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Browning inhibition and quality preservation of button mushroom (*Agaricus bisporus*) by essential oils fumigation treatment

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ABSTRACT

The effect of essential oil fumigation treatment on browning and postharvest quality of button mushrooms (*Agaricus bisporus*) was evaluated upon 16 days cold storage. Button mushrooms were fumigated with essential oils, including clove, cinnamaldehyde, and thyme. Changes in the browning index (BI), weight loss, firmness, percentage of open caps, total phenolics, ascorbic acid, microbial activity and activities of polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), and peroxidase (POD) were measured. The results indicated that all essential oils could inhibit the senescence of mushrooms, and the most effective compound was cinnamaldehyde. Fumigation treatment with 5 $\mu l\ l^{-1}$ cinnamaldehyde decreased BI, delayed cap opening, reduced microorganism counts, promoted the accumulation of phenolics and ascorbic acid. In addition, 5 $\mu l\ l^{-1}$ cinnamaldehyde fumigation treatment inhibited the activities of PPO and POD, and increased PAL activity during the storage period. Thus, postharvest essential oil fumigation treatment has positive effects on improving the quality of button mushrooms.

1. Introduction

The white button mushroom, *Agaricus bisporus*, is widely recognised for its nutritional, organoleptic and medicinal properties, and is highly popular with consumers (Beelman, Royse, & Chikthimmah, 2003). In China, the production of *A. bisporus* increased more than 7-fold (from 180,500 to 1,330,400 tonnes) during 1997–2003, largely due to the breeding of superior strains and improvements in spawn production and cultivation technology (Chang, 2005). However, button mushrooms only have a short shelf life of 3–4 days. Their commercial value is lost within a few days, due to browning, water loss, senescence and microbial attack. Mushrooms are conventionally packed in plastic punnets and overwrapped with perforated PVC film and refrigerated. High humidity, created due to the high transpiration rate of mushrooms and poor water vapour permeability of the film, causes condensation inside the package, as can be clearly seen underneath the film of the punnets being sold in the supermarket. The short shelf-life of mushrooms is an impediment to the distribution and marketing of the fresh product. Thus, prolonging post-harvest storage, while preserving their quality, would benefit the mushroom industry as well as consumers.

Essential oils (EOs) are aromatic oily extracts obtained from plant materials, such as flowers, seeds, leaves, roots, fruits, and other plant parts (Burt, 2004). EOs outstand as an alternative to chemical preservatives and their use in foods meets the demands of consumers for natural products. Several naturally occurring essential oils have been reported to have antimicrobial properties and have shown promise in reducing postharvest diseases and disorders in horticultural crops (Dorman & Deans, 2000; Serrano, Martínez-Romero, Castillo, Guillén, & Valero, 2005; Valverde et al., 2005), although their mechanism of action has not been studied in great detail. In addition, several studies have shown that some essential oils also have the potential function of enhancing antioxidant capacities in various kinds of fruits. Wang, Wang, and Chen (2008) reported that the flavonoid content and oxygen radical absorbance capacity in blueberries were enhanced by carvacrol, anethole, or perillaldehyde. Methyl jasmonate, or tea tree oil, could increase the antioxidant capacities and antioxidant enzyme activities in Chinese bayberries or raspberries (Chanjirakul, Wang, Wang, & Siriphanich, 2006; Wang et al., 2009). The natural essential oils as reducing agents of peroxidase activity in leafy vegetables were also evaluated (Ponce, Valle, & Roura, 2004). However, to our knowledge, little information is available on the response of button mushrooms to postharvest essential oil fumigation.

The purpose of this study was to investigate the effect of essential oils (including clove, cinnamaldehyde, and thyme) fumigation treatment on mushroom tissue browning and quality characteristics upon 16 d cold storage. Parameters related to tissue browning and nutritional quality, such as browning index (BI), phenylalanine ammonia lyase (PAL, EC 4.3.1.5), polyphenol oxidase (PPO, EC 1.14.18.1), and peroxidase (POD, EC 1.11.1.7) activities,

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total phenols (TP) and ascorbic acid (AA), as well as microbiological quality, were assayed at pre-storing (0), 4, 8, 12 and 16 d after storage, respectively.

