Food Packaging and Shelf Life 22 (2019) 100412

Contents lists available at ScienceDirect

Food Packaging and Shelf Life

journal homepage: www.elsevier.com/locate/fpsl

Storage quality and flavor evaluation of *Volvariella volvacea* packaged with nanocomposite-based packaging material during commercial storage condition

Fang Donglu[a], Yu Kelin[a], Deng Zilong[b], Hu Qiuhui[a], Zhao Liyan[a,*]

[a] College of Food Science and Technology, Nanjing Agricultural University, Nanjing, Jiangsu 210095, PR China
[b] State Key Laboratory Pollution Control, School of Environmental Science and Engineering, Tongji University, Shanghai, 200092, PR China

ARTICLE INFO

Keywords:
Nanocomposite-based packaging
Storage
Volvariella volvacea
Preservation
Flavor

ABSTRACT

This research was designed to demonstrate the effectiveness of nanocomposite-based packaging (NP) treatment for straw mushrooms (*Volvariella volvacea*). Without incorporating nanoparticles, *V. volvacea* was packaged with conventional polyethylene packaging (CP) material as a control. The respiratory rate, total soluble solids (TSS), firmness, colour, reactive oxygen species (ROS) and flavor properties of *V. volvacea* were investigated. The results showed that the browning, softening and respiration rate of *V. volvacea* were significantly inhibited by NP treatment. Meanwhile, NP treatment retained relatively higher TSS contents and volatile and non-volatile flavor compounds, indicating the extension of the appearance and quality of *V. volvacea*. In addition, NP significantly slowed the alteration of ROS including superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), as well as malondialdehyde (MDA), compared to the control ($P < 0.05$). These findings suggest that NP could be potentially applied to improve the postharvest storability and extend the storage shelf life of *V. volvacea*.

1. Introduction

Straw mushroom (*Volvariella volvacea*) is widely grown in most parts of Asia, especially China. Its unique flavor, high nutritional value, and medicinal properties lead to its high demand (Liu, Zhang, Lin, & Guo, 2011; Mau, Chyau, Li, & Tseng, 1997). Like other commercial mushrooms, *V. volvacea* may suffer water loss, browning, physical damage and microbiotic invasion due to its high moisture content of fruiting body without the presence of cuticles, leading to its highly perishable characteristics during storage, transportation and shelf life (Aguirre, Frias, Barry-Ryan, & Grogan, 2008). As a result, the postharvest quality deterioration of *V. volvacea* severely decreases the value of *V. volvacea* and restricts its industrial application. Therefore, it is urgent to find an effective technique to preserve *V. volvacea*.

V. volvacea is a typical tropical edible fungus which is sensitive to low temperatures, resulting in a very short shelf life after harvest (Jamjumroon et al., 2012). Thus, conventional methods such as cold storage and modified atmosphere packaging (MAP) are not applicable for *V. volvacea* preservation. Li, Kimatu, Li et al. (2017) and Li, Chen, Cui et al. (2017) developed a new combined method by using 10 min ultrasound treatment together with 95% relative humidity (RH) storage, which prolonged the postharvest quality of *V. volvacea* from 24 h or 48 h to 72 h. Dhalsamant, Dash, Bal, and Panda (2015) modified the MAP method and demonstrated the feasibility of perforation-mediated packaging to extend the shelf life of *V. volvacea*. Similar results were found in fresh fruit preservation that a proper perforating method could slow the physiological activities of the fruiting body and improve its storage stability (Cliff, Toivonen, Forney, Liu, & Lu, 2010; Kartal, Aday, & Caner, 2012).

Particles in nanophase materials possess nanometer size and high aspect ratio. As a result, they exhibit strong adsorption and oxidizing abilities (Hu, Fang, Yang, Ma, & Zhao, 2011). On the other hand, the nanophase particles together with matrix readily form complex network-like structures and restrict molecular motion (Voon, Bhat, Easa, Liong, & Karim, 2012). Therefore, nanophase materials provide not only excellent mechanical and barrier properties but also antimicrobial property, respiration inhibition and free radical scavenging ability as packaging for the food industry (Bumbudsanpharoke & Ko, 2015; He & Hwang, 2016). In our prior studies, a new type of polyethylene (PE)-based packaging material filled with nanosilver, nanotitanium dioxide and nanosilicon dioxide was developed and applied on fresh mushroom (*Flammulina velutipes*) preservation (Fang, Yang, Kimatu, Mariga et al.,

* Corresponding author.
E-mail addresses: fangdonglu@njau.edu.cn (F. Donglu), 2014108010@njau.edu.cn (Y. Kelin), zilongdeng@tongji.edu.cn (D. Zilong), qiuhuihu@njau.edu.cn (H. Qiuhui), zhlychen@njau.edu.cn (Z. Liyan).

https://doi.org/10.1016/j.fpsl.2019.100412
Received 6 January 2019; Received in revised form 2 September 2019; Accepted 26 September 2019
Available online 04 October 2019

2016). The results showed that *F. velutipes* packaged with nanocomposite-based packaging (NP) material retained optimal external and internal quality and maintained high nutrient levels after 21-day storage at 4 °C compared to those with conventional PE packaging (CP) materials. However, the effect of NP treatment on postharvest quality control of *V. volvacea* and its possible mechanisms remain unknown.

Therefore, it was hypothesized that the NP treatment could inhibit the browning, respiration rate and reactive oxygen species (ROS) accumulation, maintaining the texture and flavor characteristics and leading to a longer shelf life of *V. volvacea*. The objectives of this study were to: 1) evaluate the effect of NP on the physicochemical characteristics and flavor changes of *V. volvacea* during storage; 2) analyse the ROS metabolism, providing insight for future research.

2. Materials and methods

2.1. The preparation of packaging materials

The NP film material was prepared by incorporating three basic nanopowders, nanosilver, nanotitanium dioxide and nanosilicon dioxide, with polyethylene (PE) matrix by reference to our prior study (Fang, Yang, Kimatu, Mariga et al., 2016). The detailed formula of prepared film was shown in Suppl. Fig S1. The films with 40 μm thickness were processed into 25 × 25 cm^2 bags using a microcomputer-controlled high speed bag making machine (SDD-A500/1200, Zhaoyuan Packaging Machiner Co., Shandong, China). The conventional PE packaging (CP) material as control was made using PE matrix without any nanopowder. The physical properties of two films were provided in Suppl. Table S1. To achieve consistent perforation, two perforations were made on each side of the bags with a hole puncher. The distance between two perforations was 8 mm. The diameter of each perforation was 6 mm, based on our preliminary experiment (data not shown).

