



Fumigation with essential oils improves sensory quality and enhanced antioxidant ability of shiitake mushroom (*Lentinus edodes*)



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ABSTRACT

Several naturally occurring essential oils were evaluated for their effectiveness in maintaining sensory quality and increasing antioxidant levels and activities in shiitake (*Lentinus edodes*) mushrooms. Freshly harvested mushrooms were fumigated with 5 $\mu\text{l l}^{-1}$ clove, cinnamaldehyde and thyme oils at 10 °C for 1.5 h and the antioxidant activities determined using assays of H_2O_2 content, O_2^- production rate, DPPH, and ABTS radical scavenging activity. The results showed that the antioxidant activities of the mushrooms fumigated with cinnamaldehyde were significantly increased when compared to the controls. Moreover, cinnamaldehyde fumigation significantly delayed losses of phenolic compounds and enhanced flavonoid content. The essential oil fumigation treatment also increased the antioxidant enzyme activities of CAT, SOD, APX and GR throughout the storage periods. All the fumigation treatments were effective in retarding mushroom sensory deterioration. These results indicate that postharvest application of essential oil fumigation can extend the shelf life and enhance the antioxidant capacity of shiitake mushrooms.

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1. Introduction

Mushrooms have been used for centuries in China both as food and medicine. Shiitake (*Lentinula edodes*) mushrooms are the second most cultivated edible mushroom in the world, representing about 25% of worldwide production. Its production has increased faster than any other mushroom species (Boa, 2004). Shiitake mushroom has a high nutritional value and contains several bioactive compounds, including polysaccharides, dietary fibre, ergosterol, vitamin B1, B2 and C, folates, niacin and minerals. Among the biologically active substances present in mushrooms, phenolics have attracted much attention due to their superb properties as antioxidant, anti-inflammatory or anti-tumour agents, among others (Puttaraju, Venkateshaiah, Dharmesh, Urs, & Somasundaram, 2006). Many of the biological functions, such as anticancer, antiviral, immunopotentiating and hypolipidemic activities, are considered to be attributed to their free radical scavenging and antioxidant activity. Therefore, the secondary compounds with antioxidant activities such as phenolics and flavonoids in mushrooms are of great interest as possible protective agents to help human health.

Essential oils (EOs) are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). The antimicrobial properties of EOs derived from many plant organs have been empirically recognised for centuries, although scientific confirmation has been reviewed recently (Burt, 2004). Methyl jasmonate has been shown to suppress fungal growth in grapefruit (Droby et al., 1999), reduce decay and maintain postharvest quality of papayas (González-Aguilar, Buta, & Wang, 2003), and inhibit microbial contamination of fresh-cut celery and peppers (Buta & Moline, 1998). It was also shown that blue mould (*Penicillium expansum*) in pears was controlled by allyl isothiocyanate vapour treatment (Mari, Leoni, & Cembali, 2002). In addition, Regnier, du Plooy, Combrinck, and Botha (2008) reported that limonene, carvone and 1,8-cineole were effective against both *Botryosphaeria parva* and *Colletotrichum gloeosporioides* in mango fruit. Moreover, several studies have shown that some essential oils also have the potential function of enhancing antioxidant capacities in some fruits (Jin et al., 2012). Strawberries treated with thymol or eugenol maintained higher levels of anthocyanins, flavonoids and oxygen radical absorbance capacity compared with untreated fruits (Wang, Wang, Yin, Parry, & Yu, 2007). Linalool, carvacrol, anethole and perillaldehyde increased anthocyanins, phenolic compounds, and antioxidant activity in blueberries (Wang, Wang, & Chen, 2008). However, little information is

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available about the effect of EOs on the accumulation of antioxidant compounds in shiitake mushrooms.

The objective of this study was to evaluate the effect of essential oil (including clove, cinnamaldehyde and thyme) fumigation on sensory quality, texture, antioxidant capacities and antioxidant enzyme activities in shiitake mushroom during storage at 4 °C, with a view to better understanding how the essential oil fumigation treatments delay senescence of postharvest shiitake mushroom.

2. Materials and methods

2.1. Sample preparation, treatment and storage

Shiitake mushroom, (*Lentinula edodes*), used in this study was harvested in November from a local farm in Hangzhou, China. The mushrooms were transported to the laboratory within 1 h after picking, under refrigerated conditions, then stored in darkness at 1 ± 1 °C and 95% relative humidity (RH). The day after, mushrooms were screened for uniform size and maturity and absence of mechanical damage. In a preliminary experiment, we tested a series of concentrations of each essential oil, including clove, cinnamaldehyde and thyme, namely, 1, 5 and $10 \mu\text{l l}^{-1}$. All essential oils at the concentration of 1 or $5 \mu\text{l l}^{-1}$ significantly inhibited fruit decay, and $5 \mu\text{l l}^{-1}$ had an even better effect. However, $10 \mu\text{l l}^{-1}$ essential oil fumigation treatment caused some physiological injuries, including discolouration or a smelly flavour, in shiitake mushrooms (data not shown). Thus, a concentration of $5 \mu\text{l l}^{-1}$ was chosen for these experiments. A total of 60 shiitake mushrooms were placed in 2 l sealed polypropylene (PP) containers with a filter paper inside the cover. A total of $10 \mu\text{l}$ of each essential oil, including clove, thyme, and cinnamaldehyde, was spotted onto the filter paper. These containers were kept at 10 °C and the essential oils were allowed to vapourise within the containers. Afterward, sealed PP containers were opened and stored for 20 days at 4 ± 1 °C and 90% relative humidity (RH), and subsequently every 5 days, three replicates from each treatment group were analysed.

