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

Chong Shi[a], Yuanyue Wu[a], Donglu Fang[b], Fei Pei[a], Alfred Mugambi Mariga[c], Wenjian Yang[a,*], Qiuhui Hu[a,*]

[a] College of Food Science and Engineering/Collaborative Innovation Center for Modern Grain Circulation and Safety, Nanjing University of Finance and Economics, Nanjing, Jiangsu 210023, China
[b] College of Food Science and Technology, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China
[c] Faculty of Agriculture and Environmental Studies, Chuka University, P.O. Box 109-60400, Chuka, Kenya

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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

* Corresponding authors.
E-mail addresses: lingwentt@nufe.edu.cn (W. Yang), qiuhuihu@nufe.edu.cn (Q. Hu).

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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

0.05 mL of 2-mercaptoethanol and 4 mL mixture of 8 M urea and 5 M guanidine hydrochloride (1:1, v/v) was added to 1 mL of the sample solution. The mixture was incubated at 25 °C for 1 h and 10 mL of trichloroacetic acid (12%) was added. Then the mixture was incubated for 1 h at 25 °C and centrifuged for 10 min at 5000 rpm and the supernatant discarded. The precipitate was washed three times with 12% trichloroacetic acid, the pellets re-suspended in 10 mL of 8 M urea then 0.04 ml Ellman's reagent added. The cocktail was centrifuged for 10 min at 5000 rpm and the precipitate discarded. The supernatant's absorbance was read at 412 nm. Deionized water (1 mL) treated with the equal treatment was used as blank.

2.6. Content of cellulose, chitin and cellulase activity

Cellulose content was quantified following the method of Femenia, Garcia-Conesa, Simal, and Rosselló (1998) with slight modification. One gram (1 g) of dried sample was mixed with 70 mL of cold 60% (v/v) H_2SO_4 in ice-bath for 30 min. Then 30 mL of 60% (v/v) H_2SO_4 was added and the mixture was filtered to another beaker using a glass crucible funnel together with quartz filter leaf. The filtrate (2 mL) was mixed with 2 mL of 2% (v/v) anthraketone (0.2 g anthraketone added to 10 mL acetidin) and 5 mL of 98% (v/v) H_2SO_4, left for 1 min and measured the absorption of the mixture at 530 nm with M2E Multiskan Spectrum. The filtrate was substituted for 60% (v/v) H_2SO_4 as the blank.

Samples enriched with chitin (alcohol insoluble residue, AIR) were prepared according to the method of Domon et al. (2013) and then lyophilized. AIR was used to determine chitin content according to the method of Li, Qin, Tian, and Wang (2015) with slight modification. AIR (25 mg) was added into a 25 mL volumetric flask, mixed with 5 mL of 2 mol/L HCl, 15 mL of 98% (v/v) H_2SO_4 in ice-bath, boiled for 30 min, cooled down, and finally 25 mL of deionized water was added. Subsequently, 1 mL of hydrolyzed solution was added into a 10 mL volumetric flask, mixed with 1 mL of dihydroxybenzene (2%), 7.5 mL of 75% (v/v) H_2SO_4 sequentially. The supernatant was then boiled for 30 min, cooled down and 10 mL of deionized water was added. Finally, the absorbance of the supernatant was measured at 500 nm with M2E Multiskan Spectrum. Glucosamine was used to obtain the standard curve (0 ~ 300 mg/mL). Hydrolyzed solution was substituted for HCl as the blank.

Cellulase activity was determined by the method described by Chen et al. (2015). The reaction mixture contained 2.0 mL of 1% (w/v) solution of carboxymethyl cellulose and 0.5 mL of crude enzyme. The amount of released reducing sugar was determined using dinitrosalicylate method after reaction for 30 min at 50 °C. Carboxymethyl cellulose mixed with deionized water (0.5 mL) was used as blank. One unit of enzymatic activity was defined as production of 1 μg per minute.

2.7. Determination of H_2O_2 and $O_2^{\cdot -}$ levels

The content of super oxide anion ($O_2^{\cdot -}$) was determined according to the method of Elstner and Heupel (1976) with slight modifications. Mushroom sample (4 g) was homogenized in 36 mL of 50 mmol/L K-phosphate buffer (pH 7.8) for 30 s at 4 °C. After centrifugation for 20 min at 10,000*g* (4 °C), the supernatant (0.5 mL) were mixed with 0.5 mL of 50 mmol/L K-phosphate buffer, 1 mmol/L hydroxylamine hydrochloride and incubated at 25 °C for 60 min. Then 1 mL of 17 mmol/L p-aminophenyl sulfonic acid (in glacial acetic acid: H_2O mol/kg = 3:1) and 7 mmol/L α-naphthylamine (in glacial acetic acid: H_2O = 3:1) were added and the mixture was incubated at 25 °C for 20 min. Optical density was immediately measured at a wave length of 530 nm with M2E Multiskan Spectrum. Sodium nitrite was used for standard curve.

