Postharvest Application of Methyl Jasmonate for Improving Quality Retention of *Agaricus bisporus* Fruit Bodies

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ABSTRACT: The influence of methyl jasmonate (MeJA) on postharvest quality and enzyme activities, gene expression level, and the functional component content linked to postharvest deterioration in *Agaricus bisporus* (J.E. Lange) Imbach fruit bodies was investigated. Freshly harvested fruit bodies were treated with 0 (control), 10 and 100 μM MeJA vapor at 20 °C for 12 h and then stored at 10 °C for up to 7 days. The results indicated that treatments with 100 μM MeJA vapor maintained a high level of soluble protein and total sugar, delayed browning, promoted the accumulation of phenolics and flavonoids, and inhibited the increase of respiratory rate and membrane leakage. Furthermore, 100 μM MeJA inhibited the activities of polyphenoloxidase, increased the antioxidant enzymes activities of catalase and superoxide dismutase, and lowered relative expression levels of three genes encoding polyphenol oxidase (*AbPPO1*, *AbPPO2*, and *AbPPO3*) throughout the storage period. Comparatively, 10 μM MeJA also had a clear beneficial effect on postharvest mushroom quality maintenance but was not as effective as 100 μM MeJA treatment. These findings suggest that application of MeJA could have potential in maintaining the quality of harvested *A. bisporus* fruit bodies.

KEYWORDS: *Agaricus bisporus* (J.E. Lange) Imbach, mushroom, methyl jasmonate, postharvest quality, browning

INTRODUCTION

Agaricus bisporus (white button mushroom) is the most cultivated edible mushroom worldwide.[1] It has high concentrations of essential amino acids, vitamins, and minerals, and is also rich in polysaccharide.[2] In addition to its cultivation for food, the white button mushroom is a potential source of health-protecting and medicinal compounds.[3] However, *A. bisporus*, a climacteric fruit,[4] is highly perishable with a short postharvest shelf life of 1−3 days compared to most vegetables at ambient temperatures. It has a high respiration rate, tends to turn brown, and has limited physical barriers to water loss or microbial attack.[5] The short shelf life of the mushroom is an impediment to the distribution and marketing of the fresh product. Thus, prolonging postharvest storage would benefit the mushroom industry as well as consumers.

Methyl jasmonate (MeJA), as a naturally occurring plant growth regulator, promotes the biosynthesis of secondary metabolites, e.g., ganoderic acid,[6] induces the expression of a set of defense genes, and activates host resistance.[7] Postharvest treatment with MeJA retained higher levels of sugars and organic acids in fresh-cut kiwifruit,[8] radishes,[9] and mangoes,[10] and maintained higher levels of bioactive compounds and enhanced antioxidant capacity in blackberries, raspberries, and strawberries.[11−13] Though MeJA has been studied in mushrooms by dipping treatment[14] and combined with ethyl alcohol[15] to enhance postharvest physicochemical and microbial quality, its independent effects by vapor treatment is worth studying in more detail. Because MeJA has already been classified by the U.S. Food and Drug Administration (FDA) as Generally Recognized as Safe (GRAS) substances,[16] it may have potential commercial applications in postharvest treatments for mushroom quality maintenance. Thus, the objective of this work was to investigate the effect of a prestorage MeJA treatment on postharvest quality, physiological changes associated with postharvest deterioration, as well as antioxidant enzymes and functional components content of *A. bisporus* mushroom during storage.

MATERIALS AND METHODS

Mushroom Treatment and Storage. Fruiting bodies of *A. bisporus* at commercial maturity stage (approximately 3−4 cm) were harvested from a first flush crop from Corporation of Jiu Fa Edible Fungus in China. Sporophores were screened for uniform size and maturity and absence of mechanical damage, and then randomly divided into three lots of 180 sporophores each. Two lots were treated independently with 10 μM and 100 μM MeJA (Sigma, St. Louis, USA) vapor in an airtight container in darkness at 20 °C. One lot was kept under the same conditions without MeJA treatment. After 12 h, the three containers were opened, and the sporophores were stored at 10 ± 1 °C with a relative humidity of 80−90% for up to 7 days. There were three replicates each per treatment, and the experiment was conducted twice.

Untreated and treated fruit bodies were randomly sampled immediately following treatment or after 12, 24, 36, 48, 60, and 72 h, and subsequently 4, 5, 6, and 7 days. Eight intact sporophores per replicate were diced, frozen in liquid nitrogen, and stored at −80 °C for transcript profiles detection of *polyphenol oxidase* genes (samples at 12, 24, 36, 48, 60, and 72 h storage), and for enzyme, soluble protein, total sugar, phenolics, and flavonoids analysis (samples at each day).