2.2. Sensory evaluation

The sensory attributes that characterised mushroom deterioration were determined. These attributes were: off-odour, gill colour, gill uniformity, cap surface uniformity and presence of dark zones on the cap (Ares, Parentelli, Gámbaro, Lareo, & Lema, 2006; Jiang, Feng, Zheng, & Li, 2013). Samples were evaluated by a sensory panel of ten trained assessors. Mushrooms were served in closed, odourless plastic containers at room temperature. After opening the polyethylene bags, the mushrooms were placed in plastic containers and evaluations were performed within 2 h in order to avoid loss of off-odours. A balanced complete block design was carried out for duplicate evaluation of the samples. For scoring, 10 cm unstructured scales anchored with “nil” for zero and “high” for ten were used, except for the gill colour descriptor, for which the anchors were “white” and “brown”.

2.3. Texture measurement

A penetration test was performed on the shiitake mushroom cap using a TA.XT2i texture analyzer (Stable Micro Systems, UK), using a 5 mm diameter cylindrical probe. Samples were penetrated 5 mm in depth. The speed of the probe was 2.0 mm s^{-1} during the pretest and penetration. Force and time data were recorded with Texture Expert (version 1.0) from Stable Micro Systems. From the force vs. time curves, firmness was defined as the maximum force.

Measurements were performed in duplicate on three mushroom caps for each sample.

2.4. Determination of total phenolics and flavonoids contents

For total phenolic extraction, mushroom tissues (2 g) were homogenised with 5 ml 80% ethanol solution for 5 min. After 2 h in the dark, the slurry was filtered using Whatman No. 1 filter paper. Colour was developed by mixing $40 \mu\text{l}$ of the total phenolic extract, $50 \mu\text{l}$ ethanol, $400 \mu\text{l}$ water, $5 \mu\text{l}$ of a solution prepared by mixing 2 g of sodium carbonate and $500 \mu\text{l}$ of Folin–Ciocalteu (Sigma–Aldrich Chemical Co., St. Louis, MO, USA) reagent at half strength in 100 ml 0.1 M NaOH. After 30 min, the solution was read in a spectrophotometer set at 724 nm. Total phenolic concentration was calculated according to Singleton and Rossi (1965). The total phenolic contents were expressed as gallic acid equivalents, in mg/100 g fresh sample.

Flavonoids were measured using a colourimetric assay developed by Jia, Tang, and Wu (1999), with some modifications. Total flavonoid extraction was the same as described above. Then, 1 ml of appropriately diluted sample was added to a 10 ml volumetric flask containing 4 ml of deionised water. At time zero, 0.3 ml of 5% NaNO_2 was added to each volumetric flask; at 5 min, 0.3 ml of 10% AlCl_3 was added and at 6 min, 2 ml of 1 M NaOH was added. Each reaction flask was then immediately diluted with 3.4 ml of deionised water and mixed. After 30 min in the dark, absorbance of the mixture was determined at 510 nm against a blank of deionised water. The total flavonoid contents were then expressed as rutin equivalents, in mg/100 g fresh sample.

2.5. Scavenging activities against H_2O_2 content, O_2^- production rate, DPPH^\cdot and ABTS^\cdot radicals

The assay for H_2O_2 content was carried out by the procedure previously described by Patterson, Macrae, and Ferguson (1984). Mushroom tissues (2 g) were homogenised with 10 ml of acetone at 0 °C. After centrifugation for 15 min at 6000g and 4 °C, the supernatant phase was collected. The supernatant (1 ml) was mixed with 0.1 ml of 5% titanium sulphate and 0.2 ml ammonia, and then centrifuged for 10 min at 6000g and 4 °C. The pellets were dissolved in 3 ml of 10% (v/v) H_2SO_4 and centrifuged for 10 min at 5000g. Absorbance of the supernatant phase was measured at 410 nm. H_2O_2 content was calculated using H_2O_2 as a standard and then expressed as 10^{-6} mol/g and per g is on the fresh weight basis.

Superoxide anion production rate was measured by monitoring the nitrite formation from hydroxylamine in the presence of O_2^- , as described by Wang and Luo (1990). Mushroom tissues (4.0 g) were homogenised with 12 ml of 50 mM potassium phosphate buffer (pH 7.8) containing 1% (w/v) polyvinylpyrrolidone at 0 °C, and then centrifuged at 5000g and 4 °C for 15 min. The supernatant obtained (1 ml) was mixed with 0.9 ml of 50 mM potassium phosphate buffer (pH 7.8) and 0.1 ml of 10 mM hydroxylamine hydrochloride, and then incubated for 30 min at 25 °C. The incubated solution (1 ml) was added to 1 ml of 17 mM 3-aminobenzenesulphonic acid and 1 ml of 7 mM 1-naphthylamine, and then further kept for 20 min at 25 °C. The absorbance was recorded at 530 nm. The O_2^- production rate was expressed as $10^{-9} \text{ mol/min/g}$ and per g is on the fresh weight basis.

