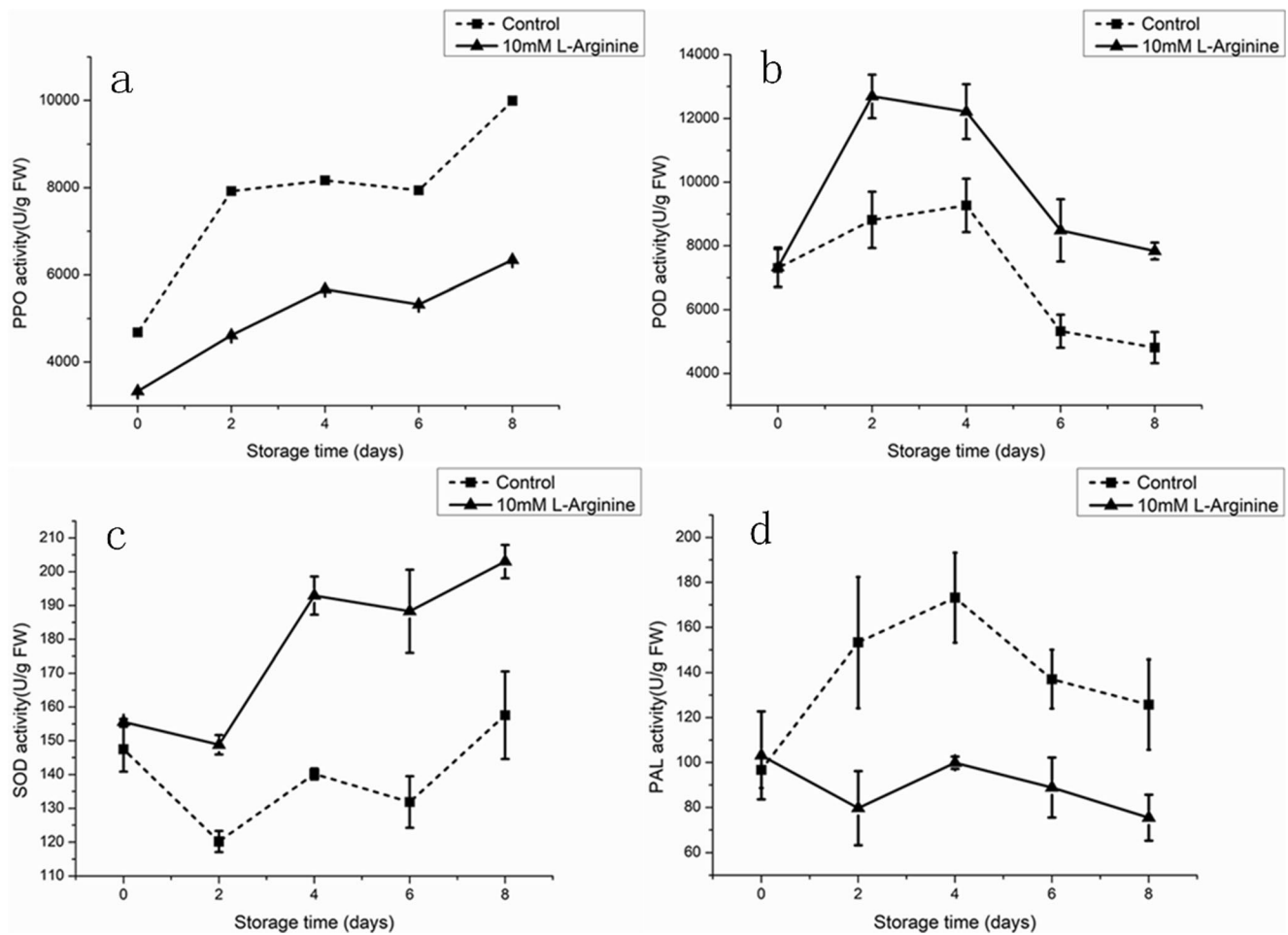
During aging of fruits and vegetables, membrane lipid peroxidation increases, and MDA is an important product of membrane lipid peroxidation (Meng 2014). MDA content is commonly used as an indicator of lipid peroxidation, resulting from oxidative stress and reflecting damage in membrane structure and the senescence level of tissue (Nejadsadeghi et al. 2015); the MDA content increased within senescence accompanied with a rapid increase in membrane permeability. In Fig. 3d, the variation of MDA content for control group and 10 mM L-arginine treatment group had the same trend. However, the MDA content of the button mushroom in the 10 mM L-arginine treatment group was significantly higher than that in the control group during storage ( $P < 0.05$ ). This concurred with the findings reported by Wang et al. (2017) and Gong et al. (2017) that the MDA content of the samples of the L-arginine-treated group was significantly lower than that of the control group.

