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Effect of gallic acid grafted chitosan film packaging on the postharvest quality of white button mushroom (*Agaricus bisporus*)

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ABSTRACT

In this study, gallic acid grafted chitosan (GA-g-CS) film was used as a novel active packaging material for the preservation of *Agaricus bisporus*. Effect of GA-g-CS film packaging on the physico-chemical parameters and enzyme activities of *A. bisporus* during cold storage was investigated. As compared to mushrooms packaged with chitosan (CS) film and commercially used polyethylene (PE) film, mushrooms packaged with GA-g-CS film showed significantly lower respiration rate, browning degree, malondialdehyde content, electrolyte leakage rate, superoxide anion production rate and hydrogen peroxide content. Among all the treatment groups, mushrooms packaged with GA-g-CS film exhibited the highest superoxide dismutase and catalase activities as well as total phenolic content, however, the lowest polyphenol oxidase activity. Our results suggested GA-g-CS film packaging could increase the antioxidant status of *A. bisporus*, which in turn maintained the postharvest quality of mushrooms.

1. Introduction

Agaricus bisporus, also known as white button mushroom, is one of the most widely cultivated mushrooms around the world. China is the largest *A. bisporus* producer in the world and its export value reached $335 million in 2011 (Xu et al., 2016). *A. bisporus* is appreciated as a health food with abundant amounts of amino acids, vitamins, minerals, polyphenols, proteins and dietary fibers (Kalač, 2013; Muszyńska et al., 2017; Rathore et al., 2017). Several studies have revealed that *A. bisporus* possesses many valuable biological properties, such as antioxidant, antibacterial, anti-inflammatory, antitumor, and immunomodulatory activities (Liu et al., 2013a; Muszyńska et al., 2018; Ruthes et al., 2016). However, because of its high respiration rate and vulnerability to browning and microbial attack, *A. bisporus* is highly perishable after harvest and usually has a short shelf life (typically 1–3 d) at room temperature (Wang et al., 2017). The short shelf life of *A. bisporus* has become the biggest limitation of its industrial development. Therefore, it is essential to apply proper preservation methods to extend the postharvest storage period and preserve the quality of *A. bisporus*.

In recent years, various different preservation approaches have been developed for *A. bisporus*, such as physical treatments (*e.g.* coating (Zalewska et al., 2018), irradiation (Lu et al., 2016; Wu et al., 2016; Yurttas et al., 2014), mild heat (Zhang et al., 2017), modified atmosphere packaging (Lin et al., 2017; Oz et al., 2015), ozone (Akata et al., 2015), pulsed light (Kalaras et al., 2011; Oms-Oliu et al., 2010), refrigeration, ultrasound and high pressure argon (Lagnika et al., 2013), and washing (Aday, 2016; Cliffe-Byrnes and O'Beirne, 2008)), chemical treatments (*e.g.* brassinolide (Ding et al., 2016), essential oil (Gao et al., 2014; Nasiri et al., 2017), glycine betaine (Wang et al., 2015), 4-methoxy cinnamic acid (Hu et al., 2015), methyl jasmonate (Meng et al., 2017), plasma activated water (Xu et al., 2016), and salicylic acid (Dokhanieh and Aghdam, 2016)) and combinations thereof (Jiang, 2013; Khan et al., 2014; Simón et al., 2010). However, these methods have some drawbacks, such as potentially toxicity, high cost and impairing the nutrition, color, texture and flavor of mushrooms (Xu et al., 2016; Zhang et al., 2017).

Active packaging is an innovative technology to extend the shelf life of the packaged food. It is based on the incorporation of antimicrobial, antioxidant and carbon dioxide emitting/generating agents in the package (Fang et al., 2017). Active packaging may act either by progressively releasing active agents to the surrounding atmosphere or by absorbing the compounds (*e.g.* oxygen or free radicals) that deteriorate food. In addition, active packaging does not require direct contact with the foodstuff to exhibit antimicrobial and/or antioxidant properties (Wrona et al., 2015). However, only a few active packaging materials have been developed for the preservation of *A. bisporus* up to now (Qin et al., 2015; Shin et al., 2013; Wrona et al., 2015).

Chitosan (CS) is a cationic polysaccharide obtained by the

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deacetylation of chitin, which is the major component of the exoskeleton of crustaceans and the second most abundant polysaccharide in nature. Due to its non-toxic, biodegradable, biocompatible, intrinsic antioxidant and film forming properties, CS is considered as an ideal component for edible films. CS film generally possesses good mechanical properties and a selective permeability to gases (Elsabee and Abdou, 2013). Moreover, the incorporation of antimicrobial and/or antioxidant compounds into CS film can further improve the physical, mechanical and biological properties of the film (Cheng et al., 2015; Liu et al., 2017b, 2017c). Thus, CS based films are promising active packaging materials for mushrooms.

In our previous study, five kinds of hydroxybenzoic acids including gallic acid, gentisic acid, protocatechuic acid, syringic acid and vanillic acid were individually grafted onto CS by carbodiimide mediated coupling reaction. The synthesized hydroxybenzoic acid grafted CS (hydroxybenzoic acid-g-CS) were further developed into films by the casting method. Results showed that gallic acid grafted CS (GA-g-CS) film exhibited the best physical, mechanical and antioxidant properties, which could be used as a promising food active packaging material (Liu et al., 2017a). In this study, the effect of GA-g-CS film packaging on the postharvest quality of *A. bisporus* was evaluated for the first time. The preservation effect of GA-g-CS film on *A. bisporus* was also compared with that of CS film and commercially used polyethylene (PE) film.

2. Materials and methods

2.1. Materials and reagents

White button mushrooms (*A. bisporus*) used in this study were harvested from Jiangsu Shangpin Modern Ecological Agriculture Co. Ltd. (Yangzhou, China) and were immediately transported to the laboratory within 1 h under refrigerated conditions. Mushrooms were screened for their uniformity in size (cap size of 4–5 cm in diameter) and color, and absence of mechanical damage and disease.

Chitosan with the average molecular weight of 1.5×10^5 Da and deacetylated degree of 90% was purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China). GA was purchased from Sigma Chemical Co. (St. Louis, MO, USA). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Maclin Biotechemical Co. Ltd. (Shanghai, China). All other reagents were of analytical grade.

