Food Packaging and Shelf Life 29 (2021) 100732

Contents lists available at ScienceDirect

Food Packaging and Shelf Life

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

Changes in cell wall metabolism and flavor qualities of mushrooms (*Agaricus bernardii*) under EMAP treatments during storage

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ARTICLE INFO

Keywords:
Agaricus bernardii
EMAP treatment
Cell wall metabolism
Flavor qualities

ABSTRACT

To clarify the dynamic changes of cell wall metabolism and flavor components in *Agaricus bernardii* packed with the packaging materials during storage. The polyethylene (PE), poly (butylene adipate-co-terephthalate)/ poly (L-lactic acid) (ECFPLA) and PBAT/PLLA/ hydrophobic silica (ECFPLASiO_2) with a different gas/water vapor permeability were used as equilibrium modified atmosphere packaging (EMAP) materials, and an appropriate gas concentration [O_2 (0.01−0.03 %), CO_2 (4.58–6.62 %)] was created inside the ECFPLASiO_2 packaging, which led to *Agaricus bernardii* with higher level of cell wall components and lower level of cell wall degrading enzymes during the storage period. For the first time application of HS-SPME–GC–MS on an extract from fresh *Agaricus bernardii*. The results showed that volatile compounds of fresh *Agaricus bernardii* mainly consists of alcohols, ketones and aldehydes, with 3-octanol, 3-octanone, 1-octene-3-alcohol and phenylcarbinol being most abundant compounds. During the storage time, the varieties and content of volatile compounds were changed in all treatments. Alcohols, aldehydes, hydrocarbons and esters contents increased during the storage while ketones declined. Among all treatment groups, ECFPLASiO_2 group showed higher most abundant compounds and organic acid content. The results suggest that ECFPLASiO_2 film could be used to reduce the changes in cell walls and flavor components after harvest and extend the shelf life of *Agaricus bernardii.*

1. Introduction

Agaricus bernardii was one of most popular mushrooms in Inner mongolia Autonomous Region with a grayish-brown cap, fish-like scales, pure black gills and thin fruiting body edges (Wang, Wang, & Sun, 2019). The protein, amino acid, fatty acid, Fe, Zn and P in *Agaricus bernardii* were higher than those in *Agaricus bisporus* (Wang et al., 2019). Mushrooms have been widely consumed long time ago, not only as foods or food flavoring materials but also for medicinal or functional purposes (Cho, Choi, & Kim, 2006; Cho, Kim, Choi, & Kim, 2006). Fresh mushrooms were very perishable agricultural products that have a limited shelf life of 1–3 days at room temperature. Thus, it was important to take post-harvest treatments to extend their shelf life (Czapski & Szudyga, 2000). Several fresh preservation techniques, including edible coating (Sedaghat & Zahedi, 2012), modified atmosphere packaging (MAP) (Ares, Lareo, & Lema, 2007; Liu, Wu, Kan, Lu, & Jin, 2013; Qin, Liu, & Wu, 2015; Villaescusa & Gil, 2003) and radiation (Xiong, Xing, Feng, Tan, & Bian, 2009) have been applied to extend the shelf life of edible mushrooms. MAP was one of the important preservation methods employed for storage of mushroom. Gantner et al. (2017) reported that medium oxygen level (50 %) and polyvinyl chloride (PVC) film with higher permeability (39 μm) was a suitable method in order to maintain an acceptable color of *Agaricus bisporus* during 14 days storage time. Gholami, Ahmadi, and Ahmadi (2020) showed that using of nanoclay film (prepared by low density polyethylene (LDPE), polyethylene-grafted maleic anhydride (LDPE-g-MA), ethylene-vinyl alcohol (EVOH)) and nanoclay film + MAP (10 % O_2 and 10 % CO_2) condition had positive effects on preserving physical, chemical, and mechanical properties of white mushroom during storage and also can extend the shelf life of mushroom till the 15th day. Shi, Wu, Fang, Ma, and Yang (2019) indicated that nanocomposite packaging material (Nano-PM) containing nano-Ag, nano-TiO_2, nano-SiO_2 and nano attapulgite alleviated postharvest senescence of *Flammulina velutipes* by regulating respiration and energy metabolism.

Quality loss in mushroom mainly occurs through color change, texture change, weight loss, stipe elongation, cap opening and off flavor development (Burton & Noble, 1993). Textural change is one of the main reason for loss of quality attributes in edible mushrooms (Olotu,

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https://doi.org/10.1016/j.fpsl.2021.100732
Received 27 February 2021; Received in revised form 14 June 2021; Accepted 19 July 2021
Available online 24 July 2021

Obadina, & Sobukola, 2015), which is mainly related to the metabolism of cell wall chemical components (Lei et al., 2015). Mushrooms show rapidly deterioration after harvest due mainly to changes in cell walls and have a very short shelf life. However, the complex relationship between cell wall chemicals and mushroom hardness and the dynamic metabolic changes associated with textural changes have not been well characterized. Only a few studied have examined the metabolic characteristics of cell wall of mushrooms. Jiang, Wang, Xu, Jahangir and Ying (2010) suggested that changes in firmness were due to differences in cellulose and chitin concentrations. Zivanovic, Busher, and Kim (2000) demonstrated that the softening of *Agaricus bisporus* is mainly caused by the intercellular space at the pilei surface, the contraction of hyphae, the rupture of the central vacuole, and the loss of protein and polysaccharides. Ni, Xu, and Bu (2017) confirmed that glucan and chitin were two important components of the cell wall of shiitake mushrooms and played an important role in the firmness of shiitake mushrooms. The content of glucan and chitin were decreased in the mushroom of softening model. In addition, all the cell wall degrading enzymes were increased significantly in the softening treatment group.

Flavor is also becoming one of the most important quality attributes of agricultural products, thus, producers regulated the flavor of fruit and vegetable crops by means of the breeding, cultivation and postharvest processes (Nicolaï et al., 2008). The widespread consumption of edible mushroom was largely due to their unique flavor. Among the 150 volatile compounds were identified from mushrooms, eight carbons compounds have been considered to be responsible for the mushroom-like odor. 1-octen-3-ol and 1-octen-3-one have been considered most important compounds, but also 1-octanol, 3-octanol, (E)-2-octen-1-ol and 3-octanone were typically present in mushrooms (Cronin & Ward, 2010; Picardi & Issenberg, 1973; Pyysalo & Suihko, 1976; Pyysalo, Berg, & Lund, 1976). Although there have been numerous reports on the flavor compounds of mushrooms (Cho, Kim et al., 2006; Pei et al., 2016; Tian, Zhao, Huang, Zeng, & Zheng, 2016), while study on *Agaricus bernardii* aroma was scarce. A lot of factors may affected the volatile components of fresh products. Storage fresh produce under modified atmospheres, as in controlled atmosphere (CA) can induce metabolic changes to flavor compounds during storage. For various apple varieties, long-term storage under ultra low oxygen conditions reduces volatile flavor substance production, resulting in poor flavor and aroma compared to apples stored in air (Dixon & Hewett, 2001; Lopez, Lavilla, Recasens, Graell, & Vendrell, 2000). Zhou, Ye, and Su (2018) reported that refrigeration extends the shelf life of peaches, but it can also lead to aroma loss. Furthermore, MAP treatment could slow down the respiratory speed and ethylene release rate, also inhibit the formation of aroma compounds (John, Jenny, & Barry, 2005; Ortiz, Graell, Lopez, Echeverría, & Lara, 2010; Thewest et al., 2017). However, systematic investigations on the influence of equilibrium modified atmosphere packaging (EMAP) treatment on changes in the aroma compounds of mushrooms were rather scarce.

