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Effect of nanocomposite packaging on postharvest senescence of *Flammulina velutipes*

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ABSTRACT

A polyethylene based packaging material containing nano-Ag, nano-TiO_2, nano-SiO_2, and attapulgite has been prepared. The effect of nanocomposite packaging material (Nano-PM) on the senescence of *Flammulina velutipes* during 15 days of postharvest storage at 4 °C and a relative humidity of 90% were analyzed. The results showed that compared with normal packaging material (Normal-PM) and no packaging (No-PM), Nano-PM improved the appearance quality, reduced weight loss and cap opening. The degree of maturity and increase in molecular weight of *F. velutipes* polysaccharides (FVP) were delayed. The content loss of proteoglycan protein was less and degree of oxidation was lower. The storage with Nano-PM reduced the fibrosis of texture, cellulase activity, the accumulation of hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\cdot -}$) by 18.9%, 48.3%, 26.6% and 27.8%, respectively ($P < .05$). The Nano-PM effectively delayed the postharvest senescence of *F. velutipes*, hence prolonged its shelf life and increased its preservation quality.

1. Introduction

Flammulina velutipes, known as golden needle mushroom and winter mushroom, is one of the edible fungi with the largest production and consumption in China. In the world, mushroom is currently ranked fourth in terms of production and consumption (Fang, Yang, Kimatu, & Mariga, 2016). Fresh *F. velutipes* fruit body has high moisture content and crisp texture. Postharvest *F. velutipes* still has high physiological activity and respiratory rate, which can rapidly lead to senescence and deterioration such as cap opening, stem fibrosis, water loss, browning, which results in the loss of edible value and overall decay (Fang, Yang, Kimatu, & An, 2016). In the production, storage, and marketing of *F. velutipes*, postharvest preservation serves as a key role in the industry. Therefore, it is critical to urgently develop a scientific, economical and convenient postharvest preservation technology, which can effectively delay senescence and deterioration of *F. velutipes* and extend shelf life.

In recent years, several studies on postharvest techniques have been conducted. These techniques are combined with refrigeration, including cold storage, modified atmosphere packaging (MAP), radiation, film packaging (Fang, Yang, Kimatu, & Mariga; Fang, Yang, Kimatu, & An, 2016) and cold plasma (Sadhu, Thirumdas, Deshmukh, & Annapure, 2017). Different preservation methods have different advantages and limitations. Hurdle technology, a combination of multiple approaches, has become a trend. Compared to traditional technologies and materials, nanomaterials have a unique performance because of their special composition and microstructure. The incorporation of nanoparticle fillers such as silver (Ag), silicon dioxide (SiO_2), titanium dioxide (TiO_2) and attapulgite to polymers may not only improve its mechanical and barrier properties but also offer other applications and functionalities in food packaging (Fang, Yang, Kimatu, & Mariga, 2016). Nanomaterials have advantageous functions in blocking CO_2, water vapor barrier properties, antimicrobial activity and ethylene elimination, which are suitable for preservation of fresh agricultural products (Kanmani & Rhim, 2014). The material has attracted increasing attention because of their potential impacts on industry and market. Thus, nano-Ag, nano-SiO_2, nano-TiO_2 and attapulgite were chosen to prepare nanocomposite packaging material (Nano-PM). Recently, there are some reports on the use of nanomaterials in

Abbreviations: AIR, alcohol insoluble residue; Ag, silver; C1, *exo*-1,4-β-D-glucanase; CX, *endo*-1,4-β-D-glucanase; ELSD, evaporative light scattering detector; FVP, *Flammulina velutipes* polysaccharides; HPSEC, high-performance size-exclusion chromatography; MAP, modified atmosphere packaging; Nano-PM, nano-packaging material; Normal-PM, normal packaging material; No-PM, no packaging material; RH, relative humidity; ROS, reactive oxygen species; SiO_2, silicon dioxide; TiO_2, titanium dioxide