Respiration Rate. The respiration rate was assessed every day during storage by the static method. Twenty random mushroom fruit

Table 1. Genes and Oligonucleotides Used in the Quantitative Real-Time PCR Experiments

	GenBank accession no.	encoded proteins	primers (5′− 3′)
AbPPO1	X85113	polyphenoloxidase	F: GGGTGTGAACGCAAAGGATAA
			R: GTATGGCTGCTGAAATGAGGC
AbPPO2	AJ223816	polyphenoloxidase	F: AGAACGAAGAGGTCAACATTACGA
			R: GATGGAAGTGATAGCGGAGGA
AbPPO3	GQ354801	polyphenoloxidase	F: GTGGTATTCACGGATTGCCC
			R: TATGCCCGATGCCAGGTAG
AbPPO4	GQ354802	polyphenoloxidase	F: CTCGTCCGCGACTACATCAA
			R: AGGGCAGGGTTCTTTCCAC
EF1-α	X97204	elongation factor 1-α	F: AAGGATATTCGACGTTGGGAAA
			525 R: CGGGTGAT GAGGACGATG

bodies per replicate were put into a gastight container of 9 L with 10 mL of 0.4 M sodium hydroxide in a Petri dish, containing ambient air as the initial atmosphere. The Petri dish was taken out and titrated with 0.2 M oxalic acid after 1 h. Respiration rate was expressed as mL CO_2 kg^{-1} fresh weight (FW) h^{-1}.[17]

Electrolyte Leakage Rate. The rate of electrolyte leakage was determined every day during storage as described by Zhao et al.[18] with some modifications. Disks (3 mm thick) of the pileus tissue were excised with a 1 cm diameter stainless steel cork borer from the top and middle part of the cap. Six disks of each replicate, excised from six caps, were first washed with distilled water to remove surface contamination and then put into 40 mL of deionized water in a 100 mL beaker. Electrical conductivity of the suspending solution was measured immediately (P_0) and again after being shaken at 100 cycles/min for 2 h (P_1) with a conductivity meter (DDS-11A, Shanghai Leici Instrument Inc., Shanghai, China). Samples were then boiled for 10 min and cooled to 20 °C, and a final conductivity measurement (P_2) was taken. The rate of electrolyte leakage was expressed as a percentage: $(P_1 - P_0)/ (P_2 - P_0) \times 100$.

Contents of Soluble Protein and Total Sugar. Protein concentration in the antioxidant enzymatic extracts was quantified according to the method of Bradford[19] with bovine serum albumin as standard. Total sugar in frozen mushroom tissues was determined according to the method of Dubois et al.[20]

Color Measurement. Mushrooms quality was assessed at days 3, 5, and 7 of storage at 10 °C by the extent of browning of the cap, measured in terms of L-value using a WSC-S Colorimeter (Shanghai precision instrument Co. Ltd., Shanghai, China). L-values of 0 and 100 represent black and white, respectively. Each mushroom was measured at three equidistant points of the cap (n = 20 measurements per replicate).

Polyphenol Oxidase (PPO) Activity. PPO (EC 1.10.3.2) activity was determined as described by Liu et al.[21] with some modifications. Frozen tissue (2 g) was extracted in 8 mL of 0.05 M phosphate buffer (pH 6.8). The homogenate was centrifuged at 12000g for 15 min at 4 °C, and the supernatants were used for the enzyme assays. An aliquot (400 μL) of the extract was reacted with 2 mL of buffered substrate (0.05 M phosphate buffer, pH 6.8) and 600 μL of 0.1 M catechol, and the change in absorbance at 420 nm was recorded over 3 min. Specific activity is expressed as U/g fresh weight (FW), where one unit is defined as an increase of 1 at OD420 per min.

Antioxidant Potential. *Total Phenolic and Flavonoids Compounds.* Total phenolic compounds content was measured according to Zheng et al.[22] by the Folin−Ciocalteu method with slight modifications. Frozen tissue (2 g) was homogenized with 5 mL of 95% ethanol. The mixture was incubated at 4 °C for 24−48 h and then centrifuged at 10000g for 10 min. A mixture was made by a combination of 1 mL of the supernatant, 1 mL of 95% ethanol, 5 mL of distilled water, and 0.5 mL of 50% Folin−Ciocalteu phenol reagent, and then was incubated for 5 min at room temperature. After that, 1 mL of 5% (w/v) sodium carbonate was added, and the mixture was vortexed briefly. The solution was read in a spectrophotometer set at 725 nm after incubation for 1 h in the dark at room temperature, and phenols were quantified by comparison to a standard curve of gallic acid. Total phenolics concentration was calculated and expressed as gallic acid equivalents (mg/100 g FW). Reported values are averages of three replicates.