The content of H_2O_2 was determined as previously described by Patterson, Payne, Chen, and Graham (1984). Mushroom sample (2 g) was homogenized in 10 mL of 100% (v/v) acetone at 4 °C. After centrifugation for 15 min at 6000 rpm (4 °C), the supernatant (1 mL) was mixed with 0.1 mL of 5% (w/v) titanium sulfate and 0.2 mL of 25% (v/v) ammonia and then centrifuged for 10 min at 6000 rpm (4 °C). The pellet was dissolved in 3 mL of 10% (v/v) H_2SO_4 and centrifuged for 10 min at 6000 rpm. The absorbance of the supernatant was measured at 410 nm with M2E Multiskan Spectrum. The H_2O_2 content was calculated using H_2O_2 as a standard and expressed as mmol/kg on a fresh weight basis.

2.8. Chemicals describing

Tween-80 (CAS number, [9005-65-6], AR), glutaraldehyde (CAS number, [111-30-8], AR), *tert*-butyl alcohol (CAS number, [75-65-0], AR), chloroform (CAS number, [67-66-3], AR), 1-butanol (CAS number, [71-36-3], AR), ethanol (CAS number, [64-17-5], AR), liquid nitrogen (CAS number, [7727-37-9], AR) were all purchased from Fisher Scientific (Pittsburgh, PA, USA).

2.9. Statistical analyses

All experiments were performed in quintuplicate and all data were expressed as the mean ± SE. Analysis of variance (ANOVA) was performed using the SPSS System. To determine statistical differences, comparisons of the means between control and treatment samples were performed using Duncan's test at a significance level of $P < .05$.

3. Results and discussion

3.1. Appearance and microstructure evaluation

The appearance quality of *F. velutipes* in different packaging materials during the postharvest period was shown in Fig. 1A, B, C. Moisture condensation was more prevalent on the inner surface of Normal-PM compared to the Nano-PM. In addition, *F. velutipes* in Normal-PM exhibited some degree of browning, wilting of the whole fruit body and cap opening after 15 days of storage. However, unpackaged mushroom showed more browning in stem and cap opening (Fig. 1A, B). Water loss causes weight loss of *F. velutipes* as well. At the end of the storage period, the weight loss of *F. velutipes* in No-PM and Normal-PM was 0.71% and 0.65% respectively. By contrast, *F. velutipes* stored with Nano-PM exhibited lower weight loss at 0.53%.

In postharvest storage, tissue structure and hyphae morphology of *F. velutipes* has changed. Previous study demonstrated that SEM was used to evaluate the microstructure changes of the blueberry fruit based on vision observation (Chu, Gao, Chen, Fang, & Zheng, 2017). Thus in our research, SEM was used to evaluate the microstructure changes of *F. velutipes* based on vision observation. The transmission electron micrographs of the samples showed the microstructural changes on the surface of *F. velutipes* during 15 days of storage. Mushroom microstructure in Nano-PM was dense after 12 days of storage and only a few pores appeared on some parts at day 15. The surface of Normal-PM and No-PM mushrooms gradually showed large pores during storage with some having fractured hyphae (Fig. 1C). In a life cycle of mushroom, cap opening is a natural development phenomenon of postharvest senescence and it can be boosted by water loss during storage.

Mushrooms have a thin epidermal structure, which does not effectively prevent rapid superficial dehydration. They also have a higher respiration rate than other vegetables, which leads to a large degree of rapid water loss (Kwon, An, & Lee, 2015). In postharvest storage, the elevated water loss could accelerate senescence and also reduce the cohesive forces between water and other hydrophilic molecules such as proteins, which are responsible for maintaining the intact positions of the caps as well as the veils (Meng, Shen, Jun, & Sheng, 2010). In our previous studies, *F. velutipes* in Nano-PM had lower respiration rate during storage at 4 °C compared to control. On the other hand, the interlayer space could be filled by composite nanoparticles. The

