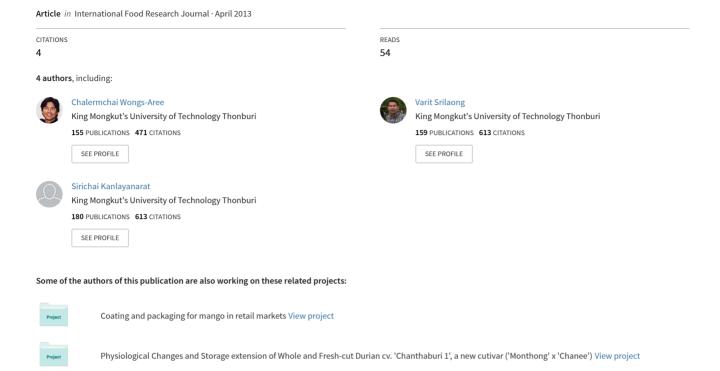
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Alleviation of cap browning of 1-MCP /High CO₂- treated straw mushroom buttons under MAP

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Abstract

Straw mushrooms (*Volvariella volvacea*) have a short shelf life at ambient temperatures due mainly to high rates of water loss and rapid cap browning. Fresh straw mushroom buttons were fumigated with 1-methylcyclopropene (1-MCP) at 0, 250, 500 and 1000 ppb in air for 6 h at 25 °C prior to storage at 15 °C on foam trays overwrapped with polyvinylchloride (PVC) film. 1-MCP concentrations above 250 ppb effectively reduced cap browning of straw mushrooms due to the inhibition of polyphenol oxidase (PPO) activity. 1-MCP treatments delayed an increase in the concentrations of malondialdehyde (MDA) a product of lipid oxidation, in the fruiting bodies, although there was a trend to increasing MDA accumulation after day 3 of storage following treatment with 1000 ppb 1-MCP. Treatment with 250 ppb 1-MCP was as effective as fumigation with 40% CO₂ for 6 h for reducing browning and increasing the shelf life of straw mushrooms stored at 15 °C.

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Introduction

The straw mushroom (Volvariella volvacea (Bull ex Fr.) Sing) is an edible tropical mushroom that is a popular ingredient in various East Asian dishes, but its quality deteriorates quickly after harvest. The fruiting body at commercial maturity (button stage) is white with a short stipe and large cap. Straw mushrooms are cultivated mainly in Southeast Asian countries (Ahlawat et al., 2008). In Thailand, straw mushrooms are grown in darkness and are white at harvest but quickly turn brown under ambient conditions. These mushrooms contain polyphenol oxidase (PPO) or polyphenolases that convert phenolic compounds to quinones that combine with amino acids to produce dark compounds (Chang and Quimio, 1980). These reactions are stimulated in plants by physical damage, water stress or senescence (Marshall et al., 2000). Browning of straw mushrooms is reported to be a water stress-independent reaction (Jamjumroon et *al.*, 2012). Jamjumroon *et al.* (2012) reported that the optimum storage temperature for straw mushroom is 15 °C.

1-methylcyclopropene (1-MCP), an inhibitor of ethylene action, is widely used to delay ripening processes and extend the storage life of fruits and vegetables, particularly climacteric species (Blankenship and Dole, 2003; Watkins, 2006). In nonclimacteric fruit, 1-MCP fumigation has been shown to reduce internal browning in whole pineapple fruit (Selvarajah et al., 2001) and browning of fresh-cut pineapple (Buda and Joyce, 2003). There have been no reports on the responses of straw mushrooms to treatment with 1-MCP. Storage atmospheres containing several percent of CO₂ are widely used to extend the shelf life of a number of perishable fruits (Agar et al., 1991; Robins and Fellman, 1993) as well as straw mushrooms (Jamjumroon et al., 2010). From our previous study, exposure of straw mushrooms to 40-60% CO₂ for 4-6 h prior to storage at 15 °C

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on foam trays overwrapped with PVC film reduced browning (Jamjumroon *et al.*, 2012). The aim of the present study was to compare the efficacy of 1-MCP and a high CO₂ shock treatment for delaying browning of straw mushrooms stored in modified atmosphere packaging (MAP) at 15 °C.

Materials and Methods

Plant Materials

Straw mushrooms at the button stage were harvested from a commercial farm in the Saraburi Province (14°32N 100°53E / 14.53°N 100.88°E) in Central Thailand between March and June 2011. The mushrooms were packed in foam boxes and transported to the Thailand Institute of Scientific and Technological Research (TISTR) laboratory, Bangkok (a trip of approximately 1 h). Mushroom buttons (20-25 g / button) free of defects were selected.

Experimental design

In the first experiment, mushroom buttons were enclosed in 60x60x60 cm³ plastic tanks and fumigated with 1-MCP (0.19% 1-MCP tablet, BioLene Co., Ltd., China) at 0, 250, 500 and 1000 ppb at 25 °C for 6 h. Mushroom buttons (approximately 250 g) from each pre-treatment were then transferred to a 15x20 cm polystyrene foam tray and overwrapped with PVC film. The packages of mushrooms were stored at 15 °C and 90-95 % RH. The experiment was performed using a completely randomised design (CRD) with 6 replications (one package/replicate).

