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Minimization of Off-Flavor Occurrence During the Storage of Modified Atmosphere Packaged *Pleurotus ostreatus*

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Abstract

This study was conducted to investigate the minimization of off-flavor occurrence and the maintenance of high quality in modified atmosphere packaged *Pleurotus ostreatus* during the storage. There are 4 treatments used to preserve high quality and for deodorization of MAP mushroom: *Artemisia princeps*, *Artemisia capillaries*, green tea and activated charcoal. The mushrooms were packed in polyethylene film with each treatment and were stored at 5 and 20°C. No difference was observed in weight loss, CO_2 and O_2 concentration, or color of mushrooms packed with or without treatment. However, the principal component analysis (PCA), electronic nose, revealed differences in off-flavor occurrence between control (MAP mushroom without treatment) and treatment groups at 5°C. This result suggested that *Artemisia princeps* and *Artemisia capillaries* was masking the off-flavor in MAP mushroom because the unique flavor of them was strongly revealed and green tea and activated charcoal might have a role of removing the off-flavor by adsorbing ethanol and acetaldehyde, which is known to cause off-flavor. The sensory test showed that *Artemisia princeps* and *Artemisia capillaries* dough treatment inhibited microbial growth.

Key words: deodorization, *Pleurotus ostreatus*, Artemisia, green tea, charcoal, electric nose

INTRODUCTION

Mushrooms are widely consumed because of their unique flavor as well as plentiful nutrients such as carbohydrates, amino acids, vitamins and minerals. Also, it is reported that mushrooms can exert antioxidative activities and antimutagenicties (1,2). However, mushrooms such as *Pleurotus ostreatus* undergo rapidly deterioration during storage and distribution. Many studies on maintaining freshness of mushrooms have been reported on modified atmosphere packaging (MAP) (3-5), controlled atmosphere (CA) (6), chill storage (7), coating treatment (8), radiation treatment (9), ozone treatment (10) and moisture absorber treatment (11,12). MAP is one of the most effective and economical ways to maintain freshness (3,13,14), but it can result in considerable losses of the unique flavor of mushrooms because the relatively large amounts of ethanol and acetaldehyde are produced in the film bag during storage (15,16). This strong off-flavor leads to deterioration of the flavor in the fresh mushrooms. The intense modified atmosphere created anaerobic condition in the packages, which probably promoted fermentation which, in turn, induced the off-flavor.

Villaescusa and Gil (17) reported that the characteristic aroma of mushrooms decreased during storage time. In addition, moderately serious off-odors were detected for mushrooms stored in PVC and LDPE packages. Lee et al. (16) also reported that the amounts of ethanol and acetaldehydes in the shiitake mushroom stored in PE film were increased proportionally to the thickness of film during the storage. Minamida et al. (15) showed that exposing the mushrooms stored in PE film in air for 6 hours decreased the off-flavor, but it brought about losses of the characteristic flavor in the mushrooms.

The off-flavors in foods may originate from environmental pollutants, the growth of microorganism, oxidation of lipids, or endogenous enzymatic decomposition (18). Many studies have been published on deodorization for removal of the odor in foods (19-22). There are the deodorization materials; β-cyclodextrin and activated charcoal as chemical materials, and green tea and Mugwort as natural materials, but little information has been published on the deodorization for MAP mushrooms. The aim of this study was to investigate the minimization of off-flavor occurrence and maintenance of high quality during the storage of *Pleurotus ostreatus* packaged with various treatments such as *Artemisia princeps*, *Artemisia capillaries*, green tea and activated charcoal, which are known to have deodorization effects.

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MATERIALS AND METHODS

Sample preparation

Fresh mushrooms (*Pleurotus ostreatus*) were obtained from a farm in Gyeonggi Province, Korea. The freshly harvested mushrooms were transported to the laboratory and were promptly placed in a cold chamber at 5 and 20°C until being packed.

The treatments for preserving high quality and deodorization were: *Artemisia princeps* (AP), *Artemisia capillaries* (AC), green tea (GT) and activated charcoal (CH). The dried AP, AC and GT were purchased at Kyoungdong market (herbal medicine market) in Seoul, Korea and were powdered in a blender (Chung-gye Industrial, Korea). Activated charcoal was purchased from Sigma. AP, AC and GT dough for treatment were mixed with proper ratios of distilled water (DW) according to the weight of each sample powder (AP powder : DW=1:3, AC powder : DW=1:2, GT : DW=1:1.5). Each amount of sample was 2 g of AP, AC powder and 5 g of GT powder, and activated charcoal, respectively to 100 g of mushroom. The amounts used in the treatments were selected based on a preliminary study.

