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Preservation of *Agaricus bisporus* freshness with using innovative ethylene manipulating active packaging paper

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ABSTRACT

Agaricus bisporus produces substantial ethylene during storage and transportation, which accelerates ripening and senescence, thereby shortening the shelf-life. In this study, a novel food packaging material with ethylene removal property was prepared to increase storage time of *Agaricus bisporus*. 1-Methylcyclopropen and molecular sieves loaded with potassium permanganate were used as ethylene scavengers to coat the fresh-keeping paper. SEM, FT-IR and DSC analyses proved that these functional components were successfully coated on the fresh-keeping paper. The qualities of the mushrooms packed by prepared functional paper were then determined. The results showed that this prepared functional paper could delay the softening, browning and weight loss of mushrooms during storage by inhibiting ethylene synthesis-related enzymes and gene expression in the mushroom fruiting body, and continuous adsorption and removal of the exogenous ethylene. Consequently, the functional paper could reduce the biochemical and physicochemical quality loss of *Agaricus bisporus*, thus prolonging its shelf-life.

1. Introduction

The mushroom *(Agaricus bisporus)* is very popular with consumers for its unique texture, flavor, and rich nutrients (Muszyńska, Kała, Sułkowska-Ziaja, Krakowska, & Opoka, 2016), and is known for prevention of hypertension, hyperlipidemia and inhibition of tumor cell activity (Manzi, Aguzzi, & Pizzoferrato, 2001). However, *Agaricus bisporus* is a climacteric vegetable. The active post ripening process will lead to many physiological and biochemical changes, such as weight loss, softening, browning, loss of nutrient values and increased membrane permeability (Li et al., 2019). Large amounts of ethylene play a key role in regulating the ripening and senescence of climacteric fruits and vegetables during the post-harvest and transportation period (Dubois, Lisa, & Inzé, 2018; Tucker, Yin, Zhang, Wang, Zhu, & Liu, 2017). Ethylene can also induce the expression of ripening related genes through signal transduction pathway (Jiang, Zheng, Li, Jing, Cai, & Ying, 2011). Therefore, the control of ethylene production is conducive to the reduction of the quality loss during the storage period and transportation of *Agaricus bisporus* (Khan et al., 2014).

At present, ethylene production is usually controlled by inhibiting the synthesis or absorption, oxidation and adsorption of ethylene during storage and transportation of fruits and vegetables (Keller, Ducamp, Robert, & Keller, 2013). 1-Methylcyclopropene (1-MCP), is an ethylene inhibitor, is widely used in postharvest handling of climacteric fruit and vegetables (Chen, Sun, Lin, Hung, Zhang, & Lin, 2016). 1-MCP destroy ethylene signal transduction and inhibit ethylene synthesis by binding to the receptor metal with its own double bond (Sisler & Serek, 1997). However, 1-MCP can only limitedly inhibit the synthesis of ethylene, and cannot remove the exogenous ethylene that has beengenerated. The adsorbent is usually used to reduce the exogenous ethylene duo to plenty of closed and interconnected pores in its inner (Jana, Bhunia, Dutta, & Koner, 2011). Additionally, adsorbents are also usually used in combination with catalysts or oxidants to destroy and remove adsorbed ethylene (Tirgar, Han, & Steckl, 2018). In this way, the storage of most of fruit and vegetables can be improved without damaging their quality characteristics during storage and transportation (Ivarez-Hernández et al., 2019).

In addition, cold chain storage and transportation system are commonly used to improve the storability of fruit and vegetables. However, the construction of cold chains in the China is insufficient, the number of refrigerated transport vehicles are small and the circulation rate is low. Therefore, most of fruit and vegetables still face the

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challenge of circulating in room temperature. As a typical climacteric vegetable, the respiration rate of *Agaricus bisporus* is strengthened with the increase of temperature. If the cold chain cannot be guaranteed at every stage of transportation, it is possible that the *Agaricus bisporus* will start to rot at this stage (Lin & Sun, 2019). For long-distance transportation, the quality of *Agaricus bisporus* can be efficiently maintained by controlled conditions (temperature and relative humidty) and other preservation methods. At present, artificial spraying or fumigation is common methods for keeping vegetables as a fresh. However, in the cold-chain logistics, this process is difficult to achieve manually, and the artificial preserves are not desired due to the safety issues. In addition, using large scale of disposable packaging could lead to serious environmental pollution (Lavoine, Givord, Tabary, Desloges, Martel, & Bras, 2014). Therefore, developing the novel packaging material with biodegradable, portable, safe and pollution-free properties has increased attention in the packaging industry (Sothornvit, 2009).

In this paper, 1-MCP, molecular sieve loaded with potassium permanganate ($KMnO_4$-MCM-41) and cinnamon essential oil (CEO) microcapsule were used to prepare the fresh-keeping paper with ethylene removal ability in order to improve the quality and prolong the shelf-life of *Agaricus bisporus*. The antibacterial effect of CEO preservative paper has been proved in the previous research (Gao, Feng, & Jiang, 2014). This study mainly focused on the effects of 1-MCP and $KMnO_4$-MCM-41 coated composite paper on the ethylene production. The chemical and physical structures of the paper were firstly characterized by SEM, FT-IR and DSC. Then, the effect of composite preservative paper on the quality characteristics of mushrooms was studied by comparing the samples packed by the CEO coated paper, 1-MCP/CEO coated paper, $KMnO_4$-MCM-41/CEO coated paper and 1-MCP/$KMnO_4$-MCM-41/CEO coated paper, respectively.

2. Materials and methods

2.1. Materials

Fresh *Agaricus bisporus* was purchased from Desheng vegetable market in Hangzhou, China. Thirty *Agaricus bisporus* were divided into 5 groups according to color and size. Each sample was measured 3 times. Food packaging paper (with a basis weight of 80 g/m^2) was supplied by Victoria Paper (Zhejiang, China). 1-Methylcyclopropen (3.5%) was purchased from Shanghai Yuanye Biotechnology Co., Ltd (Shanghai, China). Cinnamon essential oil (containing 98% *trans*-cinnamaldehyde) was purchased from Saan Chemical Technology Co, Ltd (Shanghai, China). Ethyl cellulose glycol (analytical pure) was purchased from Shanghai Maclin Biochemistry Technology Co., Ltd (Shanghai, China). Potassium permanganate (analytical pure) was purchased from Shanghai Lingfeng Reagent Co., Ltd (Shanghai, China). Molecular sieve MCM-41 was purchased from Tianjin Yuanli Chemical Co., Ltd (Tianjin, China). *Agaricus bisporus* were treated in five different ways. The fresh-keeping paper with different formula was cut into the same size and covered on the surface of plastic fresh-keeping box, then stored at room temperature (25 ± 2 ℃) for 6 days (Zhang, Li, Wang, Li, & Zong, 2017). All chemicals were of analytical reagent grade unless specified.