However, systematic investigations on the influence of EMAP treatment on changes in metabolism of cell wall and the aroma compounds of *Agaricus bernardii* has not been investigated. Hence, the present novel study was to identify the differentiation and possible pathways of cell wall metabolism and flavor changes in *Agaricus bernardii* under different EMAP treatments during postharvest storage.

2. Materials and methods

2.1. Mushrooms and experimental design

Agaricus bernardii with similar size were harvested at commercial maturity stage from a commercial farm in Hohhot, Inner mongolia, China. The pre-cooling samples about 120 ± 5 g were directly packaged in different package bags (250 mm × 200 mm) on a heat sealing machine and stored at 4 ± 1 °C and 90 % relative humidity (RH) for 19 days. Different treatments were set as follows: control group (packed in air); PE (polyethylene) group; ECFPLA (PBAT was blended with PLLA by adding radio of 30 %) group; ECFPLASiO_2 (PBAT/PLLA was blended with hydrophobic SiO_2 by adding radio of 0.5 wt%) group.

ECFPLA and ECFPLASiO_2 composite films (30 ± 2 μm thickness) were prepared using a twin-screw extruder system (PPT-3/SJ2-20-250; Guangzhou POTOP Experimental Analysis Instrument Co. Ltd., Guangzhou, China). The dry raw materials were pre-mixed according to the blending ratio and then added to the twin-screw extruder, and the extrusion temperature was set to 110~225 °C successively. Commercial PE (30 ± 2 μm thickness) was purchased from Packing Product Co. Ltd. (Shenzhen, China). Manometric gas permeability tester (Lyssy L100−500; Systech Instruments, Oxford, UK) was used to measure the oxygen transmission rate (OTR) and carbon dioxide transmission rate (CTR) of all packaged films at 23 °C followed by the ASTM 1434−82 standard; Permatran-W Model 3/61 water vapor permeability meter (Mocon Inc; USA) was used to measured water vapor transmission rate (WVTR) followed by ASTME 96 (Default Method) with 1 cm^2 mask. Measurement was carried out at 25 °C and 65 % RH. The CTR of PE, ECFPLA and ECFPLASiO_2 were 25325, 12,985 and 9873 m^3/m^2·d, respectively. The OTR were 6130, 2308, 1425 m^3/m^2·d, and the WVTR were 43.2, 1504, 1239 g/m^2·d.

2.2. Chemicals

Analytical grade Phenol, 3,5-dinitrosalicylic acid, Coomassie blue, Anthracene, Resorcinol, Hydroxymethyl cellulose sodium, *N*-acetylglucosamine, D-glucosamine hydrochloride were purchased from China National Pharmaceutical Group Corp (Shanghai, China); Organic acids (succinic acid, malic acid, tartaric acid and citric acid) standard were purchased from Yuanye Biotechnology Co., LTD (Shanghai, China), The purity of all reference compounds was greater than 99 %.

2.3. Measurements

2.3.1. Analysis of gas composition of treatment groups

The O_2 and CO_2 concentration were monitored during storage period by using a headspace O_2/CO_2 analyzer (Model 6600;Systech Instruments, UK). A needle was used to extract a gas sample from the bag and transport it to the detector for detection.

2.3.2. Preparation of alcohol insoluble residue (AIS) and determination of AIS components

For cell wall isolation, mushrooms sample (50 g) was ground with 75 mL 95 % ethanol, boiled for 5 min and settled overnight at room temperature to ensure the inactivation of enzymes and the extraction of low molecular weight solutes. The filtered insoluble material was washed with 40 mL 78 % ethanol. The insoluble material was dried overnight at 37 °C and weighed (Zivanovic et al., 2000). For each sampling date, 3 replications of AIS were prepared. Soluble protein content was measured according to the method of Bradford (1976) with bovine serum albumin as standard. The content of soluble sugar was quantitatively determined by Ye, Li, and Han (2012) with some modification. 1 g of each sample was added to 10 mL distilled water and hydrolysed at 110 °C for 30 min, and the solution was strained and diluted with water to 100 mL. 0.5 mL extraction was mixed with 1.5 mL distilled water, 1 mL 0.09 g/L the phenol solution, 5 mL concentrated sulfuric acid. The mixed solution was stand at room temperature for 30 min and absorbance was detected at 485 nm. Chitin was measured according to Kapteyn, Ter and Vink (2001) with some modifications. 0.1 g AIS sample was placed in 10 mL hydrochloric acid, and incubated at 110 °C for 4 h, 1 mL hydrolysate was blended with 1 mL 2 % resorcinol and 7.5 mL 75 % sulfuric acid, samples were incubated at 100 °C for 30 min and took constant volume to 10 mL. The absorbance at 500 nm was measured in ultraviolet spectrophotometer (UV-2450, Shimadzu, Japan). The calibration curve was prepared with D-glucosamine. The determination of cellulose was based on Jiang et al. (2010) with some modifications. AIS samples (0.1 g) was

dissolved with 60 % sulfate acid to 100 mL, 2 mL filter solution was mixed with 0.5 mL 2 g/L anthracene tung and 5 mL sulfate acid. Samples absorbance were read on a spectrophotometer at 620 nm, and a standard curve developed using purified cellulose was used to calculate concentrations.

2.3.3. Analysis of cell wall degrading enzymes

β-1,3 glucanase activity was measured according to (Leelasuphakul, Sivanunsakul, & Phongpaichit, 2006) with some modifications. Briefly, β-1,3 glucanase activity was determined using 100 μL 4 g/L laminarin in 100 μL crude enzyme at 37 °C for 40 min. 1.8 mL distilled water and 1.5 mL DNS reagent were added into reaction tube and boiled for 3 min, then the volume of reaction liquid was adjusted to 25 mL. The boiling crude enzyme solution as a control. The β-glucanase activity unit(U)was described as 1×10^{-9} mol glucose released per sec under specified conditions. For analysis of chitinase, the reaction solution was consisted of 0.5 mL enzyme solution, 0.5 mL acetic acid–sodium acetate buffer (50 mM, pH 5.2) and 0.5 mL 10 g/L chitin suspension and incubated at 37 °C for 1 h. Then, the mixed solution continued to incubate at 37 °C for 1 h after mixed with 0.1 mL of 30 g/L helicase and heated in a boiling water bath for 3 min after mixed with 0.2 mL 0.6 M potassium tetraborate. And then 2 mL dimethylaminobenzaldehyde-diaminopropane (DMAB) was added to mixed solution and incubated at 37 °C for 20 min. The deactivated crude enzyme solution was used as the control. The solution absorbance at 585 nm was detected. The chitinase activity unit (U) was defined as the weight of n-acetyl glucosamine (μg) hydrolysed by chitinase from chitin in 1 h at 37 °C (Ni et al., 2017). The cellulase activity was measured according to (Ni et al., 2017). The cellulase activity unit (U) was defined as the weight of glucose (μg) hydrolysed from cellulose by cellulase in 1 h at 37 °C.