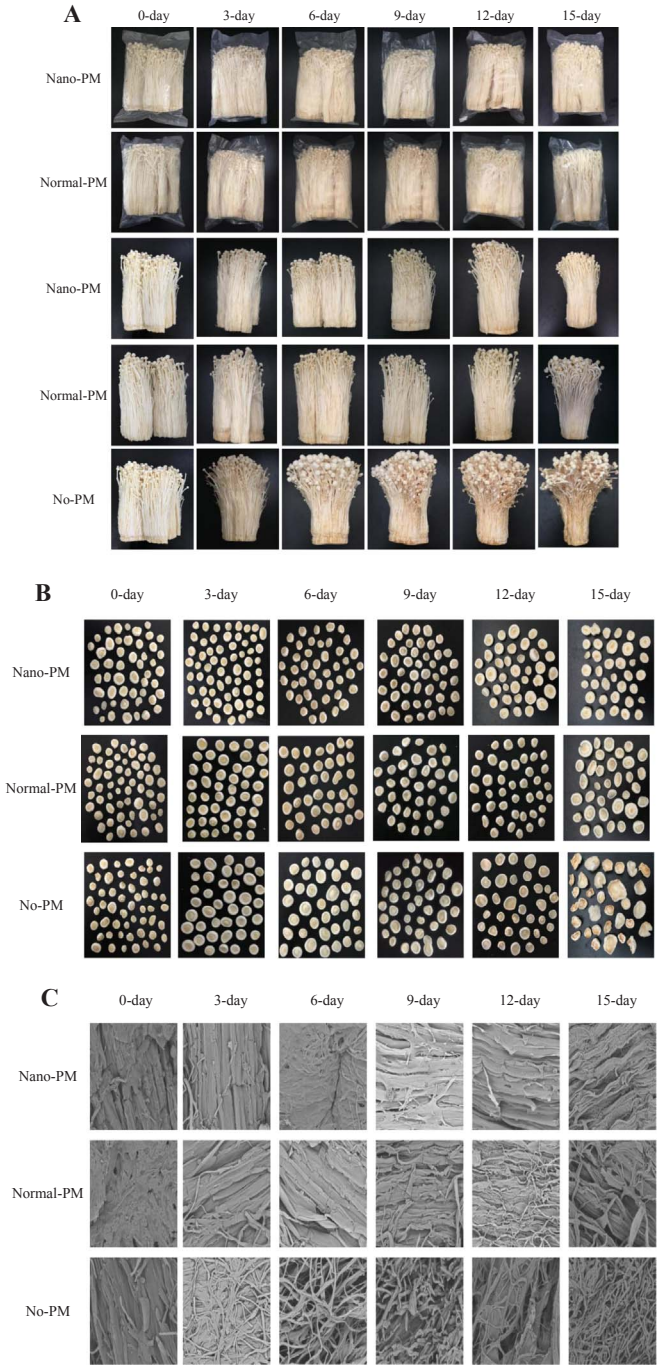

Fig. 1. Effect of different packaging materials on appearance quality and fruiting body microstructure of *F. velutipes* stored at 4 °C and 90% RH.

formation of complex network-like structure in the film resulted in the restriction on the molecular motion of the polymer chains in the matrix and facilitated the transfer of stress from the matrix to the reinforcing phase via interface. This structure led to improvement and changes in the structure of films resulting in better barrier properties against water. The package also prevented senescence and maintained the quality of appearance and enhanced commodity value (Fang, Yang, Kimatu, & Mariga, 2016).

3.2. Spore counting

Studies have shown that *F. velutipes* can produce dual-core basidiospore through sexual reproduction and mononuclear oidia through

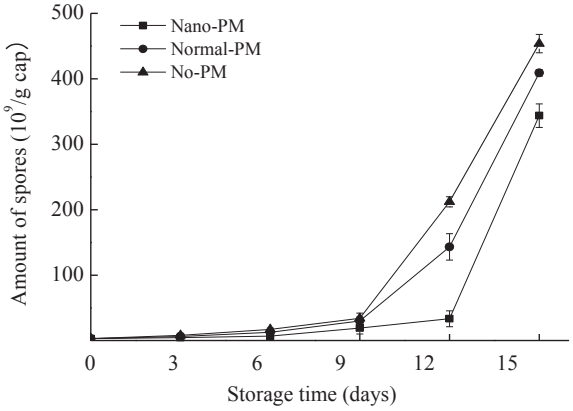

Fig. 2. Effect of different packaging materials on the amount of spores of *F. velutipes* stored at 4 °C and 90% RH.