2.2. Mushroom and storage condition

V. volvace strain V-93 was purchased from a commercial mushroom company (Jiangsu Jiangnan Biological Technology Co., LTD, China). Fresh *V. volvace* was transported from logistics centre to the laboratory in a very short time. The fresh mushrooms were chosen for the experiment based on their colour, shape, developmental stage of fruiting bodies and lack of mechanical damage and rot. After equilibration at 15 ± 1 °C and 85 ± 5% relative humidity (RH) for 1 h, the selected *V. volvace* was randomized and packaged in the NP and CP (18 bags, 135 g/bag) and the packages were sealed with a heat sealer (SF-200, Liangyungang Microwave Electric Appliance Factory, Jiangsu, China). The *V. volvace* packed with NP and CP were stored at 15 ± 1 °C and 85 ± 5% RH for 6 days.

2.3. Colour change measurement

Colour changes of mushrooms were measured with Hunter's colorimeter (Minolta CR-200, Minolta, Osaka, Japan). The mushroom sample was cut in half along the central axis and the measurement was taken in the middle of the cross section. The whiteness of samples was expressed as L value. Nine measurements were carried out for each treatment daily.

2.4. Firmness and total soluble solid (TSS) assay

Firmness of mushroom samples was analysed with a texture analyser (TA-XT Plus, Stable Micro Systems Co., England) using a 5 mm diameter cylinder probe. *V. volvace* samples were penetrated to a depth of 5 mm. The experimental parameters of speed were 1.5 mms^{-1}, 1.5 mms^{-1} and 10.0 mms^{-1} for pre-test, test and post-test. Firmness was expressed as the maximum force (in grams) in the force vs. time curves. Nine measurements were carried out for each treatment.

For TSS measurement, the mushroom samples were grinded and squeezed in a mortar. The extracted juice was measured for TSS contents at 25 °C by using a refractometer (TZ-62, Optical Instrument Factory Ltd., Guangzhou, China).

2.5. Respiration rate measurement

Three mushrooms from each treatment were selected and placed individually in a 250 mL conical flask. They were then sealed for 1 h to analyse the internal O_2 and CO_2 concentration using a gas analyser (SCY-2A, Xinrui Instrument Co., Shanghai, China). Respiration rate was expressed as CO_2 production in mg CO_2/kg/h.

2.6. Superoxide anion ($O_2^{\bullet -}$) generation rate, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content measurements

The $O_2^{\bullet -}$ generation rate and H_2O_2 contents were determined using A052 inhibition and produced superoxide anion assay kit and A064 hydrogen peroxide assay kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

MDA content was measured according to Hu et al. (2015) with some slight modifications. One *V. volvace* sample (1 g) from each treatment was homogenized and extracted in 8 mL of 10% trichloroacetic acid. Then, the mixture was centrifuged at 5000 × g for 20 min. Two millilitres mushroom extract were added with the same amount (2 mL) of 0.67% thiobarbituric acid. The mixture was then incubated in boiling water for 15 min, then cooled immediately in ice water. The aqueous phase was measured using a spectrophotometer (JH-722, Jinghua Technology and Instrument Co., Shanghai, China) at 450 nm, 532 nm and 600 nm, respectively. MDA content was calculated with the following formula:

$$MDA = \frac{V \times [6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}]}{V_0 \times m}$$

where V is the total volume of mushroom extract (mL); V_0 is the volume of the extract aqueous phase used in the analysis, and m is the mass of mushroom samples (g).

2.7. Microstructure observation

A transmission electron microscope (TEM) was applied in order to observe the structure of *V. volvace* cells after six days of storage at 15 °C. The mushroom segments were immersed into 2.5% glutaraldehyde with 0.1 mol/L phosphate buffered saline (PBS, pH 7.4) overnight at 4 °C and post-fixed with 1% osmium tetroxide for 2–3 hours. After rinsing with PBS, the samples were immersed in 10% gradient series of 50–100% ethanol and propylene oxide and gradually dehydrated. The samples were implanted in Quetol-651 resin. The blocks were sliced into ultrathin sections using a microtome, and the sections were stained using saturated uranyl acetate and lead citrate (Suzuki, Kimura, Takahashi, & Terai, 2005). The ultrastructure of the tissues in *V. volvace* was then observed under a transmission electron microscope (H-7650, Hitachi High Technologies Co., Tokyo, Japan) at an accelerating voltage of 80 kV.

2.8. Volatile flavor compounds measurement

The volatile compounds in *V. volvace* were analysed by combining headspace solid phase micro-extraction (HS-SPME) with gas chromatography-mass spectrometry (GC–MS) (Li, Kimatu, Li et al., 2017; Li, Chen, Cui et al., 2017). Two grams of mushroom samples were selected and transferred into the headspace solid-phase bottles and then sealed immediately using blue PTFE/white silicone septa (Supelco, Bellefonte, PA, USA). To extract and obtain the volatile compound, a 30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre

was applied. Before analysis, the HS-SPME fibre was preconditioned at 250 °C for 60 min to remove impurities. The fibre was then inserted into the sample vial and exposed to the headspace at 60 °C for 40 min to collect the volatile compounds. The distance between fibre tip and top of samples remained at 1–2 cm. After adsorption, the fibre was then inserted to the injection port of the GC–MS apparatus to analyse the volatile compounds.

2.9. Non-volatile flavor compounds measurement

2.9.1. Organic acid assay

Organic acids were extracted referring to the method of Li et al. (2014) with some modifications. Two grams of mushroom samples were weighed and mixed with 10 mL of 0.01 mol/L KH_2PO_4 (pH 2.86) solution. Then, the mixture was ground sufficiently and extracted at 45 °C for 30 min using an ultrasonic extraction apparatus (KQ-250DB, Jiangsu, China) and centrifuged at 5000 × *g* for 15 min. The supernatant was collected for further analysis after filtration through a 0.45 μm micro-pore filter membrane. The Zorbax Eclipse XDB C18 column (250 × 4.6 mm, 5 μm, Agilent) with the Agilent 1200 HPLC system (Agilent Technologies, Palo Alto, CA, USA) was applied. Twenty-two microliters of filtered samples were injected into HPLC. KH_2PO_4 (0.01 mol/L, pH 2.86)/methanol (95/5, v/v) was used as the mobile phase at a flow rate of 0.5 mL/min. The wavelength of the UV detector was 210 nm. An authentic standard (Yuanye Bio-Technology Co., Ltd., Shanghai, China) was used to identify each organic acid. The content of organic acids was quantified by using a calibration curve based on the external standards.

2.9.2. Soluble sugar assay

The soluble sugars were extracted and tested referring to the method of Tsai, Tsai, and Mau (2008). Six grams of mushroom samples were extracted using 50 mL of 80% aqueous ethanol and then stirred for 40 min. The residual material was obtained after centrifugation at 5000 × *g* for 15 min and conducting the extraction procedure three times. The supernatants were collected and concentrated by a rotary evaporator (EYELA OIL BATH OSB-2000, Shanghai, China). The concentrated solution was re-dissolved using 75% acetonitrile solution to ten millilitres final volume. Every extract sample was analysed after filtration through a 0.45 μm microfiltration membrane. A reference compound (Yuanye Bio-Technology Co., Ltd., Shanghai, China) and calibration curve were used to confirm and quantify.