2.3.4. Analysis of headspace volatile compounds

HS-SPME-GC–MS analysis was performed as described by Laurienzo, Stasio, Malinconico, and Volpe (2010) with some modifications. Mushroom samples (2 g) were ground in liquid nitrogen with a mortar. The powders were put into a 20 mL headspace extraction bottle. A 50/30 μm Divinylbenzene- Carboxen/polydimethylsiloxane (DVB-CAR/PDMS) fibre was used to extract volatile compounds from *Agaricus bernardii* and preconditioned at 250 °C for 2 h prior to analysis. The fibre was inserted into the sample vial through the septum and exposed to the HS for 40 min at 60 °C to collect the analytes. Agilent 7890A-5975C GC–MS (Agilent Inc.) with a capillary column (HP-5MS, 30 m × 0.25 mm × 0.25 μm; Agilent Inc.) was used to determine the content of aroma compounds. The flow of helium gas was 1 mL/min. The column temperature was kept at 50 °C for 3 min, then raised progressively to 150 °C at an increasing rate of 5 °C/min, and finally kept at 230 °C for 7 min. The electronionization source temperature was maintained at 230 °C and mass spectra were obtained by electronic impact at 70 eV. Volatile compounds were identified by comparing the mass spectral data with the spectral data of the Wiley 98 and NIST 08 Library. Relative percentages of the separated compounds were calculated from total ion chromatograms by using the computerized integrator.

2.3.5. Analysis of acid contents

Organic acids were extracted according to Li et al. (2014) with some modifications. Mushroom sample (3 g) was homogenized with 10 mL 0.1 % phosphoric acid solution under ice bath conditions, ultrasonic extraction for 1 h at 60 °C, and centrifuged for 15 min at 4500*g*. After constant volume of supernatant and 0.45 μm filter membrane, the supernatant was tested. Agilent 1100HPLC (Agilent Inc.) with a capillary column (Innoval NH_2, 250 mm × 4.6 mm × 5 μm) was used to determine the content of acids. The column temperature was 35 °C and 10 μL of sample was injected. The mobile phase was 0.1 % phosphoric acid-methanol (85:15, V/V). The detector was set at λ=210 nm. The content of individual organic acids were calculated by a standard curve and expressed in $mg.g^{-1}$ of fresh weight (FW).

2.4. Statistical analyses

The statistical analyses were performed using the SPSS 19.0 (SPSS Inc., Chicago, IL). The significant difference of the treatments were determined by Tukey's HSD test with the level of significance at $p < 0.05$. Principal component analysis (PCA) was applied to assess differences in aroma compounds among EMAP treatments groups using the SAS (V9.2, The SAS Institute, USA).

3. Results and discussion

3.1. Effects of EMAP treatments on the gas composition during storage

The changes in CO_2 and O_2 concentrations in package during the storage were displayed in Table 1. In EMAP treatment, the gas composition was depended on the gas permeability of packaging material and the respiration of mushroom. Mushroom is a highly perishable product with high metabolic rate and respiration rate, which can produce CO_2 and consume O_2 in the microenvironment of package. In the case of PE group, the CO_2 concentration rapidly increased from 0.03 % to 3.65 % by 3rd day. A slightly increase in CO_2 concentration between 11th and 19th days. However, the O_2 content decreased rapidly from 20.9 % to 0.13 % by 3rd day. The O_2 concentration inside PE group was in the rage of 0.08−0.13 % and the CO_2 concentration was 3.65–4.30 % between 3rd and 19th days.

In the case of ECFPLA group, the CO_2 concentration rapidly increased from 0.03 % to 4.76 % by 3rd day. While the O_2 concentration decreased from 20.9 % to 0.02 %. During the whole storage period, the O_2 concentration inside ECFPLA group was in the rage of 0.01−0.02 % and the CO_2 concentration was 3.35–4.76 % between 3rd and 19th days. For the ECFPLASiO_2 group, the CO_2 concentration increased to 6.62 % and O_2 concentration decreased to 0.02 % by 3rd day, and a steady gas concentration with 4.58–6.62 % CO_2 and 0.01−0.03 % O_2 were attained within the storage period. Overall, the CO_2 concentration in ECFPLA-SiO_2 group was higher than PE and ECFPLA group ($p > 0.05$). This result was related to the gas permeability of the membrane. The lower CTR of ECFPLASiO_2 film can prevent the CO_2 produced by respiration from being discharged through the membrane. From the O_2 concentration result we found that, although PE film has a higher OTR (6130 $m^3/m^2 \cdot d$), it formed a lower level of O_2 concentration in the package microenvironment, which indirectly indicated the high respiration rate of *Agaricus bernardii*.

In our preliminary study, we tried to examine the effect of MAP with different barrier packaging materials (PE, PBAT/PLLA, PBAT/PLLA/SiO_2 (0.5S SiO_2) and polyamide/PE) on the physical and selected enzyme activities of *Agaricus bernardii*, with a purpose to the better understanding how the MAP treatments delay senescence and deterioration of postharvest *Agaricus bernardii* during storage. The results indicated that the low O_2(<1 %) and moderate CO_2 levels (4–7 %)in 0.5S SiO_2 film maintained a high level of soluble protein, soluble sugar, ascorbic acid, and phenolic contents, inhibited the increase of malondialdehyde, increased the antioxidant enzymes activities of peroxidase and decreased the enzymes activities of polyphenol oxidase. The quality index of the mushroom packed in the 0.5S SiO_2 film remained a good condition and kept the market value after 15 days of storage (Wang, Chen, & Zhang, 2020). The law of gas concentration change in this study was consistent with its results. And these results have been expressed by Jafri, Jha, Bunker and Ram (2013), although the O_2 concentration below 1 % for the treatments, which could retain the better quality characteristics of *Pleurotus florida* during the whole storage period. EMAP is a powerful tool that can control the growth of putrefactive bacteria and the physiological effects of mushrooms (Li et al., 2014). It should be noted that ECFPLASiO_2 film is prepared by melt blending of biodegradable polymers and nanocomposite materials. The preparation of food packaging film by biodegradable polymers has been the focus of attention in recent years for environmental reasons.

Table 1
Changes of CO_2 and O_2 concentration in each treatment group during storage.

Storage time(d)/Sample	CO_2 concentration			O_2 concentration		
	PE	ECFPLA	ECFPLASiO$_2$	PE	ECFPLA	ECFPLASiO$_2$
0	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	20.9 ± 0.00	20.9 ± 0.00	20.9 ± 0.00
3	3.65 ± 0.35^{c}	4.76 ± 0.14^{b}	6.62 ± 0.28^{a}	0.13 ± 0.07^{a}	0.02 ± 0.01^{b}	0.02 ± 0.01^{b}
7	3.78 ± 0.01^{b}	3.81 ± 0.35^{b}	4.58 ± 0.21^{a}	0.12 ± 0.01^{a}	0.02 ± 0.00^{b}	0.03 ± 0.00^{b}
11	4.05 ± 0.49	4.08 ± 0.28	4.66 ± 0.14	0.12 ± 0.01^{a}	0.01 ± 0.00^{b}	0.02 ± 0.00^{b}
15	4.30 ± 0.56	3.45 ± 0.49	4.95 ± 0.63	0.10 ± 0.00^{a}	0.02 ± 0.00^{b}	0.01 ± 0.00^{b}
19	4.10 ± 0.42	3.35 ± 0.07	4.85 ± 0.77	0.08 ± 0.01^{a}	0.02 ± 0.00^{b}	0.01 ± 0.00^{b}

Values are mean ± stardard deviation of triplicate determination. Average in each row with different letters mean significant difference at $p < 0.05$ (comparison between different treatments on the same storage day).

3.2. Changes of AIS content and composition

Mushroom cell wall usually consisted of glucan, chitin and protein. β-1,3/1,6-Glucan was function as mucilage that connects cells together by being highly branched on the surface of the wall (Wessels, 1990).