2. Materials and methods

2.1. Sample preparation, treatment and storage

Button mushrooms (*Agaricus bisporus*) used in this study were harvested from a local farm in Hangzhou, China. The mushrooms were transported to the laboratory within one hour of picking, then stored in darkness at 4 ± 1 °C and 90% relative humidity (RH). The day after, the mushrooms were screened for their 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 μl l^{-1}. All essential oils at the concentration of 1 or 5 μl l^{-1} significantly inhibited fruit decay, and 5 μl l^{-1} had the greater effect. However, 10 μl l^{-1} essential oil fumigation treatment caused some physiological injuries, including discoloration or smelly flavour in the button mushrooms (data not shown). Thus, a concentration of 5 μl l^{-1} was chosen to use in this experiment. A total of 60 button mushrooms were placed in 2 l sealed polypropylene (PP) containers, with a filter paper inside the cover. A total of 10 μl of each essential oil, including clove, thyme, and cinnamon, was spotted onto the filter paper. These containers were stored at 10 °C and the essential oils were allowed to vapourise within the containers. Afterward, the sealed PP containers were opened and stored for 16 days at 4 ± 1 °C and 90% relative humidity (RH), and subsequently every 4 days, three replicates from each treatment group were analysed.

2.2. Texture measurement and weight loss analysis

A penetration test was performed on the button mushroom cap using a TA.XT Express-v3.1 texture analyser (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, as well as during penetration. Force and time data were recorded with Texture Expert (Version 1.0) software from Stable Micro Systems. From the force vs. time curves, firmness was defined as the maximum force.

Weight loss was determined by weighing the whole mushroom before and after the storage period. Weight loss was expressed as the percentage of loss of weight with respect to the initial weight.

2.3. Colour

The surface colour of mushroom caps was measured with a WSC-S Colourimeter (Shanghai precision instrument Co. Ltd., Shanghai, China). To analyse the L^* (light/dark), a^* (red/green) and b^* (yellow/blue) values; each mushroom was measured at three equidistant points of the cap and compared to the ideal mushroom colour values of $L^* = 97$, $a^* = -2$ and $b^* = 0$ using ΔE as described by the following equation (Ajlouni, 1991):

$$\Delta E = \left[(L-97)^2 + (a-(-2))^2 + b^2\right]^{1/2},$$

where ΔE indicates the degree of overall colour change in comparison to the colour values of an ideal mushroom.

The browning index (BI), which represents the purity of brown colour (Palou, Lopez-Malo, Barbosa-Canovas, Welti-Chanes, & Swanson, 1999), were calculated according to the following equations:

$$BI = [100(x-0.31)]/0.172,$$

where $x = (a + 1.75L)/(5.645\,L + a - 3.012b)$.

2.4. Percent open caps and overall acceptability

The criteria for judging the percentage of open caps was based on the development of the umbrella-like shape of the cap followed by detachment of veil. The percent open caps was determined from known number of mushrooms as:

$$\%\text{Open caps} = N_{oc}/N_t \times 100$$

where N_t = total number of mushrooms; N_{oc} = number of open-caped mushrooms.

The overall acceptability based on colour, texture and percent open caps was done by a panel of four judges on round table basis using a four-point scale, where 1 = poor, 2 = fair, 3 = good and 4 = excellent.

2.5. Microbiological analysis

All samples were analysed for their *mesophilic, psychrophilic, pseudomonad*, yeasts and moulds bacteria counts. Twenty-five grams of mushrooms were removed aseptically from each pack and diluted with 225 ml 0.1% peptone water. The samples were homogenised by a stomacher at high speed for 2 min. Serial dilutions (10^{-1}–10^{-9}) were made in serial dilution tubes by taking 1.0 ml with 9.0 ml of 0.1% peptone water. Aerobic counts were determined on plate count agar (PCA; Merck), following incubation at 35 °C for 2 days for *mesophilic* bacteria, and at 4 °C for 7 days for *psychrophilic* bacteria. *Pseudomonas* was counted on cephaloridin fucidin cetrimide agar (CFC; Difco), with selective supplement SR 103 (Oxoid). The incubation temperature was 25 °C and plates were examined after 48 h. Yeasts and moulds were estimated on potato dextrose agar (PDA; Merck) and incubation conditions were 28 ± 1 °C for 5–7 days.