The soluble sugars and polyols of *V. volvace* were analysed based on an Agilent 1200 HPLC system (Agilent Technologies, Palo Alto, CA, USA) loaded with Alltech 3300 evaporative light scattering detectors (ELSD, Alltech Associates Inc., Deerfield, IL, USA). A Sugar-D column (4.6 mm × 250 mm, 5 μm; Nacalai Tesque Inc., Kyoto, Japan) was applied. A mixed deionized water and LC-grade acetonitrile solution (25:75, v/v) was used as mobile phase at a flow rate of 0.8 mL/min. Twenty-two microliters of sample was injected and the working condition of ELSD was 55 °C with nitrogen as the carrier gas.

2.9.3. 5′-nucleotides assay

The contents of 5′-nucleotides were tested according to Li et al. (2018). *V. volvace* samples were mixed with 20 mL distilled water and ground. Then, the extract was boiled for 1 min. After cooling, the samples were centrifuged at 5000 × g for 15 min. The residue was re-extracted thrice following the same procedure. The supernatants were then collected, concentrated and re-dissolved using distilled water to a final volume of ten millilitres. After filtration with a 0.45 μm micro-pore filter membrane, the filtrate was tested by HPLC.

The settings and operation of the Agilent 1200 HPLC analysis system for testing 5′-nucleotides were identical to the procedure of organic acids analysis. The distilled water/methanol/acetic acid/tetrabutylammonium hydroxide (89.35/10/0.6/0.05, %) solution was treated as mobile phase and its flow rate was 0.6 mL/min based on a Zorbax Eclipse XDB C18 column (250 × 4.6 mm, 5 μm, Agilent). Twenty-two microliters of samples were injected into the HPLC and identified by the internal standard 5′-nucleotide (Yuanye Bio-Technology Co., Ltd., Shanghai, China).

2.9.4. Free amino acids assay

The free amino acids of *V. volvace* were extracted using a trichloroacetic acid (TCA) method according to the method of Fang, Yang, Kimatu et al. (2017) and Fang, Yang, Deng et al. (2017). The extracted solutions of different treatments were then analysed by an automatic amino acid analyser (L 8800, Hitachi Ltd., Japan).

2.10. Statistical analyses

All statistical analyses in this research were performed using SPSS 20.0 (SPSS Inc.). The data were expressed as the mean values ± standard deviation (SD). Significant differences between the means were determined by one-way analysis of variance (ANOVA) and Duncan's multiple comparison tests at a confidence level of $P < 0.05$.

3. Results

3.1. Sensory and microstructure evaluation

The mushrooms kept in CP exhibited severe external and internal browning at the end of storage. It was also observed that less water vapor condensation took place on the inner surface of NP, probably due to the inhibited mushroom spoilage and weakened respiration (Fig. 1). Thus, it was illustrated from the overall appearance that NP treatment could slow down the respiration, minimize browning and decrease the potential microbial issues.

The transmission electron micrographs illustrated that the microstructures of mushroom cells between the two treatments were significantly different from each other after six days of storage. The mushroom cells in NP-packed samples were more rounded and integrated compared to those of CP treatment (Fig. 1). Moreover, the cell walls of CP-packed *V. volvace* became thinner after storage, and cell membrane breakage was more severe. In addition, the intercellular space of CP-packed mushroom cells increased compared to that of NP treatment, and the mitochondria disintegrated – resulting in a vacuous scene inside the cell.

3.2. Firmness, TSS, respiration rate and colour

The firmness reductions of *V. volvacea* during storage are shown in Fig. 2A. In the first three days, there was no significant ($P > 0.05$) difference in firmness between mushrooms packed with NP and CP. However, *V. volvacea* in NP showed significantly ($P < 0.05$) higher firmness than that in CP treatment in last three days of storage. The firmness value of *V. volvacea* in NP was 517.2 g, which was 9.3% higher than that in CP (473.1 g) at the end of storage.

As shown in Fig. 2B, the TSS of mushrooms packed with NP and CP decreased along with the storage duration. The lowest TSS level (4.93%) was recorded in the CP group at the end of storage. In general, NP retained the TSS of the mushrooms in comparison with that of CP at all sampling times. More specifically, the TSS content of mushrooms packed with NP on the fifth day of storage was significantly ($P < 0.05$) higher than that of CP.

For the respiration rate in Fig. 2C, mushrooms treated with NP increased slowly in the first three days, and then fluctuated in the following days. *V. volvacea* stored in CP showed high physiological activity, which was demonstrated with a sharp increase in the respiration rate on the first day. After four days of storage, the respiration rates of mushrooms in NP and CP reached 963.87 mg CO_2/kg/h and 1008.41 mg CO_2/kg/h, respectively, indicating a slightly lower respiration rate with NP treatment.

Fig. 1. Effect of nanocomposite-based packaging (NP) and conventional packaging (CP) treatments on sensory quality and cell ultrastructure of *V. volvacea* after 6 days of storage.

In Fig. 2D, both mushroom samples showed a similar continuous reduction trend regarding lightness. The L value of *V. volvacea* treated with CP exhibited a more rapidly decreasing trend than that of NP during the entire storage time. For NP, the L values were significantly higher ($P < 0.05$) than those of CP packed mushrooms on the third, fifth and sixth days.

3.3. $O_2^{\bullet -}$ generation rate, H_2O_2 and MDA content

The $O_2^{\bullet -}$ generation rate increased during the storage time both in NP and CP treatments (Fig. 2E). Between the two treatments, NP slowed down the elevation of $O_2^{\bullet -}$ generation rate during the storage period. There were significant differences ($P < 0.05$) between NP and CP on the third, fourth and sixth days. The H_2O_2 contents in all treated mushrooms increased in the initial three days and then decreased on the fourth day (Fig. 2F). The H_2O_2 contents of NP and CP treated mushrooms on the 3rd day were 52.67 mmol/kg and 56.75 mmol/kg, respectively. At the end of storage, the H_2O_2 content of *V. volvacea* in NP was significantly ($P < 0.05$) lower than that in CP.

As the major product of membrane lipid peroxidation, MDA content continuously increased for both treatments throughout the storage period (Fig. 2G). Compared to the CP, the NP-packaged *V. volvacea* maintained overall higher MDA content. Significant ($P < 0.05$) differences were observed between NP and CP groups in MDA content of *V. volvacea* on the first day and third day.