Fig. 1 shows the effect of different treatment groups on the AIS content of *Agaricus bernardii* during cold storage. As shown in Fig. 1(A), The AIS content at harvest was 6.07 % in *Agaricus bernardii*, representing mainly the cell wall material. The AIS content in control group had a tendency to increase, with the 37.72 % increase over the 7 days storage. This was due to severe dehydration symptoms, resulting in an increase in AIS content measurement on a fresh weight basis throughout the storage period. On the other hand, a slight reduction of AIS in both EMAP treatments was found with storage time. Significant differences were found between the EMAP and control groups in terms of AIS content ($p < 0.05$). Throughout storage time, the ECFPLASiO$_2$ group maintained a steady level of AIS content. The change rule of AIS content was consistent with that of hardness (data not shown) during storage. Total protein content decreased by 45.73 % in control group at 7 days when the EMAP groups remained unchanged then decreased at the later stage (Fig. 1(B)) ($p < 0.05$). This results was accordance with the Zivanovic's result. According to Zivanovic et al. (2000), proteins extracted by both water and 1 N NaOH declined in AIS, although the amounts and changes in water-soluble protein were relatively small compared to those in the NaOH fraction. Protease activity plays an important role in the protein degradation pathway (Murr & Morris, 1975), and the liberated amino acids were probably used for synthetic substrates of cell metabolism and chitin synthesis since no amino-acid accumulation during storage has been noted (Murr & Morris, 1975).

The soluble sugar in control group was reduced by 53 % compared to EMAP treatments up to day 7 of storage (Fig. 1(C) ($p > 0.05$). Among the EMAP treatments groups, the most effective treatment was ECFPLASiO$_2$ group which showed 37.71 % decrease at the end of storage period. According to Jiang 's result, within 8 days of storage, AIS polysaccharides extracted by 10 mol/L NaOH decreased in by more than 50 % and then continued to decline as storage time increased. The purpose of extraction with 10 mol/L NaOH was to hopefully deacetylated and partially depolymerized chitin, and further completed depolymerization with 6 mol/L HCl, thereby releasing the bound polysaccharides (Sietsma & Wessels, 1979). The 10 mol/L NaOH fraction might represent wall polysaccharides that was utilized during storage. Fungal cell walls can be used as a source of carbohydrates for metabolism in the case of limited storage conditions after harvest (Peberdy & Ferenczy, 1985), and the decrease of glucose content in AIS of stored mushrooms was one of the main reasons leading to the decline of polysaccharides (Zivanovic et al., 2000). Appropriate MAP treatments reduced the loss of protein and polysaccharides, especially, packaging with LDPE film (0.04 mm thick) with two macroholes helps in maintaining steady O_2 and CO_2 level (Jiang et al., 2010). Studies have shown that the decrease of soluble protein and soluble sugar in cell walls were positively correlated with the softening of edible fungi (Zivanovic et al., 2000). The higher level of soluble sugar and soluble protein in ECFPLASiO$_2$ group was

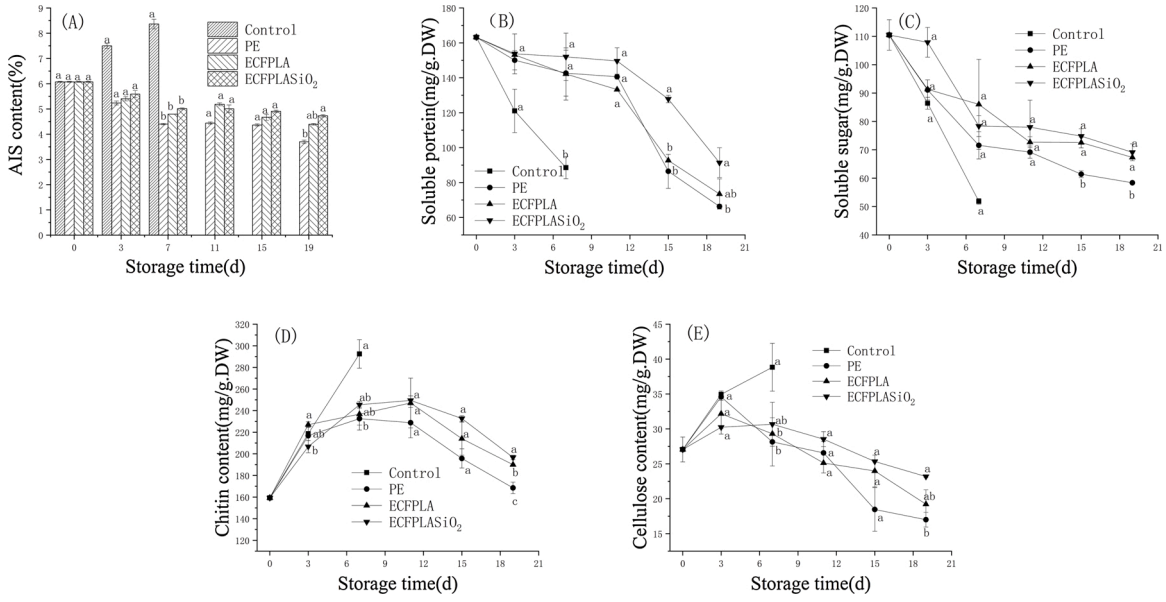

Fig. 1. Changes in AIS content (A), soluble protein (B), soluble sugar (C), chitin (D) and cellulose (E) of *Agaricus bernardii* during shelf life. The vertical bar represent the SDs of three replicates. Different letters mean significant differnece at $p < 0.05$ (comparison between different treatments on the same storage day). (600 × 600 DPI).

consistent with its higher hardness level (data not shown).

Chitin was a β-1,4-acetyl-glucosamine homopolymer that increases the strength of cell walls by forming stiff microfibrils. As shown in Fig. 1 (D), the chitin content increased by 86 % in the control group during the first 7 days, which was higher than PE, ECFPLA ($p > 0.05$) and ECFPLASiO$_2$ group ($p < 0.05$). The results indicated that EMAP treatments could inhibit the formation of chitin to some extent. Zivanovic et al. (2000) reported that increased chitin content associated with toughening during the storage ($r = 0.95$). The increase of hardness values might be realized by increase of chitin content and formation of covalent bonds between chitin and R-glucan, which enhances rigidity of the hyphal wall (Wessels, 1986). This result explained the main reason for the increase of AIS content in the control group. Comparing the packaging groups, the ECFPLASiO$_2$ group maintained a higher level of chitin content than PE group and ECFPLA group at the later stage ($p < 0.05$). It may be that ECFPLASiO$_2$ group inhibited the consumption of substrates by respiration, and the chitin content reflected the integrity of the cell wall to a certain extent.

Cellulose contents in EMAP groups increased during the first 3 days and then declined, whereas that of control group showed significantly increases during the first 7 days (Fig. 1(E)). The content of cellulose in control group was 27, 24 and 21 % higher than those in PE ($p > 0.05$), ECFPLA and ECFPLASiO$_2$ groups ($p < 0.05$) on day 7. Cellulose is generally believed to play a very important role in the development of plant cell walls. These additional cellulose contents is thought to be brought about by a rapid polymerization and epimerization of cell wall microfibrils leading to the thickening of the cell walls (An, Zhang, & Lu, 2007). Quantitative changes in the cellulose contents of mushrooms after harvest will presumably affect their textural properties (Jiang et al., 2010). EMAP treatments retarded cellulose increase, while control treatment promoted cellulose accumulation. Compared between the EMAP groups found that the cellulose content of ECFPLASiO$_2$ group was higher than PE ($p < 0.05$) and ECFPLA groups ($p > 0.05$) at the later stage. In general, moderate changes in the quantity of cellulose content occurred in EMAP groups, which was due to the fact that the crystalline nature of cellulose imparts a high resistance to chemical or enzymatic degradation (Bartz & Brecht, 2002).