For the subsequent experiment, straw mushrooms were fumigated with normal air, 40% CO $_2$ or 250 ppb 1-MCP at 25 °C for 6 h, then packed on 15x20 cm polystyrene foam trays and overwrapped with PVC film. All Treatments were stored at 15 °C and 90-95% RH. The experiment was performed in a CRD with 6 replications (one package/replicate).

Visual assessment

Ten mushroom buttons from each package were evaluated for browning. Severity of browning of the mushroom caps was scored based on the estimated brown surface area: 1=no browning, 2=browning <15%, 3=browning 15-30%, 4=browning 30-50%, 5=browning >50%. In addition, the lightness (L* Hunter scales) of the caps was measured at two places on opposite sides of the buttons with a chromameter (Model CR-300, Minolta, Japan). The buttons were photographed under standard laboratory lighting with a digital camera (Sony model DSC-W 150, Super Steady Shot).

Measurement of polyphenoloxidase (PPO) activity

PPO was extracted and assayed using the method of Luh and Phithakpol (1972). The assay medium contained 0.1 mL of enzyme extract and 1 mL of 0.04 mM catechol. PPO activity was determined by measuring absorbance at 410 nm. One unit of PPO activity was defined as the change in absorbance after 1 min at 25 °C, per g fresh weight.

Measurement of malondialdehyde (MDA) content

Ten g of mushroom fruiting bodies were taken from each treatment and homogenised in 25 mL of ice-cold extraction buffer (100 mM sodium phosphate (pH 6.4) and 0.5 g. polyvinyl polypyrrolidone (PVPP). The homogenate was centrifuged at 27,000 ×g. for 50 min at 4 °C and the resulting supernatants were used directly for assay. The MDA content was determined by adding 2 mL of 0.5% thiobarbituric acid (TBA) in 15% trichloroacetic acid (TCA) to each 1 mL sample. The solution was heated at 95 °C for 20 min, cooled in an ice-bath for 5 min, and then centrifuged at 12,000 ×g for 10 min to clarify the solution. Absorbance at 532 nm was measured and subtracted from the absorbance at 600 nm. The amount of MDA was calculated with an extinction coefficient of 155 mM/cm (Wang et al., 2005).

Statistical analysis

The data were subjected to analysis of variance (ANOVA), and the means were compared by Least Significant Differences (LSD) at P < 0.05 using SPSS software (SPSS version 17.0 for Windows, SPSS Inc., Chicago, IL, USA).

Results

Control mushroom buttons on foam trays overwrapped with PVC film turned brown quickly and were unacceptable after 3 days of storage at 15 °C. Lightness values (L* Hunter scale) (Figure 1A) were closely related to the development of browning as as shown by subjective scores (Figure 1B). All concentrations of 1-MCP were equally effective in delaying browning of the mushrooms and shelf life was extended by one day compared to untreated controls (Figure 1B). Treatment with 1-MCP delayed the increase in MDA content, a measure of lipid peroxidation (Figure 2A). MDA reached a peak of 2.0 nmol/g FW on day 2 whereas MDA remained less than 2.0 nmol/g FW in treated mushrooms throughout the storage period (Figure 2A). There was a trend to increased concentrations of MDA on Days 4 and 5 in mushrooms treated with 1000 ppb 1-MCP. PPO activity reached a maximum in untreated mushrooms by Day 2 but all1-MCP treatments delayed an

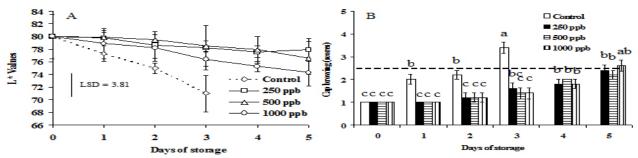


Figure 1. Changes of L* Hunter scales (A), and browning generation (B) of straw mushroom cap pre-treated with 1-MCP at 0, 250, 500 and 1000 ppb and then stored on foam trays and wrapped with PVC film at 15°C. The dashed horizontal line (B) indicates the limitation of acceptance and means with different Latin alphabet are significantly different.

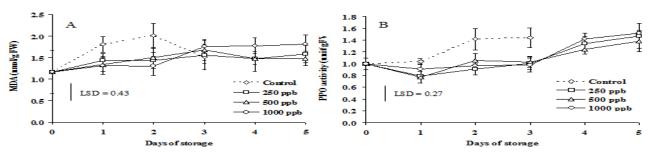


Figure 2. Changes of malondialdehyde content (A) and polyphenoloxidase activity (B) of straw mushroom pre-treated with 1-MCP at 0, 250, 500 and 1000 ppb and then stored on foam trays and wrapped with PVC film at 15°C. Vertical bars indicate standard deviation of means (n = 6).