3.4. Volatile flavor compounds

Sixty-seven volatile compounds were identified and quantified in *V. volvacea*, including 6 alcohols, 4 aldehydes, 6 ketones, 11 esters, 23 alkanes and 17 other compounds to evaluate the flavor production of *V. volvacea* with different treatments (Suppl. Table S2). 23, 25, 30, 38 and 39 kinds of volatile compounds were identified from *V. volvacea* at 0 d and 3 d with NP, 3 d with CP, 6 d with NP and 6 d with CP, respectively. Ketones and aldehydes with fruity essences and hyacinth were the dominant volatile compounds in fresh *V. volvacea*, and 50.08% and 40.63% of these were 3-octanone and benzeneacetaldehyde, respectively. The relative contents of alcohols and aldehydes decreased while ketones increased for both treatments. At the end of storage, the NP-treated *V. volvacea* showed relatively higher contents of alcohols and aldehydes compared to control. In Table S2, phenylethyl alcohol and 1-nonanol, described as floral rose and orange aromas, were detected in NP treatment but not in control. Similarly, 2-nonanone, providing fresh, sweet and green flavor (Xu et al., 2019), hadn't been found in control

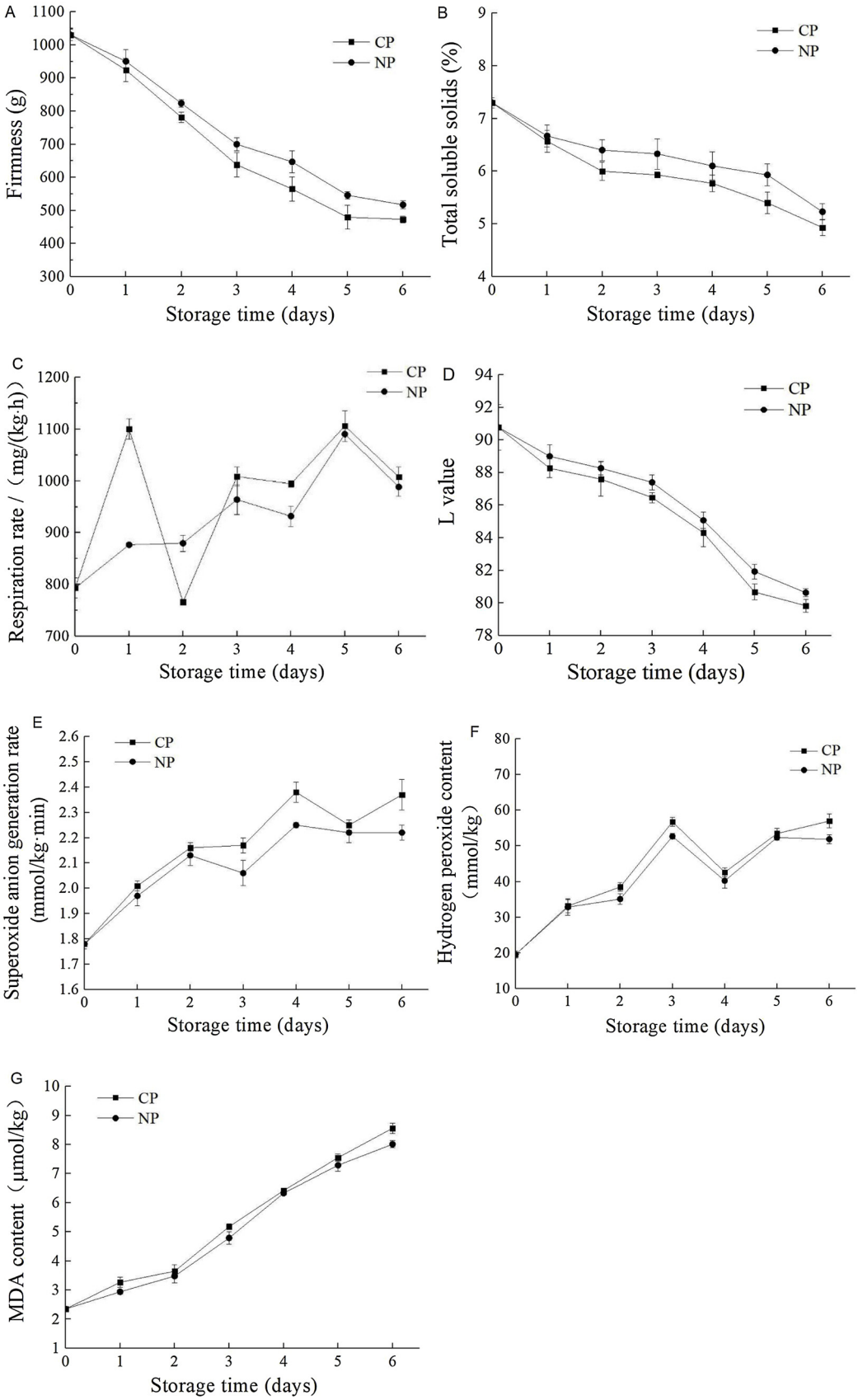

Fig. 2. Effect of nanocomposite-based packaging (NP) and conventional packaging (CP) treatments on firmness, total soluble solids, respiration rate, color, MDA and reactive oxygen species ($O_2^{\cdot -}$ and H_2O_2) of *V. volvacea* during storage. Each data point is the mean of three replicate samples.

Table 1
Organic acid changes of *Volvariella volvacea* with different packaging treatments during 6 days of storage.*

Types of Organic acid	Organic acid contents (mg/g DW)				
	Fresh samples	NP_3	CP_3	NP_6	CP_6
Malic acid	24.87 ± 0.46^{c}	32.31 ± 0.83^{a}	34.27 ± 1.55^{a}	25.42 ± 0.18^{c}	28.43 ± 0.46^{b}
Acetic acid	9.08 ± 0.62^{b}	7.15 ± 1.71^{c}	7.39 ± 1.79^{c}	11.00 ± 0.83^{a}	10.73 ± 0.57^{a}
Citric acid	9.00 ± 0.57^{a}	8.45 ± 0.30^{ab}	7.87 ± 0.09^{b}	7.55 ± 0.40^{b}	9.43 ± 0.62^{a}
Fumaric acid	3.38 ± 0.05^{c}	3.59 ± 0.04^{b}	3.49 ± 0.15^{b}	4.94 ± 0.10^{a}	5.08 ± 0.19^{a}
Succinic acid	194.83 ± 2.57^{a}	115.28 ± 1.67^{b}	113.04 ± 2.15^{b}	73.16 ± 1.08^{c}	65.41 ± 1.32^{d}
Total	241.16 ± 2.48^{a}	174.12 ± 3.64^{b}	166.05 ± 4.25^{b}	122.08 ± 0.80^{c}	119.08 ± 1.48^{d}

* Each value was expressed as the mean ± standard deviation. Means with different lower case letters within a row indicate significant differences ($P < 0.05$). DW: dry weight; NP_3: Organic acid contents of *Volvariella volvacea* treated with nanocomposite packaging (NP) on the third days of storage; CP_3: Organic acid contents of *Volvariella volvacea* treated with conventional polyethylene packaging (CP) on the third days of storage; NP_6: Organic acid contents of *Volvariella volvacea* treated with NP on the sixth days of storage; CP_6: Organic acid contents of *Volvariella volvacea* treated with CP on the sixth days of storage.

group. Moreover, an unpleasant flavor named cyclohexanol, a smell of camphor (Vera, Canellas, & Nerin, 2013), was only detected in 6 days stored *V. volvacea* packed with CP.