3.3. Changes in cell wall degradation enzymes during storage

Significant changes were observed in the activities of cell wall degrading enzymes (Fig. 2). β-glucanase is a cell-wall-degrading enzyme mainly effective on glucans. As shown in Fig. 2(A), β-1,3 glucanase activity in the control group was increased up to day 3 of storage and decreased during the rest of storage period. This trend was observed in all EMAP treatments. However, the activity in ECFPLASiO$_2$ film treated mushrooms remained at lower levels as compared to other treatments ($p < 0.05$). The glucanase may play a role in the degradation of cell wall sugars by corresponding to change in cell wall sugars during storage (Singh, Langowski, Wani, & Saengerlaub, 2010).

Chitinase in the control group was increased up to day 3 of storage and significantly higher than EMAP groups (Fig. 2(B)) ($p < 0.05$). At the early stage of storage, the chitinase of EMAP treatment groups showed little difference and reached the peak on the 7th day, then all showed a decreasing trend. The chitinase of PE was higher than ECFPLA group ($p > 0.05$) and ECFPLASiO$_2$ group ($p < 0.05$) at 19 days. Sakamoto, Nakade, and Sato (2013) identified three genes encoding chitin degrading enzyme (chi1, chi2 and chi3) in *Lentinus edoides* after harvest, and the expression of the three genes was significantly up-regulated after harvest, indicating that the increased expression of chitinase played a crucial role in the fruiting body senescence of edible fungi after harvest. ECFPLASiO$_2$ group may inhibit relative enzyme activities, reducing the degradation and depolymerization of chitin of mushroom. As shown in Fig. 2(C), the cellulase of control group was 24.21 μg/h.g at the beginning of storage, and reached its peak on the 3 day (31.45 μg/h.g), which was significantly higher than those of the EMAP groups ($p < 0.05$). As far as each EMAP group was concerned, the cellulase reached its peak on the 7 day with the range of 25.92–26.78 μg/h.g, and showed a downward trend in the later period of storage. Since cellulose is not the main component of fungal cell walls, the physiological and biochemical significance of cellulase on fungal cell walls remains to be studied. Nevertheless, cellulase is an important enzyme for mushroom growth. Mushrooms secrete cellulase to digest high-cellulose media (Ni et al., 2017). The ECFPLASiO$_2$ group maintained a lower level of cellulase than PE group and PBAT/PLLA group ($p < 0.05$), which could reduce the damage of the cell wall cellulose microfibril- hemicellulose-pectin "longitude and weft structure", and could better inhibit the cellulose degradation to ensure the integrity of the cell wall skeleton structure.

3.4. Effects of EMAP treatments on the aroma compounds during storage

Volatile compound emissions at fresh and the content for each individual compound were presented in Table 2. It was worth mentioning that the flavor composition of *Agaricus bernardii* has not been measured previously. The main chemical groups of the volatile compounds in *Agaricus bernardii* were (a) alcohols, representing 44.07 % of the total concentration of aroma compounds, followed by (b) ketones (42.13 %) and (c) aldehydes (1.02 %), other compounds such as hydrocarbons and esters were present in minor concentration. This result was consistent with Kuka's research (Kuka, Cakste, Galoburda, & Sabovics, 2014) on *Cantharellus cibarius*. The four most abundant compounds found in *Agaricus bernardii* were 3-octanol, 3-octanone, 1-octene-3-alcohol and phenylcarbinol, representing 34.26, 42.06, 4.06 and 4.82 % of total aroma concentrations respectively. The researcher shows that 1-octen-3-ol, 1-octen-3-one and 1-hexanol were the major compounds found in *Portuguese chanterelle* (De Pinho et al., 2008). ketones and alcohols were recognized as the predominant volatile compounds in fresh *F. velutipes*, and 3-octanone and 3-octanol were accounted for 43.3 % and 31.5 % of them respectively (Fang et al., 2017). These difference in the volatile aroma compounds and amounts in the present and previous studies might to due to variation between edible mushrooms.

The varieties and contents of volatile compounds from *Agaricus bernardii* were changed in control and EMAP treatments during the 19 days storage time. A significant increase of relative alcohol content was

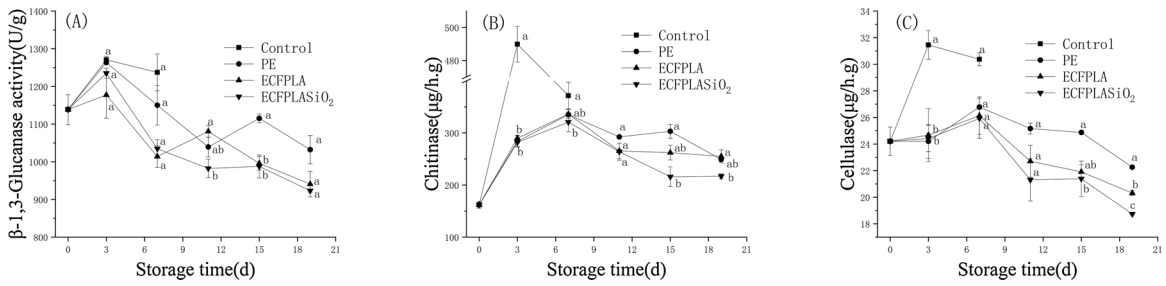

Fig. 2. Changes in β-1,3 glucanase activity (A), chitinase (B) and cellulase (C) of *Agaricus bernardii* during shelf life. The vertical bar represent the SDs of three replicates. Different letters mean significant differnece at $p < 0.05$ (comparison between different treatments on the same storage day). (600 × 600 DPI).

Table 2
Changes of volatile flavor substances in each treatment group during storage.