2.6. Determination of total phenolics and ascorbic acid

Quantification of the total soluble phenolic compounds was carried out using the method proposed by Singleton and Rossi (1965). Five grams of mushroom caps were homogenised with 20 ml of 80% ethanol for 24 h; the homogenised mix was filtered through two layers of cheesecloth, and the filtered liquid was centrifuged at 10,000*g* for 15 min. One ml of the supernatant liquid was mixed with 1 ml of Folin Ciocalteu reagent and 10 ml of sodium carbonate (7%). This was topped up to 25 ml with distilled water and left to settle for 1 h. The absorbance was then read at 750 nm. A standard curve of gallic acid was used for quantification.

The determination of total ascorbic acid was carried out as described by Hanson et al. (2004). On the basis of coupling 2,4-dinitrophenylhydrazine (DNPH) with the ketonic groups of dehydroascorbic acid through the oxidation of ascorbic acid by 2,6-dichlorophenolindophenol (DCPIP) to give a yellow/orange colour in acidic conditions. Mushroom tissues (10 g) were blended with 80 ml of 5% meta-phosphoric acid in a homogeniser and centrifuged. After centrifugation, 2 ml of the supernatant were poured into a 20 ml test tube containing 0.1 ml of 0.2% 2,6-DCIP sodium salt in water, 2 ml of 2% thiourea in 5% meta-phosphoric acid and 1 ml of 4% 2,4-DNPH in 9N sulphuric acid. The mixtures were kept in a water bath at 37 °C for 3 h followed by an ice bath for 10 min. 5 ml of 85% sulphuric acid was added and the mixtures were kept at room temperature for 30 min before reading at 520 nm.

2.7. PPO, PAL, and POD activities

For analysis of enzymatic activities, mushroom tissues (4.0 g) were homogenised with 12 ml of 50 mM K-phosphate buffer (pH

7.3), containing 1 mM EDTA and 2 mM DTT. After centrifugation for 15 min at 10,000*g* and 4 °C, the supernatant was collected and used as the crude enzyme extract for the PPO, PAL and POD assays. The protein content was determined according to the method of Bradford (1976), with bovine serum albumin used as the standard.

PPO (EC 1.10.3.2) activity was measured by incubating 0.5 ml of enzyme extract in 2.5 ml of buffered substrate (100 mM sodium phosphate, pH 6.4 and 50 mM Catechol), and then monitoring the change of absorbance at 398 nm (Wang, Tian, Xu, Qin, & Yao, 2004). One unit of activity of PPO was defined as the amount of enzyme to cause 0.01 absorbance increase per minute under the conditions of assay. The specific PPO activity was expressed as U/mg protein.

PAL (EC 4.3.1.5) activity was assayed by measuring the absorbance of trans-cinnamic acid at 290 nm (Koukol & Conn, 1961). The reaction mixture (3 ml), which contained 0.8 ml supernatant and 50 mM l-phenylalanine in sodium borate buffer (200 mM, pH 8.8), was incubated at 37 °C for 90 min and the reaction was terminated by ice water. One unit of PAL activity was defined as the amount of enzyme that caused an increase in absorbance at 290 nm of 0.01 in 1 h under the specified conditions. The specific PAL activity was expressed as U/mg protein.

POD (EC 1.11.1.7) activity was measured spectrophotometrically, using the substrate guaiacol (Moerschbacher, Noll, Flott, & Reisener, 1988). The reaction mixture for the determination of POD activity consisted of 50 mM sodium phosphate buffer (pH 6.0), 5 mM guaiacol, 5 mM H_2O_2, and 50 µl of tissue extract. One unit of POD activity was defined as the amount of enzyme that caused a change in absorbance at 470 nm of 0.01 per minute under the specified conditions. The specific POD activity was expressed as U/mg protein.

2.8. 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 texture and weight loss

Weight loss increased as the storage period progressed in all the treatments, as shown in Fig. 1A. The highest weight loss was observed in the control samples, it reached 2.57% at the end of storage, suggesting that dehydration is an important process in the loss of button mushroom quality during postharvest storage. This could be attributed to the fact that mushrooms are only protected by a thin epidermal structure, which does not prevent a quick superficial dehydration. Weight loss reductions were obtained after the fumigation of clove (2.13%), cinnamaldehyde (1.75%), and thyme (1.90%) compared to controls, and therefore delayed mushroom shriveling and quality deterioration. Nevertheless, there was no significant difference between the essential oil treated mushrooms ($P > 0.05$).