### Effects of 10 mM L-Arginine on PPO, POD, SOD, and PAL Activities

PPO is widely distributed in nature and has been detected in most fruits and vegetables. PPO enzyme promotes the synthesis of melanin in the browning of mushrooms, other vegetables, and fruits. Therefore, controlling the activity of PPO enzyme plays an important role in preservation (Meng et al. 2012). The change trend of PPO during storage is shown in Fig. 4a. During the storage period, PPO activities in both groups showed an upward trend. PPO activities were significantly higher at 2, 4, 6, and 8 days in the control group compared with the 10 mM L-arginine treatment group ( $P < 0.05$ ). During the storage period, PPO activities in both groups showed an upward trend. PPO activities were significantly higher at 2, 4, 6, and 8 days in the control group compared with the 10 mM L-arginine treatment group ( $P < 0.05$ ). This indicates that postharvest treatment with 10 mM L-arginine may reduce PPO activity in button mushrooms. Khalil et al. (2009) found that exogenous application of arginine significantly decreased PPO activity in wheat. The effect of 10 mM L-arginine treatment on PPO activity was consistent with the change in browning degree, indicating that the decrease of PPO activity by arginine treatment may be an important cause of reduced browning in button mushrooms.

POD, as one of the enzymes of browning of plants (Terefe et al. 2014), could also catalyze the synthesis of lignin in the last step for the polymerization (Chen et al. 2002). Aquino-Bolaños and Mercado-Silva (2004) found that the browning of cut jicama is related to the process of lignification in which the POD enzyme plays an important role. PPO and POD have synergistic effects on the formation of the brown polymers. It is generally accepted that browning is due to the oxidation of phenolics by PPO and POD, resulting in the formation of brown-colored substances. However, Terefe et al. (2014) also showed that POD could scavenge ROS and was one of the protective systems of plants. Some experts (Safizadeh et al. 2007; Imahori et al. 2008; Laura et al. 2010) also agreed that the coordinated action of antioxidant enzymes such as SOD, and POD, which are very important for scavenging active oxygen species (AOS) to protect cell membranes, is thought to be a major mechanism of resistance to browning. As shown in Fig. 4b, the POD activity of the samples in both groups increased and peaked and then decreased, whereas the POD activity in the samples treated with 10 mM L-arginine was significantly higher than that in the control group. The results showed that treatment with 10 mM L-arginine promoted the activity of POD, which may be one of the reasons for inhibiting browning of mushrooms. Therefore, in our study, it seems evident that POD is more suitable to act as a peroxide scavenger instead of a browning promoter and it is consistent with Gao et al. (2017).





**Fig. 4** Effects of 10 mM L-arginine on PPO (a), POD (b), SOD (c), and PAL (d) activities in white button mushrooms during storage at 4 °C for 8 days. Data are mean  $\pm$  SD,  $n = 3$