Packaging and storage

The mushrooms were sorted by size and appearance. Acceptable mushrooms were selected at random and were placed into polyethylene pouches, each pouch contained 100±2 g of mushrooms. The polyethylene pouches were LDPE (low density polyethylene) film of 20 cm×30 cm, 45.33 μm thickness, and 912.8 cc/m^2/days transfer rate (Sewang Co., Korea). Plastic cups (diameter: 5.5 cm, height: 3.0 cm) were used to hold treatments. *Artemisia princeps*, *Artemisia capillaries*, green tea dough and activated charcoal were put in the plastic cups and placed in the edge of the packaging. Packages were heat-sealed and were stored in chamber at 5 and 20°C. Samples were evaluated after the 1st, 2nd and 3rd days of storage at 20°C and after the 2nd, 4th, 6th and 8th days of storage at 5°C. The analyses were done in triplicate. Mushrooms packed without treatment were used as controls.

Electronic nose analysis

Electronic nose analysis was used to measure changes in the mushroom flavor as described through the use of PCA (principal component analysis). The electronic nose with a sensor array composed of six metal oxide sensors (Figaro Engineering Inc, Tokyo, Japan) was manufactured by Hanbit Instrument (Seoul, Korea). The injection of a gas sample was performed at the set time by opening the valve, which was controlled by computer. Data from the metal oxide sensors were also obtained via a computer. For analysis, the mushroom (5 g) was placed in a 325 mL glass bottle sealed with polyethylene. The equilibrium time for analysis was 5 min in the electronic nose. All measurements were performed at 30°C. The measurement procedures comprised of heater cleaning for 10 sec, purging the sensors and pumping clean air for 20 sec, and then headspace analysis of mushroom for 180 sec.

Instrumental analysis

The weight loss was expressed as a percentage of the initial weight at the beginning of storage. The weight loss rate (%) was calculated using the following equation.

$$\text{Weight loss rate } (\%) = [(W_1 - W_2) / W_1] \times 100$$

(W_1: initial weight, W_2: weight after storage)

For analysis of O_2 and CO_2 concentration inside film the package, oxygen analyzer (IIJIMA products M.F.G. Co. Ltd., Japan) and gas chromatography (Hewlett Packard 6890) with thermal conductivity detector (TCD) were used. Samples (1 mL) of the modified atmospheres within the packages were taken with a syringe through a tape. Analysis of CO_2 was performed on an activated charcoal column. The measurement procedures were conducted at an injection temperature of 120°C, oven temperature of 70°C, detector temperature of 150°C. The carrier gas was He (30 mL/min).

The surface color of the mushrooms was measured using a colorimeter (Minolta chromameter CR-200, Japan). Before measurement, the instrument was standardized with a white plate L=91.74, a=-0.97, b=1.46). Ten measurements were taken at random locations on the stem of each mushroom, and the results were expressed as Hunter b (yellowness) values.

Quality evaluation

The quality evaluation was determined by sensory evaluation. The sensory evaluation was conducted to determine microbial contamination by three trained panelists. Panelists scored each attribute on the extent of contamination on a 5-point scale; 5 point: no contamination (fresh mushroom), 4 point: contamination ratio <10%, 3 point: contamination ratio <30%, 2 point: contamination ratio >50%, 1 point: contamination ratio >80% (contamination serious).

Statistical analysis

Statistical analysis of the data was performed using SAS software. Analysis of variance (ANOVA) with Duncan's multiple-range test was used to test the significance of differences among means. Significance was accepted at $p<0.05$.

RESULTS AND DISCUSSION

Electronic nose analysis

The electronic nose with six metal oxide sensors was used to identify off-flavor occurrence of mushrooms during storage. The data obtained from the electronic nose were used to investigate the occurrence of off-flavor for PCA (Principal Component Analysis).