asexual reproduction. They can both become mononuclear mycelia after germination. *F. velutipes* can fissure in hypha and produce arthrospore as well (Petersen, Hughes, Redhead, Psurtseva, & Methven, 1999). The amount of spore increases as mushroom matures and the number of spores reflects the degree of *F. velutipes* maturity. As shown in Fig. 2, the amount of spores increased steadily at the first 9 days for all samples. However, in Normal-PM and unpacked groups spores increased significantly after 9th day and eventually peaked at day 15. The spore quantity of Nano-PM group had a significant increase at the 12th day. At day 15, the amount of spores in No-PM and Normal-PM groups reached 4.84×10^{11} and 4.09×10^{11}/g cap, respectively. While the Nano-PM group had 3.44×10^{11}/g cap, which was significantly lower than the other two groups ($P < .05$). Studies have found that mycelium of mushroom can produce relatively high concentrations of ethylene during hypha growth and postharvest stage, especially when fruiting body expands (Turner, Wright, Ward, Osborne, & Self, 1975; Wood & Hammond, 1977), which accelerated postharvest senescence. In our previous study, it was found that no ethylene was detected in Nano-PM during the whole storage time, inclusion of nanoparticle could possibly absorbed the released ethylene and induced exuberant respiration rate of mushrooms (Fang, Yang, Kimatu, & Mariga, 2016). This delayed metabolic processes and senescence phenomena (Luo, Wang, Wang, & Feng, 2014) including increase of the number of spores.

3.3. Content of carbonyl, disulfide and sulfhydryl groups

Postharvest senescence leads to protein oxidation. The carbonyl, sulfhydryl and disulfide bonds contents were shown in Fig. 3A, B, C. The carbonyl and the disulfide bonds contents increased while the sulfhydryl content decreased during storage. At day 15, the carbonyl content in Nano-PM, Normal-PM and No-PM packaging increased from 0.032 mg/mL to 0.051 mg/mL, 0.081 mg/mL and 0.219 mg/mL, respectively ($P < .05$). In addition, comparing with No-PM and Normal-PM, Nano-PM treatment significantly inhibited the formation of disulfide bonds from sulfhydryl thus preserved sulfhydryl content. Moreover, the disulfide content of Nano-PM group was lower than other groups ($P < .05$). Carbonyl content is the most direct indicator of the degree of oxidation while sulfhydryl is the most sensitive protein side chain group. Along with postharvest senescence and the degree of oxidation in protein, the content of carbonyl and disulfide bonds increased, whereas the sulfhydryl content decreased. The results showed that Nano-PM has better preventive property in delaying senescence by retarding the oxidation of protein. According to previous studies, proteins were mainly oxidized by ROS such as free radicals and active lipid oxidation products in postharvest stage (Wu, Lin, Hua, & Wu, 2013). Nano-PM maintained hypoxic environment resulting in delayed senescence and inhibition of protein oxidation.

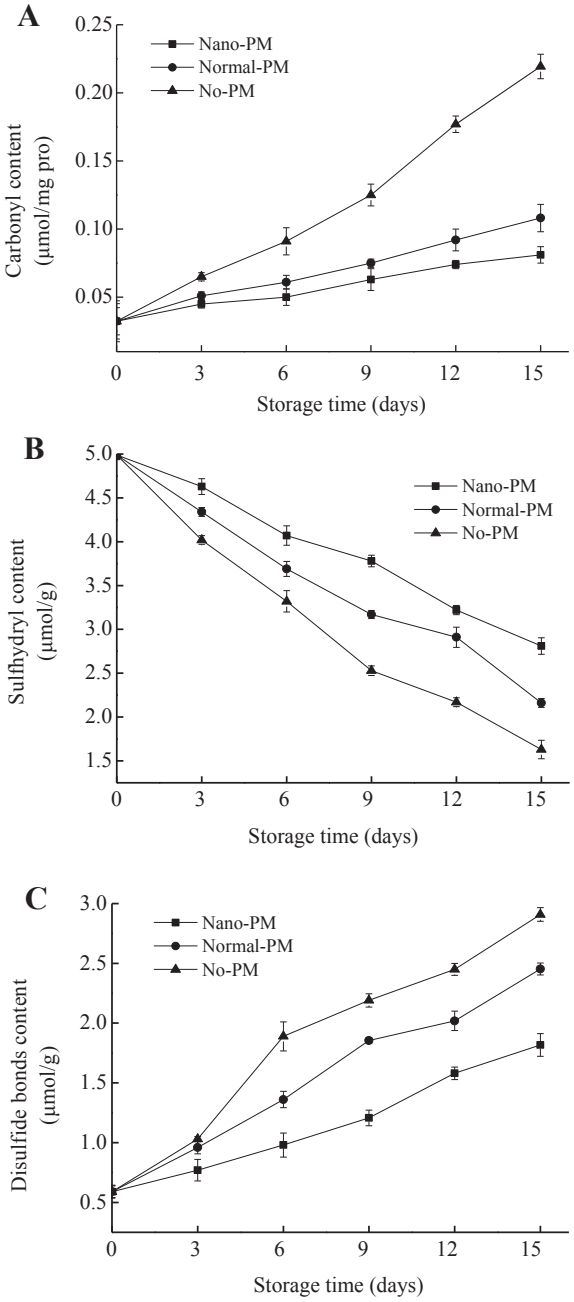

Fig. 3. Effect of different packaging materials on carbonyl content (A), sulfhydryl content (B) and disulfide bonds content (C) of *F. velutipes* stored at 4 °C and 90% RH.