Total flavonoid content was determined using a colorimetric assay as described by Lamaison and Carnat[23] with some modifications. Frozen tissue (2 g) was homogenized with 5 mL of 70% ethanol and then centrifuged at 10000g for 10 min. An aliquot (1 mL) of the supernatant was mixed with 0.3 mL of 10% aluminum chloride reagent, followed by the addition of 0.3 mL of aluminum nitrate reagent 5 min later, and the mixture was shaken gently. Two milliliters of 1 M sodium hydroxide solution was added to terminate the reaction. The absorbance of the mixture was determined at 510 nm after 10 min. Total flavonoid concentration was determined from a standard curve of vitexin and expressed as mg vitexin equivalents per g FW.

Antioxidant Enzyme Assays. To determine activities of the antioxidant enzyme, frozen tissue (2 g) was homogenized with 8 mL of 50 mM sodium phosphate buffer (pH 7.0) for superoxide dismutase (SOD) and catalase (CAT) and centrifuged at 10,000g for 10 min at 4 °C. The supernatant was diluted 12 times for enzyme assays. SOD (EC 1.15.1.1) activity was assayed by its ability to inhibit photochemical reduction of nitrotetrazolium blue chloride (NBT) at 560 nm according to Zhao et al.[24] with minor modifications. The reaction mixture (3 mL) contained 75 μM NBT, 13 mM L-methionine, 0.1 mM EDTA, and 4 μM riboflavin and 200 μL of enzyme extract in 50 mM sodium phosphate buffer (pH 7.8). One unit of SOD enzyme activity was defined as the amount of enzyme that caused 50% inhibition of nitroblue tetrazolium. The specific SOD activity was expressed as U/mg FW.

CAT (EC 1.11.1.6) activity was assayed by monitoring the initial rate of H_2O_2 disappearance at 240 nm according to the method of Maehly and Chance.[25] The reaction mixture contained 1 mL of 50 mM sodium phosphate buffer (pH 7.0), 1 mL of 0.2% H_2O_2, and 1 mL of CAT extract. One unit of CAT activity was defined as the amount of enzyme that decomposed 1 μmol H_2O_2 per min at 30 °C.

Quantitative Real-Time PCR (qPCR). Total RNA was extracted by using the trizol method as described by Zhao et al.[26] The potential contaminating genomic DNA was removed by digestion with DNase I (DNA-free; Ambion) according to the manufacturer's protocol. The cDNA was synthesized using oligo(dT)$_{18}$ and 2 μg of total RNA treated with RNase-free DNase I and M-MLV Reverse Transcriptase (Promega) according to the method of Zhao et al.[26] qPCR was carried out with SYBR Green Real time PCR MasterMix (Toyobo, Osaka, Japan) on a Chromo 4 real time PCR Detection System (Bio-Rad, Hercules, CA). *EF1-α* gene (Genbank X97204) encoding the basidiomycete elongation factor 1-α was used as the reference gene.[27] Specific primers were designed from coding sequences of each gene using Primer Express 2.0 software (Applied Biosystems) (Table 1). The PCR amplification protocol consisted of an initial denaturation at 95 °C for 2 min, followed by 40 cycles of 15 s at 95 °C and 20 s at 60 °C. The SYBR Green I fluorescence signal was measured during the 60 °C annealing step. To check the annealing specificity of each oligonucleotide, melting curve analysis (55−94 °C) was carried out at the end of amplification. All experiments were run in triplicate with different cDNAs synthesized from three biological replicates. To determine relative fold differences for each sample, the

threshold cycle (*Ct*) value was normalized to the *Ct* value for *EF1-α* and calculated relative to a calibrator using the formula $2^{-\Delta\Delta CT}$.

Data Analysis. All statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, IL). Significant differences between the means were determined by one-way analysis of variance (ANOVA) and Duncan's multiple comparisons tests. A probability of $P \leq 0.05$ was considered to be significant. All experiments were conducted in a completely randomized design with three replicates for each treatment. The data are expressed as the mean ± SD.