In the Fig. 1, the proportion of the first principal component score was 0.847 and the second principal component score was 0.143 at 20°C after 1 day. The PCA plot of fresh mushrooms (before storage) was placed on the right side (positive value of the first principal component) on the other hand that of treated mushroom and control (MAP mushroom without treatment) was placed on the left side (negative value of the first principal component). No differences in occurrence of off-flavor were found between treatments and control. It was believed to be a consequence the off-flavor of MAP mushrooms being very strong regardless of treatment after 1 day. However, at 5°C, the PCA plot of treated mushroom showed characteristic development of off-flavor at dif-

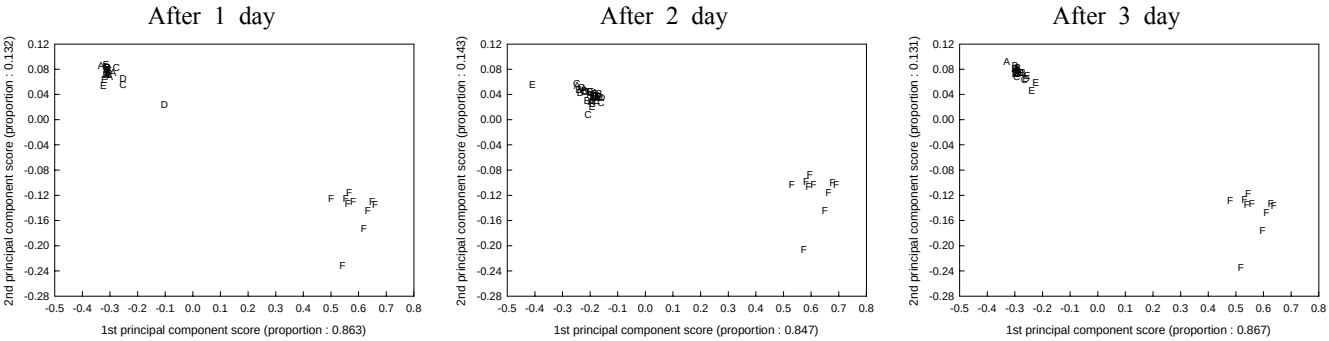

Fig. 1. Principal component analysis for *Pleurotus ostreatus* stored at 20°C.
A: control (MAP mushroom without treatment), B: MAP mushroom with *Astemisia princeps* dough, C: MAP mushroom with *Astemisia capillaries* dough, D: MAP mushroom with green tea dough, E: MAP mushroom with charcoal, F: Fresh mushroom.