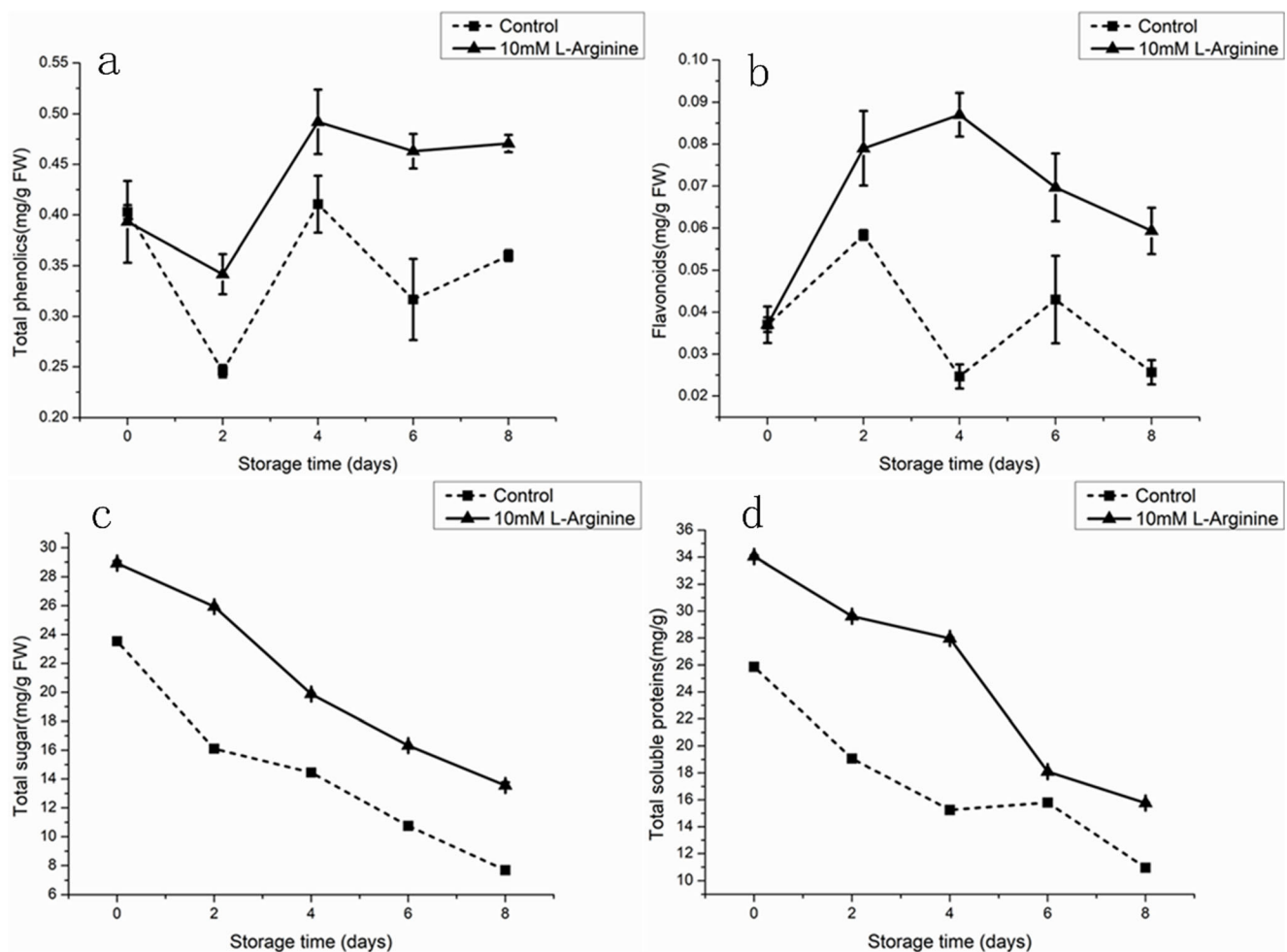
SOD plays an important role in the antioxidant mechanism, which may be the first step to prevent oxidative damage (Khan et al. 2014). Maintaining high SOD activity might be a more effective factor in the endurance of unfavorable environmental conditions by button mushrooms. As shown in Fig. 4c, the SOD activity of the 10 mM L-arginine-treated group on the 2nd, 4th, 6th, and 8th day was 23.8%, 36.6%, 42.8%, and 28.8% higher than that of the untreated control group, respectively. This indicates that L-arginine treatment could enhance SOD activity to some extent to suppress the button mushroom senescence induced by unfavorable environmental conditions which is consistent with the conclusion of Zhang et al. (2010) that arginine treatment significantly promoted the SOD activity.

PAL, a key enzyme in the synthesis of phenolic compounds and lignin, catalyzes the deamination of L-phenylalanine to produce ammonia and trans-cinnamic acid, with phenolic compounds produced during the process (Benoit et al. 2000). As shown in Fig. 4d, the PAL activity of the 10 mM L-arginine treatment group was significantly lower than that of the control group during storage ( $P < 0.05$ ). This indicates

that the PAL enzyme activity is reduced by 10 mM L-arginine treatment, which delays the lignification process of the mushroom and plays a certain role in preservation. The decreased PAL activity in turn inhibits the formation of lignin, leading to the accumulation of total phenol formation. This concurred with the findings reported by Wang et al. (2017) and Gong et al. (2017) that the activity of PAL enzyme could be significantly reduced by L-arginine treatment.