■ RESULTS AND DISCUSSION

Effect of MeJA Treatment on Respiratory and Electrolyte Leakage Rates in *A. bisporus* Fruit Bodies during Storage at 10 °C. The change of respiration rate in the MeJA treatments and nontreated controls is given in Figure 1A. *A.*
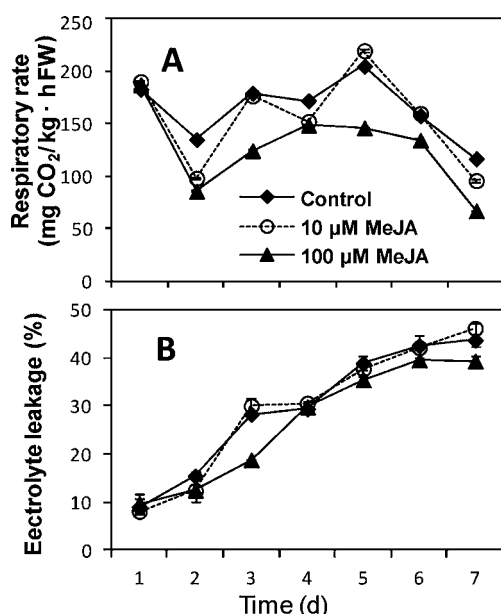

Figure 1. Effects of MeJA on respiratory rate (A) and electrolyte leakage rate (B) in *A. bisporus* fruit bodies during storage at 10 °C for 7 days. Data represent the means ± SD, *n* = 3.

bisporus is a climacteric fruit that exhibits an initial high respiration rate (approximately 190 mg/kg·h). This was probably caused by harvesting stress because of the removal of nutrients and waters. The respiratory rate in both control and MeJA treated samples decreased rapidly from the initial high value, then increased to a peak, and finally decreased. The occurrence and timing of the peak roughly coincides with the cap opening of the mushroom. Similar respiratory behavior in *Pleurotus* mushrooms was also reported by Villaescusa and Gil.[28] The respiration rate in 100 μM MeJA treated samples was significantly inhibited (*p* < 0.05) during the whole storage period, and was 57%−86% lower than that in nontreated controls (Figure 1A). However, 10 μM MeJA was less effective. The lower respiration rate in 100 μM MeJA-treated fruit may result in lower consumption of sugars and hence better retention of their contents.

Electrolyte leakage is an index of the semipermeable properties of cell membranes. It was shown in Figure 1B that the electrolyte leakage increased with storage time in all samples, which indicated that membrane systems became more vulnerable to leakage. Hildebrand[29] reported that a reduction in membrane integrity resulting from lipid peroxidation increases

membrane leakage and enhances cell senescence. However, Braaksma et al.[30] did not find lipid peroxidation on *A. bisporus* mushrooms stored for 3 days at 20 °C. Electrolyte leakage could be due to another type of degradation. Anyway, throughout storage, 100 μM MeJA slowed the increase rate in electrolyte leakage as compared with nontreated fruit bodies. Particularly, the electrolyte leakage in nontreated mushrooms increased to 28.3% on day 3, while only 18.8% of that was observed in 100 μM MeJA-treated mushrooms. At the end of storage, electrolyte leakage was 43.7% and 39.3%, respectively, in nontreated and 100 μM MeJA-treated mushrooms. However, there is no significant difference between controls and 10 μM MeJA treatments (*P* > 0.05), indicating that effects of MeJA on mushroom quality retention are probably concentration dependent.

Effect of MeJA Treatment on Soluble Protein and Total Sugar Content of *A. bisporus* Fruit Bodies during Storage at 10 °C. Fruit bodies of *A. bisporus* contain approximately 13.5 mg/g FW soluble protein and, after harvesting, these serve as a nutrient source to support continuing metabolic activity. A decline in soluble protein concentration is considered to be an important indicator of tissue senescence.[31] Soluble protein content declined both in MeJA treatments and nontreated controls during the 7-day storage period (Figure 2A). However, during the first 5 days,
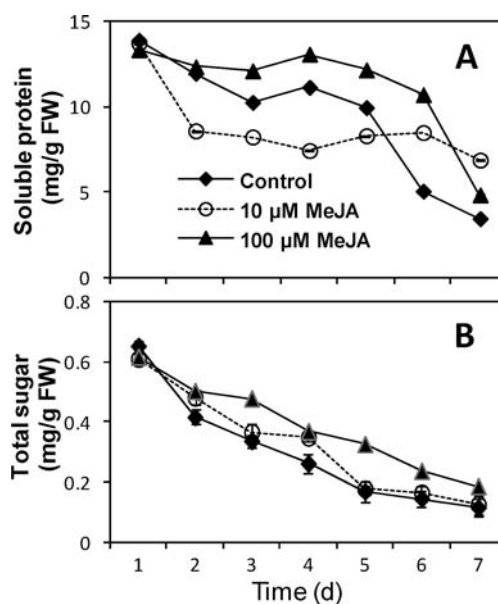