Texture is a critical factor for button mushroom quality and reflects metabolic changes and changes in water content. Firmness at harvest was 17.32 N and during cold storage, button mushrooms suffer a rapid loss in firmness, which contributes greatly to its short postharvest life and susceptibility to fungal contamination. However, the firmness diminution was significantly lower in those treated with essential oil fumigation than in the controls. The

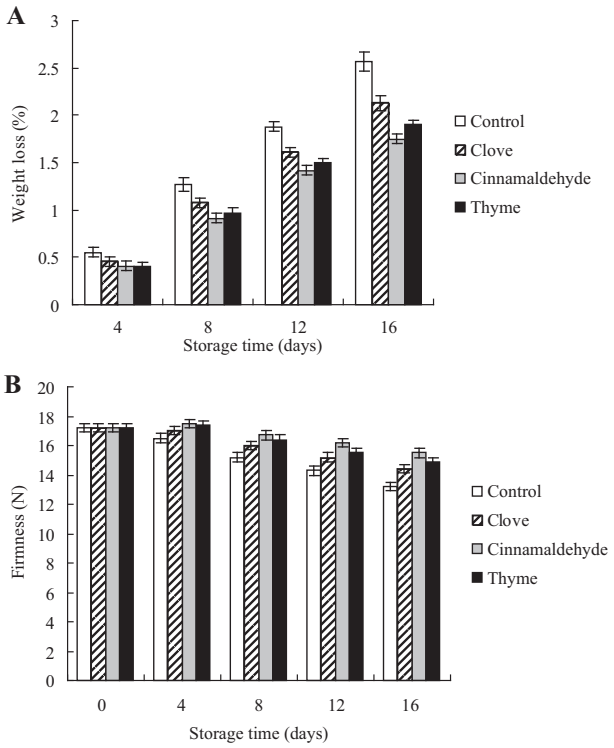

Fig. 1. Effect of essential oil fumigation treatment on weight loss (A) and firmness (B) change of button mushrooms stored at 4 °C for 16 days. Each data point is the mean of three replicate samples. Vertical bars represent standard errors of means.

greatest reduction of mushroom firmness was obtained for those mushrooms treated with cinnamaldehyde (Fig. 1B). Softening can occur due to the degradation of cell walls in postharvest mushrooms by bacterial enzymes and increased activity of endogenous autolysins (Zivanovic, Buescher, & Kim, 2000). Microorganisms, such as *Pseudomonas*, degrade mushrooms by breaking down the intracellular matrix and reducing the central vacuole, resulting in partially collapsed cells and a loss of turgor. This kind of bacterial-induced softening was observed in the control samples but was inhibited by essential oils fumigation treatment. Moreover, the results showed that these natural compounds somehow could reduce the action of cell-wall degrading enzymes. However, the mechanism by which these essential oils led to a reduction in weight loss and delay of the softening process is still unknown and further study is required.

3.2. Effect of essential oils fumigation on colour

It is widely accepted that the most important parameter in determining button mushroom acceptability by consumers is colour. Changes in the external colour were monitored by measuring lightness (L), total colour variation (ΔE) and browning index (BI) (Jiang, 2013). Table 1 shows the different values obtained after application of essential oil fumigation, compared to the control treatment. From this table, higher L values and a lower ΔE was observed in essential oils fumigation compared to control treatment after 4 days. The L value of the control samples decreased sharply after the first 4 days, and was 80.4 at the 8th day and 77.3 by the 12th day, the last value may not be considered as commercially acceptable if a *L* value of 80 for wholesalers was taken into account (López-Briones et al., 1992). The BI of the mushrooms was higher in the control than in those treated by essential oils fumigation during whole storage. At the end of the storage, mushrooms fumigated with cinnamaldehyde browned slightly, but they also had

Table 1
Effect of essential oil fumigation treatment on colour changes of button mushrooms stored at 4 °C for 16 days.[a,b]