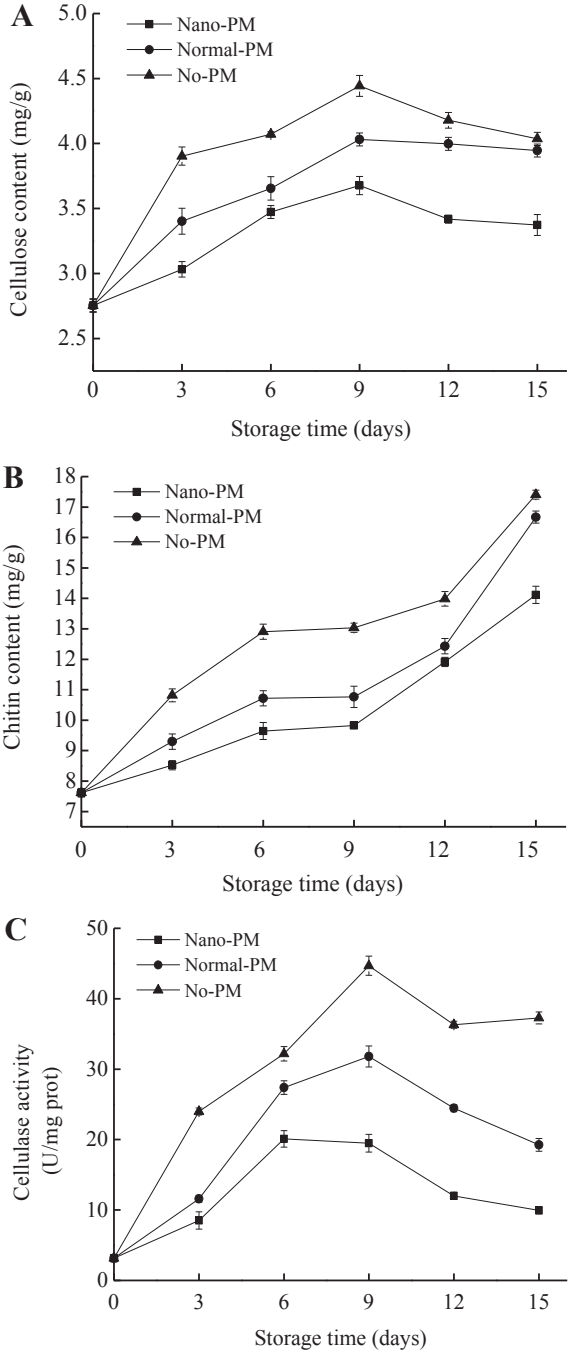

Fig. 4. Effect of different packaging materials on the cellulose content (A), chitin content (B), cellulase activity (C) of *F. velutipes* stored at 4 °C and 90% RH.

3.4. Content of cellulose and chitin and cellulase activity

Cellulose content in the cell wall of *F. velutipes* is an important marker of senescence. The cellulose content in all the groups increased to peak and then decreased, but the levels were higher than that of the initial content. In three groups, cellulose content reached the peak on the 9th day. At the 15th day, cellulose content of Nano-PM was 3.37 mg/g, which was significantly lower than that of other groups ($P < .05$). Cellulose is an important frame in cell wall composition. Its content in Nano-PM mushrooms was significantly lower than other groups (Fig. 4A). The result showed that Nano-PM retarded senescence and reduced accumulation of cellulose and metabolism rate in *F. velutipes* cell wall during storage which could delay mushroom aging.

Chitin content continuously increased during the storage process. It increased rapidly in the first six days and became steady for 3 days, then increased rapidly again for the remaining 6 days. In Nano-PM, Normal-PM and No-PM, the content of chitin was 14.1, 16.7 and 17.4 mg/g respectively. Thus, in Nano-PM chitin content was significantly lower than that in other groups (Fig. 4B). Chitin is a unbranched polymer consisting of β-1,4-*N*-acetylglucosamine as a unit and a main component in the cell wall of fungi. Chitin and cellulose constitute fibrous microfilaments in the cell wall and play a supporting role (Munro, 2013). In general, postharvest senescence causes fibrosis in tissue texture, which is mainly a combined result of the mechanical change of cell wall and the turgidity of cells (Sharma, Barman, & Siddiqui, 2016). Nano-PM maintained low respiration rate of *F. velutipes* and therefore delayed postharvest senescence, decreased the loss of moisture during storage and maintained a low degree of fibrosis in tissues.