3.5. Non-volatile flavor compounds

Organic acids are strongly connected with the synthesis and metabolism of amino acids, phenols, esters and aromatic compounds (McMahon et al., 2014). In Table 1, succinic acid was indicated as the major organic acid (194.83 mg/g DW) in fresh *V. volvacea*, followed by malic acid (24.87 mg/g DW), acetic acid (9.08 mg/g DW), citric acid (9.00 mg/g DW) and fumaric acid (3.38 mg/g DW). The total organic acid of *V. volvacea* was significantly ($P < 0.05$) decreased from 241.16 mg/g DW (fresh mushrooms) to 166.05 mg/g DW in the CP group after three days of storage. After six days of storage, the total organic acid content of NP-treated *V. volvacea* was 122.08 mg/g DW, which was significantly ($P < 0.05$) higher than that of control.

For soluble sugars in Table 2, fresh *V. volvacea* contained high amounts of trehalose (337.83 mg/g DW). Mushrooms in both NP and CP treatments showed a decreasing trend in the contents of mannitol and trehalose throughout the entire period. After six days of storage, the trehalose content of NP-treated mushrooms was 79.34 mg/g DW, which was significantly ($P < 0.05$) higher than that of CP-packed mushrooms (75.16 mg/g DW).

In Table 3, the 5′-nucleotide content of fresh *V. volvacea* was 4.74 mg/g DW, which was significantly ($P < 0.05$) lower than other treated samples. 5′-GMP was the predominant flavor nucleotide in *V. volvacea*. After six days of storage, the NP-treated mushrooms maintained significantly higher contents of total 5′-nucleotide compared to that of the CP-treated mushrooms. The contents of flavor 5′-nucleotides including 5′-GMP and 5′-UMP in control were 17.1% and 38.7% lower than those of NP, respectively.

Edible mushrooms offer strong umami and pleasant sweet flavors owing to the presence of free amino acids (FAAs) (Li et al., 2014). In Table 4, the initial amount of total FAAs in fresh *V. volvacea* was 179.37 mg/g DW. A continuously decreasing trend was observed during the storage period for all treatments. Phenylalanine was the predominant FAA in fresh *V. volvacea*, accounting for 32% of total FAAs. After six days of storage, threonine became the main FAA both in NP- and CP-treated mushrooms. Moreover, NP-packed *V. volvacea* maintained higher FAA contents than control groups.

4. Discussion

Fresh edible fungus is highly perishable after harvest, and the conventional MAP technology under low temperature (4 °C) is effective for most types of mushrooms except *V. volvacea* (Duan, Pang, & Zhang, 2000). When exposed to low temperature (4 °C), the fruiting bodies of *V. volvacea* are generally softened, liquefied and readily rot. Our preliminary study showed that 15 °C was the preferred temperature for *V. volvacea* preservation. The respiration and metabolism of *V. volvacea* increase dramatically under 15 °C, leading to oxygen starvation inside the package (Dhalsamant et al., 2015). Additionally, it was demonstrated that NP treatment was effective for preservation of other mushrooms including *F. velutipes* (Fang, Yang, Kimatu, Mariga et al., 2016). Therefore, the NP combined with perforation was developed to extend the shelf life of *V. volvacea*. It was shown that treatment with the NP prolonged the retention of nutrient substances of *V. volvacea* and inhibited browning and ROS accumulation, indicating that NP slowed down the metabolism in comparison with the CP control during storage.

Nanocomposite-based packaging technology could be regarded as a kind of active packaging alternatives and regulated the gas composition including O_2, CO_2 and ethylene inside the package, resulting in lower respiration rate and slower metabolic processes (Shi et al., 2018). During the preparation, nanoparticles embedded in the PE matrix and their interaction formed compact network structures, strengthening mechanical and barrier properties (Fang, Yang, Kimatu, Mariga et al., 2016; Poisson et al., 2008). On the other hand, the formation of complex network-like structure in the film resulted in the restriction on the molecular motion of polymer chains in the matrix and facilitated the

Table 2
Soluble sugar (polyols) changes of *Volvariella volvacea* with different packaging treatments during 6 days of storage.*

Types of soluble sugar (polyols)	Soluble sugar (polyols) contents (mg/g DW)				
	Fresh samples	NP_3	CP_3	NP_6	CP_6
Mannitol	8.80 ± 0.04^{a}	7.48 ± 0.15^{b}	7.15 ± 0.09^{b}	6.43 ± 0.02^{c}	6.25 ± 0.03^{d}
Trehalose	337.83 ± 2.03^{a}	145.71 ± 0.72^{b}	141.76 ± 0.49^{c}	79.34 ± 0.48^{d}	75.16 ± 0.61^{e}
Total	346.63 ± 2.04^{a}	153.19 ± 0.58^{b}	148.91 ± 0.48^{c}	85.78 ± 0.49^{d}	81.40 ± 0.61^{e}

* Each value was expressed as the mean ± standard deviation. Means with different lower case letters within a row indicate significant differences ($P < 0.05$). DW: dry weight; NP_3: Soluble sugar (polyols) contents of *Volvariella volvacea* treated with nanocomposite packaging (NP) on the third days of storage; CP_3: Soluble sugar (polyols) contents of *Volvariella volvacea* treated with conventional polyethylene packaging (CP) on the third days of storage; NP_6: Soluble sugar (polyols) contents of *Volvariella volvacea* treated with NP on the sixth days of storage; CP_6: Soluble sugar (polyols) contents of *Volvariella volvacea* treated with CP on the sixth days of storage.