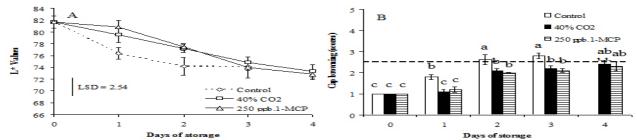


Figure 3. Changes of L* Hunter scales (A), and browning generation (B) of straw mushroom pre-treated with normal air, 40% CO₂ or 250 ppb 1-MCP for 6 h prior to storage on foam trays wrapped with PVC film at 15°C. The dashed horizontal line (B) indicates the limitation of acceptance and means with different Latin alphabet are significantly different.

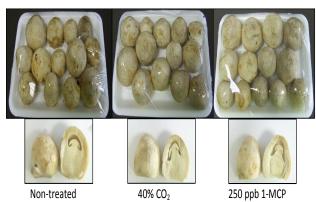


Figure 4. Appearance of straw mushroom pre-treated with 40% $\rm CO_2$ or 250 ppb 1-MCP for 6 h prior to storage on foam trays wrapped with PVC film at 15°C 90-95% RH for 3 days.

increase in activity until after Day 3 (Figure 2B). A second experiment was conducted to compare the responses of straw mushrooms to treatment with

250 ppb 1-MCP and with a 40% CO₂ at treatment. Untreated mushrooms quickly turned brown and became unacceptable within 2 days whereas the 250 ppb 1-MCP and 40% CO₂ treatments slowed the rate of decline in lightness (Figure 3A) and significantly delayed the development of browning (Figure 3B). Changes in the internal appearance of the mushrooms were also slowed by 1-MCP and high CO₂ treatments (Figure 4). Both of these treatments significantly delayed the increases in MDA concentrations and PPO activity by 2 and 3 days respectively (Figure 5).

Discussion

1-MCP fumigation has been shown to reduce postharvest changes and disorders in some nonclimacteric produce such as yellowing in lime (Win

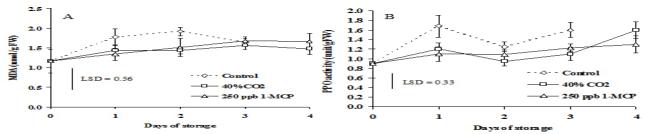


Figure 5. Changes of malondialdehyde content (A) and polyphenoloxidase activity (B) of straw mushroom pre-treated with 40% CO₂ or 250 ppb 1-MCP for 6 h prior to storage on foam trays wrapped with PVC film at 15°C. Vertical bars indicate standard deviation of means (n = 6).

et al., 2006), peel disorders and incidence of decay in other citrus fruit (Dou et al., 2006), internal browning in pineapple (Selvarajah et al., 2001), ripening of grape (Chervin et al., 2004) and firmness and colour changes inf strawberry (Jiang et al., 2001). In the present study fumigation with 250 ppb 1-MCP for 6 h at 25°C was sufficient to reduce PPO activity and delay browning of the fruiting bodies of straw mushrooms subsequently stored at 15°C. Fumigation with 1000 ppb 1-MCP may actually be toxic to straw mushrooms because this treatment did not appear to be as effective as lower concentrations for retarding the rate of decrease in lightness and the increase in MDA concentrations (Figures 1A and 2A). Relatively high concentrations of 1-MCP have been previously reported to induce more severe postharvest disorders in lime fruit (Win et al., 2006). PPO has been shown to be involved in the induction of browning reactions in many products including fresh straw mushrooms (Jamjumroon et al., 2010; Jamjumroon et al., 2012). The results of the present study support earlier findings on the inhibitory effects of storage in high CO₂ concentrations (Tian et al., 2005, Jamjumroon et al., 2010) and pre-storage treatments with high CO₂ (Jang et al., 2002; Jamjumroon et al., 2012) on PPO activity and the development of browning. Tyrosine and pyrocatechol were reported to be the preferred substrates for enzymic browning of straw mushroom (Jamjumroon et al., 2012). Tyrosinase has been reported to be involved in browning in many species of mushrooms (Espin et al., 1998; Seo et al., 2003). Protection of membrane lipids appears to be an important response to pre-storage treatments with both 1-MCP and high CO₂ since the increases in MDA associated with senescence of straw mushrooms were delayed (Figures 2B and 5B).

In this study, we found that pre-storage treatments with either 1-MCP or 40% CO₂ retards browning of straw mushrooms but treatment with 1-MCP would be easier to apply commercially.

Conclusions

Fumigation with 250 ppb 1-MCP for 6 h at 25°C

was sufficient to reduce cap browning and increase the shelf life of straw mushrooms stored at 15 °C and 90-95%RH on foam trays overwrapped with PVC. This treatment was found to be as effective as a prestorage treatment with 40% CO₂ or 1-MCP at 250 ppb for 6 h before storage at 15 °C

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