2.2. Preparation of GA-g-CS film and mushroom treatments

The preparation of GA-g-CS film was carried out according to our previously established method (Liu et al., 2017a). First, GA-g-CS was synthesized by grafting GA onto CS *via* an EDC/NHS coupling reaction. Then, 1.7 g of GA-g-CS was completely dissolved in 170 mL of 1% (v/v) acetic acid solution overnight to obtain film forming solution. Afterwards, 1 mL of glycerol was added into the solution as a plasticizer and the obtained mixture was ultrasonically treated to remove air bubbles. The resultant film forming solution (150 mL) was cast onto a self-designed Plexiglas plate (24 cm × 24 cm) and dried at 30 °C with 50% relative humidity for 48 h in a humidity chamber. Finally, the dried GA-g-CS film (30 μm of thickness, O_2 permeability of 1.0×10^{-11} mL m^{-1} s^{-1} Pa^{-1}, CO_2 permeability of $1.6 \times 10^{-1°}$ mL m^{-1} s^{-1} Pa^{-1}, and water vapor permeability of 1.1×10^{-10} g m^{-1} s^{-1} Pa^{-1} at 25 °C and 90% relative humidity) was peeled from the plate and stored at 25 °C in desiccators containing saturated $Ca(NO_3)_2$ solution (50 ± 2% relative humidity) for at least 72 h before use. CS film (30 μm of thickness, O_2 permeability of 1.2×10^{-11} mL m^{-1} s^{-1} Pa^{-1}, CO_2 permeability of $1.7 \times 10^{-1°}$ mL m^{-1} s^{-1} Pa^{-1}, and water vapor permeability of 1.8×10^{-10} g m^{-1} s^{-1} Pa^{-1} at 25 °C and 90% relative humidity) was prepared in the same way without grafting GA. At 5 g L^{-1} of film equivalent, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of CS and GA-g-CS films was 16.05% and 68.47%, respectively. The low density polyethylene (PE) film (20 μm of thickness, O_2 permeability of 2.6×10^{-11} mL m^{-1} s^{-1} Pa^{-1}, CO_2 permeability of 8.1×10^{-11} mL m^{-1} s^{-1} Pa^{-1}, and water vapor permeability of 1.2×10^{-12} g m^{-1} s^{-1} Pa^{-1} at 25 °C and 90% relative humidity) purchased from Jieda Chemical Plastic Co. Ltd. (Suzhou, China) was also used in this study.

Mushrooms were randomly divided into four different treatment groups: (1) control (without any film packaging), (2) PE film packaging, (3) CS film packaging and (4) GA-g-CS film packaging. For each treatment group, mushrooms were placed in a plastic box (17.5 cm × 12 cm × 7 cm) and were sealed with different films by a heat sealer. All mushrooms were then stored at 4 ± 1 °C with 90% relative humidity for 15 d. Fifteen replicates were included in each treatment group, and three replicates were randomly selected from each treatment group and analyzed every 3 d.

2.3. Weight loss

Weight loss was determined by weighing mushrooms every 3 d throughout the storage period. Results were expressed as the percentage of weight loss with respect to the initial weight.

2.4. Firmness

The firmness of mushroom cap was measured by a TMS-PRO texture analyzer (Food Technology Co., USA) equipped with a 6 mm diameter cylindrical probe. Samples were penetrated 5 mm in depth with a speed of 2.0 mm s^{-1}. Firmness was defined as the maximum force from the force *vs* time curves.

2.5. Respiration rate

The respiration rate of mushroom was measured according to the method of Li et al. (2006) with some modifications. Firstly, mushroom was taken out from each package and exposed to ambient condition for 1 h to allow the CO_2 accumulated in the tissue to diffuse into the air. Afterwards, mushroom was put into an air-tight jar with 10 mL of 0.4 mol L^{-1}NaOH solution in a Petri dish. Two drops of phenolphthalein were added after 30 min and the obtained mixture was titrated with 0.2 mol L^{-1}oxalic acid. Respiration rate of mushroom was expressed as CO_2 production rate using following equation:

$$\text{Respiration rate (mg CO}_2\text{ kg}^{-1}\text{ s}^{-1}) = \frac{(V_1 - V_2) \times c \times 44}{W \times t} \quad (1)$$

where V_1 is the volume of oxalic acid control (mL); V_2 is the volume of oxalic acid of sample (mL); c is the concentration of oxalic acid (mol L^{-1}); 44 is the molecular weight of CO_2 (g mol^{-1}); W is the sample weight (kg); and t is time (s).

2.6. Headspace gas composition

The concentrations of O_2 and CO_2 in the package headspace were detected by an SCY-2 A O_2/CO_2 analyzer (Xinrui Instrument Co. Ltd., Shanghai, China). Gas sample was taken from the package with a 20 mL syringe.

2.7. Browning degree

The browning degree of mushroom was measured according to the method of Liu et al. (2013b). Mushroom cap (5 g) was ground with 20 mL of 0.2 mol L^{-1} sodium phosphate buffer (pH 6.8) containing 2.5% (w/v) polyvinylpolypyrrolidone (PVPP) and 0.15 mol L^{-1} NaCl in an ice bath. The mixture was centrifuged at 10,000 × *g* and 4 °C for 10 min. The absorbance of supernatant was measured at 420 nm and was used to reflect browning degree.

2.8. Malondialdehyde (MDA) content

MDA content was measured according to the method of Liu and Wang (2012) with some modifications. Mushroom sample (1 g) was ground with 5 mL of 5% (w/v) trichloroacetic acid (TCA) in an ice bath and then centrifuged at 10,000 × g and 4 °C for 20 min. Supernatant (1 mL) was mixed with 1 mL of 10% (w/v) TCA containing 0.67% (w/v) thiobarbituric acid (TBA) and incubated in boiling water for 20 min. The mixture was cooled quickly and centrifuged at 10,000 × g for 10 min. The absorbance of supernatant was measured at 532 nm, 600 nm and 450 nm, respectively. The MDA content in mushroom was calculated and expressed in μmol kg^{-1} fresh weight.