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postharvest storage. New types of nanomaterial packaging have been developed and applied in the preservation of several agricultural products. In our previous studies, a polyethylene packaging material containing nano-Ag, nano-TiO_2, nano-SiO_2, and attapulgite was prepared. Studies have showed that the Nano-PM regulated oxygen and carbon dioxide level, eliminated ethylene and inhibited the growth of microbes. Treatment with the Nano-PM improved nutrient retention and flavor protection of the fresh *F. velutipes* (Fang, Yang, Kimatu, & Mariga; Fang, Yang, Kimatu, & An, 2016). Many results revealed that Nano-PM delayed the postharvest senescence of *F. velutipes*, but the underlying mechanism is still unclear.

In this study we have evaluated the effects of Nano-PM on postharvest senescence including changes of appearance quality and microstructure. The amount of spores, oxidation degree in protein, fibrosis of cell walls, reactive oxygen species (ROS) levels, molecular weight of *F. velutipes* polysaccharide (FVP) and protein content of proteoglycan were also determined. Furthermore, the effect of Nano-PM on senescence in postharvest and the influence of Nano-PM on cell wall fibrosis, FVP molecular weight, protein oxidation and free radical metabolism have been also analyzed.

2. Materials and methods

2.1. Preparation of F. velutipes

F. velutipes used in this study was obtained from a wholesale market (a commercial farm) in Jiangsu Province, in China, and transported to the laboratory within one hour upon harvesting. The mushrooms were selected according to their whiteness, development stage, closed veil and shape (stipe length of 10–15 cm). After storage in the darkness at 4 ± 1 °C and 90% relative humidity (RH) for 24 h, mushrooms (320 g) were randomly packaged in Nano-PM (15 bags, 20 cm × 15 cm) and normal packaging material (Normal-PM, 15 bags, 20 cm × 15 cm). On the other hand, the unpacked mushroom was separated into 15 parts and placed on wound gauze as no packaging material (No-PM) group. The Nano-PM used in this study was prepared according to our earlier study (Fang, Yang, Kimatu, & Mariga, 2016). Polyethylene was used as the matrix materials with nano-powders (0.34% of nano-Ag, 0.39% of nano-TiO_2, 0.28% of attapulgite, and 0.11% of nano-SiO_2). Normal-PM was polyethylene bag of the same thickness and size but without the nanocomposite masterbatch nano-powder. The permeability of water vapor and oxygen were determined and the results were 6.24 g/m^2/24 h and 3017.5 cm^3/(m^2/24 h/0.1 MPa), respectively (Fang, Yang, Kimatu, & Mariga, 2016). The samples were then sealed with a heat sealer (ShouChuang Instrument Corporation, Wenzhou) and stored at 4 ± 1 °C and 90% RH for 15 days. Three bags in each packaging sample were used to analyze the physiological and biochemical indexes every 3 days during the storage. The samples were freezed by liquid nitrogen and stored at −80 °C.

2.2. Spore count

Spores occurrence is a senescence phenomenon of *F. velutipes* and the amount of spore is an indication of maturity degree. The collection of *F. velutipes* spores was carried out according to the method of Fischer, Stolze-Rybczynski, Cui, and Money (2010) with slight modification. Caps were washed with Tween-80, centrifuged at 1000 rpm for 15 min at 4 °C and the spores were collected. The sediment was dissolved in deionized water and the number of spores was counted by blood cell counting chamber.

2.3. Microstructure observation

Microstructure observation was followed the method of Fang, Yang, Kimatu, & Mariga (2016) with slight modification, ultrathin sections of *F. velutipes* were pre-fixed in 4% (w/w) glutaraldehyde with 0.1 mol/L phosphate buffer (pH 7.8) for 1 h and rinsed in phosphate buffer 3 times (15 min each time). The samples were further dehydrated in *tert*-butyl alcohol (at concentrations of 50%, 70%, 80%, and 90% sequentially, 5 min each) and lyophilized. They were coated with a layer of gold by a sputter coater (BAL-TEC AG, Balzers, Liechtenstein) and then examined and photographed using JEOLJSM-6390LV (Japan Electron Optics Laboratory Corporation) scanning electron microscope.