#### Effects of 10 mM L-Arginine on Total Phenolic, Flavonoid, Total Sugar, and Total Soluble Protein Contents

Phenolic compounds are major antioxidant components in mushrooms, with widely reported beneficial effects on health maintenance as well as in the prevention of cancer and cardiovascular diseases (Gao et al. 2014). In Fig. 5a, compared to the control, total phenolic contents from the samples treated with 10 mM L-arginine showed significantly higher levels throughout the storage. The total phenolic content of 10 mM L-arginine was 30.5% higher than that in the control mushroom



**Fig. 5** Effects of 10 mM L-arginine on total phenolic (a), flavonoid (b), total sugar (c), and total soluble protein (d) contents in white button mushrooms during storage at 4 °C for 8 days. Data are mean  $\pm$  SD,  $n = 3$

at the last day of the storage ( $P < 0.05$ ). The increase in total phenolic content may be caused by L-arginine inhibiting PPO activity. Our results are consistent with the conclusions of Zheng et al. (2011) and Zhang et al. (2010).

Flavonoids are also a class of antibacterial and antioxidant substances. As shown in Fig. 5b, the total flavonoid content of button mushrooms in 10 mM L-arginine treatment group was significantly increased compared to the untreated control group during storage ( $P < 0.05$ ). The total flavonoid content in 10 mM L-arginine treatment group reached peaked 2.62 times higher than that in control mushrooms on day 4. That suggests that L-arginine may improve the antioxidant status of the mushroom by positively affecting phenolic metabolism. Similar conclusion was obtained about tomato fruits treated by L-arginine reported by Zheng et al. (2011).

### Total Sugar and Soluble Protein Contents

Total soluble sugar concentrations in harvested plant products are also considered important indicators of postharvest deterioration (Meng et al. 2012). Soluble sugar content is an

important indicator of the quality of fruits and vegetables post-harvest, and the changes in its content may reflect physiological and biochemical changes as well as postharvest decay. Total sugar content of button mushrooms in both treatment groups showed an overall decreasing trend with storage time, as shown in Fig. 5c. Ten-millimolar L-arginine treatment significantly slowed the decline rate in total sugar levels compared with that in control group ( $P < 0.05$ ). This suggests that L-arginine treatment can slow down the reduction of soluble sugar.

Total soluble protein content exhibited a continuous decrease during storage in button mushrooms, as a key indicator of tissue senescence (Wu et al. 2017; Cui 2010). Protein synthesis decreases with age. Decreased soluble protein content after harvest is considered an important indicator of tissue aging (Meng 2014). As shown in Fig. 5d, total soluble protein contents from the samples treated with 10 mM L-arginine presented a higher level compared to the control. This indicates that L-arginine treatment can slow down soluble protein loss in button mushrooms to some extent, which is consistent with the conclusion of Wang et al. (2017) that L-arginine treatment can slow down the degradation of soluble proteins.

## Conclusions

In our current study, three concentrations of L-arginine (5 mM/L, 10 mM/L, and 25 mM/L) were used to assess post-harvest preservation of button mushrooms. Among them, 10 mM L-arginine treatment can more effectively slow down browning of button mushrooms and maintain the surface color of mushrooms. Therefore, this concentration was used for further analysis. Compared to the control, 10 mM L-arginine treatment can effectively delay mushroom enzymatic browning resulting from changes in PPO activities. Meanwhile, 10 mM L-arginine treatment can not only improve mushroom antioxidant capacity by promoting the POD and SOD activity to some extent, but also delay the lignification process of the mushroom by inhibiting the PAL activity. The data shows that 10 mM L-arginine treatment keeps the firmness of the mushroom. This may be related to inhibition of electrolyte leakage rate and MDA accumulation. The 10 mM L-arginine treatment also slowed down the decrease of the total phenolics, total flavonoids, total sugar, and total soluble protein content. Therefore, application of proper L-arginine could be an important strategy to extend postharvest shelf life and maintain the quality of button mushrooms' fruit bodies.

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