



## 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  $1.5 \times 10^5$  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 Biotechnical 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<sub>2</sub> permeability of  $1.0 \times 10^{-11}$  mL m<sup>-1</sup> s<sup>-1</sup> Pa<sup>-1</sup>, CO<sub>2</sub> permeability of  $1.6 \times 10^{-10}$  mL m<sup>-1</sup> s<sup>-1</sup> Pa<sup>-1</sup>, and water vapor permeability of  $1.1 \times 10^{-10}$  g m<sup>-1</sup> s<sup>-1</sup> Pa<sup>-1</sup> at 25 °C and 90% relative humidity) was peeled from the plate and stored at 25 °C in desiccators containing saturated Ca(NO<sub>3</sub>)<sub>2</sub> solution (50 ± 2% relative humidity) for at least 72 h before use. CS film (30 μm of thickness, O<sub>2</sub> permeability of  $1.2 \times 10^{-11}$  mL m<sup>-1</sup> s<sup>-1</sup> Pa<sup>-1</sup>, CO<sub>2</sub> permeability of  $1.7 \times 10^{-10}$  mL m<sup>-1</sup> s<sup>-1</sup> Pa<sup>-1</sup>, and water vapor permeability of  $1.8 \times 10^{-10}$  g m<sup>-1</sup> s<sup>-1</sup> Pa<sup>-1</sup> at 25 °C and 90% relative humidity) was prepared in the same way without grafting GA. At 5 g L<sup>-1</sup> 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<sub>2</sub> permeability of  $2.6 \times 10^{-11}$  mL m<sup>-1</sup> s<sup>-1</sup> Pa<sup>-1</sup>, CO<sub>2</sub> permeability of  $8.1 \times 10^{-11}$  mL m<sup>-1</sup> s<sup>-1</sup> Pa<sup>-1</sup>, and water vapor permeability of  $1.2 \times 10^{-12}$  g m<sup>-1</sup> s<sup>-1</sup> Pa<sup>-1</sup> 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<sup>-1</sup>. 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<sub>2</sub> 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<sup>-1</sup> 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<sup>-1</sup> oxalic acid. Respiration rate of mushroom was expressed as CO<sub>2</sub> 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<sup>-1</sup>); 44 is the molecular weight of CO<sub>2</sub> (g mol<sup>-1</sup>);  $W$  is the sample weight (kg); and  $t$  is time (s).

### 2.6. Headspace gas composition

The concentrations of O<sub>2</sub> and CO<sub>2</sub> in the package headspace were detected by an SCY-2 A O<sub>2</sub>/CO<sub>2</sub> 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<sup>-1</sup> sodium phosphate buffer (pH 6.8) containing 2.5% (w/v) polyvinylpyrrolidone (PVPP) and 0.15 mol L<sup>-1</sup> 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.

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