NO	Compounds name	Formula	Relative time (min)	Relative content(%)										
				Fresh	Control-7	PE-7	ECFPLA -7	ECFPLASiO$_2$-7	PE-11	ECFPLA -11	ECFPLASiO$_2$-11	PE-15	ECFPLA -15	ECFPLASiO$_2$-15
Alcohols				44.04	46.78	42.44	44.49	34.77	45.48	76.15	40.08	80.37	65.17	66.25
1	1-pentanol	$C_5H_{12}0$	3.49	0.05	1.05	1.14	1.90	1.1	2.14	16.51	1.89	11.95	10.58	12.33
2	2-methyl-butanol	$C_5H_{12}O$	3.54	0.03	0.41	0.42	0.92	0.36	1.00	10.29	0.79	7.29	7.88	8.03
3	2,3-butanediol	$C_4H_{10}O_2$	5.051	–	0.58	2.43	3.71	4.27	9.04	29.03	4.47	32.59	20.01	15.63
4	1-hexanol	$C_6H_{14}O$	6.439	0.05	0.04	0.07	0.15	0.07	0.08	0.44	0.24	0.43	0.63	1.06
5	n-heptanol	$C_7H_{16}O$	9.446	–	0.09	0.03	0.05	–	1.30	0.08	0.07	0.08	0.10	0.13
6	3-methiopropyl alcohol	$C_4H_{10}OS$	9.874	–	0.08	0.02	0.04	0.06	0.04	0.15	0.09	0.24	0.12	0.29
7	1-octene-3-alcohol	$C_8H_{16}O$	9.741	4.06	–	3.72	2.25	1.24	1.37	1.59	1.10	1.77	1.40	2.37
8	3-octanol	$C_8H_{18}O$	10.238	34.26	23.48	27.53	27.78	22.62	13.01	3.90	20.2	4.95	1.95	7.69
9	isooctyl alcohol	$C_8H_{18}O$	11.277	–	0.05	0.11	0.24	0.03	0.03	0.88	0.20	1.26	1.10	0.84
10	phenylcarbinol	$C_9H_{12}O_3$	11.617	4.82	18.63	4.37	5.66	3.42	11.85	6.54	8.77	16.07	15.29	13.09
11	n-octyl alcohol	$C_8H_{18}O$	12.576	0.73	0.25	1.75	0.70	0.52	1.09	0.33	0.62	0.24	0.32	0.44
12	2-nonyl alcohol	$C_9H_{20}O$	13.492	–	0.01	0.01	0.02	0.13	0.55	0.14	0.02	0.14	0.16	0.06
13	phenethyl alcohol	$C_8H_{10}O$	13.979	0.04	2.11	0.84	1.07	0.95	3.98	6.27	1.62	3.36	5.63	4.29
Ketones				42.13	31.79	53.28	54.00	60.17	49.47	13.21	54.97	11.47	20.71	27.94
14	2-heptyl ketone	$C_7H_{14}O$	7.029	0.01	0.02	0.04	0.06	0.15	0.18	0.22	0.03	0.12	0.13	0.36
15	3-octanone	$C_8H_{16}0$	9.953	42.06	31.54	52.79	53.47	57.05	45.08	12.00	54.66	10.5	19.48	25.81
16	acetophenone	C_8H_8O	12.463	0.01	0.03	0.03	0.04	0.04	0.05	0.11	0.04	0.12	0.08	0.07
17	2-nonyl ketone	$C_9H_{18}O$	13.221	–	0.08	0.32	0.30	2.71	3.76	0.56	0.05	0.27	0.80	1.14
18	2-undecanone	$C_{11}H_{22}O$	19.088	0.01	0.03	0.04	0.03	0.09	0.20	0.07	0.03	0.05	0.05	0.22
19	geranylacetone	$C_{13}H_{22}O$	23.272	0.04	0.09	0.06	0.1	0.13	0.20	0.25	0.16	0.41	0.17	0.34
Aldehydes				1.02	2.84	1.17	1.01	1.30	2.90	2.80	2.12	3.58	2.46	1.57
20	benzaldehyde	C_7H_6O	9.165	1.02	2.84	1.17	1.01	1.30	2.90	2.80	2.12	3.58	2.46	1.57
Hydrocarbon				0.39	1.92	1.65	1.22	1.71	2.35	1.99	2.02	7.06	2.36	2.07
21	3-ethyl hexane	C_8H_{18}	8.575	–	–	–	–	–	0.01	0.03	0.03	0.03	0.01	0.01
22	anti-2,3-epoxy butane	C_4H_8O	8.755	–	–	–	–	–	0.01	0.03	0.03	–	–	0.04
23	hexamethylcy	$C_6H_{18}O_3Si_3$	12.931	–	0.01	0.19	0.18	0.20	0.06	0.10	0.05	0.09	0.14	0.10
24	decamethylcyclopentasiloxane	$C_{10}H_{30}O_5Si_5$	15.096	0.13	0.13	0.14	0.06	0.09	0.22	0.05	0.17	0.16	0.29	0.21
25	dodecamethylcyclohe xasiloxane	$C_{12}H_{36}O_6Si_6$	19.959	0.16	0.42	0.54	0.23	0.45	0.70	0.29	0.75	2.37	0.94	0.51
26	dodecamethylpentasiloxane	$C_{12}H_{36}O_4Si_5$	24.507	0.08	0.31	0.41	0.21	0.47	0.68	0.47	0.52	4.31	0.62	0.46
27	styrene	C_8H_8	7.049	0.01	1.04	0.06	0.04	0.04	0.14	0.73	0.43	0.09	0.23	0.42
28	cyclooctatetraene	C_8H_8	7.029	0.01	0.01	0.06	0.1	0.16	0.12	0.20	0.03	–	–	0.17
29	1-eleven ene	$C_{11}H_{22}$	13.221	–	–	0.25	0.40	0.30	0.41	0.09	0.01	0.01	0.13	0.15
Esters				0.14	0.44	0.18	0.35	0.45	0.96	2.19	0.52	1.84	2.55	1.81
30	ethyl isobutyrate	$C_6H_{12}O_2$	3.874	–	0.36	–	–	0.03	–	0.06	–	0.04	0.13	0.15
31	ethyl butyrate	$C_6H_{12}O_2$	4.706	0.01	–	0.01	0.01	0.01	0.02	0.24	0.03	0.48	0.44	0.25
32	isoamyl acetate	$C_7H_{14}O_2$	6.635	0.02	0.02	0.01	0.02	0.02	0.05	0.56	0.17	0.49	0.59	0.22
33	methylbutyl acetate	$C_{14}H_{20}O_3$	6.704	0.02	0.02	0.01	0.02	0.01	0.03	0.45	0.09	0.31	0.24	0.14
34	butyl propyl lactone	$C_4H_6O_2$	7.95	0.01	0.02	0.02	0.01	0.02	0.01	0.05	0.09	0.04	0.02	0.07
35	isoamyl butyrate	$C_9H_{18}O_2$	12.104	0.03	–	0.01	0.07	0.17	0.01	0.01	0.01	0.01	0.23	0.01
36	iso-amyl n-valerate	$C_{10}H_{20}O_2$	13.398	0.01	–	0.04	0.13	0.07	0.51	0.01	0.01	0.01	0.07	0.02
37	benzyl acetate	$C_9H_{10}O_2$	15.431	0.02	0.01	0.01	–	0.01	0.03	0.02	0.03	0.04	0.06	0.02
38	ethyl caprylate	$C_{10}H_{20}O_2$	16.351	0.02	0.01	0.05	0.06	0.06	0.16	0.21	0.02	0.16	0.10	0.19
39	ethyl3-phenylpropionate	$C_{11}H_{14}O_2$	20.634	–	–	0.01	–	0.02	–	–	–	–	0.03	0.02
40	decanoic acid, ethyl ester	$C_{12}H_{24}O_2$	21.78	–	–	–	0.01	–	0.02	0.03	–	0.02	0.06	0.10
41	ethyl laurate	$C_{14}H_{28}O_2$	27.5	–	–	–	–	–	0.02	0.13	0.01	0.05	0.2	0.19
42	ethyl tetradecate	$C_{16}H_{32}O_2$	31.324	–	–	–	–	–	0.02	0.11	0.01	0.05	0.12	0.21
43	ethyl palmitate	$C_{18}H_{36}O_2$	33.874	–	–	0.01	0.01	0.02	0.06	0.23	0.03	0.11	0.19	0.15
44	ethyl linoleate	$C_{20}H_{36}O_2$	35.961	–	–	–	0.01	0.01	0.01	0.08	0.02	0.03	0.07	0.07
Other compounds				0.12	0.56	0.43	0.39	0.42	0.73	0.69	0.41	0.67	0.53	0.57
45	o-isopropyl benzene	$C_{10}H_{14}$	11.134	–	0.02	0.01	0.01	0.01	0.03	0.03	0.02	0.07	0.04	0.03
46	benzoic acid	C_6H_5COOH	15.628	0.1	0.19	0.15	0.13	0.18	0.2	0.28	0.2	0.17	–	0.17
47	naphthalene	$C_{10}O_8$	16.036	0.02	0.21	0.2	0.18	0.22	0.34	0.3	0.17	0.34	0.4	0.29
48	5-fluorine indole	C_8H_7N	17.272	–	0.03	0.03	0.02	0.01	0.04	0.03	0.01	0.04	0.03	0.02
49	2, 6-D itert-butyl paracresol	$C_{15}H_{24}O$	25.068	–	0.05	0.03	0.04	–	0.1	0.04	0.01	0.03	0.05	0.03
50	dimethoxybenzene	$C_8H_{10}O_2$	14.909	–	0.06	0.01	0.01	–	0.02	0.01	–	0.02	0.01	0.03