The capacity of the mushrooms with different treatments to scavenge the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH^\cdot) was monitored according to the method reported (Hatano, Kagawa, Yasuhara, & Okuda, 1988) with minor modification. Briefly, 0.3 ml of the methanolic extract of mushrooms was mixed with 2.7 ml of a methanolic solution containing DPPH^\cdot radicals ($6 \mu\text{mol/l}$). The mixture was shaken vigorously and kept for

60 min in the dark (until stable absorption values were obtained). The reduction of the DPPH[•] radical was measured by monitoring the decrease of absorption at 517 nm continuously. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH[•] discolouration using the equation: $RSA (\%) = 100 (1 - A_c/A_D)$, where A_c is the absorbance of the solution with methanolic extract of the mushrooms and A_D is the absorbance of the DPPH[•] solution.

2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS^{•+}) scavenging activity was measured as previously described by Goh, Barlow, and Yong (2003), with slight modifications. ABTS^{•+} diammonium salt (75 mM) and potassium persulphate (1.225 mM) were mixed overnight. The mixture was diluted 10-fold with 99.5% ethanol before use. 3 ml of the diluted ABTS^{•+} solution was added to 1 ml methanolic extract of mushrooms. The absorbance of the resultant mixture was measured after 60 min at 414 nm. A blank sample (without antioxidants), containing the same amount of ethanol and ABTS^{•+} was prepared and measured daily. The scavenging ability of antioxidants was calculated as: $ABTS^{•+}$ scavenging activity (%) = $[(A_0 - A)/A_0] \times 100$, where A_0 is the absorbance of the control reaction and A is the absorbance in the presence of samples.

2.6. Antioxidant enzyme measurements

For analysis of enzymatic activities, mushroom tissues (4.0 g) were homogenised with 12 ml of 50 mM potassium phosphate buffer (pH 7.3) containing 1 mM EDTA and 2 mM DTT. After centrifugation for 15 min at 10,000g and 4 °C, the supernatant was collected and used as the crude enzyme extract for the CAT, SOD and GR assays. The APX enzyme extract was prepared as above except that the extraction buffer contained 1 mM ascorbic acid in addition to other ingredients. Protein content was determined according to the method of Bradford (1976), with bovine serum albumin used as the standard.

Catalase (CAT; EC 1.11.1.6) activity was determined according to the method of Candan and Tarhan (2003). It was assayed in a reaction mixture containing 50 mM phosphate buffer (pH 7.0), 10 mM H₂O₂ and enzyme. One unit of CAT activity was defined as the amount of enzyme which decomposes 1 μmol H₂O₂ per minute at 25 °C and which causes changes in the substrate (on a molecular weight basis). The specific CAT activity was expressed as mol/min/g and per g is on the protein basis.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed by its ability to inhibit the photochemical reduction of nitrotriazolium blue chloride (NBT) at 560 nm (Beuchamp & Fridovich, 1971). The assays were performed at 25 °C. The reaction mixture (3 ml) contained 33 μM NBT, 10 mM l-methionine, 0.66 mM EDTA-Na₂ and 0.0033 mM riboflavin in 0.05 M sodium phosphate buffer (pH 7.8). The reaction was started by adding riboflavin and placing the tubes containing the reaction mixture under 300 μmol m⁻² s⁻¹ irradiance at 25 °C for 10 min. The absorbance was recorded at 560 nm. One unit of SOD enzyme activity was defined as the quantity of enzyme that reduced the absorbance reading of samples to 50% in comparison with tubes lacking enzymes, and causes changes in substrate (on a molecular weight basis). The specific SOD activity was expressed as mol/g and per g is on the protein basis.

Ascorbate peroxidase (APX; EC 1.11.1.11) activity was determined according to Nakano and Asada (1981). The assay depends on the decrease in absorbance of ascorbic acid at 290 nm because of oxidation of ascorbic acid to monodehydroascorbic acid and dehydroascorbic acid. The reaction mixture contained 0.05 M sodium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM EDTA Na₂, 1.2 mM H₂O₂, and 0.1 ml enzyme extract in a final assay volume of 1 ml. The reaction was started with the addition of hydrogen peroxide. The decrease in absorbance at 470 nm was

recorded for 3 min. One unit of enzymatic activity was defined as the amount of the enzyme that caused a change of 0.01 in absorbance per minute and causes changes in substrate (on a molecular weight basis). The specific APX activity was expressed as mol/min/g and per g is on the protein basis.

Glutathione reductase (GR; EC 1.6.4.2) activity was assayed according to Foyer and Halliwell (1976). The reaction mixture contained 0.025 M sodium phosphate buffer (pH 7.8), 0.5 mM GSSG, 0.12 mM NADPH-Na₄ and 0.1 ml enzyme extract in a final assay volume of 1 ml. The decrease in absorbance at 340 nm was recorded for 3 min. One unit of enzymatic activity was defined as the amount of the enzyme that caused a change of 0.01 in absorbance per minute and causes changes in substrate (on a molecular weight basis). The specific GR activity was expressed as mol/min/g and per g is on the protein basis.

2.7. Statistical analysis

Experiments were performed using a completely randomised design. Data were subjected to one-way analysis of variance (ANOVA). Mean separations were performed by Duncan's multiple range test (DPS version 6.55). Differences at $P < 0.05$ were considered significant.

3. Results and discussion

3.1. Effect of essential oils fumigation on sensory attributes

Changes in sensory qualities of off-odour, gill colour, gill uniformity, cap uniformity and dark zones of shiitake mushroom during 20 days of storage are presented in Table 1. Off-odour intensity

Table 1
Effect of essential oil fumigation treatments on sensory attributes change of shiitake mushrooms stored at 4 °C for 20 days^{a,b}.