2.2. Preparation of coating solution and coating papers

After boiling the saturated potassium permanganate solution for 15 min, 5 g molecular sieve was added to the solution (100 mL) and soaked for 60 min, and then filtrate was drained at 70 °C to prepare potassium permanganate molecular sieve. The coating solutions of 1-methylcyclopropen (1-MCP) (0.20 mg/mL1-MCP in ethyl cellulose solution), cinnamon essential oil (CEO) microcapsule (36.0 mg/mL CEO in ethyl cellulose solution), and potassium permanganate molecular sieve (20.0 mg/mL1-MCP in ethyl cellulose solution) were prepared, respectively. By coating filter papers with different prepared coating solutions, four coated papers were obtained: CEO coated paper, 1-MCP/CEO coated paper, $KMnO_4$-MCM-41/CEO coated paper and 1-MCP/$KMnO_4$-MCM-41/CEO coated paper.

2.3. Characterization and measurement of coated papers

2.3.1. Scanning electron microscopy (SEM)

The papers surface morphology was analyzed using scanning electron microscopy (SU8010, Hitachi instruments, Tokyo, Japan). The sample surface was coated with gold for 40 s. The accelerating voltage of 10 kV and magnification was 200 times and 1000 times, respectively. The images was analyzed using Image-Pro Plus software.

2.3.2. Fourier-transform infrared spectroscopy (FTIR)

FTIR spectra was recorded on FTIR spectrophotometer (AVATAR 370, Nicolet, USA). For the average value of each FTIR signal, a total of 32 scans were accumulated with a resolution of 10 cm^{-1}. The spectra of the samples were recorded in the wave number range of 500–4000 cm^{-1} and the wavelength range of 2.5–20 μm. Signal processing used OMNIC spectrum software (Zhou, Wang, Hu, & Luo, 2016).

2.3.3. Differential scanning calorimetry (DSC)

Thermal properties of the coated paper were analyzed by a DSC apparatus (Water-TA, Q20, USA). The sample (5–10 mg) was sealed in an aluminum pans (Jingyi Chemical, Shanghai, China). To eliminate the thermal history, a reaped pretreatment procedure by increasing and decreasing temperature was used. Finally, the heating process was increased from 50 ℃ to 250 ℃ at a scan rate of 10 ℃/min a nitrogen 99 flow of 20 mL/min was used (Liu, Huang, Zheng, Liu, & Liu, 2020). An empty aluminum pan was used as a reference.

2.4. Physical characteristics of mushrooms

2.4.1. Color measurement

The surface color of mushroom cap was determined by a CR-400 colorimeter (ColorQuest XE, Hunter Lab, USA). The CIE L*a*b* color system, a* red-green (+a*=red; −a*=green), b* yellow-blue (+b*=yellow; −b*=blue). Six *Agaricus bisporus* were measured repeatedly in each treatment, each sample was measured 3 times. The total color differences (△E) were calculated using the following equation (Gao, Feng, & Jiang, 2014):

$$\Delta E = \left[(L-97)^2 + (a-(-2))^2 + b^2\right]^{\frac{1}{2}}$$

where ΔE represents the overall change of color compared with the color value of the ideal mushroom. The browning index (BI) representing the purity of brown was calculated according to the following equation.

$$BI = \frac{100(X-0.31)}{0.172}$$

$$X = \frac{a+1.75L}{5.645+a-3.012b}$$

2.4.2. Firmness

The firmness of the *Agaricus bisporus* was tested by a texture analyzer (TA-XT Plus, Stable Micro System Ltd., London, UK) equipped with the p/2 probe with the diameter of 2 mm. The samples were tested on XT/DIB of the texture analyzer. The puncture speed was set at 10 mm/s and the puncture depth was set as 5 mm. The initial maximum pressure during the puncture indicated the firmness of the epidermis of the *Agaricus bisporus*. Each sample was measured in triplicate (Kaur, Sandhu, Arora, & Sharma, 2015).

2.4.3. Weight loss

The weight loss was calculated as follows:

$$\text{Weight loss}\,(\%) = \frac{W_1 - W_2}{W_1} \times 100$$

where W_1 is the initial weight of the mushroom and W_2 was the weight after storage different period (2, 4 and 6 days).

2.5. Determination of cell membrane permeability in mushrooms

2.5.1. Determination of electrolyte permeability

The conductivity was measured by a digital display conductometer (DDS-11A, Shanghai Lei Magnetism Chuangyi Instrument Co., Ltd., China). After cutting mushroom into four cylinders at the equator, 40 mL of deionized water was added. Then the conductivity was immediately measured and recorded as P_0. The conductivity was again measured as P_1 after 10 min. After the water solution was boiled for 10 min, cooled it to room temperature, the solution was again added to the primary volumn and thendetermined the conductivity as P_2. The calculation formula of cell membrane permeability is as follows (Liu, Liu, Zhang, Kan, & Jin, 2019).

$$\text{Cell membrane performance}\,(\backslash\%)) = \frac{P_1 - P_0}{P_2 - P_0} \times 100\,\backslash\%$$

2.5.2. Determination of malondialdehyde (MDA)

Agaricus bisporus sample (1 g) was ground with 5 mL of 100 g/L trichloroacetic acid (TCA) in an ice bath and then centrifuged at 10000 × g at 4 °C for 20 min. The supernatant (2 mL) was mixed with 2 mL of 100 g/L TCA containing 0.67% thiobarbituric acid (TBA) and incubated in boiling water for 20 min. The mixture was quickly cooled and then centrifuged at 10000 × g for 10 min (Liu and Wang, 2012). The absorbance of supernatant was measured at 532 nm, 600 nm and 450 nm by UV spectrophotometer, respectively. The content of MDA in the reaction mixture was calculated according to the following formula.

$$c = 6.452(A_{532} - A_{600}) - 0.56A_{450}$$

The MDA content of *Agaricus bisporus* was expressed as μmol kg^{-1} fresh weight according to the following formula.

$$\text{MDA content}\left(\mu\ \text{mol}\,\text{kg}^{-1}\right) = \frac{c \times V}{V_S \times m}$$

where c is the concentration of MDA in the reaction mixture (μ mol L^{-1}); V was the total volume of the extraction solution (mL); Vs was the volume of sample extraction solution taken for the determination (mL); and m was the sample quality (g).

2.6. Ascorbik acid content

The content of ascorbic acid was determined by 2,6-dichloroindophenol titration. *Agaricus bisporus* (10 g) was ground in an ice bath with a small amount of 20 g/L oxalic acid solution, and then the sample was transferred into a 100 mL volumetric flask (Lopes, Silva, Canuto, Silva, & Miranda, 2016). After the volume was fixed with the oxalic acid solution, shaken and filtered. The filtrate (10 mL) was titrated to red with 2,6-dichlorophenol-indophenol, and the oxalic acid solution without sample was used as a control. The mass of ascorbic acid in 100 g fresh weight was calculated according to the following formula:

$$\textit{Ascorbic acid content}(mg/100g) = \frac{V \times (V_1 - V_0) \times \rho}{V_S \times m} \times 100$$

where V_1 was the dye volume consumed by sample titration (mL); V_0 was the dye volume consumed in blank titration (mL); ρ was the 1 mL dye solution equal to the mass of ascorbic acid (mg/mL); V_S was the volume of sample solution taken out during titration (mL); V was the total volume of sample extract (mL); m was the sample quality (g).

2.7. Total soluble solids (TSS)

The mushroom sample (5 g) were added with 10 mL of deionized water (pH 7), and then was stirred in a mortar and filtered with four layers of gauze to remove the filter residue. Subsequently, the supernatant was used to measure the content of soluble solid (°Brix) with a refractometer (A1701161, ATAGO, Japan).