2.9. Membrane permeability

Membrane permeability of mushroom was measured by the electrolyte leakage rate (Kaya et al., 2002). Mushroom sample (2 g) was washed with deionized water for three times, dried with filter paper and suspended in 25 mL of deionized water. The initial electrical conductivity of solution (C_1) was immediately recorded by a DDSJ-308 A electrical conductivity meter (Leici Instrument Co., Shanghai, China). Then, mushroom stipe was boiled for 10 min, cooled to room temperature, restored to original volume and its final electrical conductivity (C_2) was recorded. The electrolyte leakage rate was calculated by the following formula:

$$\text{Leakage rate (\%)} = C_1/C_2 \times 100 \quad (2)$$

2.10. Superoxide radical ($O2^{\cdot}$ -) production rate

$O_2^{\cdot -}$ production rate was determined according to the method of Liu and Wang (2012) with some modifications. Mushroom sample (2 g) was homogenized with 5 mL of 50 mmol L^{-1} phosphate buffer (pH 7.8) and then centrifuged at 10,000 × g and 4 °C for 15 min. Supernatant (0.5 mL) was mixed with 0.5 mL of 50 mmol L^{-1} phosphate buffer (pH 7.8) and 1 mL of 1 mmol L^{-1} of hydroxylamine hydrochloride. After incubation at room temperature for 1 h, 1 mL of 50 mmol L^{-1} *p*-aminophenylsulfonic acid and 1 mL of 7 mmol L^{-1} *α*-naphthylamine were added, and the mixture was incubated at room temperature for 20 min. The absorbance of the reaction mixture was immediately measured at 530 nm. $O_2^{\cdot -}$ production rate was calculated and expressed as mmol s^{-1} kg^{-1} fresh weight.

2.11. Hydrogen peroxide (H_2O_2) content

H_2O_2 content was determined according to the method of Khan et al. (2014) with some modifications. Mushroom sample (1 g) was homogenized with 5 mL of 0.1% (w/v) TCA in an ice bath and then centrifuged at 10,000 × g and 4 °C for 20 min. Supernatant (1 mL) was mixed with 1 mL of 0.1 mol L^{-1} phosphate buffer (pH 7.0) and 1 mL of 1 mol L^{-1} potassium iodide. The reaction mixture was allowed to stand at room temperature in the dark for 1 h. The absorbance of reaction mixture was measured at 390 nm and calibrated to a standard curve generated using known concentration of H_2O_2. H_2O_2 content was calculated and expressed as mmol kg^{-1} fresh weight.

2.12. Enzyme activities

To determine enzyme activities, mushroom tissue (2 g) was ground with 5 mL of 50 mmol L^{-1} phosphate buffer (pH 7.8) containing 1% (w/v) PVPP, 1 μmol L^{-1} EDTA and 0.3% (w/v) Tween-20 in an ice bath. The homogenate was centrifuged at 10,000 × g and 4 °C for 20 min. The obtained supernatant was used as the crude enzyme extract.

Superoxide dismutase (SOD) activity was measured according to the method of Li et al. (2013) with slight modifications. First, 0.1 mL of enzyme extract was mixed with 2.4 mL of 50 mmol L^{-1} phosphate buffer (pH 7.8), 0.2 mL of 130 mmol L^{-1} methionine, 0.2 mL of 750 μmol L^{-1} NBT, 0.2 mL of 100 μmol L^{-1} EDTA and 0.1 mL of 20 μmol L^{-1} riboflavin. The reaction mixture was then placed under 4000 Lx irradiance at 25 °C for 60 min. The absorbance of reaction mixture was determined at 560 nm. One unit (U) of SOD was defined as the amount of enzyme required to inhibit the initial rate of NBT photoreduction by 50%. SOD activity was expressed in U kg^{-1} fresh weight.

Catalase (CAT) activity was measured according to the method of Aebi (1984) with some modifications. First, 0.1 mL of enzyme extract was mixed with 1 mL of 0.1 mol L^{-1} H_2O_2 and 2.5 mL of 0.05 mol L^{-1} phosphate buffer (pH 7.8). The absorbance of the reaction mixture was then determined at 240 nm at an interval of 30 s for 3 min. One U of CAT was defined as the amount of enzyme that decreased in absorbance of 0.01 at 240 nm per minute. CAT activity was expressed in U kg^{-1} fresh weight.

Polyphenol oxidase (PPO) activity was determined by the method of Galeazzi et al. (1981) with some modifications. First, 0.1 mL of enzyme extract was mixed with 4 mL of 0.1 mol L^{-1} phosphate buffer (pH 7.0) and 1 mL of 50 mmol L^{-1} catechol. The absorbance of reaction mixture was then recorded at 420 nm at an interval of 30 s for 3 min. One Unit of PPO was defined as the amount of enzyme that increased in absorbance of 0.01 at 420 nm per minute. PPO activity was expressed in U kg^{-1} fresh weight.

2.13. Total phenolic content

The total phenolic content was measured by the method of Dokhanieh and Aghdam (2016). Briefly, 2 g of mushroom sample was homogenized with 5 mL of methanol. The obtained mixture was centrifuged at 10,000 × g and 4 °C for 25 min. Then, 1 mL of supernatant was mixed with 1 mL of Folin-Ciocalteu reagent and allowed to react at room temperature for 5 min in the dark. Then, 5 mL of saturated Na_2CO_3 solution was added and the mixture was allowed to stand for 2 h. Finally, the absorbance of the reaction mixture was recorded at 760 nm. GA was used to calculate the standard curve and the results were expressed as GA equivalents on a fresh weight basis (g kg^{-1}).

2.14. Statistical analysis

Data were expressed as mean ± standard deviation. Data were analyzed by one-way analysis of variance (ANOVA) followed by the Duncan's multiple-range comparisons. Difference was considered to be statistically significant if $p < 0.05$. All statistical analyses were carried out by using SPSS software package (Version 13.0, SPSS, Chicago, IL).

3. Results and discussion

3.1. Weight loss

Effect of different packages on the weight loss of mushrooms during storage is shown in Fig. 1A. Weight loss of mushrooms in the control, CS film and GA-g-CS film treatment groups increased with the duration of storage. The weight loss of mushrooms is due to the fact that the thin epidermal structure of mushroom cannot prevent its quick superficial dehydration and high transpiration rate (Guillaume et al., 2010). Notably, mushrooms packaged with PE film exhibited the lowest weight loss ($p < 0.05$). The low weight loss of mushrooms packaged with PE film was probably attributed to the low water vapor permeability of PE film (Gholami et al., 2017). So, condensed water could be observed in the inner surface of PE film. Similar phenomena were also observed in mushrooms packaged with other films (Guillaume et al., 2010; Liu et al., 2015; Qin et al., 2015). After 12 d of storage, the inhibitory effect on weight loss of mushrooms packaged with different films decreased in the order of PE film > GA-g-CS film > CS film ($p < 0.05$). Although