"-" Means no detection.

observed in all treatment groups during storage duo to the increase of 2,3-butanediol, 1-pentanol and 2-methyl-butanol compounds content. But the relative content of main compound 3-octanol progressively declined over 19 days storage due to the participation of oxidative reaction processes (Niponsak, Laohakunjit, & Kerdchoechuen, 2015), and the same pattern was found for 1-octene-3-alcohol. 1-octene-3-alcohol has been considered as contributing a typical raw mushroom odor, which was enzymatically formed from linoleic acid (Pyysalo et al., 1976; Tressl, Bahri, & Engel, 1982). Compared to initial value (4.06 %), PE, ECFPLA and ECFPLASiO$_2$ group reduced to 1.77, 1.40 and 2.37 % respectively at the end of storage. Comparison between EMAP treatment groups, the content of 3-octanol and 1-octene-3-alcohol of ECFPLASiO$_2$ group were higher than those of other EMAP groups. The biosynthesis of ethanol fermentation-derived volatiles is responsible for the "alcoholic" and "fermented" off-flavor and impaired the sensory acceptability of fresh produce (Guzel-Seydim, Seydim, & Greene, 2000). It was worth mentioning that no ethanol was produced in each EMAP group during the whole storage period.

The ketones was the second major volatile compounds in *Agaricus bernardii*. The ketones level in all treatment groups was declined during storage, and the same pattern was found for 3-octanone. The content of 3-octanone was increased in the early stage and then decreased at the later stage in all treatment groups, which was generally in agreement with the finding of Zhang, Pu, and Sun (2018). The ketones content of ECFPLASiO$_2$ group was higher than other treatment groups.

On the opposite, aldehydes, hydrocarbons and esters in all treatments group were increased during storage. The increase observed in aldehydes was due to the increase of benzaldehyde, which came from microbial catabolism amino acid (Pripis-Nicolau, de Revel, Bertrand, & Maujean, 2000). Compared to initial value (1.02 %), control, PE, ECFPLA and ECFPLASiO$_2$ group increased to 2.84, 1.17, 1.01 and 1.30 % respectively after 7 days storage, and ECFPLASiO$_2$ group showed lowest content at the end of storage. The relative ester content of fresh mushroom was 0.14 %, which has the single species. With the extension of the storage period, the more and more species of ester substance were detected. Compared to initial value, the ester content of PE, ECFPLA and ECFPLASiO$_2$ group increased to 1.84, 2.55 and 1.81 % respectively at the end of storage, which mainly depends on the increases of ethyl-butyrate, isoamylacetate and acetic acid-2 methyl butyl ester content. Most of the preconditions for the synthesis of ester substances the production of esters by carbohydrates in the presence of oxygen (Luis, Feijoo, Villanueva, Lagaron, & Giménez, 2006). In Zhou' s research (2018), appropriate MAP treatment (O_2: 3 %–5 %, CO_2: 3 %–5 %, and N_2: 90 %–94 %) could maintain the higher content of flavor of peach, such as sugar, acids and aroma compounds. To sum up, ECFPLASiO$_2$ group was the best EMAP treatment, providing final *Agaricus bernardii* with the highest contents of main volatile compounds.

To provide a general overview of volatile compounds evolution in different treatments during storage period. GC—MS data were compressed and represented by PCA. The PCA model created with identified volatile compounds demonstrates the overall volatile profile differences (Fig. 3). The variance contribution rate of the first and second PCs was 76.6 % and 19.96 % respectively. Treatment groups at different storage period could be visually grouped into three different clusters. The first group included all treatment groups except the ECFPLA at 11 days and all treatments groups at 15 days. These results indicated that the volatile components in each treatment group did not change significantly during the first 11 days storage period. The second group included ECFPLASiO$_2$ group at 15 days. The third group included the ECFPLA at 11 days and PE, ECFPLA groups at 15 days. Compared between treatment groups found that ECFPLASiO$_2$ group was also relatively close to the fresh sample during the storage period.

3.5. Effects of EMAP treatments on acid content during storage

Acids took crucial role in the flavor and physiological metabolic process, which were consumed as respiration substrates (Wu, Quilot, Genard, Kervella, & Li, 2005). The changes of acid content of *Agaricus bernardii* in different treatments were shown in Fig. 4. It could be seen that succinic acid (14.20 mg.g/FW) was the major organic acid, followed by malic acid (9.78 mg.g/FW), citric acid (9.38 mg.g/FW)and tartaric acid (7.15 mg.g/FW). All of these organic acids were also found

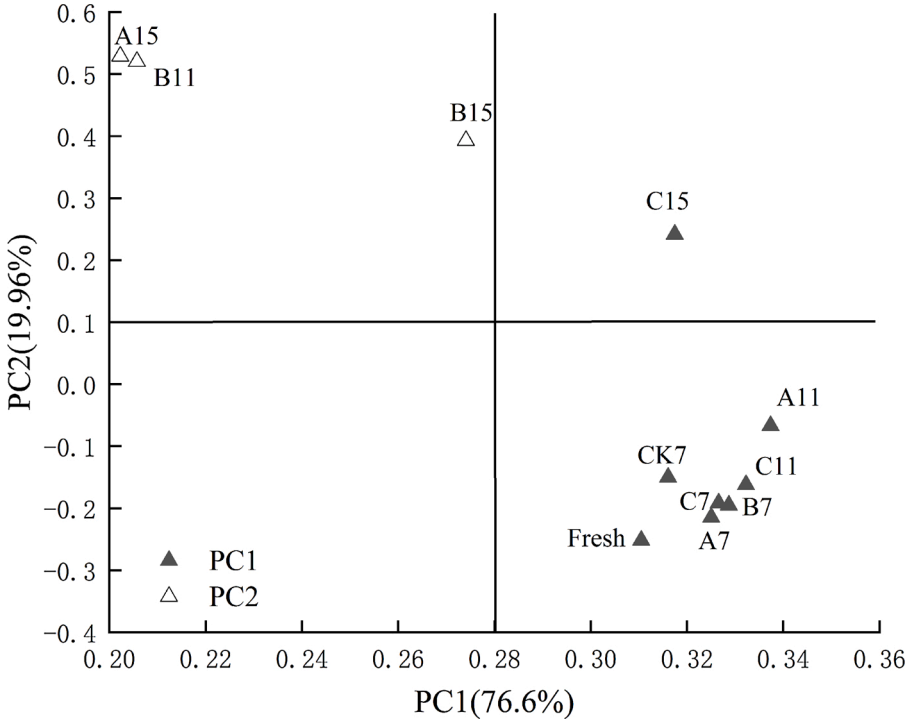

Fig. 3. PCA of GC—MS data for *Agaricus bernardii* with different treatments at different storage time. CK, A, B and C refers to Control, PE, ECFPLA and ECFPLASiO$_2$ group respectively. Numbers refer to days of storage.