Days at 4 °C	Control	Clove	Cinnamaldehyde	Thyme
<i>Off-odour</i>				
0	0	0	0	0
5	1.87 ± 0.12 ^a	1.31 ± 0.04 ^b	1.25 ± 0.06 ^b	1.22 ± 0.05 ^b
10	2.92 ± 0.11 ^a	2.50 ± 0.05 ^{a,b}	2.42 ± 0.13 ^b	2.45 ± 0.03 ^b
15	5.53 ± 0.17 ^a	4.26 ± 0.22 ^b	3.90 ± 0.26 ^d	4.11 ± 0.12 ^c
20	7.84 ± 0.32 ^a	6.45 ± 0.18 ^b	5.74 ± 0.21 ^c	5.86 ± 0.25 ^c
<i>Gills colour</i>				
0	0	0	0	
5	1.56 ± 0.07 ^a	1.36 ± 0.10 ^b	1.30 ± 0.08 ^b	1.35 ± 0.06 ^b
10	3.32 ± 0.21 ^a	2.62 ± 0.15 ^b	2.36 ± 0.22 ^c	2.33 ± 0.12 ^c
15	5.74 ± 0.10 ^a	4.93 ± 0.18 ^b	4.20 ± 0.17 ^d	4.58 ± 0.15 ^c
20	7.97 ± 0.16 ^a	6.67 ± 0.17 ^b	5.74 ± 0.32 ^d	6.11 ± 0.16 ^c
<i>Gills uniformity</i>				
0	10	10	10	
5	8.42 ± 0.26 ^b	8.70 ± 0.16 ^a	8.68 ± 0.14 ^a	8.65 ± 0.30 ^a
10	6.50 ± 0.20 ^d	7.02 ± 0.23 ^c	7.33 ± 0.17 ^a	7.16 ± 0.24 ^b
15	4.94 ± 0.14 ^d	5.86 ± 0.30 ^c	6.42 ± 0.23 ^a	6.11 ± 0.23 ^b
20	3.73 ± 0.19 ^d	4.77 ± 0.13 ^c	5.10 ± 0.06 ^a	4.93 ± 0.17 ^b
<i>Cap uniformity</i>				
0	10	10	10	10
5	8.26 ± 0.33	8.44 ± 0.33 ^b	8.52 ± 0.21 ^a	8.50 ± 0.42 ^a
10	6.05 ± 0.31 ^d	6.53 ± 0.26 ^c	6.86 ± 0.20 ^a	6.74 ± 0.34 ^b
15	4.95 ± 0.17 ^d	5.49 ± 0.10 ^c	5.94 ± 0.13 ^a	5.65 ± 0.30 ^b
20	3.56 ± 0.24 ^d	4.50 ± 0.07 ^c	5.12 ± 0.07 ^a	4.67 ± 0.21 ^b
<i>Dark zones</i>				
0	0	0	0	0
5	1.46 ± 0.05 ^a	1.30 ± 0.12 ^b	1.23 ± 0.10 ^c	1.32 ± 0.04 ^b
10	2.73 ± 0.10 ^a	2.11 ± 0.05 ^b	1.78 ± 0.13 ^d	1.90 ± 0.07 ^c
15	4.13 ± 0.11 ^a	2.85 ± 0.03 ^b	2.53 ± 0.12 ^d	2.72 ± 0.16 ^c
20	6.22 ± 0.13 ^a	4.43 ± 0.17 ^b	3.64 ± 0.23 ^d	3.88 ± 0.12 ^c

^a Mean of ten replications ± SD.

^b Means in same row with different letters are significantly different ($P < 0.05$).

significantly increased after 10 days of storage in control samples. The colour of mushroom gills gradually became browner and less uniform with time for all the evaluated conditions. The gills of control mushrooms showed a colour intensity of 5.74 and uniformity 4.94 at the 15th day of storage. A better trend was observed for the uniformity of the cap surface and the presence of dark stains on the cap in cinnamaldehyde treated samples. These results suggest that cinnamaldehyde fumigation treatment was more effective in retarding mushroom sensory deterioration. Considering the development of the evaluated sensory attributes, mushrooms treated with cinnamaldehyde showed the lowest deterioration rate, followed by those treated with thyme and finally those treated with clove and the control treatment.

3.2. Effect of essential oils fumigation on firmness

As shown in Fig. 1, the firmness of shiitake mushroom showed varying degrees of reduction over time. By day 20, the firmness of the treated samples were significantly higher than those of the control samples ($P < 0.05$). It was clear that the texture of oil fumigated mushrooms was better retained than that of the control mushrooms during storage, showing that these natural compounds somehow could reduce the action of cell-wall degrading enzymes. In addition, the kind of bacterial-induced softening was observed in control samples but was inhibited by essential oil fumigation treatments. In fact, a number of EOs and several of their individual components exhibit antibacterial activity against food borne pathogens *in vitro* and, to a lesser extent, in foods. Valverde et al. (2005) found that the addition of eugenol, menthol or thymol inside packages was effective in reducing the microorganism proliferation in table grapes, the effect being higher for yeast and moulds than for mesophilic aerobes. These results showed beneficial effects of these essential oils, especially cinnamaldehyde, on increasing the shelf life of shiitake mushrooms, since it has been postulated that mushroom softening and texture changes during storage determine mushroom storability and shelf life, as well as reduced incidence of decay and less susceptibility to mechanical damage (Gao, Feng, & Jiang, 2014).