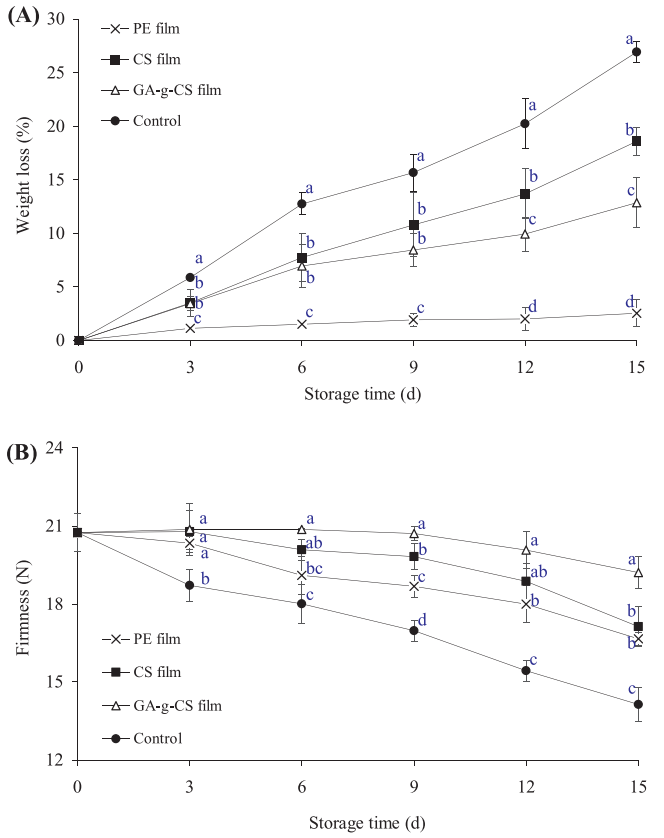

Fig. 1. Effect of different packages on the weight loss (A) and firmness (B) of mushrooms stored at 4 ± 1 °C for 15 d. Vertical bars represent the standard deviations of triplicate assays. Different lower case letters indicate the statistically significant difference among treatments at the same storage time ($p < 0.05$).

mushrooms packaged with GA-g-CS film showed a higher weight loss than those packaged with PE film ($p < 0.05$), the weight loss of mushrooms packaged with GA-g-CS film was still within the acceptable range of market values (5–10%) in 12 d of storage (Mahajan et al., 2008).

3.2. Firmness

Ageing of mushrooms resulted in a soft and spongy texture characterized by a softening of mushroom tissues (Guillaume et al., 2010). So, firmness is a critical factor reflecting the quality of mushrooms. Effect of different packages on the firmness of mushrooms during storage is shown in Fig. 1B. The firmness of mushrooms in all the groups gradually decreased with the duration of storage. Similar phenomenon was observed by many other researchers (Gholami et al., 2017; Guillaume et al., 2010; Liu et al., 2015; Qin et al., 2015; Singh et al., 2018). The reduction of firmness was related to protein and polysaccharide degradation, hyphae shrinkage and central vacuole disruption in mushrooms due to biochemical and microbial processes (Liu et al., 2015). Notably, mushrooms packaged with GA-g-CS film were significantly firmer than those packaged with PE film after 6 d of storage ($p < 0.05$), indicating GA-g-CS film could protect mushrooms from senescence (Guillaume et al., 2010). Liu et al. (2015) reported packaging film with low water vapor permeability could result in high relative humidity inside package and further increase microbial growth. By contrast, the grafted GA moiety in GA-g-CS film could inhibit microbial growth (Lee and Je, 2013; Liu et al., 2017), and thus mushrooms packaged with GA-g-CS film showed higher firmness than those packaged with PE film. Other researchers suggested that essential oils could

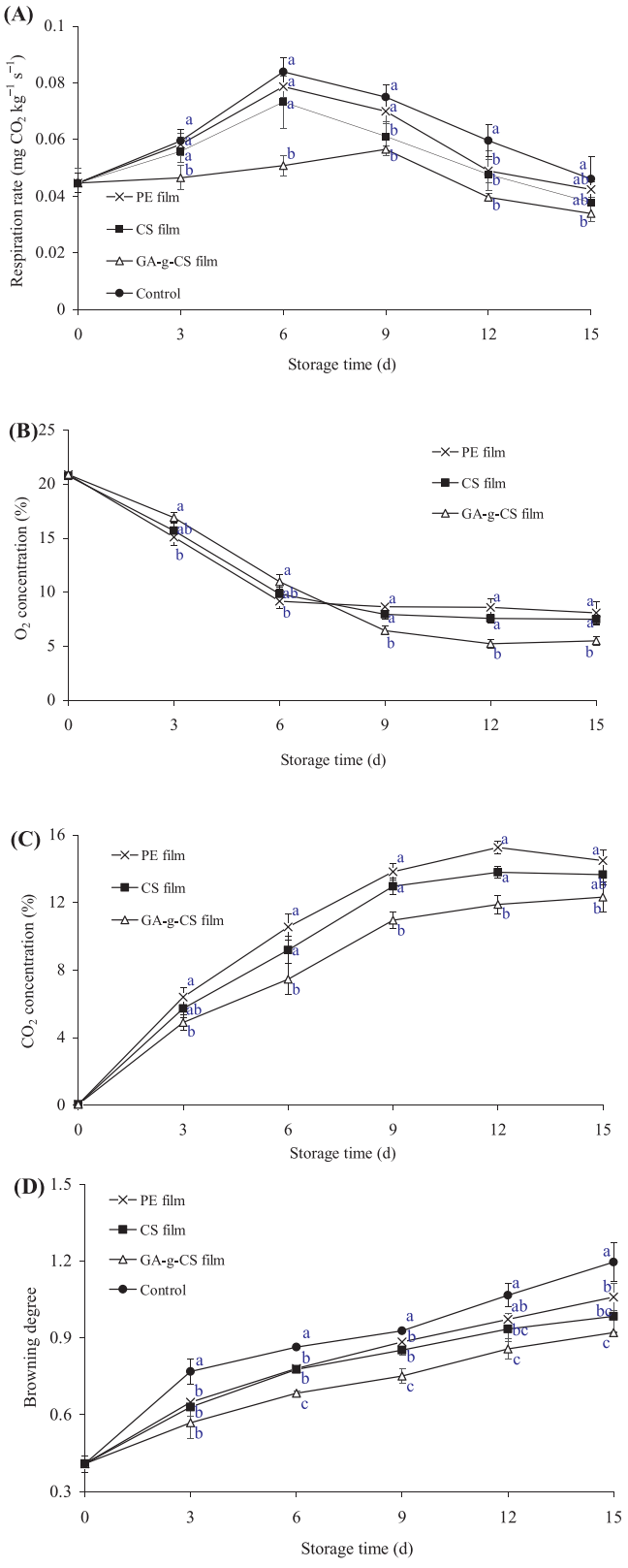

Fig. 2. Effect of different packages on the respiration rate (A), O_2 concentration (B), CO_2 concentration (C) and browning degree (D) of mushrooms stored at 4 ± 1 °C for 15 d. Vertical bars represent the standard deviations of triplicate assays. Different lower case letters indicate the statistically significant difference among treatments at the same storage time ($p < 0.05$).

