**Effect of 1-methylcyclopropene (1-MCP) on quality of button mushrooms *(Agaricus bisporus)* packaged in different packaging materials**

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**Abstract:** The effects of 1-MCP treatment on the quality of *Agaricus bisporus* mushrooms packed in three different packaging films, i.e., low permeable packaging (LPP), medium permeable packaging (MPP) and high permeable packaging (HPP), were evaluated. Quality factors included weight loss, color, texture, and sensory. Results show that 1-MCP can slow down respiration rate of mushrooms. In LPP and MPP, where O2 supply was limited, respiration rate of mushrooms can be reduced by around 25 %, while in HPP where there was constant O2 supply, respiration rate reduced by around 2 %. The best effectiveness was obtained from combination of 1-MCP and MPP, which created headspace composition of less than 0.1 % of O2, and 5-10 % CO2, providing more than 15 d of shelf life. This combination provided several benefits for mushroom quality including highest sensory quality, maintaining weight and firmness, as well as altering the formation of flavor nucleotides process to improve umami taste.

**Keywords: *Agaricus bisporus*; 1-MCP; MAP; Shelf life**

**1. Introduction**

*Agaricus bisporus* mushroom (also known as button mushrooms or white mushroom) is popular in the global food market, accounting for 30% of total mushroom production in the world, because of its nutritional value, sensory properties and medicinal attributes (Ban, et al., 2014; Gholami et al., 2017; Royse & Singh, 2014). One challenge in its distribution and retail is that *A. bisporus* is highly perishable with only shelf life of 1–3 d at ambient temperature and 5–8 d under refrigerated conditions (Jiang, 2013; Zhang et al., 2018). Major quality limiting factors include weight loss, discoloration, browning, texture change, microbial spoilage, and off-flavor development. (Gholami, et al., 2017; Mollazade, 2017; Oz, et al., 2015; Yang, et al., 2016).

One problem associated with mushroom quality is that ethylene can cause browning of mushroom caps (Hardenburg, 2016). Although mushroom has very low ethylene production, it needs to be kept separately from other ethylene generating produces during storage, shipping and distribution. It is particularly challenging for shipping mushrooms as it is not always possible to have a full load of mushrooms in a shipping truck, and a mixed load of produce is usually practiced. Due to the browning effect of ethylene, it is inconvenient for growers and shippers to arrange for shipping mushrooms.

One approach commonly applied to mitigate the adverse effect of ethylene is 1-methylcyclopropene (1-MCP). Jamjumroon et al. (2013) reported a study of packing 1-MCP treated straw mushrooms (*Volvariella volvacea*) in low oxygen permeable packages, and showed that this treatment reduced cap browning and extended shelf life. In another study by Choi et al. (2012), packing 1-MCP treated king oyster mushroom in microperforated film was effective to inhibit browning and maintain sensory quality. But these are so far the only studies of 1-MCP on mushroom; however, the effect of 1-MCP on mushroom quality has not been well established, and no study has been conducted for button mushroom. There is a need for qualitative and quantitative evaluation for practical application of 1-MCP.

Modified atmosphere packaging (MAP) also has been evaluated extensively for mushroom storage. Literature data showed that reduced O2 decreased the metabolism of mushroom and CO2 accumulation inside the package acted as a preservative against the detrimental decay reactions. Treating 1-MCP on MAP packaged produce has also been shown effective on a number of crops such as apples, papayas, and bananas, providing multiple benefits to delay or prevent respiration and ethylene induced quality deterioration. The success of 1-MCP and MAP on other crops and the scarcity of knowledge of 1-MCP on mushroom provide us with the incentive to investigate the effects of 1-MCP and MAP on mushroom. Therefore, the objective of this paper is to evaluate the effect of 1-MCP on mushroom quality including color, texture, and taste under different modified atmosphere created by different packaging films.

**2. Materials and methods**

**2.1. Materials**

1-MCP was generated from1-MCP tablet with 3.3% concentration (Xianyang Xiqin Biotechnology Co., Ltd., Xianyang, China). Standard of 5'-nucleotide was purchased from Shanghai Yuanye Bio-Technology Co., Ltd, Shanghai, China. Methanol was purchased from Xilong Scientific Co., Ltd., Guangdong, China.