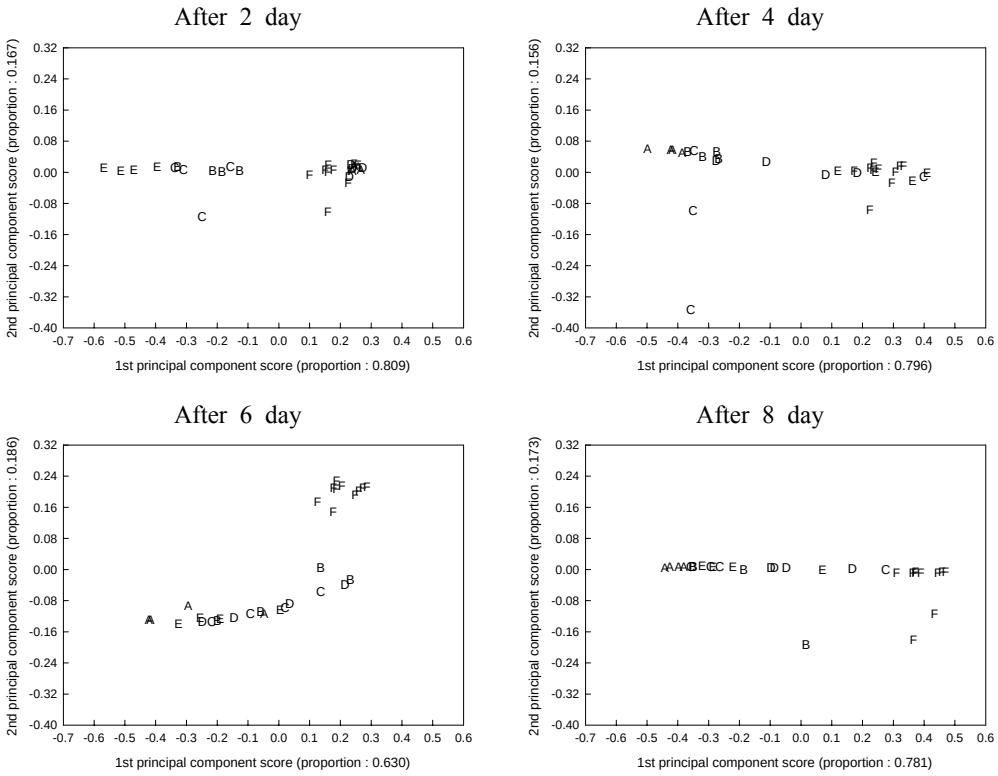

Fig. 2. Principal component analysis of *Pleurotus ostreatus* stored at 5°C.
A: control (MAP mushroom without treatment), B: MAP mushroom with *Astemisia princeps* dough, C: MAP mushroom with *Astemisia capillaries* dough, D: MAP mushroom with green tea dough, E: MAP mushroom with charcoal, F: Fresh mushroom.

ferent levels among groups. Fig. 2 showed the first principal component in mushroom packed with green tea dough, and control had a positive value whereas mushrooms packed with *Artemisia princeps* or *Artemisia capillaries* dough or activated charcoal had negative values after 2 days storage. After 4 days, the PCA plot of fresh mushroom was placed on the right side and control on the left side and that of mushroom packed with treatment was located between the fresh mushrooms and control, and changes appearance were observed during storage at 5°C.

The results suggested that the strong flavors of *Artemisia princeps*, *Artemisia capillaries* masked the off-flavor of MAP mushroom, and that green tea and activated charcoal might remove the off-flavor by adsorbing ethanol and acetaldehyde which are known to produce off-flavor.

Kee and Park (21) reported that activated charcoal added to onion juice might remove onion flavor by absorbing volatile flavor compounds in onion. Asao and Wang (22) also reported the charcoal particles combined with H_3PO_4 removed ammonia and trimethylamine odors. In addition, Urabe et al. (20) reported that green tea was exhibited deodorant activity against methylmercaptan that was the specifically offensive main volatile of porcine small intestines used as a human food.

Weight loss

The weight loss in mushrooms is due to moisture loss by diffusion through the film and respiratory substrates loss by respiration. Fig. 3 shows the differences in the weight loss between mushrooms packed with treatment and without treatment during storage. All groups lost weight during the storage, but weight loss of all groups (including control) was less than l% at 20°C and 0.6% at 5°C. There were no significant differences among control and treatments, but the storage temperature of 20°C or 5°C did have an effect. The results showed an effect of modified atmosphere (MA) that decrease transpiration rate and respiration activity by modified gas content in bag.

Loss of 3～6% of weight of fresh vegetable and fruit is usually enough to cause a marked deterioration of quality for most kinds of products and for mushrooms the acceptable weight loss is about 2% (13,23).

Package atmosphere

Fig. 4 shows the changes in CO_2 and O_2 concentration in the PE film bag. The CO_2 concentration in the bag increased rapidly, between 11% and 13%, within l day at 20°C and 2 days at 5°C, in all groups regardless of treatments. The O_2 concentration decreased to 1% and remained at less than 1% throughout storage.

Some authors have reported an optimum in-package O_2 concentration of 1% without creating anaerobic conditions (3), and that the accumulation of CO_2 inside the mushroom package can also prevent deterioration. On the other hand, excessive accumulation of CO_2 (>20%) inside package can have a damaging effect, producing ethanol and acetaldehyde as well as potential growth of anaerobic pathogens causing anaerobic respiration (3, 6,14,23). In our case, the off-flavor was detected in the mushrooms stored in packages and it was believed to

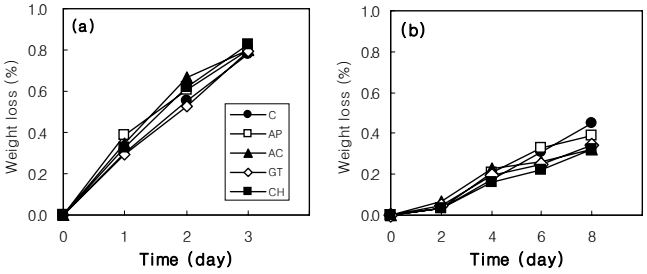

Fig. 3. Changes in weight loss (%) in modified atmosphere packaged (MAP) *Pleurotus ostreatus* during storage at 20 (a) and 5°C (b). C: control (MAP mushroom without treatment), AP: MAP mushroom with *Astemisia princeps* dough, AC: MAP mushroom with *Astemisia capillaries* dough, GT: MAP mushroom with green tea dough, CH: MAP mushroom with charcoal.