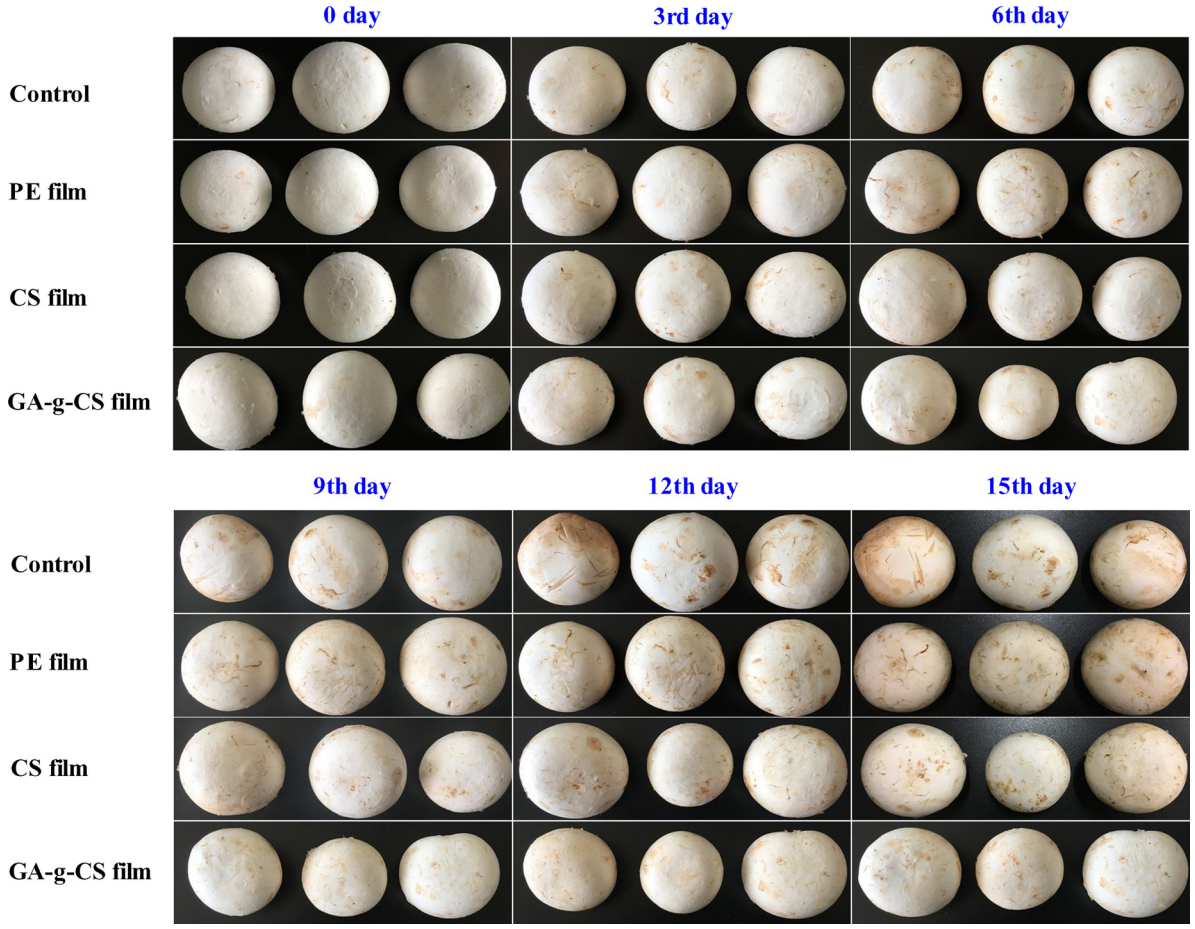

Fig. 3. Effect of different packages on the physical appearance of mushrooms stored at 4 ± 1 °C for different times.

also delay the softening process of mushrooms (Gao et al., 2014; Nasiri et al., 2017).

3.3. Respiration rate

Respiration involves the oxidation of energy-rich organic molecules in cells (*e.g.* starch, sugar and organic acids) into simpler molecules (*e.g.* CO_2 and H_2O) with the concurrent production of energy (Xu et al., 2016). The high respiration rate is related with the postharvest quality loss of mushrooms. Changes in the respiration rate of mushrooms packaged with different films are shown in Fig. 2A. The respiration rate of mushrooms in all the groups first increased and then decreased during storage. Similar results were also reported by other researchers (Qin et al., 2015; Xu et al., 2016). The respiration rate of mushrooms packaged with PE film was much higher than that packaged with GA-g-CS film in 9 d of storage ($p < 0.05$). The high respiration rate of mushrooms packaged with PE film could accelerate the consumption of nutrients and initiate the spoilage of mushrooms (Gholami et al., 2017). At the end of 15 d of storage, the respiration rate of mushrooms packaged with GA-g-CS film accounted for only 80.2% and 89.7% of those packaged with PE and CS films, respectively. The occurrence of the respiration peak was delayed to the 9th day in mushrooms packaged with GA-g-CS film. These results indicated GA-g-CS film was very effective in reducing the respiration rate of mushrooms.

3.4. Headspace gas composition

Fresh mushrooms consume O_2 and produce CO_2 during storage. The combined effect of mushroom respiration and gas permeability of film can influence the headspace gas composition in the package (Oz et al., 2015). Changes in O_2 concentration of mushrooms packaged with different films are presented in Fig. 2B. In the first 9 d of storage, O_2 concentration of mushrooms decreased rapidly in all three packages. However, the extent of decrease in O_2 concentration varied with packaging film. Notably, O_2 concentration of mushrooms packaged with PE film decreased more rapidly than those packaged with GA-g-CS film ($p < 0.05$) because of high respiration and O_2 consumption of mushrooms packaged with PE film. After 9 d of storage, O_2 concentration reached equilibrium in all the treatment groups, indicating gas diffusion through film exactly compensated the O_2 consumption by mushrooms (Gholami et al., 2017). The finally O_2 concentration of mushrooms packaged with different films was higher than 5%, which could effectively avoid anaerobic respiration in mushrooms (Qin et al., 2015). Furthermore, the final O_2 concentration of mushrooms packaged with GA-g-CS film were lower than those packaged with other films ($p < 0.05$). This was probably because the grafted GA moiety in GA-g-CS film could act as an O_2 scavenger to remove the O_2 permeated through film into the package and reduce the residual O_2 trapped inside the package (Ahn et al., 2016). In addition, GA-g-CS film possessed the lowest O_2 permeability amongst the three packaging films.