Treatments	*L*	△*E*	*BI*
0 d			
Control	91.5 ± 0.14 a	17.5 ± 0.38 a	20.0 ± 0.32 a
Clove	90.4 ± 0.07 b	17.0 ± 0.29 a	18.4 ± 0.21 b
Cinnamaldehyde	91.2 ± 0.13 a	16.5 ± 0.30 b	18.0 ± 0.38 bc
Thyme	90.7 ± 0.05 b	16.4 ± 0.22 b	17.8 ± 0.20 c
4 d			
Control	85.5 ± 0.26 c	21.4 ± 0.21 a	24.1 ± 0.62 a
Clove	86.2 ± 0.25 b	20.9 ± 0.17 b	23.6 ± 0.37 b
Cinnamaldehyde	87.8 ± 0.33 a	19.1 ± 0.14 d	21.3 ± 0.18 d
Thyme	87.3 ± 0.17 a	20.2 ± 0.08 c	22.8 ± 0.36 c
8 d			
Control	80.4 ± 0.06 d	25.2 ± 0.04 a	27.6 ± 0.48 a
Clove	82.2 ± 0.24 c	23.3 ± 0.32 b	25.3 ± 0.72 b
Cinnamaldehyde	85.4 ± 0.32 a	20.9 ± 0.26 d	22.7 ± 0.33 d
Thyme	84.3 ± 0.42 b	22.1 ± 0.13 c	24.6 ± 0.63 c
12 d			
Control	77.3 ± 0.19 d	32.7 ± 0.29 a	42.6 ± 0.52 a
Clove	79.2 ± 0.20 c	30.7 ± 0.42 b	39.4 ± 0.28 b
Cinnamaldehyde	83.5 ± 0.15 a	26.9 ± 0.20 d	34.0 ± 0.49 d
Thyme	81.2 ± 0.18 b	29.0 ± 0.15 c	37.1 ± 0.30 c
16 d			
Control	74.3 ± 0.42 d	36.2 ± 0.40 a	49.9 ± 0.46 a
Clove	76.5 ± 0.38 c	33.5 ± 0.21 b	44.3 ± 0.86 b
Cinnamaldehyde	81.4 ± 0.25 a	30.3 ± 0.36 c	39.6 ± 0.72 c
Thyme	79.3 ± 0.37 b	30.9 ± 0.11 c	40.1 ± 0.77 c

[a] Mean of three replications ± standard deviation.
[b] Means in same column with different letters are significantly different ($P < 0.05$).

commercial value and edibility. Compared with control, essential oils fumigation treatments can inhibit the browning of the button mushrooms. There is no evidence of the role of these natural compounds on this issue, but the well known antioxidant activity reported for these essential oils may reduce dehydration and occurrence of browned polymers responsible for the mushrooms browning and shriveling.

3.3. Effect of essential oils fumigation on percent open caps and overall acceptability

The percentage of open-cap mushrooms increased as the storage period advanced in all the treatments, and was higher in control samples. The percentage of open-cap mushrooms in the control was 82.1% after 16 days of storage (Table 2). On the other hand, the percentage of open caps mushrooms subjected to clove, cinnamaldehyde, and thyme fumigation were in the range of 64.3–77.5% after 16 days. Among treatments, the minimum percentage (64.3%) was recorded for the cinnamaldehyde treated samples. The cap opening of mushrooms is related to the dryness of mushrooms as a result of water loss during storage. The increased water loss during storage causes a decrease in cohesive forces of water and other hydrophilic molecules, such as proteins, responsible for the intact position of the caps and veil in mushrooms. As essential oils fumigation reduced water loss, the cap opening of mushrooms was less, particularly in cinnamaldehyde. The overall acceptability based on colour, texture and percentage of open caps of the mushrooms decreased as the storage period advanced, in all the treatments. Based on the judgements made by members of a sensory panel, the control samples were unacceptable after 12 days of storage. However, mushrooms in cinnamaldehyde and thyme treatment did not exhibit these characteristics even on day 16, the cinnamaldehyde samples were acceptable and in marketable condition and recorded an overall acceptability of 2.12 after 12 days of storage. These results suggest that cinnamaldehyde fumigation was effective in retarding mushroom sensory deterioration.