Cellulase activity increased initially and peaked at the 6th day for Nano-PM and 9th day for both the Normal-PM and No-PM groups followed by a rapid decrease. The increase rate of cellulase activity in Normal-PM and No-PM group was higher than that of Nano-PM. The cellulase activity in Nano-PM was significantly lower than that in the other groups during the whole storage period ($P < 0.05$). In Nano-PM, Normal-PM and No-PM the activity was 9.95, 19.3 and 37.3 U/(mg protein) respectively at day 15 (Fig. 4C). Cellulase is a complex enzyme consisting of *exo*-1,4-β-D-glucanase (C1), *endo*-1,4-β-D-glucanase (CX) and β-glucosidase which can degrade cellulose into glucose synergistically. Among these enzymes, C1 can hydrolyze natural cellulose into amorphous cellulose. Amorphous cellulose can then be hydrolyzed into fiber oligosaccharides by CX, and ultimately into glucose by β-glucanase (Wei et al., 2010). Previous studies found that softening process could be delayed via the inhibition of cell wall degrading enzymes including cellulase, which confirmed that cellulase played an important role in postharvest senescence such as softening (Vicente, Costa, Martínez, Chaves, & Civello, 2005). Nano-PM effectively inhibited the senescence of mushroom by decreasing cellulose activity, delaying softening and maintaining appearance quality.

3.5. Content of H_2O_2 and $O_2^{\cdot -}$

The H_2O_2 content in each group increased during storage and peaked at day 15. In No-PM group, Normal-PM group and Nano-PM group, the content was 78.1, 56.2 and 44.4 mmol/(g protein) respectively at day 15 (Fig. 5A). Both the content and increase rate of H_2O_2 in Nano-PM group were significantly lower than those in other groups ($P < .05$). $O_2^{\cdot -}$ content had a marked increase in all groups during the first 9 days of storage. Afterwards, the $O_2^{\cdot -}$ content in Nano-PM began to be steady, while those in other groups continued to rise slowly till day 15 (Fig. 5B). Nevertheless, the content was significantly lower in Nano-PM than that in other groups. Previous studies showed that senescence led to the accumulation of ROS, which would reduce the storage quality and marketability of fruits and vegetables (Morales & Munné-Bosch, 2016). The results showed that compared with other groups, Nano-PM markedly inhibited the accumulation of H_2O_2 and $O_2^{\cdot -}$ and therefore delayed senescence during storage time.

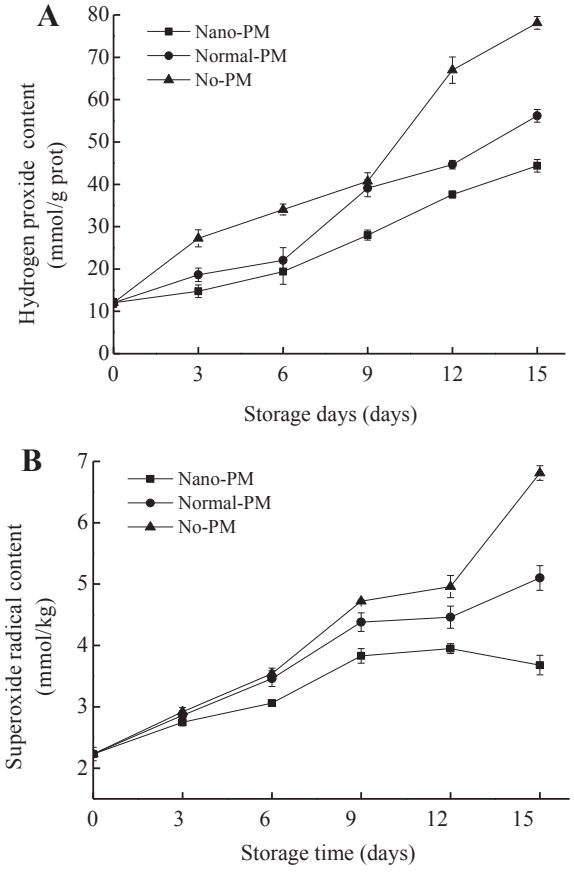

Fig. 5. Effect of different packaging materials on H_2O_2 content (A) and $O_2^{\cdot -}$ content (B) of *F. velutipes* stored at 4 °C and 90% RH.