As presented in Fig. 2C, CO_2 concentration in headspace of mushrooms packaged with different films gradually increased during storage. Mushrooms packaged with PE film showed higher CO_2 concentration than those packaged with GA-g-CS film ($p < 0.05$), which was due to the combined effect of high mushroom respiration and low CO_2 permeation of film. Ban et al. (2014) suggested that the rate of CO_2 production by mushrooms was much higher than CO_2 permeation rate of PE film. Thus, the high CO_2 barrier property of PE film caused a build-up of CO_2 produced by the respiration of mushrooms. By contrast, mushrooms packaged with GA-g-CS film exhibited the lowest CO_2

concentration throughout the storage period ($p < 0.05$). Similar changes in headspace gas composition were also reported by many other researchers (Ban et al., 2014; Gholami et al., 2017; Han et al., 2015; Liu et al., 2015; Qin et al., 2015). Qin et al. (2015) suggested that the accumulation of CO_2 was one of the major problems resulting in mushroom softening. CO_2 levels above 12% had a negative effect on the firmness and flavor of mushrooms. This could further explain why mushrooms packaged with PE film had lower firmness than those packaged with CS and GA-g-CS films.

3.5. Browning degree

The color of mushrooms is the most important parameter for consumer acceptance. After harvest, the color of mushrooms gradually turns from white to brown. This unfavorable browning was mainly due to enzyme oxidation, senescence and microbial growth, resulting in the loss of nutritional quality and the shortage of shelf life in fresh mushrooms (Oz et al., 2015). Changes in the browning degree of mushrooms packaged with different films are shown in Fig. 2D. The increasing browning degree was observed in mushrooms regardless of the film packaged. At the end of 15 d of storage, the browning degrees of mushrooms packaged with PE, CS and GA-g-CS films were 1.06, 0.98 and 0.92, respectively. As compared with PE film, GA-g-CS film significantly retarded mushroom browning after 6 day of storage ($p < 0.05$); indicating GA-g-CS film could retain the freshness of mushrooms. These results were also supported by the physical appearance of mushrooms storage for different times (Fig. 3). The protective effect of GA-g-CS film against mushroom browning might be due to several reasons. First, the presence of phenolic groups in GA-g-CS film could inactivate some bacteria on the surface of mushrooms (Qin et al., 2015). Secondly, due to its strong antioxidant activity, GA-g-CS film could protect phenolic compounds in mushrooms from being oxidized. Finally, the relatively lower respiration rate in mushrooms packaged with GA-g-CS film could avoid damage to the mushroom cap surface tissue, which resulted in a low browning degree (Gholami et al., 2017).

3.6. MDA content and electrolyte leakage rate

Lipid peroxidation is an oxidative alteration of polyunsaturated fatty acids in the cell membranes that generates a number of degradation products. MDA is one of the products of lipid peroxidation, which is widely accepted as the biomarker of lipid peroxidation (Hu et al., 2015). As presented in Fig. 4A, MDA content significantly increased in mushrooms after 9 d of storage in all the treatment groups. Notably, mushrooms packaged with GA-g-CS film exhibited lower MDA contents than those packaged with PE film ($p < 0.05$); indicating GA-g-CS film could alleviate oxidative injury during storage. The relatively slower accumulation of MDA in mushrooms packaged with GA-g-CS film could be attributed to the protection effect of GA-g-CS on cell membrane against free radical's damage (Wang et al., 2015). Several researchers suggested that many other treatments, such as high CO_2, electron beam irradiation, mild heat, 4-methoxy cinnamic acid, glycine betaine and methyl jasmonate treatments could also prevent MDA accumulation during postharvest storage of mushrooms (Duan et al., 2010; Hu et al., 2015; Lin et al., 2017; Meng et al., 2017; Wang et al., 2015).

Membrane permeability of mushrooms is often reflected by the electrolyte leakage rate of mushrooms (Hu et al., 2015). As shown in Fig. 4B, the electrolyte leakage rate of mushrooms in all the groups increased with the duration of storage, indicating a reduction in membrane integrity. Moreover, mushrooms packaged with GA-g-CS film exhibited a significantly lower electrolyte leakage rate than those packaged with other films ($p < 0.05$), which could be attributed to the antioxidant activity of GA-g-CS. Notably, the increase of electrolyte leakage in mushrooms was consistent with the increase of browning degree and MDA content. Other researchers also suggested the loss of membrane integrity was closely associated with the browning of mushrooms (Aday et al., 2016; Ding et al., 2016; Dokhanieh and Aghdam, 2016).

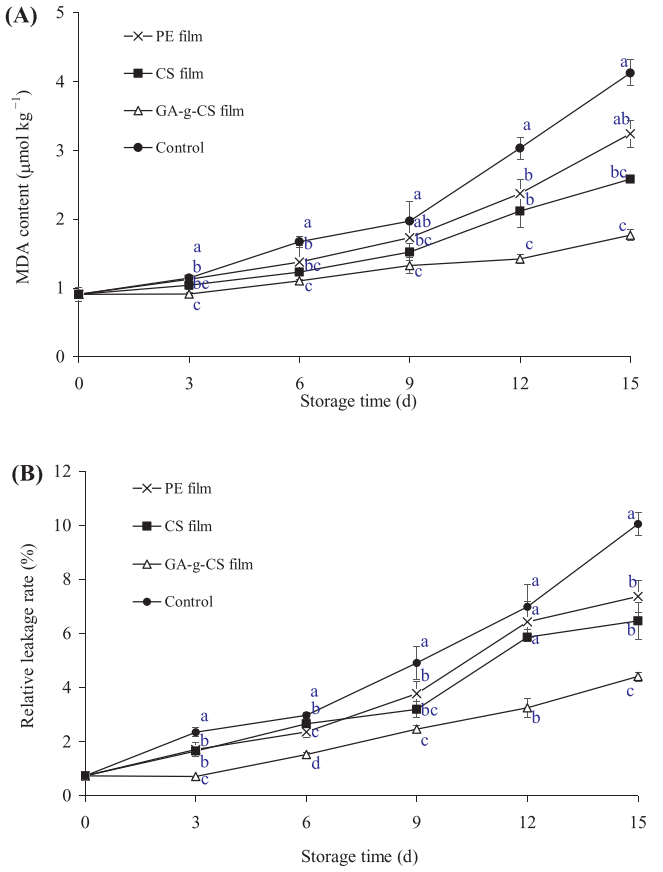

Fig. 4. Effect of different packages on MDA content (A) and relative leakage rate (B) of mushrooms stored at 4 ± 1 °C for 15 d. Vertical bars represent the standard deviations of triplicate assays. Different lower case letters indicate the statistically significant difference among treatments at the same storage time ($p < 0.05$).