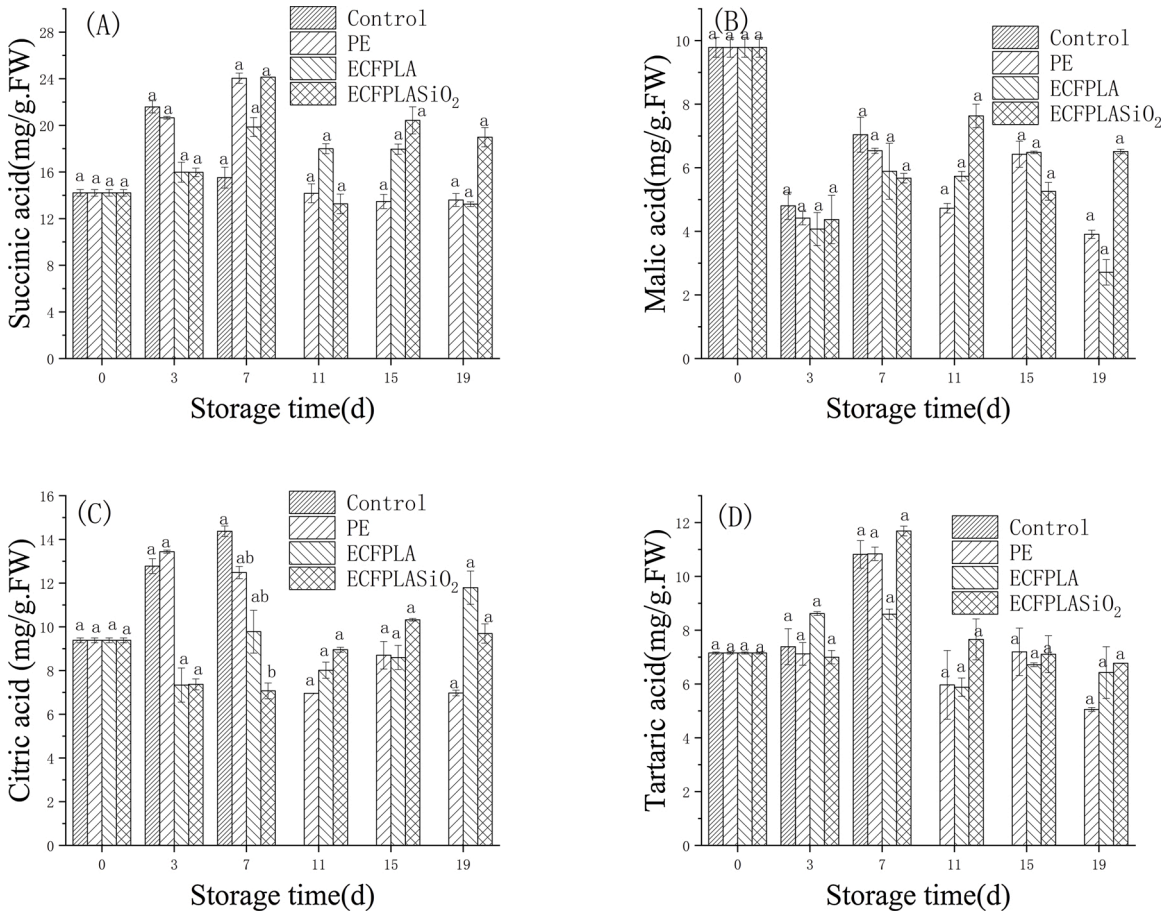

Fig. 4. Changes in the contents of succinic acid (A), malic acid (B), citric acid (C) and tartaric acid (D) during shelf life. The vertical bar represent the SDs of three replicates. Different letters mean significant differnece at $p < 0.05$ (comparison between different treatments on the same storage day). (600 × 600 DPI).

by Pei et al. (2014) in *Agaricus bisporus*. The organic acid in each treatment group showed a trend of increasing at the early stage and then decreasing at the later stage expect malic acid. As shown in Fig. 4(A), the succinic acid contents of EMAP groups were higher than that of control group at the early stage ($p > 0.05$). The succinic acid content of ECFPLASiO$_2$ group was higher than other EMAP groups at 19 days ($p > 0.05$). The malic acid content of all treatments decreased and all EMAP treatments were lower than that of control group at the early stage (Fig. 4(B)) ($p > 0.05$). The content of malic acid of ECFPLASiO$_2$ group was 6.67 mg.g/FW at 19 days and was higher than PE and ECFPLA group (3.90 and 2.72 mg.g/FW) ($p > 0.05$). The citric acid is produced by fungal fermentation and is the second largest fermentation product after industrial ethanol. As shown in Fig. 4(C), the citric acid in control and PE groups increased to 1.53 and 1.33 times of initial value respectively at 7 days storage, on the contrary, citric acid of ECFPLA and ECFPLASiO$_2$ group changed more gently. Citric acid of all treatment groups showed the tendency to decrease at the later stage. ECFPLA group has the highest content of citrate acid at 19 days, which was higher than those of PE group and ECFPLASiO$_2$ group ($p > 0.05$). The tartaric acid content of all treatments decreased from initial value 7.15 mg.g/FW to 5.05–6.77 mg.g/FW at the end of storage period (Fig. 4(D)). The presence of organic acids in edible fungi not only affects its flavor, but is related to its stability and the length of its freshness (Begic'Akagic' et al., 2014). The presence of organic acids in natural substrates can also reduce oxidative stress (Wu, Cheng, & Wang, 2017). Among all treatment groups, ECFPLASiO$_2$ group showed higher organic acid and could inhibit the consumption of organic acid during storage, which was consistent with the report that fruits stored at appropriate concentration of O_2 and CO_2 had a higher acid content compared with control group (Liu, Wang, Qin, & Tian, 2016).

4. Conclusions

The cell wall metabolism and flavor components in *Agaricus bernardii* packed with the different packaging materials were analysed. Due to afford appropriate gas concentration [low O_2 (0.01−0.03 %), moderate CO_2 (4.58–6.62 %) in package], The ECFPLASiO$_2$ treatment significantly reduced the changes in cell wall components and cell wall degrading enzyme activities. Besides, this study provided the first determination of the aroma-active compounds in *Agaricus bernardii*. The most predominant aroma compounds in fresh *Agaricus bernardii* were 3-octanol, 3-octanone, 1-octene-3-alcohol and phenylcarbinol. The varieties and content of volatile compounds from *Agaricus bernardii* in all treatments became abundant during the storage time. Alcohols, aldehydes, hydrocarbons and esters content increased during the storage while ketones declined. Among all treatment groups, ECFPLASiO$_2$ group could inhibit the loss of flavors such as aroma compounds (especially predominant aroma compounds) and acids. This study provided theoretical basis for the metabolism of cell wall and flavor production during post-harvest *Agaricus bernardii* storage.

Author's contributions

L.M. Wang conducted most of the experiments, completed statistical analysis of data, interpreted results and edited the manuscript. Y.F. Zhou and Y.Y. Wang participated in the analyze of flavor compounds under EMAP packaging materials. H.Y. Bu participated in the analyze of cell wall metabolism. T. Dong supervised data collection and processing, and

revised the manuscript.

Declaration of Competing Interest

The authors report no declarations of interest

Acknowledgement

This research was supported by the Natural Science Foundation of Inner Mongolia (no.2019 MS03062).

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