3.6. Changes of molecular weights and protein content in FVP

FVP is an important active substance and research on its biological activity has been conducted (Yang et al., 2007; Zhang, Xiao, Deng, He, & Sun, 2012). Conversely, there are few investigations focusing on changes of FVP molecular weight during storage. In this study, we have investigated the changes of FVP molecular weight in the process of storage. HPSEC chromatograms of FVP-1 peaked between 8th and 9th min, that of FVP-2 peaked between 14th and 16th and that of FVP-3 peaked between 17th and 18th min. During storage, HPSEC chromatograms of FVP-1 in Nano-PM peaked with low intensity for the first 12 days, but peaked with high intensity at day 15 (Fig. 6A). That of FVP-1 in Normal-PM and No-PM peaked between 8th and 9th min with high intensity on day 6 and day 3 respectively (Fig. 6B and C). HPSEC chromatograms of FVP-2 in Nano-PM showed similar trend as that of FVP-1 in Nano-PM and that of FVP-2 in No-PM peaked with high intensity on day 9. These results indicated accumulation of FVP with larger molecular weight. Contrarily, HPSEC chromatograms of FVP-3 in Nano-PM peaked with low intensity on day 15 while that of FVP-3 in Normal-PM and No-PM peaked with low intensity on day 6 and day 3 respectively. The molecular weight of FVP in Nano-PM and Normal-PM gradually increased during storage though in No-PM it increased to a peak and then decreased (Fig. 6D, E and F). The molecular weight of FVP-1 in Nano-PM, Normal-PM and No-PM increased from 124 kDa at day 0 to 137, 141 and 138 kDa at day 15, respectively. The molecular weight of FVP-2 in Nano-PM, Normal-PM and No-PM increased from 20.7 kDa at day 0 to 21.0, 21.1 and 21.3 kDa at day 15, respectively. In addition, the molecular weight of FVP-3 in Nano-PM, Normal-PM and No-PM increased from 7.18 kDa at day 0 to 7.39, 7.49 and 7.56 kDa at day 15, respectively. The molecular weight of FVP-1, FVP-2 and FVP-3 in Nano-PM was significantly lower than that in other groups ($P < .05$). Polysaccharides have active hydroxyl groups associated with other intermolecular forces, which could lead to polymerization of FVP (Tolstoguzov, 2003). Senescence in postharvest may accelerate the polymerization of FVP and the increase in molecular weight. Compared with other groups, nanocomposite materials have demonstrated a capacity to inhibit the oxidation of FVP through decreased respiration rate during the 15 days of storage.

During storage, proteoglycan protein contents in other groups declined more rapidly than that in Nano-PM. There were the lowest protein levels at the end of the storage period in the three treated groups of mushroom. The protein content of proteoglycan in the Nano-PM mushrooms was 23.9 mg/g after 15 days of storage at 4 °C, which was significantly higher ($P < .05$) than the proteins in the Normal-PM (19.5 mg/g) and the No-PM (15.6 mg/g) in Fig. 6G. Proteoglycans, also known as mucin, are extremely important active constituents with large molecular weight, mainly made of carbohydrate through covalent connection of glycosaminoglycans and proteins that form a complex polymer. Studies showed that proteoglycan contains 6–20 percent protein and 80–90 percent polysaccharide chain, sometimes up to 95 percent. Proteoglycan is widely distributed in the extracellular matrix and on the surface of the cells, and has important biological functions in organisms. In previous studies, Fang, Yang, Kimatu, and Mariga (2016) studied the effect of Nano-PM on the change in soluble protein contents, which was considered a sensitive indicator for tissue destruction. Protein content changes in proteoglycan showed similar result. During senescence, some factors such as enzymatic hydrolysis led to decreased protein content which may also result in the loss of protein in