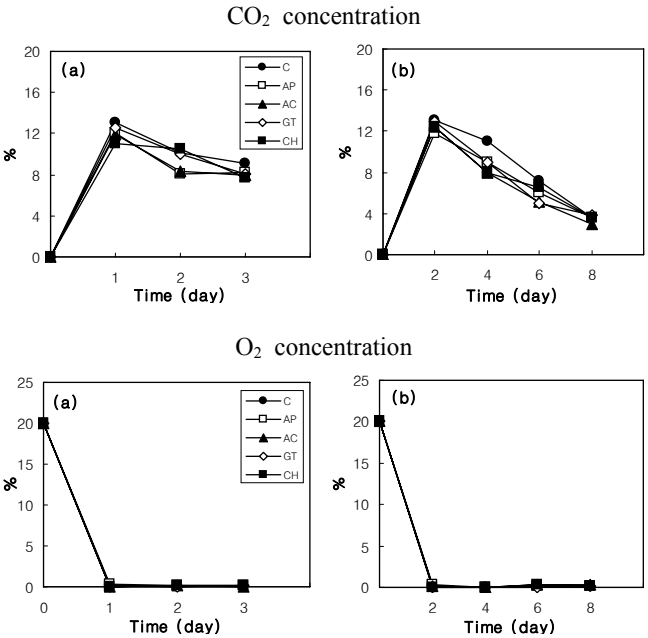

Fig. 4. Changes in packaging atmosphere of modified atmosphere packaged (MAP) *Pleurotus ostreatus* during storage at 20 (a) and 5°C (b). C: control (MAP mushroom without treatment), AP: MAP mushroom with *Astemisia princeps* dough, AC: MAP mushroom with *Astemisia capillaries* dough, GT: MAP mushroom with green tea dough, CH: MAP mushroom with charcoal.

Table 1. Changes in color (Hunter b value) of MAP *Pleurotus ostreatus*

Temperature	Storage time (days)	Treatment[1)]				
		C	AP	AC	GT	CH
20°C	0	16.65	16.65	16.65	16.65	16.65
	1	17.73^{ab}	16.39^{a}	18.76^{b}	18.30^{b}	18.64^{b}
	2	19.76^{a}	18.93^{a}	18.59^{a}	19.65^{a}	19.40^{a}
	3	20.88^{b}	19.86^{ab}	18.40^{a}	20.49^{b}	19.21^{ab}
5°C	0	16.65	16.65	16.65	16.65	16.65
	2	17.62^{a}	18.51^{a}	18.77^{a}	17.57^{a}	17.52^{a}
	4	17.67^{a}	17.14^{a}	19.15^{a}	19.17^{a}	17.55^{a}
	6	18.78^{a}	19.03^{a}	19.99^{a}	19.51^{a}	19.21^{a}
	8	19.79^{a}	19.79^{a}	19.64^{a}	19.69^{a}	19.86^{a}

[1)]C: control (MAP mushroom without treatment), AP: MAP mushroom with *Astemisia princeps* dough, AC: MAP mushroom with *Astemisia capillaries* dough, GT: MAP mushroom with green tea dough, [2)]CH: MAP mushroom with charcoal.
[a,b]Means (the average of ten replicates) within the same row bearing the same superscript are not significantly different ($p<0.05$).

be caused by anaerobic respiration. The CO_2 concentration decreased gradually after reaching a peak by 24 hours of storage (Fig. 4). According to Lee (24), this appearance was considered to be a consequence of film permeability characteristics-OTR (oxygen transfer rate), CTR (carbon dioxide transmission rate).

Color

Duckworth and Coleman (25) observed that the activity of tyrosinase, responsible for mushroom browning was depending on O_2 concentration. The yellowness of mushrooms during storage is shown in Table 1. At 5°C, the b value (yellowness) was generally increased from 16.65, initial b value of fresh mushrooms before storage. At 20°C, the b value of AC dough treatment increased less comparing to others.