3.3. Effect of essential oils fumigation on total phenolic and flavonoid contents

Phenolics and flavonoids are considered to be beneficial antioxidants as they exhibit scavenging activity of harmful active oxygen species. The effects of essential oil fumigation on phenolic and flavonoid content in the mushrooms have been examined in this work. As shown in Fig. 2A and B, the content of phenolic and

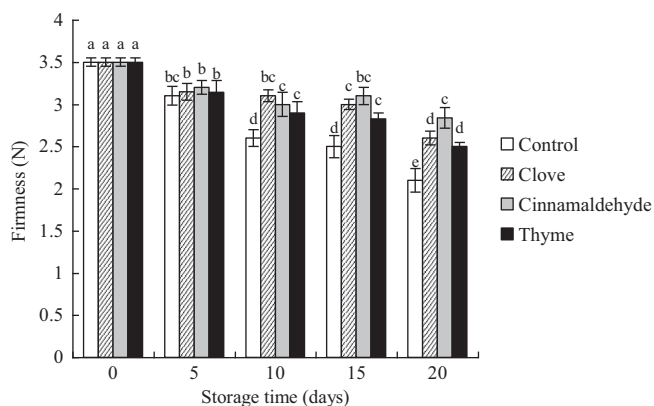


Fig. 1. Effect of essential oil fumigation treatments on firmness change of shiitake mushrooms stored at 4 °C for 20 days. Data are means \pm SD of three replications.

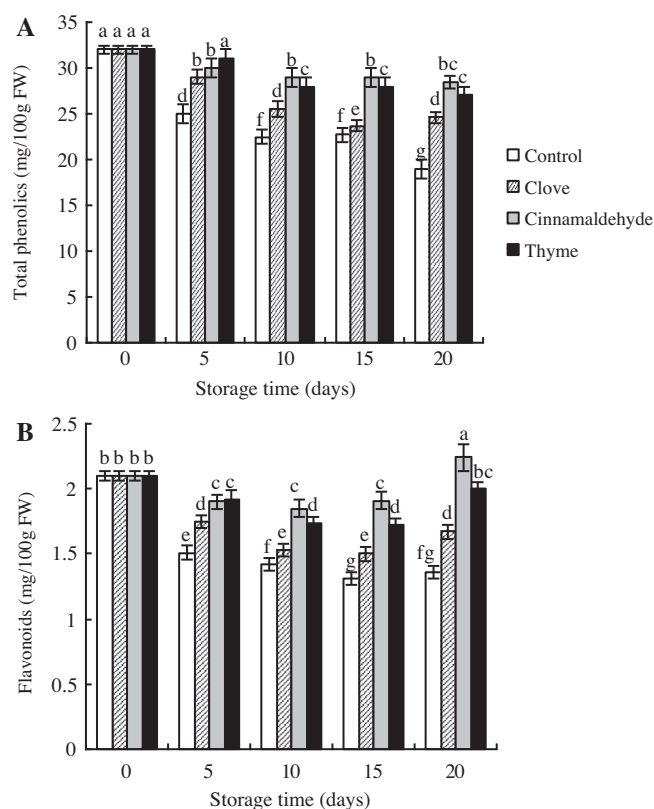


Fig. 2. Effect of essential oil fumigation treatments on total phenolic (A) and flavonoid (B) changes in shiitake mushrooms stored at 4 °C for 20 days. Data are means \pm SD of three replications.

flavonoid compounds diminished in the control mushrooms during storage. However, essential oil fumigation induced a lower rate of decrement in phenolic content, and cinnamaldehyde fumigation even stimulated flavonoid accumulation in the mushroom compared with the other treatments at the end of storage. This is similar to the findings by Wang et al. (2008) in blueberries. Accumulation of secondary metabolites such as flavonoids and phenolics is one of the common responses of plants to many biotic and abiotic stresses (Dong, Zhang, Lu, Sun, & Xu, 2012). For example, UV-C irradiation and ozone fumigation have been reported to stimulate flavonoid accumulation in shiitake mushroom and plant cells (Jiang, Muhammad, Jiang, Lu, & Ying, 2010; Xu et al., 2011). It is hypothesised that essential oils would act as “signalling compounds” that trigger a signal that resembles a mild stress to the fruit. As a defense response, fruit produces additional phenolic compounds and flavonoids and increases their antioxidant activities (Sharma & Tripathi, 2006). Some studies have shown that the accumulation of phenols and anthocyanins paralleled the increase in phenylalanine ammonia-lyase (PAL) activity in Chinese bayberry (Wang et al., 2009).