2.8. Enzyme activity of 1-aminocyclopropane-1-carboxyl synthetase (ACS) and 1-aminocyclopropane-1-carboxyl oxidase (ACO)

The mushroom tissue (5 g) was weighed and mixed with 5 mL ACS extraction buffer (1 mmol/L Ethylene Diamine Tetraacetic Acid, 1 mmol/L Phenylmethylsulfonyl fluoride, 4 mmol/L Dithiothreitol, 3% Crosslinked Polyvinylpyrrolidone and 10 μmol/L Pyridoxal phosphate) and 5 mL ACO extraction buffer (10% Glycerine, 5% Crosslinked Polyvinylpyrrolidone, 5.0 mmol/L Dithiothreitol, 30 mmol/L L-Ascorbic Acid Sodium Salt, 0.1 mmol/L $FeSO_4$), respectively. Then the homogenate was ground in ice bath and centrifuged (12000 × g) at 4 ℃ for 30 min, then the supernant was collected and used as an enzyme extract. The enzyme activity of ACS and ACO was determined by Jiangsu Jingmei ELISA Kit (Jiangsu Jingmei Biological Technology Co., Ltd., China).

2.9. Gene expression of ACS and ACO

The extraction of total RNA from *Agaricus bisporus* or mycelia was carried out following a protocol described elsewhere (Meng, Song, Shen, Zhang, & Sheng, 2012). The expression level of ACS and ACO related genes in *Agaricus bisporus* of different treatment groups was analyzed by quantitative RT-PCR (Zhang et al., 2016). The primers (nucleotide sequence (5′–3′)) used were listed as follows: Aco-F: ATGGAA CCCACCCAGAACC; Aco-R: TTGCGATGAGAGAGGAAT; ACS1-F: CACATCGCTGTGACCCTAT; ACS1-R: CATCGTAGATA GACTGGGA.

2.10. Statistical analysis

Data were expressed as mean ± standard deviation. Data were analyzed by one-way analysis of variance (ANOVA) followed by the Duncan's multiple-range comparisons. Difference was considered to be statistically significant if $p < 0.05$. All statistical analyses were carried out by using SPSS software package (Version 13.0, SPSS, Chicago, IL). Thirty *Agaricus bisporus* were divided into 5 groups according to color and size. Each sample was measured 3 times.

3. Results and discussion

3.1. Characterization of the coated papers

3.1.1. Scanning electron microscopy (SEM)

The SEM of coated papers obtained by different treatment groups was showed in Fig. S1. The uncoated paper had smooth surface and obvious cellulose fiber network structure. After the coating treatment, the paper network structure still existed, but the fiber pores of the covering part were filled and became denser. The surfaces of all coated papers were rough, and preservative particles were evenly distributed on the surface and inside of the fiber. Especially, CEO microcapsule, 1-MCP and modified molecular sieve preservative particles were also observed on the composite preservative paper (Fig. S1).

3.1.2. FTIR analysis

The interaction between base paper and coating material was evaluated by the FT-IR analysis. The FT-IR spectrum of coated paper was shown in Fig. 1A. The characteristic peak at 3440 cm^{-1} (3050–3770 cm^{-1}) corresponded to the stretching of the free hydroxyl group on the base paper and the remaining physical absorption of water, while the band at 1632 cm^{-1} was attributed to the O—H bending mode of water

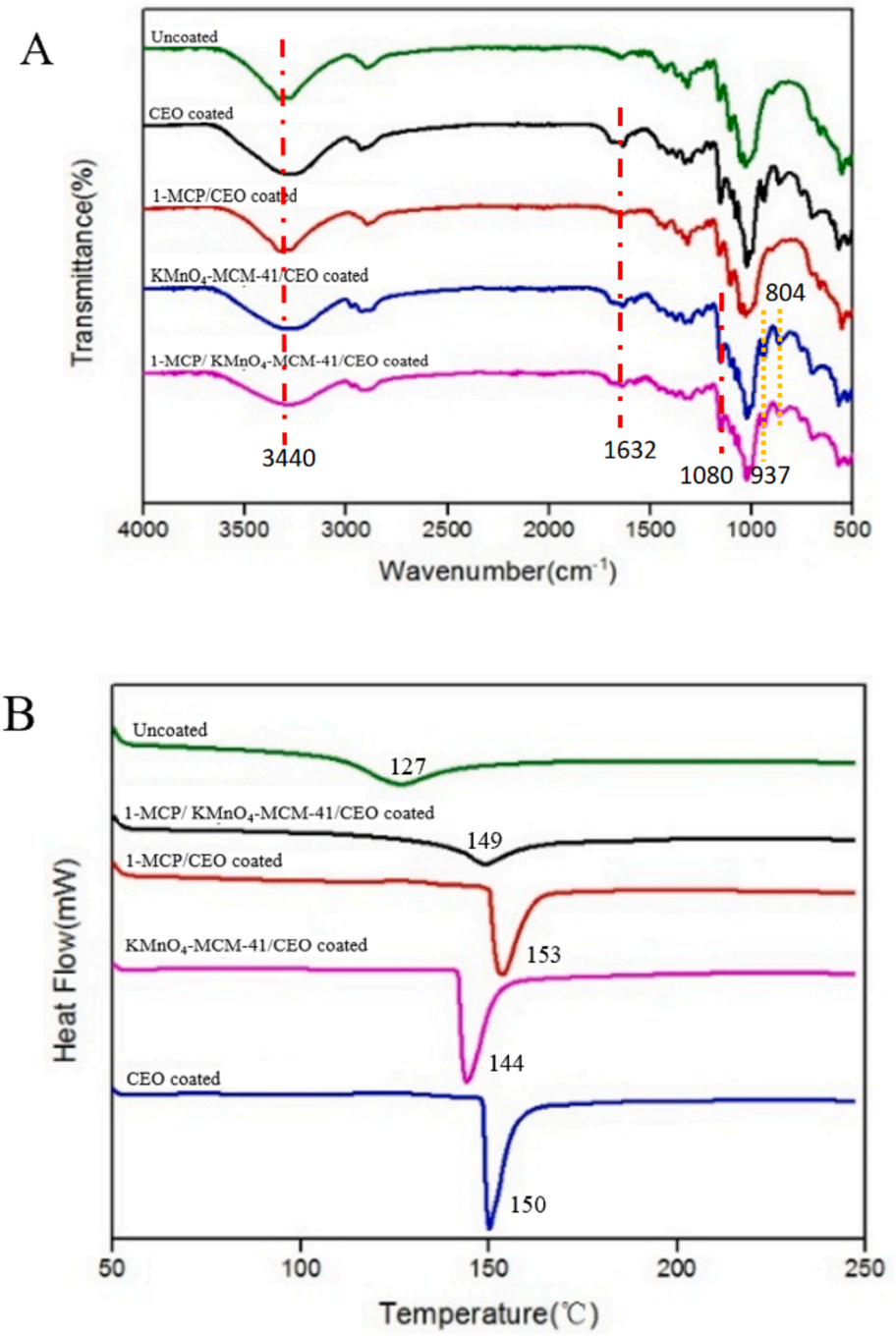

Fig. 1. (A) FTIR spectra of Uncoated paper, CEO coated paper, 1-MCP/CEO coated paper, $KMnO_4$-MCM-41/CEO coated paper and 1-MCP/$KMnO_4$-MCM-41/CEO coated paper; (B) DSC curves of Uncoated paper, CEO coated paper, 1-MCP/CEO coated paper, $KMnO_4$-MCM-41/CEO coated paper and 1-MCP/$KMnO_4$-MCM-41/CEO coated paper.