**2.2. 1-MCP treatment**

Mushrooms were supplied by Yucheng Fungi Co., Ltd. (Kuandian, China). The first flush mushrooms were harvested according to uniform shape and size (35～40 mm diameter). Samples were then stored at 5°C and treated with 5 μL L-1 1-MCP in a closed plastic container for 12 h.

**2.3. Packaging materials**

Both 1-MCP treated and untreated mushrooms were selected for uniform size and color. Then the mushrooms (approximately 100 g) were placed in biaxially-oriented polypropylene (BOPP) trays (L × W × H = 223 × 133 × 40 mm) and heat-sealed with the three polymeric films and stored in an incubator (MIR-254-PC, SANYO, Japan) at 5°C for 15 d. The mushrooms without 1-MCP treatment were also packaged and stored as control. For each treatment, three replicates were performed.

The three films used in the study were: (1) low oxygen permeable packaging (LPP), which is a laminated nylon (PA)/polyethylene (PE) film; (2) medium oxygen permeable packaging (MPP), which is a PE film; and (3) high oxygen permeable packaging (HPP), which has the same structure as LPP with one perforation (0.5 mm). The film thickness and permeable properties are summarized in Table 1.

**2.4. Headspace analysis**

The headspace O2 and CO2 concentrations inside the packages were determined everyday using a checkmate O2/CO2 instrument (PBI Dansensor, Denmark). The results of headspace analysis were used to calculate respiration rate based on O2 consumption rO2 (mL kg-1 L-1) using the equation below.

where *V* is void volume of package (0.755 L), *L* is thickness (m), *W* is product weight (0.1 kg), *S* is package surface area (0.027 m2), *p* is atmospheric pressure (1.01 × 105 Pa), *PO2* is oxygen permeability of the film (mL m-1 s-1 Pa-1), where were known parameters of the package and product. [O2] is oxygen concentration (%) and t is time (h), which were obtained from experiment.

**2.5. Firmness**

Firmness was measured after 0, 4, 8, 11, 13 and 15 d of storage and determined by penetration test using a Texture Analyzer XT2 (BrookWeld, USA). After removing stem of the mushroom, the cap was compressed with a probe (diameter 0.5 mm) at 1.5 mm s-1. The peak stress at 70 % compression and the maximum force for extrusion were used to determine firmness of the mushrooms (Matser, et al., 2000).

**2.6. Weight loss**

Weight loss was determined by weighing the mushrooms in the packages before and after the storage period, which was expressed as the percentage of weight loss with respect to the initial weight.

where is the initial weight of the mushroom and the weight at time t (4, 8, 11, 13, and 15 d).

**2.7. Anti-browning effect**

Internal and external browning of mushroom samples were evaluated visually. For external browning, both cap and stipe were evaluated. For internal browning, samples were sliced vertically in half for evaluation. Evaluation was done for 0, 4, 8, 11, and 15 d after storage.

**2.8. Sensory analysis**

The sensory quality of the mushrooms was evaluated by a panel of five testers. Samples were evaluated in terms of: color, cap opening, stickiness, odor and flavor and the results were expressed in score values on a discrete scale from 0 to 5 (Table 2).

**2.9. Electronic tongue measurement**

Analyses were conducted according to the method used in our previous study (Zhang, et al., 2019). The Taste-Sensing System SA 402B (Intelligent Sensor Technology, Inc., Kanagawa, Japan), consisting of auto-sampler, reference electrodes, and multichannel lipid/polymer membrane electrodes was used. The mushroom samples were measured (thrice) after the electric potentials of all membranes had been balanced in standard solutions.

**2.10. 5′-Nucleotides Assay**

5′-Nucleotides analysis was the same as that used in our previous study (Zhang, et al., 2019). Different samples were weighed (5.0 g), milled, and extracted with 25 mL of distilled water. The suspension was boiled for 1 min, cooled subsequently, and then centrifuged at 4500 × g for 15 min. The residues were extracted with 20 mL of distilled water, and the combined filtrates were concentrated by rotary evaporation and re-dissolved in distilled water to a final volume of 50 mL and filtered using a 0.45 μm micro-pore filter membrane before analysis.