3.4. Effect of essential oil fumigation on H_2O_2 content, O_2^- production rate, DPPH $^{\cdot}$ and ABTS $^{\cdot+}$ radicals

Fruit and vegetable ripening has been described as an oxidative phenomenon which requires a turnover of reactive oxygen species (ROS), such as H_2O_2 and O_2^- . In the present study, the increases in both H_2O_2 content and O_2^- production rate were observed during 20 days of storage (Fig. 3A and B). The control treatment does not seem to substantially contribute to enhancement of the antioxidant capacity of the mushrooms. On the other hand, essential oil fumigation generally reduced the increase in both H_2O_2 content

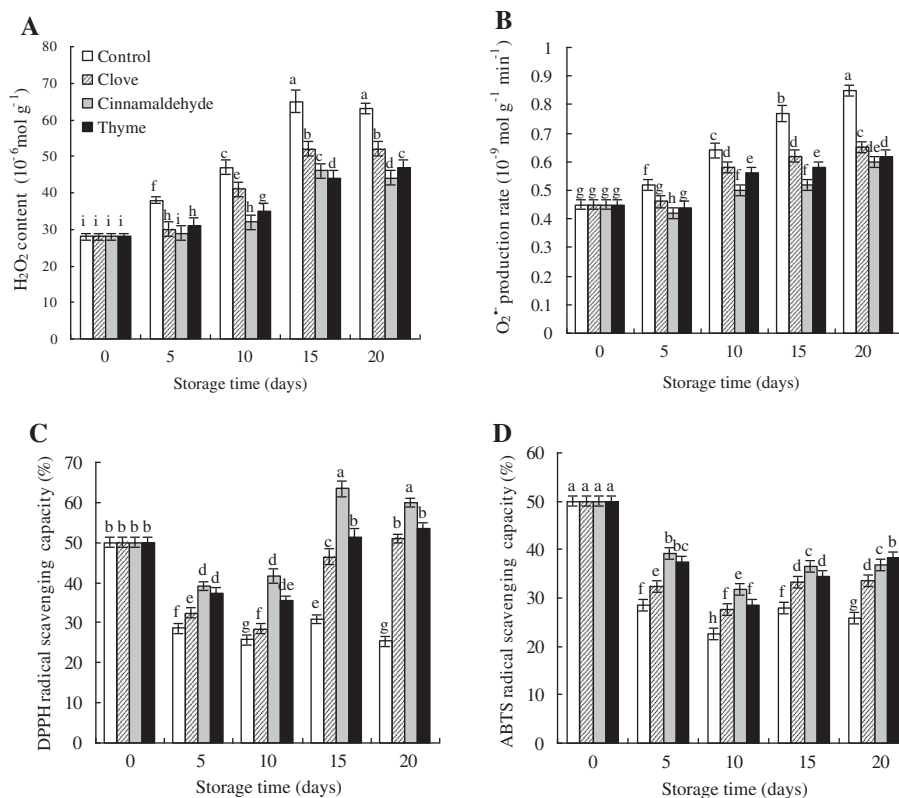


Fig. 3. Effect of essential oil fumigation treatments on H₂O₂ content (A), O₂⁻ production rate (B), DPPH[•] (C) and ABTS^{•+} (D) scavenging capacity of shiitake mushrooms stored at 4 °C for 20 days. Data are means ± SD of three replications.

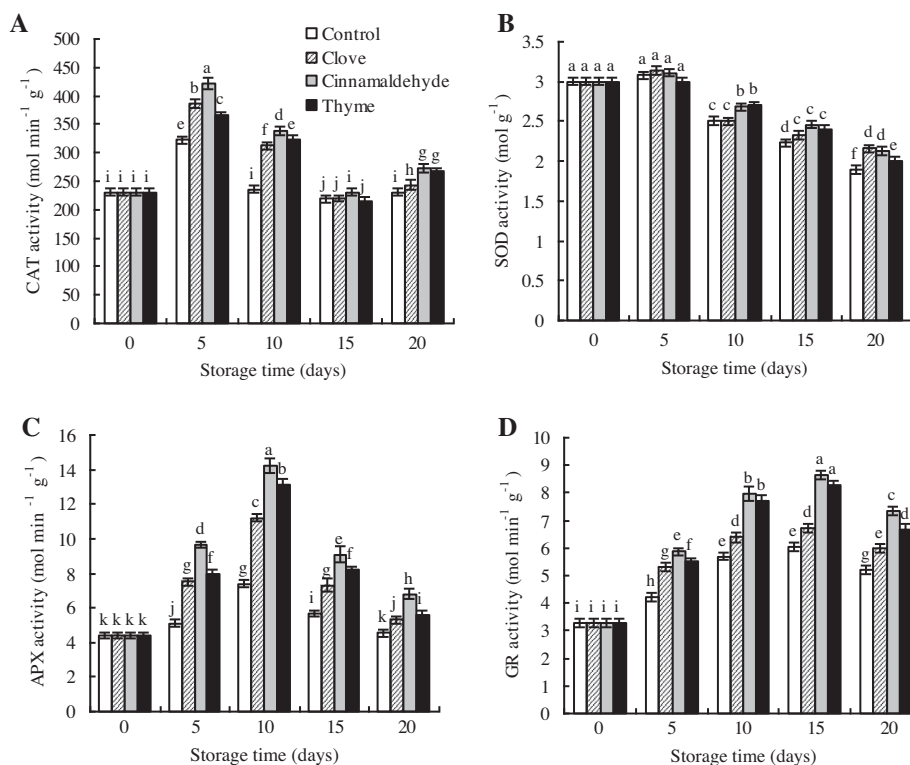


Fig. 4. Effect of essential oil fumigation treatments on CAT (A), SOD (B), APX (C) and GR (D) activities of shiitake mushrooms stored at 4 °C for 20 days. Data are means ± SD of three replications.