(Heidari, Younesi, Mehraban, & Lausanne, 2009). In addition, for $KMnO_4$-MCM-41/CEO and 1-MCP/$KMnO_4$-MCM-41/CEO coated paper, the absorption bands at 1080 cm^{-1} and 804 cm^{-1} may be related to the asymmetric and symmetric stretching of Si-O-Si groups in the molecular sieve, respectively. The absorption peaks at 937 cm^{-1} corresponded to the asymmetric stretching of Si-OH groups in the molecular sieve (Mohammad et al., 2017). These results indicated that the molecular sieve MCM-41 exists on the coated paper. However, 1-MCP was difficult to observe by FT-IR because it was a small molecule with low content in paper and easily covered by other substances. Thus, in order to further prove the existence of 1-MCP, $KMnO_4$ and CEO, DSC analysis was carried out for all coated papers below.

3.1.3. DSC analysis

The DSC images of coated paper were illustrated in Fig. 1B. The thermal degradation temperature of CEO coated paper was 150 °C. However, the temperature for 1-MCP/CEO coated paper and $KMnO_4$-MCM-41/CEO coated paper was 153 °C and 144 °C, respectively. Noticeably, the thermal degradation temperature of 1-MCP /$KMnO_4$-MCM-41/CEO composite preservative paper (149 °C) was between 1-MCP/CEO coated paper and $KMnO_4$-MCM-41/CEO coated paper, and also different from that of CEO coated paper. This showed that 1-MCP and $KMnO_4$ existed on the corresponding coated paper.

3.2. Changes in physical characteristics of mushrooms treated with different coating materials

3.2.1. Weight loss

Weight loss values of mushrooms increased during storage time (0–6 days). At the end of the 4th day of storage, the weight loss rate of mushrooms treated with uncoated paper was the highest (6.51%). Compared to samples treated with CEO alone, the weight loss rate of 1-MCP/CEO coated paper treated samples was significantly caused to slow down of weight loss. The main reason of this statement 1-MCP can reduce the biosynthesis of ethylene and delay the maturation and aging of mushrooms, therefore resulting in the decrease of water loss caused by respiration rate during the storage period. In addition, the papers coated with $KMnO_4$-MCM-41/CEO and 1-MCP/$KMnO_4$-MCM-41/CEO can also effectively reduce the weight loss of mushrooms, in which 1-MCP/$KMnO_4$-MCM-41/CEO coated paper was able to minimize the weight loss of *Agaricus bisporus* after six days of storage (4.66%). This was probably because molecular sieve MCM-41 can adsorb ethylene produced during the storage of postharvest mushrooms, while the potassium permanganate can convert the adsorbed ethylene into carbon dioxide and water, therefore reducing the ethylene content in the packaging. The decrease of ethylene content could reduce the water loss due to the decrease of respiration rate during storage period. Ivarez-Hernández et al. (2019) also observed that sepiolite-loaded potassium permanganate ethylene scavenger can reduce the ethylene content and keep the weight of apricot stable during storage. In addition, Wang, Zhou, Zhou, Wei, & Ji (2018) found that potassium permanganate can inhibit the weightlessness of blueberries during cold storage. In conclusion, the composite preservative paper played a good role in delaying the weight loss of mushroom.

3.2.2. Firmness

Loss of firmness may affect the quality of mushroom in the sales process, and thus hardness is a key factor reflecting the quality of mushrooms (Jiang, 2013). The changes of firmness of mushrooms are shown in Table 1. During the storage period, the firmness of all samples rapidly decreased. After the 4th day storage, the softening rate of the *Agaricus bisporus* in the uncoated control group reached the highest (64.3%), and the treatment with CEO coated paper significantly reduced (44.6%) the softening rate. While the hardness of *Agaricus bisporus* treated with 1-MCP or $KMnO_4$-MCM-41 coated paper was maintained better than that of the control group, in which the softening rate was 28.7% and 33.0% after the 6th day storage in room temperature, respectively (Table 1). Inhibition of ethylene production by 1-MCP and $KMnO_4$-MCM-41 probably contributed to the results. The result was consistent with Jeong, Huber, Sargent, & Technology (2003), who demonstrated that ethylene plays a key role in regulating softening related metabolic activities.

3.2.3. Color parameters

Color is the most important factor for the quality of *Agaricus bisporus*, in terms of consumer acceptance. After harvest, the color of mushrooms gradually changes from white to brown, which is a sign of losing nutritional value (Mishra, Gautam, & Sharma, 2013). The change of color parameters (brihgtness, browning index and color change) are shown in Table 1. L* values of control group and treatment group decreased gradually, and ΔE and BI values showed an upward trend with storage time prolonging. The coating treatment significantly slowed down the increase of ΔE and BI value. The L* value of samples treated with uncoated paper decreased from 96.23 to 80.87 after 4th day storage. The L * value of the mushrooms treated with composite preservative paper was the highest, indicating that the mushroom freshness was maintained best. This showed that 1-MCP and modified molecular sieve could delay the loss of L* value of *Agaricus bisporus*. The browning index of the samples treated with 1-MCP (41.43) and modified molecular sieve (41.19) was lower than that of the control group after 6 days of storage. It can be concluded that 1-MCP and $KMnO_4$-MCM-41 can reduce the ethylene content in packaging, delay the ripening and aging process of the mushroom, and thus inhibit browning (Han, Qin, Liu, Chen, Li, & Yuan, 2015). These indexes for color change were also

Table 1
Weight loss, Firmness and Lightness (L) of mushrooms stored: Uncoated paper, CEO coated paper, 1-MCP/CEO coated paper, $KMnO_4$-MCM-41/CEO coated paper, 1-MCP/$KMnO_4$-MCM-41/CEO coated paper. All samples are stored in room temperature.