Figure 2. Effects of MeJA on contents of soluble protein (A) and total sugar (B) in *A. bisporus* fruit bodies during storage at 10 °C for 7 days. Data represent the means ± SD, *n* = 3.

the decline of soluble protein levels in fruit bodies treated with 100 μM MeJA (91.2% of initial levels) was smaller compared with that in nontreated controls (71.6% of initial levels), whereas, at lower concentration levels (10 μM MeJA), the rate of decline during this period increased (60.5% of initial levels). However, after 7 days of storage, residual soluble protein levels in fruit bodies treated with MeJA (100 μM and 10 μM) was 36.3% and 50.1% of initial values compared with 24.9% in nontreated controls, respectively, which suggests that an appropriate concentration of MeJA may improve maintaining mushroom quality during postharvest storage.

Total and soluble sugar concentrations in harvested plant products are also considered important indicators of post-harvest deterioration.[4] Total sugar levels gradually declined with storage time both in MeJA treatments and nontreated controls (Figure 2B). Steady decreases in total sugar content were also reported in *A. bisporus* mushrooms stored at 12 °C for 12 days.[32] As shown in Figure 2B, both 100 μM and 10 μM MeJA significantly slowed ($P < 0.05$) the decline rate in total sugar levels as compared with that in nontreated controls. However, 10 μM MeJA was shown to be less effective, and 26.1% of the initial levels were retained on the fifth day compared with 53.0% in 100 μM MeJA treatments. MeJA treatment, applied either pre- or postharvest, has also been reported to maintain higher levels of sugars in a number of horticultural crops, including fresh-cut kiwifruit, radish, and mangoes.[8−10] Since sugars are the main substrates of respiratory metabolism, the maintenance of their levels by MeJA could be due to the inhibition of mushroom respiration (Figure 1A).

Effect of MeJA Treatment on the Browning of *A. bisporus* Fruit Bodies during Storage at 10 °C. Mushroom browning, particularly in the case of white mushroom, is one of the main features considered in the quality spectrum defined by Gormley and MacCanna,[33] and assumes greater importance in determining market ability and consumer acceptability. According to Gormley,[34] mushrooms with L-values <80 or <69 were considered as unacceptable from a whiteness point of view at wholesale or consumer levels respectively. Figure 3A shows the different L-values obtained after application of MeJA (10 and 100 μM), compared to the control without any treatment. From this figure, higher L-values were observed in MeJA (10 and 100 μM) compared to the control after 7 days. The L-value in the control samples sharply decreased to 82.7 (day 3), and then to 79.2 (day 5), which may not be considered as wholesale-acceptable.[34] Compared with the control, the L-value in 10 μM MeJA treated fruit bodies was 80.04 on day 5, which showed that 10 μM MeJA significantly ($P < 0.05$) inhibited the browning of button mushrooms. Comparatively, 100 μM MeJA was observed to be a more competitive white color protector, which was demonstrated by the much lower L-value (80.8) at the end of the storage. One hundred micromolar MeJA significantly ($P < 0.05$) slowed the decline rate of the L-value and maintained mushroom whiteness value until the seventh day.