and O_2^- production rate of mushrooms during storage. Mushrooms treated with cinnamaldehyde presented higher antioxidant capacity, compared with thyme and clove. The antioxidant capacities (including DPPH and ABTS radical scavenging capacities) of mushroom extracts during 20 days of storage are shown in Fig. 3C and D. Mushrooms treated with clove, cinnamaldehyde or thyme had markedly lower radical signals for DPPH and ABTS than those of the control, indicating that the treated mushroom had higher scavenging activities for these radicals. Cinnamaldehyde treated mushroom in particular had the highest scavenging activities among all the treatments. Mushrooms are well known to possess antioxidant activity. This is mainly correlated with their phenolic and flavonoid compounds (Chen, Xia, Zhou, & Qiu, 2010). The mechanisms of action of flavonoids and phenolics are through scavenging and chelating processes or as free radical terminators (Cook & Samman, 1996). Our data suggested that essential oil fumigation significantly improved the antioxidant capacities of the mushrooms. The antioxidant properties were evaluated using the ethanol extract of the mushrooms, which is a complex mixture of phytochemicals with additive and synergistic effects. It has been reported that the H_2O_2 content, O_2^- production rate, DPPH and ABTS radical scavenging activity are mainly related to the content of total phenolics and flavonoids in mushrooms. Though other antioxidants were probably present in the mushroom extract, the amounts of ascorbic acid, β -carotene and lycopene found in the mushroom extract were very low (Chen et al., 2010). Therefore, phenolic compounds and flavonoids are considered to make a significant contribution to the mushrooms' antioxidant activity (Chen et al., 2010). Our results showed that essential oil fumigation treatments significantly delayed losses in phenolic compounds, and increased flavonoid contents in the mushrooms. Thus, it is suggested that essential oil fumigation may stimulate the antioxidant activities of the mushrooms by enhancing phenolic and flavonoid contents; an increase in the antioxidant capacity and free radical scavenging activity would reduce the physiological deterioration and enhance the resistance of tissue against microbial invasion and reduce the spoilage of shiitake mushroom (Dong et al., 2012).

3.5. Effect of essential oil fumigation on antioxidant enzyme activities

The antioxidant system plays a crucial role in the ripening process. This system includes CAT, SOD, some peroxidases and the enzymes involved in the ascorbate–glutathione cycle: APX and GR. These enzymatic components, together with the low-molecular-weight antioxidants ascorbic acid (AA) and glutathione (GSH), ultimately scavenge H_2O_2 at the expense of NADPH or NADH (Foyer & Halliwell, 1976). In general, all essential oil fumigation treatments enhanced the antioxidant enzyme activities of mushrooms compared to control (Fig. 4), in agreement with the reduced ROS production (Fig. 3A and B). Mushrooms from cinnamaldehyde fumigation treatment had the highest activities for APX and GR. However, no significant differences ($P > 0.05$) were observed between essential oil treatments for SOD activity. These findings are in agreement with the results of Chanjirakul, Wang, Wang, and Siriphanich (2006), who reported that antioxidant enzyme activities in raspberries were increased by methyl jasmonate treatment.

4. Conclusion

The results indicate that postharvest essential oil fumigation not only maintained mushroom sensory quality during storage, but also exhibited the capability to increase antioxidant activity and levels. Essential oils, such as cinnamaldehyde, have positive effects on increasing antioxidant capacities, enhancing antioxidant

enzyme activities, and maintaining higher total phenolic and flavonoid content. Thus, cinnamaldehyde has the potential to preserve the quality and safety of shiitake mushroom.