Storage time	Treatment	Weight loss(%)	Firmness (kgf)	L	△E	BI
0d	Uncoated	0	1.15 ± 0.03^{a}	96.23 ± 0.35^{a}	14.82 ± 0.32^{a}	17.44 ± 0.15^{a}
	CEO	0	1.01 ± 0.06^{a}	97.29 ± 0.28^{a}	21.08 ± 0.17^{c}	23.26 ± 0.18^{b}
	1-MCP/CEO	0	1.01 ± 0.09^{a}	96.10 ± 0.13^{a}	22.63 ± 0.33^{d}	27.86 ± 0.28^{c}
	$KMnO_4$-MCM-41/CEO	0	1.03 ± 0.01^{a}	95.46 ± 0.15^{a}	23.49 ± 0.21^{d}	29.07 ± 0.17^{c}
	1-MCP/$KMnO_4$-MCM-41/CEO	0	1.10 ± 0.07^{a}	96.78 ± 0.19^{a}	19.10 ± 0.18^{b}	22.09 ± 0.19^{b}
2d	Uncoated	3.32 ± 0.14^{d}	0.68 ± 0.05^{a}	87.62 ± 0.12^{a}	30.04 ± 0.22^{d}	41.75 ± 0.25^{d}
	CEO	2.64 ± 0.13^{c}	0.82 ± 0.04^{b}	89.34 ± 0.27^{b}	28.27 ± 0.18^{c}	37.97 ± 0.17^{c}
	1-MCP/CEO	2.13 ± 0.12^{b}	0.91 ± 0.08^{c}	93.92 ± 0.13^{d}	26.36 ± 0.25^{b}	33.90 ± 0.25^{b}
	$KMnO_4$-MCM-41/CEO	2.34 ± 0.14^{b}	0.87 ± 0.07^{c}	92.19 ± 0.25^{d}	28.42 ± 0.15^{c}	37.71 ± 0.21^{c}
	1-MCP/$KMnO_4$-MCM-41/CEO	1.92 ± 0.09^{a}	0.95 ± 0.05^{d}	91.56 ± 0.19^{c}	22.44 ± 0.26^{a}	28.53 ± 0.29^{a}
4d	Uncoated	6.51 ± 0.12^{d}	0.41 ± 0.09^{a}	80.87 ± 0.29^{a}	31.13 ± 0.34^{d}	42.37 ± 0.19^{d}
	CEO	4.54 ± 0.09^{c}	0.71 ± 0.05^{b}	87.12 ± 0.21^{c}	30.48 ± 0.25^{d}	42.01 ± 0.24^{d}
	1-MCP/CEO	3.45 ± 0.17^{b}	0.83 ± 0.07^{c}	85.21 ± 0.23^{b}	26.89 ± 0.35^{b}	35.43 ± 0.15^{b}
	$KMnO_4$-MCM-41/CEO	3.87 ± 0.15^{b}	0.79 ± 0.06^{c}	87.76 ± 0.31^{c}	29.41 ± 0.27^{c}	40.14 ± 0.21^{c}
	1-MCP/$KMnO_4$-MCM-41/CEO	3.12 ± 0.16^{a}	0.88 ± 0.06^{d}	90.33 ± 0.17^{d}	24.91 ± 0.19^{a}	32.39 ± 0.26^{a}
6d	Uncoated	/	/	/	/	/
	CEO	6.13 ± 0.25^{c}	0.56 ± 0.04^{a}	80.10 ± 0.17^{a}	32.20 ± 0.24^{c}	44.24 ± 0.25^{c}
	1-MCP/CEO	5.02 ± 0.16^{b}	0.72 ± 0.03^{b}	83.45 ± 0.18^{b}	30.45 ± 0.35^{b}	41.43 ± 0.18^{b}
	$KMnO_4$-MCM-41/CEO	5.32 ± 0.14^{b}	0.69 ± 0.05^{b}	81.43 ± 0.19^{a}	30.66 ± 0.21^{b}	41.19 ± 0.29^{b}
	1-MCP/$KMnO_4$-MCM-41/CEO	4.66 ± 0.23^{a}	0.79 ± 0.03^{c}	86.11 ± 0.17^{c}	27.33 ± 0.24^{a}	36.18 ± 0.16^{a}

The letters of the superscripts in each column indicate significant differences ($P < 0.05$).

supported by the mushroom appearance images at different storage periods, as shown in Fig. 2. After the 4th day storage in the room temperature, the sample surface in the control group appeared black spots and obvious browning, however the sample in treated groups kept freshness better.

3.3. Membrane permeability and MDA content

Malondialdehyde (MDA) is one of the products of lipid peroxidation and widely be regarded as a biomarker of lipid peroxidation (Hu, Chen, Xu, Cui, Yu, & Gao, 2015). The accumulation of MDA can cause damage to the plasma membrane and organelle of the fruit, especially changing the cell membrane permeability (Tao, Zhang, & Yu, 2007). As shown in Fig. 3A and 3B, the cell membrane permeability and MDA content of *Agaricus bisporus* gradually increased with storage time increasing. The initial values of membrane permeability and MDA content of all samples were about 9.5% and 0.18 μmol/g, respectively. Compared with the control group, 1-MCP, $KMnO_4$-MCM-41 and composite (1-MCP/$KMnO_4$-MCM-41/CEO) coating significantly ($P < 0.05$) delayed the increase of cell membrane permeability and MDA content, in which the composite preservative paper showed the highest efficiency with cell membrane permeability of 23.1% and MDA content of 0.34 μmol/g after the 6th day storage (Fig. 3). It was speculated that the decrease of ethylene content caused by 1-MCP and $KMnO_4$-MCM-41 can inhibit the increase of cell membrane permeability and MDA content by reducing respiratory rate and delaying lipid peroxidation during storage (Aday, 2016).

3.4. Ascorbic acid content in mushrooms

Ascorbic acid (AA) is a water-soluble strong antioxidant that can inhibit or reduce oxidative damage in fruits and vegetables. The AA initial value in all samples was 4.5 mg/100 g and AA content gradually decreased during storage (Fig. 3C). After the 6th day storage, the retention of AA in samples treated with $KMnO_4$-MCM-41/CEO coated paper and 1-MCP/CEO coated paper was 66.7% and 68.9%, respectively. The 1-MCP/$KMnO_4$-MCM-41/CEO coated paper treated mushroom samples showed the highest AA retention rate (75.6%). However, the AA retention rate in the control group was only 55.6% after the 4th day storage (as the mushrooms in control group had decayed after the 4th day, thus there was no data for the 6th day). This is probably because the decrease of ethylene synthesis due to the presence of 1-MCP and $KMnO_4$-MCM-41 in coating materials reduced the loss of AA.

3.5. Content of soluble solids in mushrooms

The content of soluble solids in *Agaricus bisporus* showed a downward trend with the increase of storage days, as shown in Fig. 3D. All samples of *Agaricus bisporus* had a similar initial value of 5°Brix. The soluble solid content of in all samples rapidly decreased at the first 4 days of storage, this may be due to its high metabolic activity and high respiratory rate. After the 4th day of storage in room temperature, the soluble solid content in control group decreased to 2°Brix. However, the loss of soluble solids in all treated groups was effectively alleviated. After the 6th of storage, the contents of soluble solids in the mushroom samples treated with CEO coated paper, $KMnO_4$-MCM-41/CEO coated paper, 1-MCP/CEO coated paper, 1-MCP/$KMnO_4$-MCM-41/CEO coated paper