3.4. Effect of essential oils fumigation on microbiological quality

At harvest, the button mushroom had 4.30, 3.35, 4.82 and 3.31 $\log_{10}$ cfu/g for mesophilic, psychrophilic, *pseudomonad*, yeasts and moulds, respectively. Following 16 days of cold storage, the microbial counts in the essential oils fumigation treatments were drastically reduced, the reduction being more effective for yeasts and moulds counts (below 4.50 $\log_{10}$ cfu/g for cinnamaldehyde treatment). On the contrary, increases in microbial populations were observed in the control mushrooms (Fig. 2). Therefore, microbial degradation resulting in changes, such as browning and softening, was clearly delayed in the cinnamaldehyde treated samples. According to Eastwood and Burton (2002), the organisms usually responsible for spoilage of mushrooms are gram-negative, psychrotrophic bacteria, belonging particularly to the *Pseudomonasae* family, due to contamination of the product from compost. Essential oils were effective in reducing food-spoiling microorganisms, food-borne pathogens, spoilage and mycotoxigenic fungi, pathogenic and dimorphic yeasts (Dorman & Deans, 2000; López, Sanchez, Batlle, & Nerín, 2005; López, Sanchez, Batlle, & Nerín, 2007; Manso, Cacho-Nerín, Becerril, & Nerín, 2013); Rodríguez, Nerín, & Batlle, 2008. The antimicrobial effects of these natural compounds are well established and the mechanism of action has been related to damage to the membrane integrity (Bagamboula, Uyttendaele, & Debevere, 2004).

3.5. Effect of essential oils fumigation on total phenolics and ascorbic acid

Fig. 3A shows the variation in the total phenolics content of button mushrooms stored at 4 °C under the four treatments. There

Table 2
Effect of essential oil fumigation treatment on percentage of open caps and overall acceptability change of button mushrooms stored at 4 °C for 16 days.[a,b]

Treatments	Storage time (d)				
	0	4	8	12	16
Percent open caps					
Control	0	13.9 ± 0.42 a	34.3 ± 0.41 a	58.3 ± 0.52 a	82.1 ± 1.72 a
Clove	0	12.4 ± 0.32 b	31.4 ± 0.30 b	52.4 ± 0.61 b	77.5 ± 0.50 b
Cinnamaldehyde	0	11.5 ± 0.18 c	25.3 ± 0.52 d	42.5 ± 0.21 d	64.3 ± 1.32 c
Thyme	0	11.7 ± 0.21 c	27.6 ± 0.32 c	45.2 ± 0.30 c	68.5 ± 0.83 c
Overall acceptability					
Control	5	3.60 ± 0.13 c	2.68 ± 0.14 d	1.57 ± 0.16 d	1.20 ± 0.06 c
Clove	5	3.72 ± 0.05 b	2.83 ± 0.11 c	1.82 ± 0.06 c	1.53 ± 0.08 b
Cinnamaldehyde	5	3.98 ± 0.06 a	3.35 ± 0.04 a	2.36 ± 0.04 a	1.84 ± 0.12 a
Thyme	5	3.90 ± 0.10 a	3.18 ± 0.15 b	2.14 ± 0.10 b	1.73 ± 0.05 ab

[a] Mean of three replications ± standard deviation.
[b] Means in same column with different letters are significantly different ($P < 0.05$).