Quality evaluation

Bacterial growth on the surface and internal tissue of freshly harvested mushrooms is a cause of deterioration during post harvest storage (26). Fig. 5 shows the changes in the extent of contamination in modified atmosphere packaged mushrooms during storage. At 20°C, the mushrooms packed with *Artemisia princes* (AP) or *Artemisia capillaries* (AC) dough exhibited little microbial contamination (the score of AP and AC treatment was 4.76 and 4.86, respectively) while the mushroom packed with green tea (GT) dough, activated charcoal (CH) and without treatment had severe microbial contamination (the score of GT, CH and control was 2.76, 2.79 and 2.78, respectively). For that reason, the mushrooms packed with green tea (GT) dough, activated charcoal (CH) and without treatment lost their value as commodities after 3 days. At 5°C, the sensory score of treatment groups as compared with that of control were high. After 8 days, the scores of control, AP, AC, GT and CH were 3.86, 4.10, 4.31, 4.36 and 4.40, respectively. These results showed that the microbial contamination

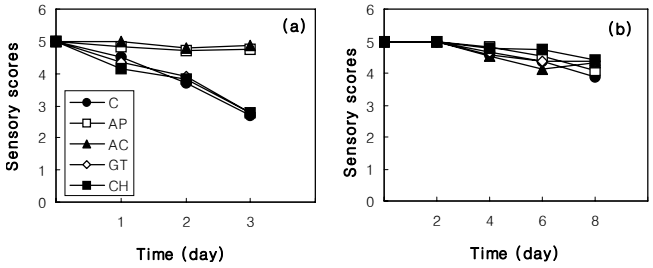

Fig. 5. Changes in sensory score (microbial contamination) of MAP *Pleurotus ostreatus* during storage at 20 (a) and 5°C (b). C: control (MAP mushroom without treatment), AP: MAP mushroom with *Astemisia princeps* dough, AC: MAP mushroom with *Astemisia capillaries* dough, GT: MAP mushroom with green tea dough, CH: MAP mushroom with charcoal.

of mushrooms during storage was effected by temperature and kinds of treatments. In the case of storage temperature, the higher temperature was, the greater the microbial contamination. On the other hand, treatment with *Artemisia princeps* (AP) and *Artemisia capillaries* (AC) showed antimicrobial activity regardless of storage temperature and that of green tea (GT) dough and activated charcoal (CH) were more effective at 5°C. But there were no statistically significant differences among the treatments.

The inhibition of microbial activity has previously been reported for *Artemisia* species (27-29). Lee and Seo (28) reported the acetone extracts of *Artemisia capillaries* showed antibacterial activity against food-borne pathogens and Cho et al. (29) reported that antibacterial constituents were isolated from whole *Artemisia princeps*. It showed a clear inhibitory effect against human intestinal bacteria. Fabien et al. (30) also reported that the essential oil of the aerial parts of *Artemisia annua*, consisting of camphor (44%), germacrene D (16%), trans-pinocarveol (11%), β-selinene (9%), β-caryophyllene (9%) and artemisia ketone (3%), was screened for its antimicrobial activity and remarkably inhibited the growth

of tested gram-positive bacteria and fungi.

It has also been reported green tea exhibits antimicrobial effects as well as deodorization activities (20). Polyphenols and extract of green tea have been shown to have antibacterial activities against disease-related bacteria (31) and food-borne bacteria (32).

In our study, *Artemisia princeps* and *Artemisia capillaries* dough exhibited the strongest antimicrobial properties when packed with mushrooms. These results showed that the contamination of mushroom was effected by storage temperature and treatments.

CONCLUSION

The biochemical changes and occurrence of off-flavor were observed in mushrooms packed with treatments (AP, AC, GT dough and CH) and without treatment. No differences in weight loss, gas concentration or color were found between mushrooms packed with and without treatment. However, the principal component analysis (PCA) using the electronic nose showed the differences in principal components between control and treatment group at 5°C. This result demonstrated that *Artemisia princeps*, *Artemisia capillaries* masked the off-flavor of MAP mushroom, due to their strong characteristic flavors. The results also suggested that green tea and activated charcoal might have a role in removing the off-flavor by adsorbing ethanol and acetaldehyde, which is known as the component of off-flavor. The sensory result revealed that *Artemisia princeps* and *Artemisia capillaries* treatment inhibited microbial growth in mushrooms.

ACKNOWLEDGEMENT

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