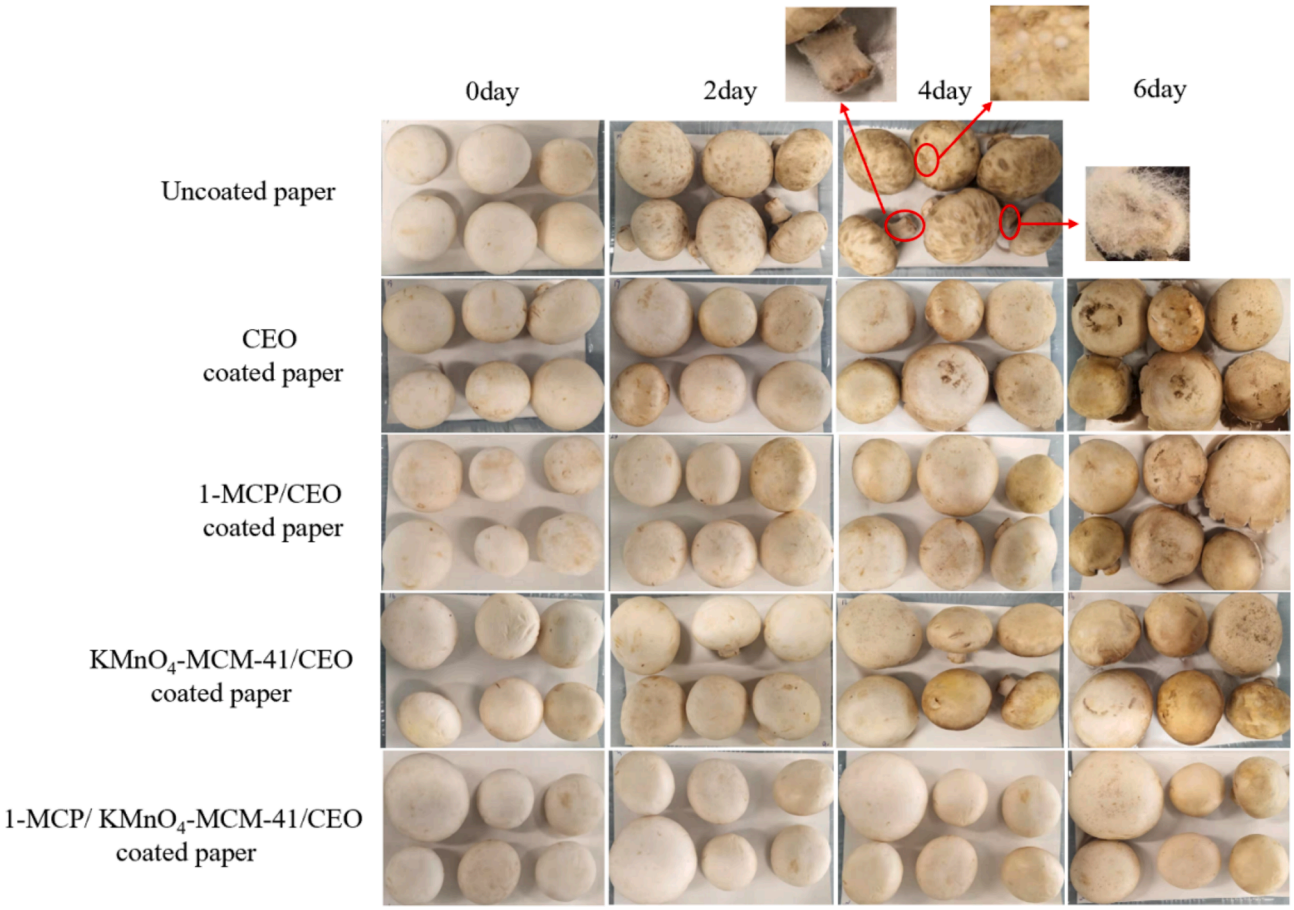

Fig. 2. Appearance of *Agaricus Bisporus* stored in room temperature for 6 days with uncoated paper, CEO coated paper, 1-MCP/CEO coated paper, $KMnO_4$-MCM-41/CEO coated paper, 1-MCP/$KMnO_4$-MCM-41/CEO coated paper.

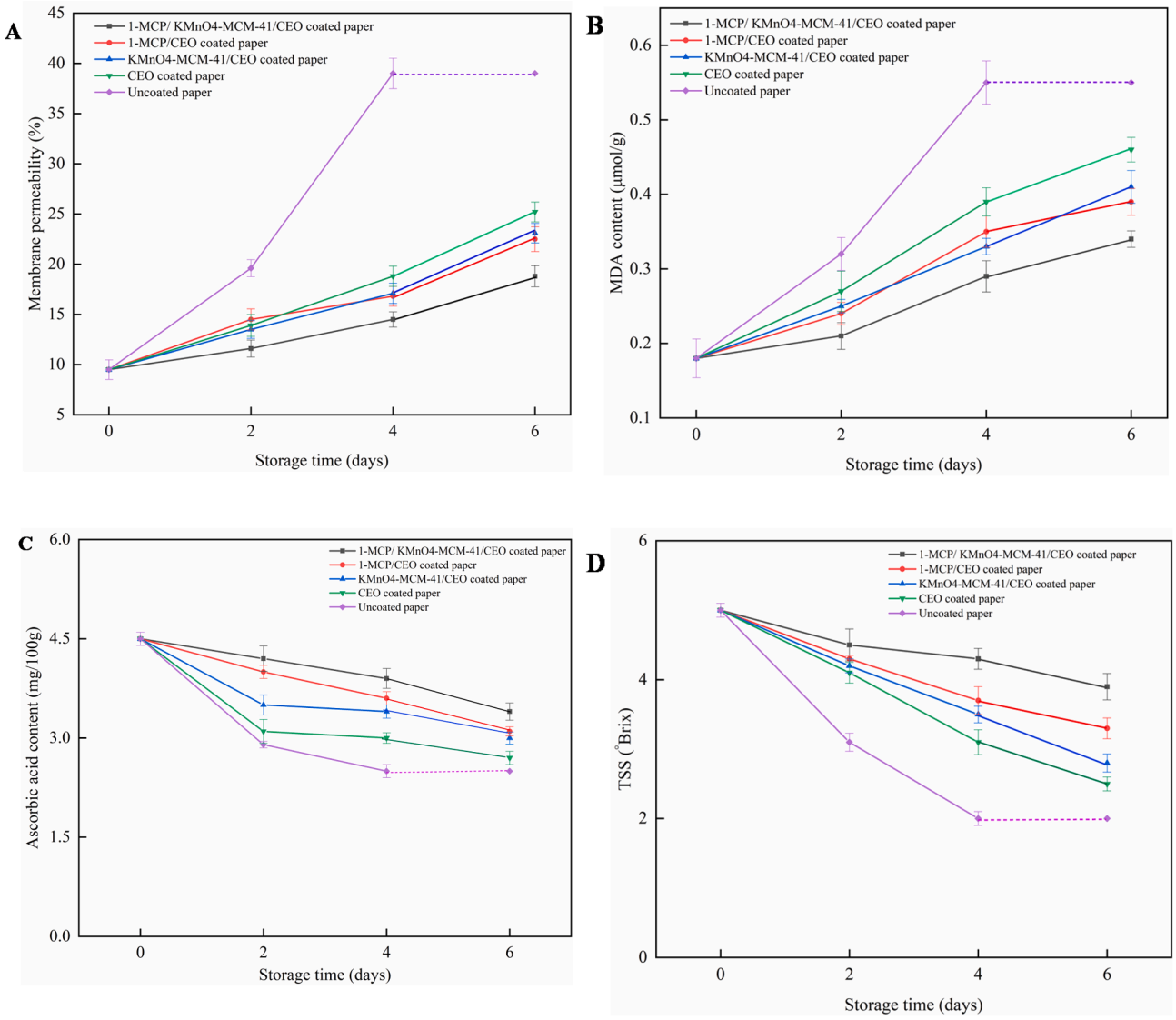

Fig. 3. (A) Cell membrane permeability, (B) malondialdehyde content, (C) contents of ascorbic acid, and (D) soluble solids of *Agaricus bisporus* stored in room temperature for 6 days with uncoated paper, CEO coated paper, 1-MCP/CEO coated paper, $KMnO_4$-MCM-41/CEO coated paper, 1-MCP/$KMnO_4$-MCM-41/CEO coated paper (As the mushrooms of uncoated paper group had decayed on the 4th day and had no edible value, there was no data on the 6th day. In the figure, it is indicated by dotted line).

were 2.5°Brix, 2.8°Brix, 3.3°Brix, 3.9°Brix, respectively. Obviously, 1-MCP/$KMnO_4$-MCM-41/CEO coated paper treatment was the most effective on reducing the loss of soluble solid content in mushrooms. It has been reported that the good antibacterial effect of CEO can help to reduce the consumption of soluble solids in *Agaricus bisporus* (Ponce Cevallos, Buera, & Elizalde, 2010).