Table 3
5′-nucleotiides changes of *Volvariella volvacea* with different packaging treatments during 6 days of storage.*

Types of 5'-nucleotiides	5′-nucleotiides contents (mg/g DW)				
	Fresh samples	NP_3	CP_3	NP_6	CP_6
5'-CMP	1.35 ± 0.04^{c}	1.33 ± 0.01^{c}	1.76 ± 0.07^{a}	1.26 ± 0.03^{d}	1.51 ± 0.01^{b}
5'-AMP	1.32 ± 0.01^{c}	1.50 ± 0.01^{b}	1.13 ± 0.04^{d}	1.77 ± 0.12^{a}	1.65 ± 0.12^{ab}
5'-GMP	1.72 ± 0.02^{e}	2.74 ± 0.09^{a}	2.45 ± 0.05^{b}	2.23 ± 0.03^{c}	1.85 ± 0.03^{d}
5'-UMP	0.30 ± 0.001^{c}	0.72 ± 0.06^{a}	0.65 ± 0.09^{b}	0.62 ± 0.05^{b}	0.38 ± 0.02^{c}
5'-IMP	0.05 ± 0.001^{d}	0.06 ± 0.002^{c}	0.07 ± 0.002^{b}	0.06 ± 0.003^{c}	0.10 ± 0.007^{a}
Total	4.74 ± 0.03^{d}	6.36 ± 0.15^{a}	6.06 ± 0.09^{ab}	5.93 ± 0.07^{b}	5.48 ± 0.13^{c}

* Each value was expressed as the mean ± standard deviation. Means with different lower case letters within a row indicate significant differences ($P < 0.05$). DW: dry weight; NP_3: 5′-nucleotiides contents of *Volvariella volvacea* treated with nanocomposite packaging (NP) on the third days of storage; CP_3: 5′-nucleotiides contents of *Volvariella volvacea* treated with conventional polyethylene packaging (CP) on the third days of storage; NP_6: 5′-nucleotiides contents of *Volvariella volvacea* treated with NP on the sixth days of storage; CP_6: 5′-nucleotiides contents of *Volvariella volvacea* treated with CP on the sixth days of storage.

Table 4
Free amino acids changes of *Volvariella volvacea* with different packaging treatments during 6 days of storage.*

amino acids	Free amino acids	Contents (mg/g DW)				
		Fresh samples	NP_3	CP_3	NP_6	CP_6
Essential amino acid	Thr	22.08	26.20	23.73	34.58	29.34
	Val	7.41	7.02	5.90	3.26	2.39
	Met	–	2.19	1.45	–	–
	Ile	4.59	4.50	3.44	3.77	2.68
	Leu	4.30	10.33	7.80	1.20	1.49
	Phe	57.42	21.02	20.06	23.73	23.54
	Lys	6.80	3.29	2.80	3.19	2.91
Non-essential amino acid	Asp	9.68	4.43	2.79	5.87	4.20
	Ala	12.34	11.63	9.94	7.05	6.21
	Ser	7.94	7.11	6.21	7.70	6.26
	Glu	15.58	14.37	12.02	15.31	13.34
	Gly	1.44	1.37	1.14	1.30	0.99
	Cys	3.54	2.45	2.09	1.59	1.56
	Tyr	15.18	6.28	5.98	6.53	5.12
Semi-essential amino acid	His	4.69	3.00	2.27	3.44	3.23
	Arg	6.38	4.21	3.37	4.61	3.45
	Total	179.37	129.40	111.00	123.12	106.72

* DW: dry weight; NP_3: Free amino acids contents of *Volvariella volvacea* treated with nanocomposite packaging (NP) on the third days of storage; CP_3: Free amino acids contents of *Volvariella volvacea* treated with conventional polyethylene packaging (CP) on the third days of storage; NP_6: Free amino acids contents of *Volvariella volvacea* treated with NP on the sixth days of storage; CP_6: Free amino acids contents of *Volvariella volvacea* treated with CP on the sixth days of storage.

transfer of stress from the matrix to the reinforcing phase via interface (Voon et al., 2012). This structure leaded to improvement on the mechanical properties and stability of the resulting nanocomposite-based films, reducing the risk of nano-particles migration.

Included nanophase silver and titanium dioxide were able to scavenge ethylene, a plant hormone that accelerates ripening and senescence of agricultural produce (Li et al., 2009; Yang et al., 2010). This could explain the obtained results that the NP-packed *V. volvacea* postponed the peak of respiratory rate during storage as compared to the control (Fig. 2C). Similar results, that the application of NP could decompose or oxidize ethylene produced by Chinese jujube into water and carbon dioxide, were delivered by Li et al. (2009).

Colour and appearance are critical parameters impacting consumer acceptance of fresh fruits and vegetables (Barrett, Beaulieu, & Shewfelt, 2010). In this research, reduced browning issues and quality deterioration of *V. volvacea* were indicated in NP than in CP (Figs. 1 and 2D). Browning of edible mushrooms is mainly due to the oxidation of phenolic compounds catalysed by tyrosinase and polyphenoloxidase (PPO) (Oliveira, Sousa-Gallagher, Mahajan, & Teixeira, 2012). NP could directly inactivate the enzyme activities, including that of PPO, and retain the whiteness inside the fruiting body of *V. volvacea* (Yang et al., 2010). Moreover, the attack from spoilage microorganisms in later storage time would promote mushroom browning (Simón, Elena, & Vanesa, 2005). NP was proved to be effective in inhibiting the growth of microbes benefitting the whiteness maintenance of *V. volvacea.*

The process of mushroom senescence along with the continuous strengthening of oxidation could result in ROS accumulation and generate oxidative damage (Li et al., 2013). The present research showed that the ROS (including $O_2^{\bullet -}$ and H_2O_2) production rate was slowed in NP-treated *V. volvacea* during storage (Fig. 2 E & F). Fang, Yang, Kimatu, An et al., 2016) reported that NP treatment was effective in controlling ROS accumulation via activating the antioxidant defence system in mushroom cells, consequently decreasing oxidative damage and slowing senescence. Furthermore, ROS accumulation in mushroom cells damaged membrane structure and function, triggered membrane lipid peroxidation and induced membrane breakdown (Juan, Wang, & Jin, 2011). For MDA, a product of membrane lipid peroxidation, NP-treated *V. volvacea* had significantly lower content than that packed with CP after six days of storage. This was consistent with the ROS level and TEM demonstrating that NP treatment was more effective in delaying the cell autolysis and senescence of *V. volvacea* induced by ROS. The results of differential proteomics experiment from Fang, Yang, Kimatu et al. (2017) and Fang, Yang, Deng et al. (2017) also proved that NP could inhibit the protein expression involving in carbohydrate and energy metabolism bioprocess, promote amino acids biosynthesis, enhance antioxidant system, and improve its resistance to stress, resulting in a further extended shelf life of mushrooms.

Mushrooms accumulate a variety of secondary metabolites leading to their diversity in flavors (Lin et al., 2017). The typical flavor of mushrooms, which consist of volatile and non-volatile compounds, was an important factor to determine its quality and public acceptance. The flavoring compounds containing eight carbon atoms made up the main volatile constituent (Fang, Yang, Kimatu et al., 2017; Fang, Yang, Deng et al., 2017). The non-volatile components including organic acid, soluble sugar, 5′-nucleotides and free amino acids were conducive to the formation of umami taste in mushroom (Manninen, Rotola-Pukkila, Aisala, Hopia, & Laaksonen, 2018).