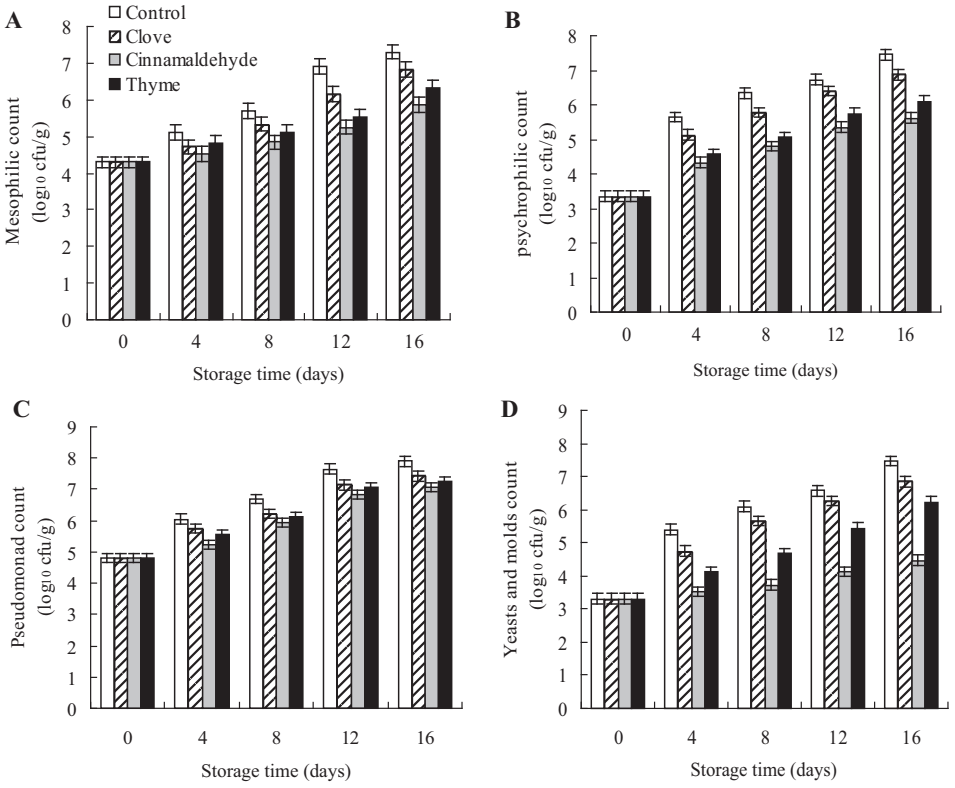

Fig. 2. Effect of essential oil fumigation treatment on mesophilic (A), psychrophilic (B), *Pseudomonad* (C) and yeasts and molds (D) counts ($\log_{10}$ cfug^{-1}) change of button mushrooms stored at 4 °C for 16 d. Each data point is the mean of three replicate samples. Vertical bars represent standard errors of means.

was no significant difference ($P > 0.05$) between the essential oils fumigation treatments. However, a lower phenolics content was found for the control treatment mushrooms, compared to the essential oils fumigation treatment, which might be explained by the participation of polyphenols in the browning synthesis during the storage. It appears that lower levels of browning correlated fairly well with the phenolic content, which seems to be the limiting factor of the discoloration process. Phenolic compounds have been reported as the major antioxidant components in mushrooms. These antioxidant compounds have been widely reported to have beneficial effects on the maintenance of health and the prevention of cancer and cardiovascular diseases. It is hypothesised that essential oils would act as "signaling compounds" that trigger a signal that resembles a mild stress to the fruit (Gutiérrez, Batlle, Sánchez, & Nerín, 2010; Montero-Prado, Bentayeb, & Nerín, 2012; Pezo, Salafranca, & Nerín, 2007). As a defense response, the fruit produces additional phenolic compounds, thus flavonoids and increases their antioxidant activities (Sharma & Tripathi, 2006).

Fig. 3B shows changes in the ascorbic acid (AA) content of button mushrooms during 16 days storage under different treatments. The initial AA content of the button mushrooms was 33.6 mg/kg. Although the AA content of both the treated and control samples decreased throughout storage, the use of cinnamaldehyde fumigation significantly reduced the loss of AA in mushroom samples. After 16 days of storage, the AA retention of mushrooms treated with clove, cinnamaldehyde, and thyme was 17.8, 25.5 and 21.5 mg/kg, respectively, whereas the control samples maintained 16.7 mg/kg of the initial AA content. In our study, the total phenolics and AA contents in button mushrooms increased by the treatment with different essential oils. This is consistent with the findings by Wang et al. (2008) in blueberries.

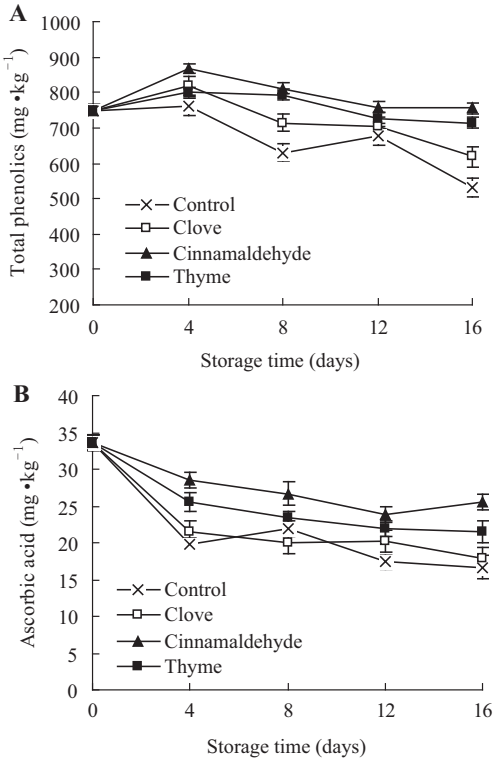

Fig. 3. Effect of essential oil fumigation treatment on total phenolics (A) and ascorbic acid (B) changes in button mushrooms stored at 4 °C for 16 days. Each data point is the mean of three replicate samples. Vertical bars represent standard errors of means.