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References

- Ares, G., Parentelli, C., Gámbaro, A., Lareo, C., & Lema, P. (2006). Sensory shelf life of shiitake mushrooms stored under passive modified atmosphere. *Postharvest Biology and Technology*, 41, 191–197.
- Beuchamp, C., & Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*, 44, 276–287.
- Boa, E. (2004). *Wild edible fungi: A global overview of their use and importance to people*. Non-wood forest products series No. 17. Rome: FAO.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein–dye binding. *Analytical Biochemistry*, 72, 248–254.
- Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods – A review. *International Journal of Food Microbiology*, 94, 223–253.
- Buta, J. G., & Moline, H. E. (1998). Methyl jasmonate extends shelf life and reduces microbial contamination of fresh-cut celery and pepper. *Journal of Agricultural and Food Chemistry*, 46, 1253–1256.
- Candan, N., & Tarhan, L. (2003). Relationship among chlorophyll-carotenoid content, antioxidant enzyme activities and lipid peroxidation levels by Mg^{2+} deficiency in the *Mentha pulegium* leaves. *Plant Physiology and Biochemistry*, 41, 35–40.
- Chanjirakul, K., Wang, S. Y., Wang, C. Y., & Siriphanich, J. (2006). Effect of natural volatile compounds on antioxidant capacity and antioxidant enzymes in raspberries. *Postharvest Biology and Technology*, 40, 106–115.
- Chen, X. H., Xia, L. X., Zhou, H. B., & Qiu, G. Z. (2010). Chemical composition and antioxidant activities of *Russula roseocarpa* sp. nov. *Journal of Agricultural and Food Chemistry*, 58, 6966–6971.
- Cook, N. C., & Samman, S. (1996). Flavonoids – chemistry, metabolism, cardioprotective effects, and dietary sources. *Nutritional Biochemistry*, 7, 66–76.
- Dong, J., Zhang, M., Lu, L., Sun, L., & Xu, M. (2012). Nitric oxide fumigation stimulates flavonoid and phenolic accumulation and enhances antioxidant activity of mushroom. *Food Chemistry*, 135, 1220–1225.
- Droby, S., Porat, R., Cohen, L., Weiss, B., Shapiro, B., Philosophas, S., et al. (1999). Suppressing green mold decay in grapefruit with postharvest jasmonate application. *Journal of the American Society for Horticultural Science*, 124, 184–188.
- Foyer, C. H., & Halliwell, B. (1976). The presence of glutathione and glutathione reductase in chloroplast: A proposed role in ascorbic acid metabolism. *Planta*, 133, 5–21.
- Gao, M., Feng, L., & Jiang, T. (2014). Browning inhibition and quality preservation of button mushroom (*Agaricus bisporus*) by essential oils fumigation treatment. *Food Chemistry*, 149, 107–113.
- Goh, L. M., Barlow, P. J., & Yong, C. S. (2003). Examination of antioxidant activity of *Ginkgo biloba* leaf infusions. *Food Chemistry*, 82, 275–282.
- González-Aguilar, G. A., Buta, J. G., & Wang, C. Y. (2003). Methyl jasmonate and modified atmosphere packaging (MAP) reduce decay and maintain postharvest quality of papaya 'sunrise'. *Postharvest Biology and Technology*, 28, 361–370.
- Hatano, T., Kagawa, H., Yasuhara, T., & Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: Their relative astringency and radical scavenging effects. *Chemical and Pharmaceutical Bulletin*, 36, 2090–2097.
- Jia, Z., Tang, M., & Wu, J. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64, 555–559.
- Jiang, T., Feng, L., Zheng, X., & Li, J. (2013). Physicochemical responses and microbial characteristics of shiitake mushroom (*Lentinus edodes*) to gum arabic coating enriched with natamycin during storage. *Food Chemistry*, 138, 1992–1997.
- Jiang, T., Muhammad, M. J., Jiang, Z., Lu, X., & Ying, T. (2010). Influence of UV-C treatment on antioxidant capacity, antioxidant enzyme activity and texture of postharvest shiitake (*Lentinus edodes*) mushrooms during storage. *Postharvest Biology and Technology*, 56, 209–215.
- Jin, P., Wu, X., Xu, F., Wang, X., Wang, J., & Zheng, Y. (2012). Enhancing antioxidant capacity and reducing decay of Chinese bayberries by essential oils. *Journal of Agricultural and Food Chemistry*, 60, 3769–3775.
- Mari, M., Leoni, O., & Cembali, M. (2002). Antifungal vapour-phase activity of allyl-isothiocyanate against *Penicillium expansum* on pears. *Plant Pathology*, 51, 231–236.
- Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiology*, 22, 867–880.

- Patterson, B. D., Macrae, E. A., & Ferguson, I. B. (1984). Estimation of hydrogen peroxide in plant extracts using titanium (IV). *Analytical Biochemistry*, 139, 487–492.
- Puttaraju, N. G., Venkateshaiah, S. U., Dharmesh, S. M., Urs, S. M. N., & Somasundaram, R. (2006). Antioxidant activity of indigenous edible mushrooms. *Journal of Agricultural and Food Chemistry*, 54, 9764–9772.
- Regnier, T., du Plooy, W., Combrinck, S., & Botha, B. (2008). Fungitoxicity of *Lippia scaberrima* essential oil and selected terpenoid components on two mango postharvest spoilage pathogens. *Postharvest Biology and Technology*, 48, 254–258.
- Sharma, N., & Tripathi, A. (2006). Fungitoxicity of the essential oil of citrus sinensis on postharvest pathogens. *World Journal of Microbiology and Biotechnology*, 2006(22), 587–593.
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16, 144–158.
- Valverde, J. M., Guillén, F., Martínez-Romero, D., Calvador, S., Serrano, M., & Valero, D. (2005). Improvement of table grapes quality and safety by the combination of modified atmosphere packaging (MAP) and eugenol, menthol, or thymol. *Journal of Agricultural and Food Chemistry*, 53, 7458–7464.
- Wang, A. G., & Luo, G. H. (1990). Quantitative relation between the reaction of hydroxylamine and superoxide anion radicals in plants. *Plant Physiology Communications*, 26, 55–57.
- Wang, C. Y., Wang, S. Y., & Chen, C. T. (2008). Increasing antioxidant activity and reducing decay of blueberries by essential oils. *Journal of Agricultural and Food Chemistry*, 56, 3587–3592.
- Wang, C. Y., Wang, S. Y., Yin, J. J., Parry, J., & Yu, L. L. (2007). Enhancing antioxidant, antiproliferation, and free radical scavenging activities in strawberries with essential oils. *Journal of Agricultural and Food Chemistry*, 55, 6527–6532.
- Wang, K. T., Jin, P., Cao, S. F., Shang, H. T., Yang, Z. F., & Zheng, Y. H. (2009). Methyl jasmonate reduces decay and enhances antioxidant capacity in Chinese bayberries. *Journal of Agricultural and Food Chemistry*, 57, 5809–5815.
- Xu, M., Zhu, Y., Dong, J., Jin, H., Sun, L., Wang, Z., et al. (2011). Ozone induces flavonol production of *Ginkgo biloba* cells dependently on nitrate reductase-mediated nitric oxide signaling. *Environmental and Experimental Botany*, 75, 114–119.