PPO is widely distributed in nature and has been detected in most fruits and vegetables, and its involvement in enzymatic browning is thought to be the main factor of discoloration of many fruits and vegetables.[35] Hence, the control of PPO activity is of importance in preventing the synthesis of melanin in the browning of mushrooms and other vegetables and fruits. As shown in Figure 3B, though PPO activity in *A. bisporus* fruit bodies increased gradually in both MeJA-treated samples and nontreated controls with storage time, enzyme levels in the latter were 1.09−1.31 and 1.06−1.90 times higher compared with that of 10 and 100 μM MeJA-treated fruit bodies, respectively. One hundred micromolar MeJA treatment significantly ($P < 0.05$) prevented the increase in PPO activity, 10 μM MeJA treatment also inhibited the PPO activity; however, the effect was not as good as the 100 μM MeJA treatment. Besides, a high positive correlation ($R^2 = 0.9857$) between polyphenol oxidase and browning in *A. bisporus* fruit bodies was found in Figure 3C. Increased PPO activity was associated with mushroom browning, suggesting that the
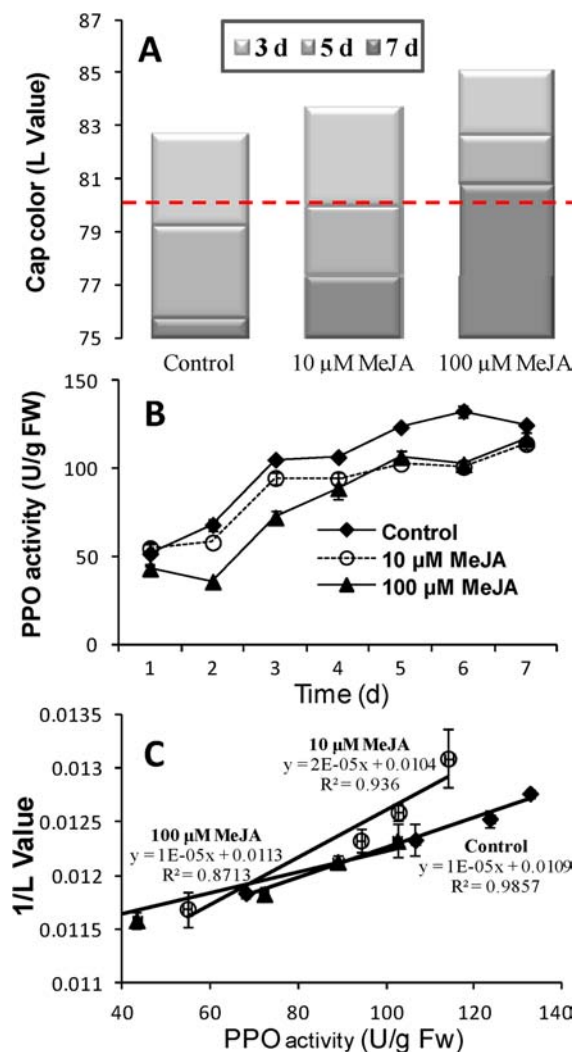

Figure 3. Effects of MeJA on cap color (A) and PPO activity (B) and their correlation (C) in *A. bisporus* fruit bodies during storage at 10 °C for 7 days. Data represent the means ± SD, $n = 3$.

decrease of browning as a result of the MeJA treatment was associated with lower PPO activity (Figure 3C). All these results confirmed the beneficial effects of MeJA in delaying enzymatic discoloration and thus maintaining mushroom commercial value.

Effect of MeJA Treatment on Antioxidant Potential of *A. bisporus* Fruit Bodies during Storage at 10 °C. Phenolic compounds have been reported as the major antioxidant components in mushrooms,[36] and they may produce beneficial effects by scavenging free radicals. Thus, these antioxidant compounds have been widely reported to maintain health and prevent cancer and cardiovascular diseases.[37] In the present study, the total phenolic content in control fruit bodies decreased from the initial day, whereas total phenolic content in 10 and 100 μM MeJA-treated fruit exhibited a slight increase over the first 5 and 4 days, respectively, and then gradually decreased (Figure 4A). A significant ($P < 0.05$) higher level of total phenolics was observed in MeJA-treated samples in the first 6 days compared to control samples. Moreover, increased MeJA concentration from 10 to 100 μM resulted in an increase in phenolics content.