3.7. ROS production

Reactive oxygen species (ROS), such as $O_2^{\cdot -}$ and H_2O_2, are the by-products of cellular metabolism in normal mitochondrial respiration. ROS can oxidize and destroy the cytoplasmic membrane, thereby accelerating lipid peroxidation, membrane damage and senescence. So, the overproduction of ROS is an intrinsic feature of senescence (Zhang et al., 2017). Effect of different packages on $O_2^{\cdot -}$ production rate of mushrooms during storage is shown in Fig. 5A. A significant increase in $O_2^{\cdot -}$ production rate was observed after 6 d of storage for all the groups. However, there was no statistical difference in $O_2^{\cdot -}$ production rate between PE and CS film packaging after 6 d of storage ($p > 0.05$). Among all the treatment groups, mushrooms packaged with GA-g-CS film showed the slowest $O_2^{\cdot -}$ production rate ($p < 0.05$), indicating GA-g-CS film could greatly inhibit the increase of $O_2^{\cdot -}$ production rate in mushrooms. Such decrease in $O_2^{\cdot -}$ production rate was also reported in mushrooms treated with 4-methoxy cinnamic acid, brassinolide and mild heat (Ding et al., 2016; Hu et al., 2015; Zhang et al., 2017). As shown in Fig. 5B, H_2O_2 content in mushrooms packaged with PE film significantly increased after 6 d of storage. After 9 d of storage, mushrooms packaged with GA-g-CS film maintained relatively lower H_2O_2 contents than those packaged with PE film ($p < 0.05$). Above results indicate that GA-g-CS film could reduce ROS levels that were directly involved in the oxidation process and cell degradation.

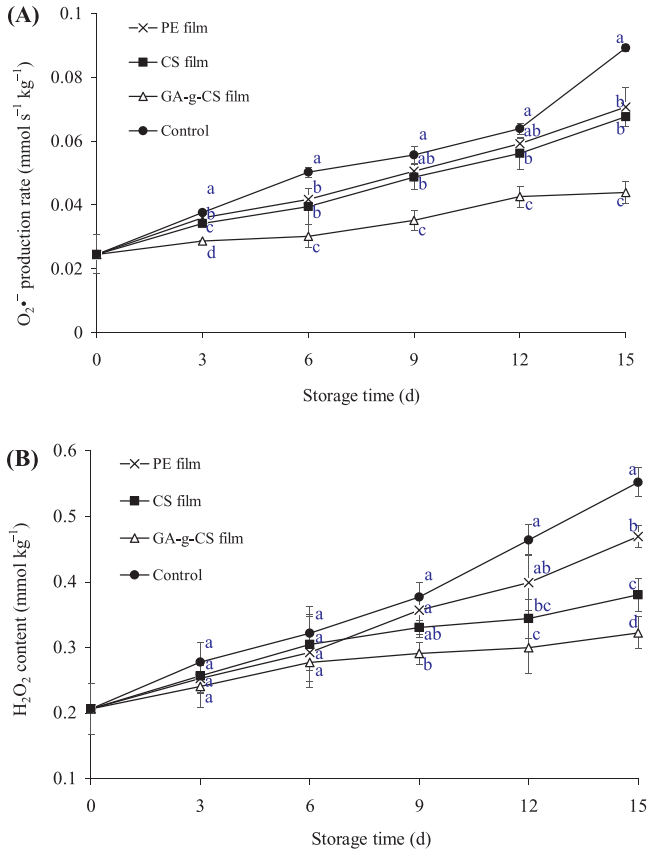

Fig. 5. Effect of different packages on $O_2^{\cdot -}$ production rate (A) and H_2O_2 content (B) of mushrooms stored at 4 ± 1 °C for 15 d. Vertical bars represent the standard deviations of triplicate assays. Different lower case letters indicate the statistically significant difference among treatments at the same storage time ($p < 0.05$).

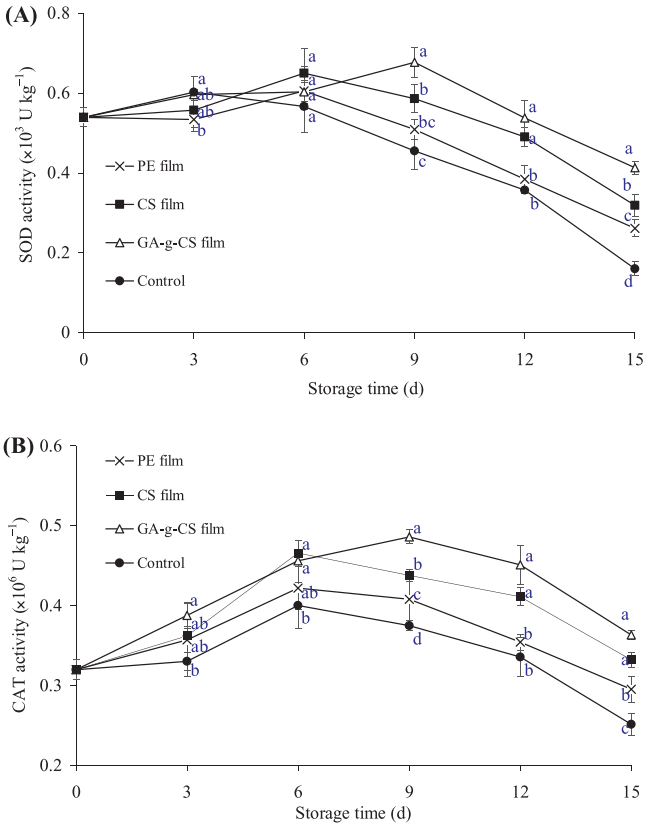

Fig. 6. Effect of different packages on SOD (A) and CAT (B) activities of mushrooms stored at 4 ± 1 °C for 15 d. Vertical bars represent the standard deviations of triplicate assays. Different lower case letters indicate the statistically significant difference among treatments at the same storage time ($p < 0.05$).