2.4. Determination of FVP molecular weights and protein content of proteoglycan

FVP was prepared by the method of Du et al. (2016) with slight modification. *F. velutipes* tissue (50 g) was extracted by 1 L of deionized water with stirring for 4 h at 80 °C. The extraction was collected by centrifugation and then deproteinized three times with Sevag reagent (chloroform: 1-butanol, 4:1). FVP extract was then mixed with 4-fold volume anhydrous ethanol at 4 °C. After centrifugation at 10,000 rpm for 20 min, the precipitate was collected and lyophilized as FVP.

Molecular weights determination employed the method described by Yang et al. (2012) with slight modification. Samples were added into water to make 1.5 mg/mL solution and filtered through a 0.45 μm filter membrane. The samples were then analyzed on a high-performance size-exclusion chromatography (HPSEC) on an Agilent 1200 system equipped with a TSK gel G4000 PWXL column (300 mm × 7.8 mm) and an evaporative light scattering detector (ELSD). Twenty microliter of sample solution was injected and eluted with deionized water at a flow rate of 0.6 mL/min. The linear regression was calibrated with *T*-series dextrans standards (T-500, T-200, T-70, T-40 and T-10). Consequently, the protein content of proteoglycan was determined by the method of Yanu and Jakmunee (2017). The sample (0.2 g) was mixed a catalyst of 0.5 g of copper sulfate and 4.5 g of potassium sulfate and 3 mL of concentrated sulfuric acid. It was digested for 2.5 h. Then the digested solution was cooled down and determined by titration.

2.5. Content of total carbonyl, disulfide, sulfhydryl

Protein extraction followed the method of Xia et al. (2012) with a minor modification. The *F. velutipes* protein was prepared using a base-acid extraction method, first with 0.1 M NaOH alkali solution then 0.01 M HCl of acid solution. The protein was collected, washed twice with distilled water and lyophilized.

Total carbonyl content was determined by previously reported method (Wu, Wu, & Hua, 2010). Protein powder (70 mg) was centrifuged in 5 mL of 0.85% saline for 10 min at 2500 rpm. Four aliquots of 200 μL of the supernatant were dispensed. Two aliquots were treated with 400 μL of 2 M HCl (blank) and the others were treated with equal volume of 10 mM DNPH dissolved in 2 M HCl. All samples reacted in the dark for 1 h at 37 °C. Afterwards, 500 μL of ice cold 0.2% (w/v) trichloroacetic acid was added. The mixture was washed three times with 1 mL of ethyl acetate: ethanol (1:1, v/v) and centrifuged for 15 min at 10,000 rpm. The pellets were re-suspended in 1.25 mL of 6 M guanidine hydrochloride and centrifuged. The precipitate was discarded and the absorbance was measured at 370 nm with M2E Multiskan Spectrum. The content of carbonyl was expressed as nmol of carbonyl per mg of protein.

Sulfhydryl and disulfide content was determined according to the method of Huang, Hua, and Qiu (2006). The sulfhydryl group (SH) were determined by titration with 5, 5′-„dithio-bis-(2-nitrobenzoic acid) (DTNB). Protein solution (0.75%) was prepared in 5 M of guanidine hydrochloride as a sample solution. To 1 mL of the solution, 4 mL mixture of 8 M urea and 5 M guanidine hydrochloride (1:1, v/v) and 0.05 mL of 1 mM DTNB aqueous solution (Ellman's reagent) was added. The cocktail was centrifuged at 5000 rpm for 10 min and the precipitate discarded. The supernatant's absorbance was measured at 412 nm.

To determine disulfide bond content, a solution consisting of