The mushroom flavor variation was a dynamic process and influenced by complex factors including storage time, respiration, microbial spoilage and other physiological metabolism of mushrooms after harvest. In this research, 3-octanone, benzeneacetaldehyde, succinic acid, trehalose and 5′-GMP were indicated to be the characteristic flavor compounds in *V. volvacea*. Similar major flavor compounds were found in a study regarding the volatile compounds of *F. velutipes* conducted by Yang et al. (2016). Volatile compounds of fresh *F. velutipes* were mainly composed of ketones, aldehydes and alcohols. The major flavor compound was 3-octanone.

The development of unpleasant smells from mushrooms during storage is related to their respiration, spoilage, growth of

microorganisms, and metabolism (Breheret, Talou, Rapior, & Bessière, 1997). They are usually packaged and sealed in vacuum condition after postharvest during delivering and transportation. This treatment will cause anaerobic respiration from mushroom inside the package and the yeasty smell is perceived after bag opening (Fang, Yang, Kimatu et al., 2017; Fang, Yang, Deng et al., 2017).

NP treatment in our study reduced the physiological disorder induced by anaerobic respiration and inactivated the key enzymes including polyphenol oxidase, lipoxidase and peroxidase to avoid flavor components from over oxidation or degradation (Yang et al., 2019). Meanwhile, the existence of nano-Ag and nano-TiO_2 within the NP material was able to inhibit the growth of microbes in the later stages of mushroom storage due to the antibacterial property of active nanoparticles (De Azeredo, 2013). Therefore, the NP-treated *V. volvacea* better retained flavor compared to control.

In conclusion, the developed NP postharvest treatment for *V. volvacea* reduced the browning degree, respiration rate, MDA content and ROS levels compared to those of CP treatment. Mushrooms packed in NP retained firmness, total soluble solids and flavor compounds. Therefore, it was demonstrated that NP delayed the senescence of *V. volvacea*. As a minimal processing technology, NP could be one of the potential commercial approaches for the postharvest mushroom industry to improve storability and maintain shelf life.

Authors contributions

Fang Donglu was responsible for experiment implementation, data collection, and manuscript composition and revision. Yu Kelin worked on data analysis and manuscript revision. Dr. Deng worked on manuscript composition and grammar check. Dr. Hu and Dr. Zhao provided instructions and facilities for this study. Dr. Zhao supported the revision of the manuscript.

Declaration of Competing Interest

None.

Acknowledgements

The authors acknowledge financial support from the Fundamental Research Funds for the Central Universities (Grant No. KJQN201939) and a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fpsl.2019.100412.

References

Aguirre, L., Frias, J. M., Barry-Ryan, C., & Grogan, H. (2008). Assessing the effect of product variability on the management of the quality of mushrooms (*Agaricus bisporus*). *Postharvest Biology and Technology, 49*(2), 247–254.

Barrett, D. M., Beaulieu, J. C., & Shewfelt, R. (2010). Color, flavor, texture, and nutritional quality of fresh-cut fruits and vegetables: Desirable levels, instrumental and sensory measurement, and the effects of processing. *Critical Reviews in Food Science and Nutrition, 50*(5), 369–389.

Breheret, S., Talou, T., Rapior, S., & Bessière, J. M. (1997). Monoterpenes in the aromas of fresh wild mushrooms (Basidiomycetes). *Journal of Agricultural and Food Chemistry, 45*(3), 831–836.

Bumbudsanpharoke, N., & Ko, S. (2015). Nano-food packaging: An overview of market, migration research, and safety regulations. *Journal of Food Science, 80*(5), 910–923.

Cliff, M. A., Toivonen, P. M. A., Forney, C. F., Liu, P., & Lu, C. (2010). Quality of fresh-cut apple slices stored in solid and micro-perforated film packages having contrasting O_2 headspace atmospheres. *Postharvest Biology and Technology, 58*, 254–261.

De Azeredo, H. M. (2013). Antimicrobial nanostructures in food packaging. *Trends in Food Science & Technology, 30*(1), 56–69.

Dhalsamant, K., Dash, S. K., Bal, L. M., & Panda, M. K. (2015). Effect of perforation mediated MAP on shelf life of mushroom (*Volvariella volvacea*). *Scientia Horticulturae, 189*, 41–50.

Duan, X., Pang, X., & Zhang, Z. (2000). Low-temperature storage of straw mushroom and its physiological changes. *Chinese Journal of Tropical Crops, 4*, 012.

Fang, D., Yang, W., Kimatu, B. M., Mariga, A. M., Zhao, L., An, X., et al. (2016). Effect of nanocomposite-based packaging on storage stability of mushrooms (*Flammulina velutipes*). *Innovative Food Science & Emerging Technologies, 33*, 489–497.

Fang, D., Yang, W., Kimatu, B. M., An, X., Hu, Q., & Zhao, L. (2016). Effect of nanocomposite packaging on postharvest quality and reactive oxygen species metabolism of mushrooms (*Flammulina velutipes*). *Postharvest Biology and Technology, 119*, 49–57.

Fang, D., Yang, W., Kimatu, B. M., Zhao, L., An, X., & Hu, Q. (2017). Comparison of flavor qualities of mushrooms (*Flammulina velutipes*) packed with different packaging materials. *Food Chemistry, 232*, 1–9.

Fang, D., Yang, W., Deng, Z., An, X., Zhao, L., & Hu, Q. (2017). Proteomic investigation of metabolic changes of mushroom (*Flammulina velutipes*) packaged with nanocomposite material during cold storage. *Journal of Agricultural and Food Chemistry, 65*, 10368–10381.

He, X., & Hwang, H. (2016). Nanotechnology in food science: Functionality, applicability, and safety assessment. *Journal of Food and Drug Analysis, 24*, 671–681.

Hu, Q., Fang, Y., Yang, T., Ma, N., & Zhao, L. (2011). Effect of nanocomposite-based packaging on postharvest quality of ethylene-treated kiwifruit (*Actinidia deliciosa*) during cold storage. *Food Research International, 44*, 1589–1596.

Hu, Y., Chen, C., Xu, L., Cui, Y., Yu, X., Gao, H., et al. (2015). Postharvest application of 4-methoxy cinnamic acid for extending the shelf life of mushroom (*Agaricus bisporus*). *Postharvest Biology and Technology, 104*, 33–41.

Jamjumroon, S., Wongs-Aree, C., McGlasson, W. B., Srilaong, V., Chalermklin, P., & Kanlayanarat, S. (2012). Extending the shelf-life of straw mushroom with high carbon dioxide treatment. *Journal of Food, Agriculture & Environment, 10*(1), 78–84.