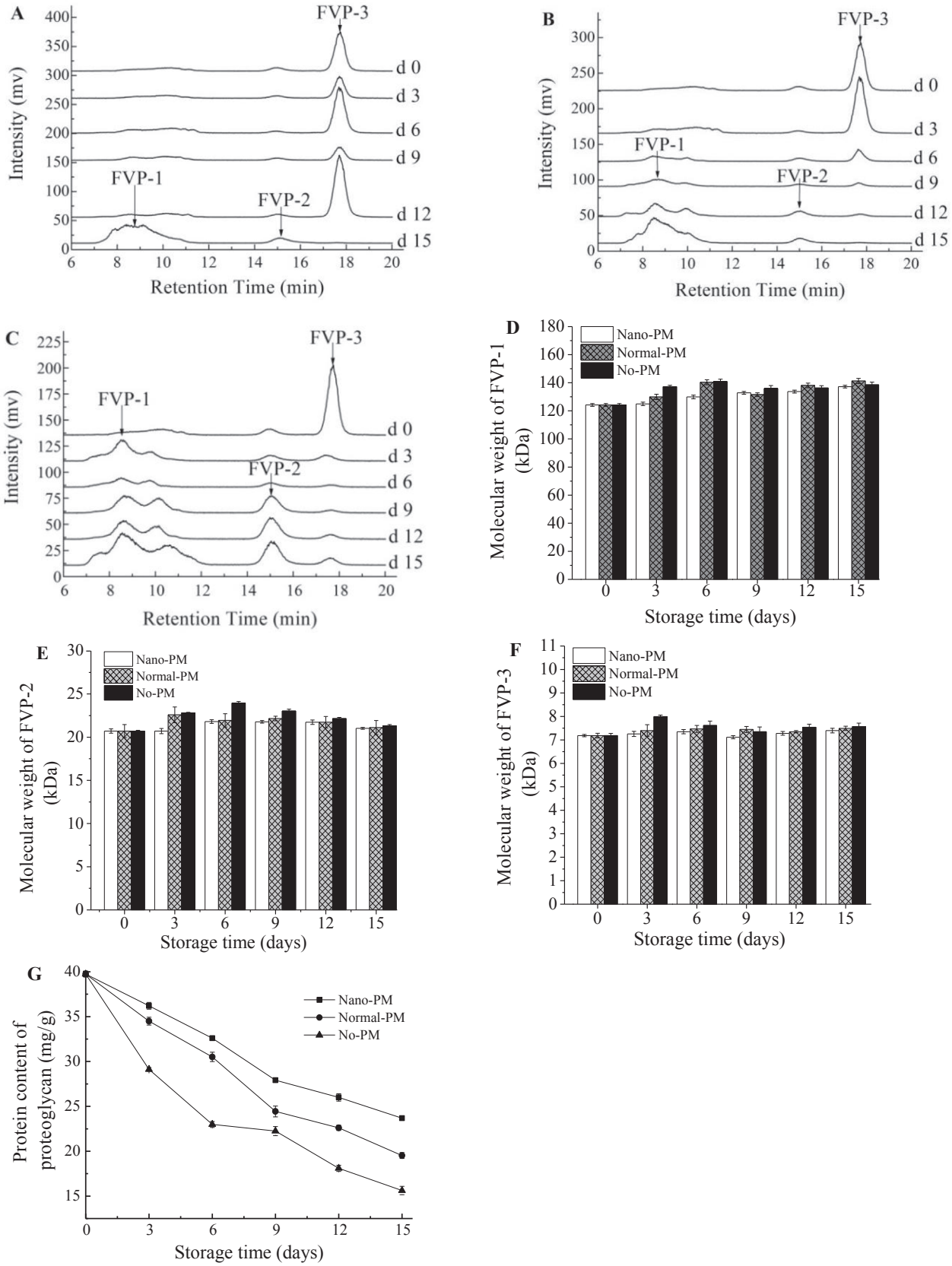

Fig. 6. Effect of different packaging materials on changes of molecular weight of FVP and protein content of proteoglycan of *F. velutipes* stored at 4 °C and 90% RH. (A) Nano-PM, (B) Normal-PM, (C) No-PM, (D) FVP-1, (E) FVP-2, (F) FVP-3, (G) protein content of proteoglycan.

proteoglycan. In addition, the loss of protein in proteoglycan may help explain the change in molecular weight of FVP, which decreased after it reached the peak, but the mechanism still need further exploration. In general, Nano-PM decreased the loss of protein content in proteoglycan, which indicated inhibition of senescence and superior preservation of proteins.

4. Conclusion

In this study, nano-Ag, nano-TiO_2, nano-SiO_2, and attapulgite were added to a polyethylene packaging material to inhibit the senescence of *F. velutipes* and extend shelf life. Our results have demonstrated that as a storage material of *F. velutipes*, Nano-PM had advantages over Normal-PM and No-PM, i.e., less negative changes in appearance quality such as browning, wilting and cap opening as a result of low respiration rate. The quantity of spores in Nano-PM was significantly lower than that in other groups ($P < .05$), which indicated a senescence inhibition and a maturity rate reduction. The degree of fibrosis in cell wall and tissue, cellulase activity, protein oxidation and levels of ROS of *F. velutipes* in Nano-PM were lower than those in other groups. Besides, mushrooms treated in Nano-PM effectively decreased the accumulation of FVP with large molecular weight as well as retarding the loss of protein content in proteoglycan. For safety of Nano-PM, our previous study showed that no nano-Ag particles was observed in the *F. velutipes*, indicating of no migration of nano-Ag particles from packaging film into *F. velutipes* at 4 °C after 14 days of storage (Fang, Yang, Kimatu, & Mariga, 2016). In summary, Nano-PM have advantages in inhibiting senescence in *F. velutipes*. Nano-PM provided an attractive alternative to maintain the quality of stored mushrooms by delaying senescence during postharvest period, good appearance quality, nutritional value, prolong the shelf life and has a wide commercial application prospect.

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Conflict of interest

There are no conflicts of interests to declare.

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