Flavonoids act in plants as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellents, and light
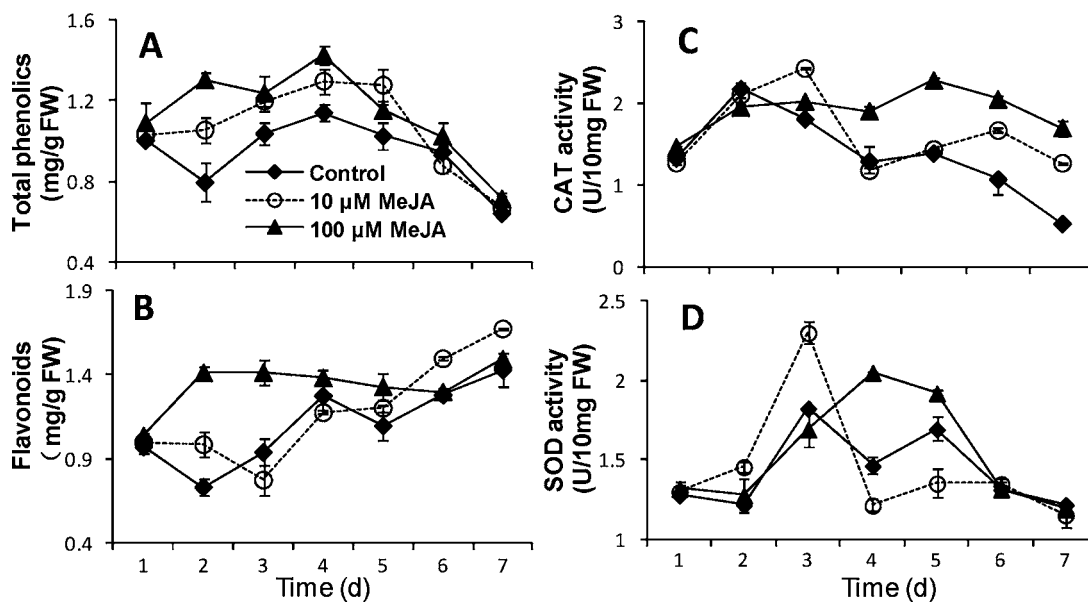
Figure 4. Effects of MeJA on total phenolics (A) and flavonoids (B) contents, and CAT (C) and SOD (D) activities in *A. bisporus* fruit bodies during storage at 10 °C for 7 days. Data represent the means ± SD, $n = 3$.
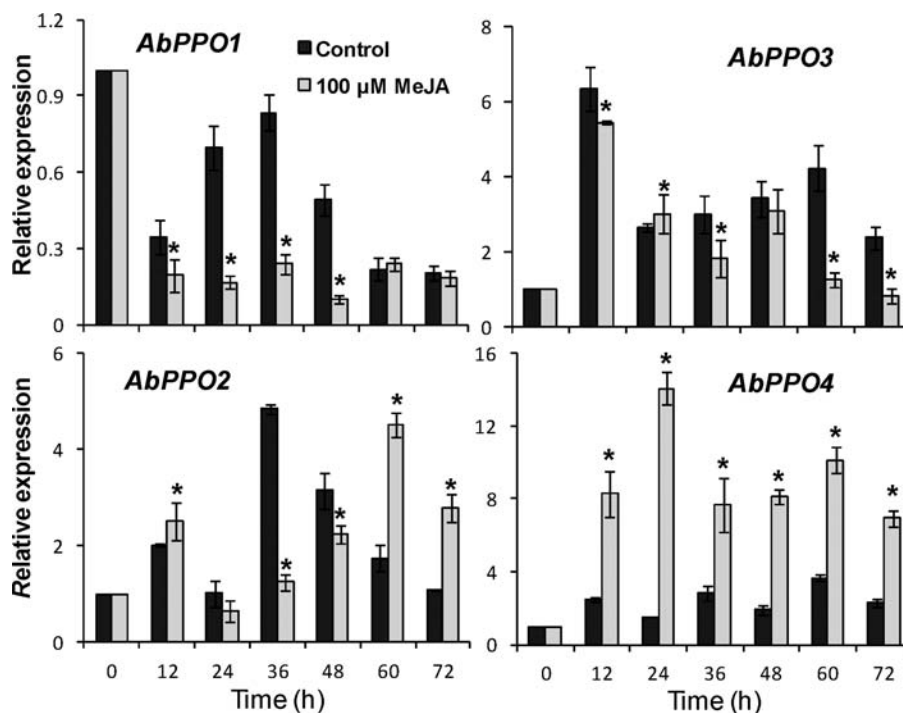

Figure 5. Effects of MeJA on gene expression of polyphenol oxidase in *A. bisporus* fruit bodies during storage at 10 °C for 7 days. The expression levels of *AbPPO1, AbPPO2, AbPPO3*, and *AbPPO4* encoding polyphenol oxidase were evaluated by quantitative real-time PCR, normalized to the *EF1-α* gene, and set relative to 0 h control samples according to the $2^{-\Delta\Delta CT}$ method. Data represent the means ± SD, $n = 3$. * indicates significant differences at $P = 0.05$ by Duncan's multiple range tests.

screening. One hundred micromolar MeJA treatment markedly increased total flavonoids content and maintained a significant ($P < 0.05$) higher level of total flavonoids than nontreated controls during the first 5 days of storage (Figure 4B). However, 10 μM MeJA treatment was not as effective in maintaining total flavonoids content as 100 μM MeJA treatment during this storage period, but total flavonoids content in 10 μM MeJA-treated samples began increasing from day 5 and was 1.17 times higher than that of the control at the end of the storage. *A. bisporus* fruit bodies treated with MeJA

exhibited significant ($P < 0.05$) higher levels of total phenolics and flavonoids compared to the control, which suggests that MeJA may improve the antioxidant status of the mushroom by positively affecting phenolic metabolism.

Antioxidant enzymes such as SOD and CAT play a crucial role in antioxidant defense during the fruit ripening process. SOD and CAT protect cells from the damaging effects of reactive oxygen species (ROS) and represent key components of cellular antioxidant defense systems, and the two enzymes are thought to extend food freshness by protecting the integrity

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of membranes. CAT activity in controls gradually decreased throughout the postharvest period from 132.8 to 52.6 U/g FW after 7 days (Figure 4C). Large increases in CAT activity were recorded in samples treated with 10 and 100 μM MeJA during the postharvest storage period, and markedly higher enzyme activities were present in samples treated with 100 μM MeJA. Although enzyme levels in treated samples also decreased during later postharvest storage, residual levels in 10 and 100 μM MeJA-treated samples were still 3.2 and 2.4 times higher than the nontreated controls at the end of the storage, respectively.