3.6. ACS and ACO gene expression and enzyme activity in mushroom fruiting bodies

Li et al. (2019) found that the three predicted plant ethylene response elements in ACO promoter region could be expressed in transgenic onion epidermal cells induced by exogenous ethylene. In ethylene-induced mushrooms at postharvest stage, ACO genes were also highly expressed, suggesting that ethylene signal transduction systems may also exist in the mushroom. The last two steps of ethylene biosynthesis: (i) S-adenosylmethionine is converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthetase (ACS); (ii) then ACC is converted to ethylene by ACC oxidase (ACO). The ethylene production index is always positively associated with transcription products of the ethylene synthesis-related enzymes (ACS or ACO) gene (Zhang et al., 2016). To further investigate the effects mechanism of coating materials 1-MCP, $KMnO_4$-MCM-41 and CEO on the quality traits of *Agaricus bisporus* during storage, the activity and gene expression of ethylene synthesis-related enzymes in the fruiting body of *Agaricus bisporus* were also investigated.

3.6.1. Gene expression

As shown in Fig. 4A and 4B, the gene expression of ACS and ACO in *Agaricus bisporus* increased after the 6th day storage in room temperature. The initial gene expression values of ACO and ACS enzymes were 0.1 and 0.5 for all groups, respectively. The ACO and ACS gene expression of *Agaricus bisporus* treated with $KMnO_4$-MCM-41/CEO coated paper increased from 0.7 to 0.9, and from 0.9 to 1.45 during 2–4 days of storage, respectively. Compared with the control group, 1-MCP/CEO coated paper also inhibited the gene expression of ACO and ACS. During 2–4 days of storage, the ACO and ACS gene expression of *Agaricus bisporus* treated with 1-MCP/CEO coated paper increased from 0.25 to 0.65, and from 0.7 to 1.4, respectively. Noticeably, 1-MCP/$KMnO_4$-MCM-41/CEO coated paper was the most effective way to reduce gene

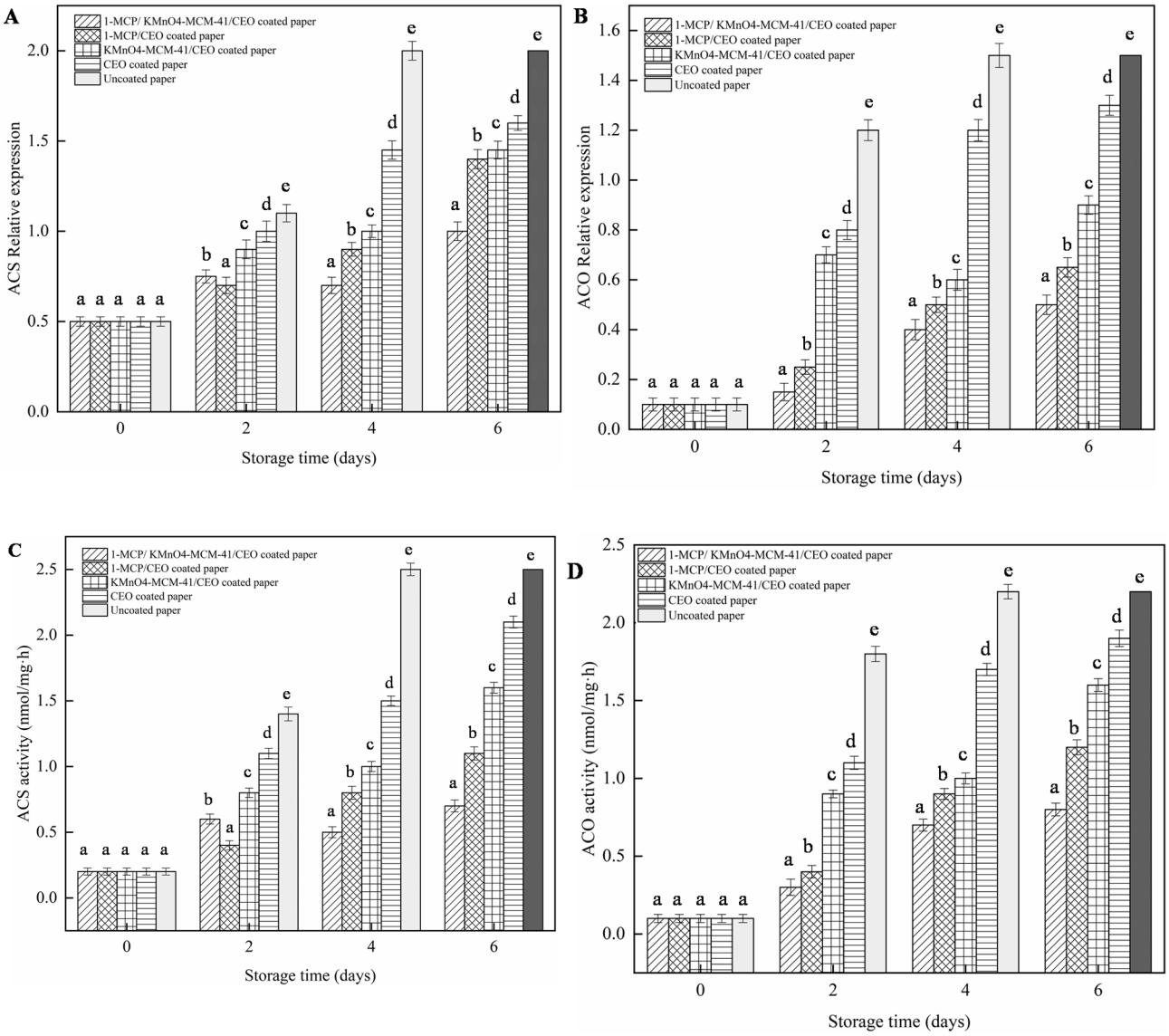

Fig. 4. (A) ACS Relative expression, (B) ACO Relative expression, (C) ACS activity and (D) ACO activity of *Agaricus bisporus* stored in room temperature for 6 days with uncoated paper, CEO coated paper, 1-MCP/CEO coated paper, $KMnO_4$-MCM-41/CEO coated paper, 1-MCP/$KMnO_4$-MCM-41/CEO coated paper (As the mushrooms of uncoated paper group had decayed on the 4th day and had no edible value, there was no data on the 6th day. In the figure, it is indicated by shadow). The letters in each group indicate significant differences ($P < 0.05$).

expression of ethylene synthesis-related enzyme after the 6th day of storage. The reason is could be associated with ethylene production, ethylene perception also plays an important role in mushroom senescence. Ethylene signaling regulates gene expression of ethylene response through a range of biochemical conditions, and then induces ethylene biosynthesis, eventually leading to mushroom senescence. The results showed that 1-MCP combined with $KMnO_4$-MCM-41 can very effectively inhibit the expression of ethylene responsive genes and control ethylene biosynthesis, which is beneficial to maintain the storage quality of postharvest *Agaricus bisporus*.