Waters 1525 HPLC system equipped with UV detector (Waters Corporation, Shanghai, China) was used for the determination of 5′-Nucleotides. The analysis was completed on a Li-Chrospher RP-18 column (250 mm × 4.6 mm, Merck) at a flow rate of 1.0 mLmin-1.

The mobile phase consisted of methanol (solvent A) and phosphate buffer (pH 4.2, solvent B). The 5′-Nucleotides were monitored at 254 nm, with injection volume of 20 μL and oven temperature of 30 °C. Quantification of 5'-Nucleotides was achieved using an external calibration curve of 5'-nucleotide standards.

**2.11. Statistical analysis**

Data were expressed as the mean ± SD (standard deviation) of three replications. One-way analysis of variance (ANOVA) was performed by SPSS (IBM SPSS Statistics for Windows version 20, Armonk, NY) and Duncan multiple comparison method was used to compare the significant difference (P<0.05).

**3. Results and discussion**

**3.1. Changes in Headspace Composition**

Changes of O2 and CO2 concentrations in all treatments are shown in Figure 1. Both 1-MCP treatment and permeation properties of packaging materials significantly affected the gas composition inside the packages. For LPP, O2 depleted in the first 3 d, reaching level below 0.1 %. CO2 constantly raised for 15 d, eventually reaching 26 % and 30.5 % in 1-MCP/LPP and CON/LPP, respectively, indicating occurrence of fermentation. For MPP, gas composition equilibrium and steady state respiration were reached on 3 d, with less than 0.1 % O2 and around 12 % CO2. For HPP, the gas composition equilibrium and steady state respiration were reached in 2-3 d, with O2 around 15 % (1-MCP /HPP) and 11 % (CON/HPP), and CO2 around 5 % (1-MCP/HPP) and 11 % (CON/HPP).

1-MCP treatment reduced respiration rate of mushrooms in all packaging variables (Table 3). Respiration rates were calculated only for the initial 24 h of the study for comparison purpose, because in the first 24 h, headspace change was governed by only aerobic respiration and film permeation, without any interference of fermentation. For LPP and MPP where limited O2 was available, the respiration rate reduced around 25 % by 1-MCP treatment; for HPP which allowed more O2 transmission, respiration rate reduced around 2.2 % by 1-MCP. Thus, it suggests that more effect of 1-MCP on respiration and sample quality could be expected when O2 supply is limited.

For LPP and MPP, the respiration of mushroom is the major factor contributing to the headspace change in the package. The headspace composition change caused by O2 permeation was almost negligible compared to the respiration. Although both LPP and MPP had similar final headspace O2 close to 0 %, they have completely different dynamic in the headspace. Table 3 shows that samples packed in MPP and LPP had similar respiration rates, and so they have similar O2 depletion, but LPP had much higher CO2 accumulation than MPP. High CO2 content has been associated with several quality and safety issues in mushroom such as poor texture and anaerobic microbial growth. In the case of HPP, the headspace change was controlled by both O2 transmission through the perforation and respiration of mushroom. O2 supply was able to match the respiration of the packed mushroom to achieve gas equilibrium in the headspace.

**3.2. Weight loss**

Figure 2 shows the weight loss under different treatments. Water loss maintained at low level (less than 2.4 %) for all treatments, and 1-MCP treated samples had consistently less weight loss than control (no 1-MCP treatment), because 1-MCP reduced respiration and water generated as a respiration product of treated samples as shown in Table 3. It is also worth mentioning that although the weight loss of different treatments was significantly different statistically, they are practically equivalent as all treatments had weight loss below 2.4 %, which was below the limit of acceptance of 5 % (Mahajan, et al., 2007).

**3.3. Anti-browning effects**

Figure 3 shows the external browning of mushrooms under different treatments. Samples treated by 1-MCP had less degree of browning on both cap and stipe sides than control right after 1-MCP treatment (0 d). Among 1-MCP treated samples, 1-MCP/LPP and 1-MCP/MPP had no obvious browning on both sides through 15 d, while 1-MCP/HPP treatment group showed some browning on the stipe side after 11 d, and no browning on cap side. The results suggest that 1-MCP delayed the browning initially for 1-MCP/LPP and 1-MCP/MPP, and fast O2 depletion due to high respiration of mushrooms provided a low O2 environment to further inhibit browning. But in 1-MCP/HPP treatment group, a relatively high O2 level was maintained throughout the test, leading to more browning on stipe side after 11 d of storage.