Juan, K., Wang, H. M., & Jin, C. H. (2011). Changes of reactive oxygen species and related enzymes in mitochondrial respiration during storage of harvested peach fruits. *Agricultural Science of China, 10*(1), 149–158.

Kartal, S., Aday, M. S., & Caner, C. (2012). Use of microperforated films and oxygen scavengers to maintain storage stability of fresh strawberries. *Postharvest Biology and Technology, 71*, 32–40.

Li, B., Kimatu, B. M., Li, C., Pei, F., Hu, Q., & Zhao, L. (2017). Analysis of volatile compounds in *L. Edodes* blanched by hot water and microwave. *International Journal of Food Science & Technology, 52*(7), 1680–1689.

Li, B., Kimatu, B. M., Pei, F., Chen, S., Feng, X., Hu, Q., et al. (2018). Non-volatile flavor components in *Lentinus edodes* after hot water blanching and microwave blanching. *International Journal of Food Properties*. https://doi.org/10.1080/10942912.2017.1373667.

Li, H., Li, F., Wang, L., Sheng, J., Xin, Z., Zhao, L., et al. (2009). Effect of nano-packing on preservation quality of Chinese jujube (*Ziziphus jujuba* Mill. Var. Inermis (Bunge) Rehd). *Food Chemistry, 114*(2), 547–552.

Li, N., Chen, F., Cui, F., Sun, W., Zhang, J., Qian, L., et al. (2017). Improved postharvest quality and respiratory activity of straw mushroom (*Volvariella volvacea*) with ultrasound treatment and controlled relative humidity. *Scientia Horticulturae, 225*, 56–64.

Li, P., Zhang, X., Hu, H., Sun, Y., Wang, Y., & Zhao, Y. (2013). High carbon dioxide and low oxygen storage effects on reactive oxygen species metabolism in *Pleurotus eryngii*. *Postharvest Biology and Technology, 85*, 141–146.

Li, W., Gu, Z., Yang, Y., Zhou, S., Liu, Y., & Zhang, J. (2014). Non-volatile taste components of several cultivated mushrooms. *Food Chemistry, 143*, 427–431.

Lin, Q., Lu, Y., Zhang, J., Liu, W., Guan, W., & Wang, Z. (2017). Effects of high CO_2 in-package treatment on flavor, quality and antioxidant activity of button mushroom (*Agaricus bisporus*) during postharvest storage. *Postharvest Biology and Technology, 123*, 112–118.

Liu, Z., Zhang, K., Lin, J. F., & Guo, L. (2011). Breeding cold tolerance strain by chemical mutagenesis in *Volvariella volvacea*. *Scientia Horticulturae, 130*(1), 18–24.

Manninen, H., Rotola-Pukkila, M., Aisala, H., Hopia, A., & Laaksonen, T. (2018). Free amino acids and 5′-nucleotides in Finnish forest mushrooms. *Food Chemistry, 247*, 23–28.

Mau, J. L., Chyau, C. C., Li, J. Y., & Tseng, Y. H. (1997). Flavor compounds in straw mushrooms *Volvariella volvacea* harvested at different stages of maturity. *Journal of Agricultural and Food Chemistry, 45*(12), 4726–4729.

McMahon, D. J., Oberg, C. J., Drake, M. A., Farkye, N., Moyes, L. V., Arnold, M. R., et al. (2014). Effect of sodium, potassium, magnesium, and calcium salt cations on pH, proteolysis, organic acids, and microbial populations during storage of full-fat Cheddar cheese. *Journal of Dairy Science, 97*(8), 4780–4798.

Oliveira, F., Sousa-Gallagher, M. J., Mahajan, P. V., & Teixeira, J. A. (2012). Development of shelf-life kinetic model for modified atmosphere packaging of fresh sliced mushrooms. *Journal of Food Engineering, 111*, 466–473.

Poisson, C., Guerengomba, J., Lacrampe, M. F., Krawczak, P., Gupta, B., Miri, V., et al. (2008). Mechanical, optical and barrier properties of PA6/nanoclay-based single-and multilayer blown films. *Polymers & Polymer Composites, 16*(6), 349.

Shi, C., Wu, Y., Fang, D., Pei, F., Mariga, A. M., Yang, W., et al. (2018). Effect of nanocomposite packaging on postharvest senescence of *Flammulina velutipes*. *Food Chemistry, 246*, 414–421.

Simón, A., Elena, G., & Vanesa, T. (2005). The sensory and microbiological quality of fresh sliced mushroom (*Agaricus bisporus* L.) packaged in modified atmospheres. *International Journal of Food Science & Technology, 40*(9), 943–952.

Suzuki, Y., Kimura, T., Takahashi, D., & Terai, H. (2005). Ultrastructural evidence for the inhibition of chloroplast-to-chromoplast conversion in broccoli floret sepals by ethanol vapor. *Postharvest Biology and Technology, 35*, 237–243.

Tsai, S. Y., Tsai, H. L., & Mau, J. L. (2008). Non-volatile taste components of *Agaricus blazei*, *Agrocybe cylindracea* and *Boletus edulis*. *Food Chemistry, 107*(3), 977–983.

Vera, P., Canellas, E., & Nerin, C. (2013). Migration of odorous compounds from

adhesives used in market samples of food packaging materials by chromatography olfactometry and mass spectrometry (GC-O-MS). *Food Chemistry, 145*, 237–244.
Voon, H. C., Bhat, R., Easa, A. M., Liong, M., & Karim, A. (2012). Effect of addition of halloysite nanoclay and SiO_2 nanoparticles on barrier and mechanical properties of bovine gelatin films. *Food and Bioprocess Technology, 5*(5), 1766–1774.
Xu, X., Xu, R., Jia, Q., Feng, T., Huang, Q., Ho, C. T., et al. (2019). Identification of dihydro-β-ionone as a key aroma compound in addition to C8 ketones and alcohols in *Volvariella volvacea* mushroom. *Food Chemistry, 293*, 333–339.
Yang, F. M., Li, H. M., Li, F., Xin, Z. H., Zhao, L. Y., Zheng, Y. H., et al. (2010). Effect of nano-packing on preservation quality of fresh strawberry (*Fragaria ananassa* Duch. Cv Fengxiang) during storage at 4 °C. *Journal of Food Science, 75*(3), 236–240.
Yang, W., Shi, C., Hu, Q., Wu, Y., Fang, D., Pei, F., et al. (2019). Nanocomposite packaging regulate respiration and energy metabolism in *Flammulina velutipes*. *Postharvest Biology and Technology, 151*, 119–126.
Yang, W., Yu, J., Pei, F., Alfred, M. M., Ma, N., Fang, Y., et al. (2016). Effect of hot air drying on volatile compounds of *Flammulina velutipes* detected by HS-SPME–GC–MS and electronic nose. *Food Chemistry, 196*, 860–866.