SOD catalyzes the dismutation of superoxide anions to produce hydrogen peroxide, which is then removed by catalase. In *A. bisporus*, SOD has been associated with stress tolerance, and the gene encoding the enzyme is reported to be up-regulated in postharvest sporophores.[38] Time courses of SOD activity in MeJA treated samples and nontreated controls are shown in Figure 4D. A rapid increase in the 10 μM MeJA-treated samples was observed during the first 3 days, and a higher peak of SOD activity (230.1 U/g FW) was found on day 3 compared with a lower peak activity of the control (182.6 U/g FW). However, after 3 days, the SOD activity of 10 μM MeJA-treated samples decreased sharply to a 0.83 times lower level than that of the control and then remained at a relatively constant level. After 4 days of postharvest storage, a relatively large increase in enzyme activity was recorded in 100 μM MeJA-treated samples (55.3%) compared with control samples (14.6%). SOD levels then exhibited a gradual downward trend over the next 3 days. The results obtained seem to indicate that MeJA, as the plant growth regulator, positively affects the response to the oxidative stress associated with mushroom senescence.

Effect of MeJA Treatment on Gene Expression of Polyphenol Oxidase in *A. bisporus* Fruit Bodies during Storage at 10 °C. It has been shown that 100 μM MeJA treatment significantly ($P < 0.05$) inhibited mushroom browning due to the marked decrease of PPO activity (Figure 3A,B,C). However, Koussevitzky et al.[39] pointed out that MeJA treatment increased the activity and expression of *PPO* in tomatoes. The expression pattern of *AbPPO1* or *AbPPO2* or *AbPPO3* or *AbPPO4* encoding polyphenol oxidase in 100 μM MeJA-treated samples was similar to that in its corresponding control samples during storage (Figure 5). The transcript level of *AbPPO1* was significantly ($P < 0.05$) decreased by 100 μM MeJA treatment, in agreement with the significant decrease of PPO activity (Figure 3B). The peak expression level of *AbPPO1* in 100 μM MeJA-treated samples was only 28.8% of that in control samples. The transcript level of *AbPPO2* in the control samples sharply increased from the 24 h level (1.01) to the peak level (4.84), but this increase rate was slowed by 100 μM MeJA. As compared with the control, the transcript level of *AbPPO2* in the 100 μM MeJA-treated samples increased at a lower rate from the 24 h level (approximately 0.66) to the peak level (4.52), and the peak time was delayed by 1 day. Though the transcript level of *AbPPO3* was also inhibited by 100 μM MeJA treatment, the degree of the decrease was not as good as *AbPPO1*. There is no obvious difference ($P > 0.05$) of *AbPPO3* relative expression between the control and 100 μM MeJA treatment samples in the first 24 h, but the transcript level of *AbPPO3* in the 100 μM MeJA treatment samples was 9.30%–65.22% lower than that of control samples from 36 h to the termination of the experiment. Different from *AbPPO1–3*, the transcript level of *AbPPO4* was 2.75–9.28-fold induced by 100

μM MeJA treatment, which was probably due to the different roles of *polyphenol oxidase* genes in the mushroom browning. Thus, the role of MeJA in PPO activity-related fruit browning needs further investigation.

In summary, postharvest application of MeJA maintained higher levels of soluble protein, total sugar, total phenolics, and total flavonoids as well as antioxidant activity than control samples. In addition, MeJA reduced the respiratory rate, electrolyte leakage rate, cap browning, PPO activity, and relative expression of *AbPPO1−3*. In all cases of parameters except for soluble protein content at the later storage time, 100 μM MeJA treatments produced greater beneficial effects compared to the 10 μM MeJA treatments. Thus, application of MeJA could be an important strategy to extend storage life and maintain the quality of harvested *A. bisporus* fruit bodies.

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■ **ABBREVIATIONS USED**

MeJA, methyl jasmonate; PPO, polyphenol oxidase; SOD, superoxide dismutase; CAT, catalase; ROS, reactive oxygen species; FW, fresh weight; NBT, nitrotetrazolium blue chloride; EDTA, ethylenediaminetetraacetic acid; PVPP, cross-linked polyvinylpyrrolidone; qPCR, quantitative real-time PCR

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