Among control samples (no 1-MCP treatment), there was no obvious difference among different packages, and browning was observed from the beginning of the test due to the initial O2 content in the headspace. Browning on the cap side did not progress during test, while on the stipe side browning was intensified after 8 d.

However, due to the presence of high CO2 concentration, the mushrooms in LPP treatment after 8 d showed more browning at surface than other treatments. In addition, slight yellowing in LPP packed samples was observed(as shown in Fig. 3). It was different from browning and was likely due to damage of the mushrooms’ surface tissue. Lin et al., (2017) had concluded that high CO2 concentration caused damage to the mushroom cap surface tissue and result in high browning index. In this experiment, mushrooms under low O2 (<0.1 %) and high CO2 (>20 %) atmosphere for more than 3 d were found to show yellowing at surface. Nevertheless, there is limited information of the causes of mushroom yellowing and further research is needed.

Figure 4 shows the internal browning of all treatments. There was no obvious browning in all samples except for 1-MCP/HPP and HPP treatments, due to the high O2 in HPP throughout the test. In addition, 1-MCP did not help inhibit internal browning, suggesting that anti-browning effect of 1-MCP was short term and on the external surface.

**3.4. Sensory analysis**

Color, surface stickiness, and odor varied greatly during the shelf life, with color change having the most variation among different treatments (Table 4). MPP groups had the highest scores, especially the 1-MCP-treated mushrooms did not have surface stickiness and odor during 15 d test period, and the color was brighter than other treatments. HPP and LPP began to show noticeable browning after 11 d accompanied by slight off-odor and surface stickiness. No cap opening was observed in all treated samples. Briones et al. (1992) also showed that controlled atmosphere storage particularly high CO2 was beneficial to prevent cap and veil opening, suggesting that the internal atmosphere obtained from the treatments in this study was effective in this regard. Sensory analysis indicated that 1-MCP/MPP had the highest consumer acceptance throughout the whole shelf life among all treatments.

**3.5. Texture**

Figure 5 shows the changes in firmness by different treatments. After 1-MCP treatment, the initial mushroom firmness was higher than the untreated samples. For HPP and LPP samples, the effect of 1-MCP diminished over time, while for MPP samples, 1-MCP treated samples had consistently higher firmness than untreated samples. The reason why 1-MCP treatment for LPP and HPP was not as effective as MPP could be due to that for LPP, high CO2 level damaged the mushroom tissue (Lin, et al., 2017); for HPP, the high O2 level and respiration rate were maintained, leading to faster physiological changes and reduced firmness. Thus, gas composition played a more important role in maintaining firmness in HPP and LPP packed samples than 1-MCP. Thus, 1-MCP provides initial benefits for maintaining firmness, and needs appropriate gas composition for further protection.

**3.6. Electronic tongues measurement**

Table 5 shows that 1-MCP did not affect the flavor profile of mushroom. Among different tastes, umami intensity was the only one that increased with the number of storage days, and the other four tastes decreased slightly. In this study, umami intensity increased significantly from 4.06 to 6.14 (1-MCP treatment) and from 4.01 to 6.79 (no 1-MCP treatment) during the testing period. Saltiness and sourness showed negative scores, with the saltiness almost close to the human sensory threshold (-6), and the sourness was much lower than the threshold (-13), indicating that these two tastes were barely present in the mushroom during the entire testing period. Overall, the scores of umami, saltiness and sourness in this study agreed with literature data that umami intensity of seventeen edible mushrooms ranged from 8.45 to 14.35 and all tested mushrooms showed negative scores in saltiness and sourness (Phat et al., 2016). It was also found that umami intensity decreased as permeable property increased, indicating that O2 and respiration are associated with the biochemical process of formation of umami compounds.