3.8. Antioxidant enzyme activity

Antioxidant enzymes such as SOD and CAT play a crucial role in antioxidant defense during the ripening process of mushrooms (Meng et al., 2012). SOD can catalyze the dismutation of $O_2^{\cdot -}$ to produce H_2O_2, which is then removed by CAT. As shown in Fig. 6A, a slight increase in SOD activity was observed in mushrooms packaged with CS and GA-g-CS films after 6 d of storage. Then, SOD activity in these two treatment groups decreased sharply. Similar results were reported by many other researchers (Ding et al., 2016; Dokhanieh and Aghdam, 2016; Khan et al., 2014; Meng et al., 2012; Wang et al., 2015). The slight increase in SOD activity was observed in mushrooms packaged with GA-g-CS film at 9 d of storage. After 9 d of storage, mushrooms packaged with GA-g-CS film exhibited higher SOD activity as compared with PE film packaging ($p < 0.05$).

In case of CAT, it can reduce the oxidative damage caused by H_2O_2 in plant tissues (Hu et al., 2015). As shown in Fig. 6B, CAT activity of mushrooms packaged with PE and CS films increased in the first 6 d of storage and decreased thereafter. A similar tendency was also observed by other researchers (Ding et al., 2016; Dokhanieh and Aghdam, 2016; Hu et al., 2015; Khan et al., 2014; Meng et al., 2012). In comparison, GA-g-CS film packaging prolonged the increase of CAT activity in mushrooms to 9 d. Mushrooms packaged with GA-g-CS film showed higher CAT activity than those packaged with PE film after 9 d of storage ($p < 0.05$). Notably, the decrease of SOD and CAT activities in mushrooms was closely associated with the increase of $O_2^{\cdot -}$ production rate and H_2O_2 content. These indicated the reduction of ROS levels by GA-g-CS film packaging might be due to the enhancement of antioxidant defense system in mushrooms.

3.9. PPO activity and total phenolic content

PPO is one of the key enzymes in enzymatic browning of mushrooms. It can catalyze the oxidation of mono- and di-phenols to *o*-quinones, and these quinones then polymerize to produce brown pigments (Han et al., 2015). As shown in Fig. 7A, mushrooms packaged with GA-g-CS film showed the lowest PPO activity among all the treatment groups ($p < 0.05$). This result suggested GA-g-CS film packaging was effective to suppress PPO activity and thus reduced enzymatic browning of mushrooms.

Changes in the total phenolic content of mushrooms packaged with different films during storage period are shown in Fig. 7B. The total phenolic content of mushrooms in all the groups first increased and then gradually decreased. A similar tendency was also observed by other researchers (Ding et al., 2016; Dokhanieh and Aghdam, 2016; Gao et al., 2014; Wang et al., 2015). Among all the treatment groups, mushrooms packaged with GA-g-CS film showed the highest total phenolic content ($p < 0.05$). These results revealed that the accumulation of phenols in mushrooms during storage is effectively boosted by GA-g-CS film packaging.

In general, phenols play dual functions in mushrooms (Dokhanieh and Aghdam, 2016; Gao et al., 2014). On one hand, phenols can be oxidized by PPO, which leads to browning, as the main postharvest stress symptom in mushrooms. On the other hand, phenols accumulated in mushrooms possess strong antioxidant capacity, which can delay the lipid peroxidation *via* inhibiting the initiation or propagation of oxidizing chain reactions. Our study suggested that GA-g-CS film packaging greatly stimulated the accumulation of phenols. The accumulated phenols showed antioxidant activity, which may contribute to the browning alleviating in mushrooms through maintaining membrane

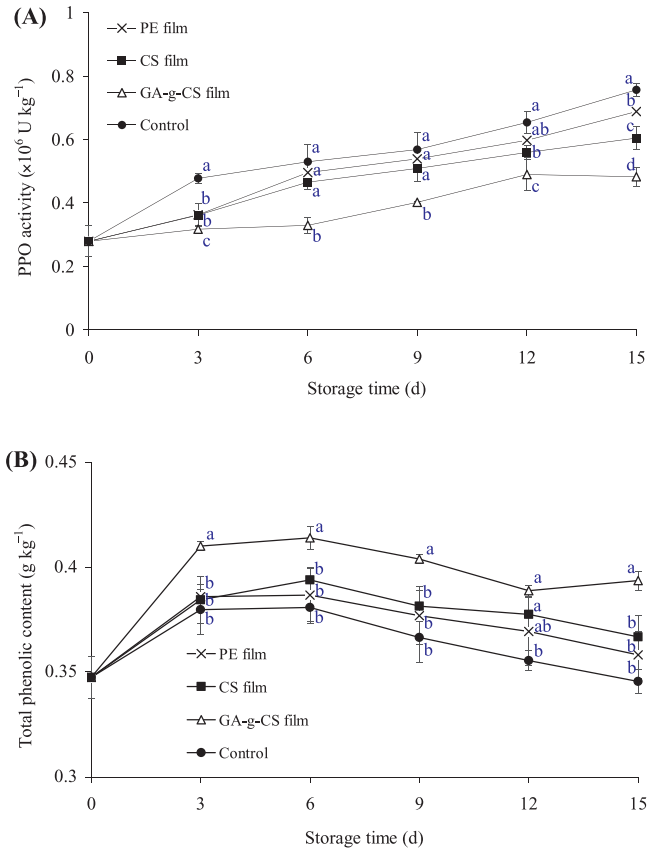

Fig. 7. Effect of different packages on PPO activity (A) and total phenolic content (B) of mushrooms stored at 4 ± 1 °C for 15 d. Vertical bars represent the standard deviations of triplicate assays. Different lower case letters indicate the statistically significant difference among treatments at the same storage time ($p < 0.05$).

integrity. In addition, the relatively lower PPO activity in mushrooms packaged with GA-g-CS film could lead to higher levels of phenols because less phenolic substrates were consumed in enzymatic browning reactions. Dokhanieh and Aghdam (2016) also observed that salicylic acid treated mushrooms displayed a significantly higher total phenolic content but lower PPO activity than the control. Zhang et al. (2017) reported the mild heat treatment could enhance the total phenolic content but decrease PPO activity in fresh-cut mushrooms.

4. Conclusion

Our results have shown that GA-g-CS film packaging has a positive effect on maintaining the postharvest quality of *A. bisporus*. The preservation effect of GA-g-CS film could be attributed to its intrinsic antioxidant property as well as ideal water vapor and CO_2 transmission rates. Thus, mushrooms packaged with GA-g-CS film showed lower respiration rate and browning degree but higher antioxidant status than those packaged with CS and PE films. These indicated that GA-g-CS film could be explored as a novel active packaging material for the postharvest storage of *A. bisporus*. In future, the preservation effect of GA-g-CS film on other mushrooms can be also evaluated.

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