3.6.2. Enzyme activity

The ACS and ACO enzyme activity of *Agaricus bisporus* during six days of storage in room temperature were shown in Fig. 4C and 4D. ACO and ACS enzyme activities increased during storage of *Agaricus bisporus*. The initial values of ACO and ACS enzyme activities in all groups were about 0.1 and 0.2 (nmol/mg·h), respectively. The activities of ACO and ACS in the treated groups were lower than the control group during the storage time. These enzyme activities of 1-MCP/CEO group were lower than those of $KMnO_4$-MCM-41/CEO group. This phenomenon might be resulted from 1-MCP blocked ethylene signal transduction by binding to the receptor metal with its own double bond and inhibited ethylene synthesis, while the modified molecular sieve can regulate enzyme activity by controlling the ethylene in the environment. Therefore, the composite paper (1-MCP/$KMnO_4$-MCM-41/CEO) probably kept the enzyme activity at a low level through the synergistic action of inhibiting ethylene synthesis and eliminating exogenous ethylene.

3.7. Preservation mechanism and appearance diagram of active packaging paper

1-MCP and CEO were gradually released from the composite paper (1-MCP/$KMnO_4$-MCM-41/CEO) in the process of preservation (Fig. 5). CEO can inhibit the infection of microorganisms on mushroom. 1-MCP reduces the production of ethylene in the mushroom by reducing the gene expression and enzyme activities of ACS and ACO due to inhibiting the combination of ethylene and the receptor. In addition, the potassium permanganate loaded in the molecular sieve can oxidize the ethylene

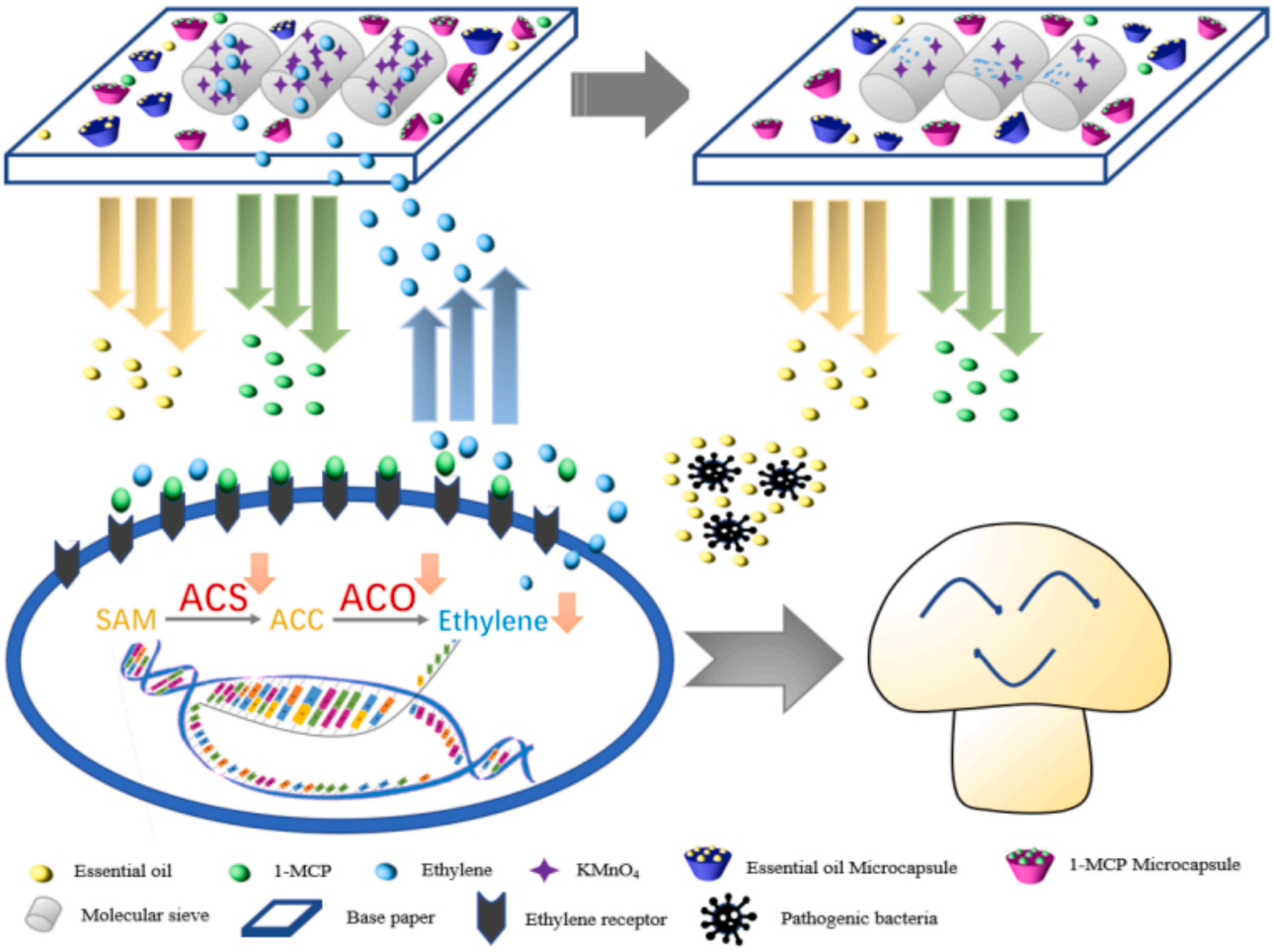

Fig. 5. Preservation mechanism of active packaging paper.

absorbed by the molecular sieve. These chain reactions are able to avoid the adsorption saturation, achieve continuous adsorption and removal by oxidation, and finally effectively reduce the ethylene content in the packaging environment and slow down the maturity and aging of the mushroom.

4. Conclusions

In this study, a novel paper-based food packaging materials with four functional components (1-MCP/$KMnO_4$-MCM-41/CEO) was successfully prepared and characterized by SEM, FT-IR and DSC. Among packaging materials, 1-methylcyclopropen (1-MCP) and molecular sieve loaded with potassium permanganate ($KMnO_4$-MCM-41) acted as ethylene scavengers by inhibiting ACO and ACS enzyme activities and the related gene expression, also continuous adsorption and removal of ethylene production in the packaging atmosphere, respectively. The functional paper was able to effectively delay the softening, browning and weight loss of the mushrooms during storage period. The mushroom even had an acceptable quality with weight loss of 4.66%, firmness of 0.79 kgf, lightness of 86.11 and brown index of 36.18 after the 6th day of storage. Therefore, this prepared composite packaging paper shows a great potential to extend the shelf life of *Agaricus bisporus* during the storage period and transportation process.

CRediT authorship contribution statement

Xiaoyu Ni: Writing - original draft, Data curation. **Jiahao Yu:** Data curation. **Ping Shao:** . **Jiang Yu:** Methodology. **Hangjun Chen:** Methodology. **Haiyan Gao:** Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.128757.

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