**3.7. 5’-Nucleotides Assay**

Table 6 shows that four nucleotides 5′-guanosine monophosphate (5′-GMP), 5′-ionosine monophosphate (5′-IMP), 5′-xanthosine monophosphate (5′-XMP), and 5′-adenosine monophosphate (5′-AMP) were detected in the tested samples. The contribution of these compounds to umami taste decreases in the order of 5′-GMP > 5′-IMP > 5′-XPM > 5′-AMP (Yamaguchi, et al., 2010). Their contents decreased in the order of 5′-AMP > 5′-GMP > 5′-XMP > 5′-IMP in the tested samples. Overall, the content of flavor 5′-nucleotides, summation of 5’GMP, 5’-XMP, and 5’-IMP (5’-AMP was excluded due to its low contribution), in each treatment group did not show significant difference until 8 d, while on 15 d, MPP packed samples had more flavor nucleotides than LPP and HPP, which agreed with results from electric tongue experiments. In addition, 1-MCP treatment enhanced the amount of flavor nucleotides, which was not shown by the electric tongue results, because each nucleotide contributes differently to umami taste, and combined nucleotide compounds may not show difference in umami taste.

It is also worth mentioning that 1-MCP treatment altered the formation process of nucleotides. For LPP, 1-MCP treated samples had flavor nucleotides remained around the same level, while samples without 1-MCP had constant declined. For MPP, nucleotides in 1-MCP treated samples increased initially and declined, while samples without 1-MCP had remained at similar level initially and increased later. For HPP, 1-MCP treated sample was at the peak in the beginning and declined later, while samples without 1-MCP had constant increase. In general, 1-MCP treated samples had nucleotides formed earlier than without 1-MCP treatment. It is also reasonable to expect that different 1-MCP concentrations would have different effects on the formation of nucleotides, which worth of further investigation.

**3.8. Practical implications**

The results of this paper provide a potential postharvest quality management system for button mushrooms. This system consists of treating mushrooms with 1-MCP after harvest, followed by packing the treated mushrooms in trays covered with PE film. The initial 1-MCP treatment inhibits browning and slows down the respiration rate of mushrooms, resulting in products with better color and firmness quality. The PE film has O2 transmission property that limits O2 supply to further inhibit browning and slow down respiration, and sufficient CO2 transmission to avoid CO2 accumulation in the headspace and thus prevent high CO2 related deterioration such as texture softening. Although 1-MCP alters the process of flavor compounds formation, its practical benefits in this regard still requires further investigation.

It is also important to note that the volume of the tray, weight of mushroom and film surface area reported in this paper were designed based on available resources, and thus validation using commercial parameters is needed. More importantly, microbial safety of mushroom under this treatment requires additional scrutiny because *Clostridium botulinum* is usually associated with low O2 condition similar to this study (Cutter, 2002). Further study on perforation size is also needed. Reducing the perforation size can reduce the O2 level to the point which does not raise microbial safety concern or cause browning. Combining 1-MCP with this condition bears more value for commercial application.

The time of 1-MCP treatment is also worth further investigation. 1-MCP treatment without packaging was employed in this study. This process can be combined with the pre-cooling step in mushroom postharvest handling. Alternatively, treatment 1-MCP after packaging may be practiced because 1-MCP needs to be treated in a closed area which would be challenging for some mushroom harvesting facilities where the products are packed. Assuming treating 1-MCP after packaging is equally effective as treating 1-MCP before packaging, the former method allows more flexibility in practice, because 1-MCP can be treated in shipping trucks and distribution centers if it cannot be treated at the harvest facilities. To ensure the effectiveness of this alternative, 1-MCP transmission through packaging films needs to be well understood to calculate the treatment time. In addition, it should be recommended that 1-MCP needs to be treated as soon as possible.

**4. Conclusions**

1-MCP can slow down respiration rate of mushroom under different headspace compositions. When mushrooms were packed in low permeable (PA/LDPE laminate) and medium permeable (LDPE) films, where O2 supply was limited, respiration rate of mushrooms can be reduced by around 25 % in the first 24 h after packing; while in high permeable (perforated) films, respiration rate reduced by around 2 %. To achieve the best effectiveness based on the experimental condition of this study, 1-MCP treatment needs to combined with medium permeable film (LDPE), which had limited O2 supply, creating gas composition of less than 0.1 % of O2, and 5-10 % CO2. This combination provided several benefits for mushroom quality including highest sensory quality, maintaining weight and firmness, as well as altering the formation of flavor nucleotides process to improve umami taste. For practical application, the microbial safety of mushroom under this treatment bear additional scrutiny in the future.

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