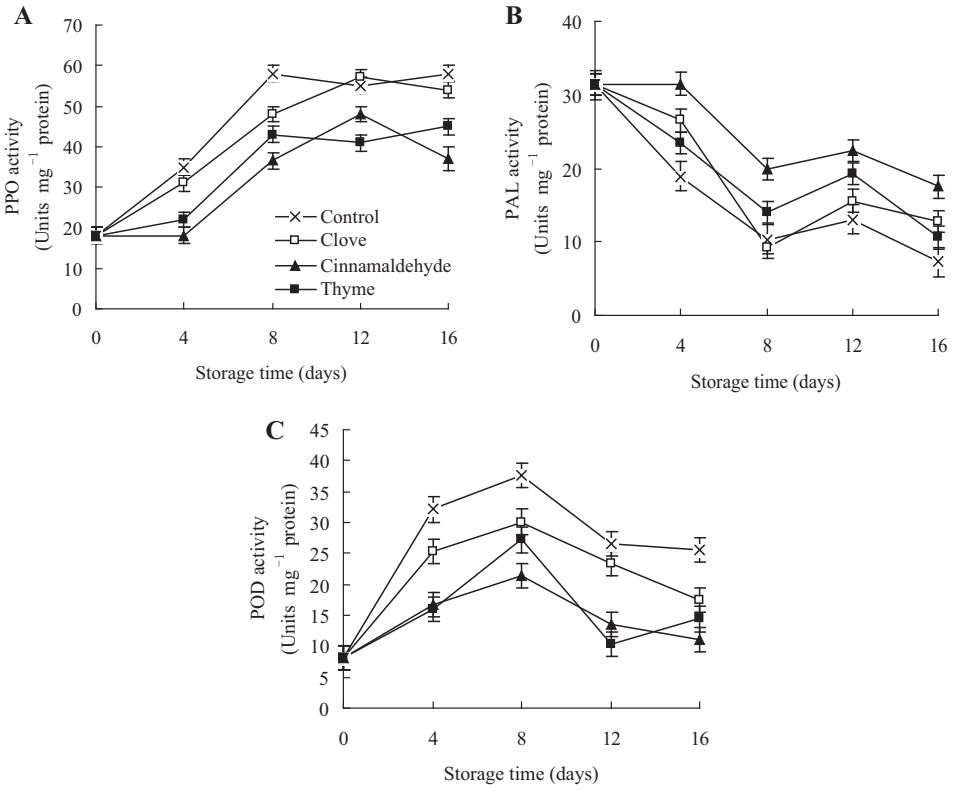

Fig. 4. Effect of essential oil fumigation treatment on PPO (A), PAL (B), and POD (C) activities of button mushrooms stored at 4 °C for 16 days. Each data point is the mean of three replicate samples. Vertical bars represent standard errors of means.

3.6. Effect of essential oil fumigation on PPO, PAL, and POD activities

During storage, the PPO activity in the control mushrooms increased rapidly without peaks and was significantly higher than that in the samples subjected to essential oils fumigation treatment (Fig. 4A). Unlike PPO, PAL activities showed a continuous decrease in response to the different treatments (Fig. 4B). The POD activity in samples exposed to cinnamaldehyde and thyme fumigation were significantly lower than that of the control samples (Fig. 4C). Nevertheless, the POD evolution was similar over time, regardless of the treatment, which increased and reached a peak and then decreased. It is generally accepted that browning is due to the oxidation of phenolics by PPO and POD, resulting in the formation of brown-coloured substances. PPO and POD have synergistic effects on the formation of the brown polymers. Treatment with essential oils fumigation, inhibited the activities of POD and PPO, which may account for the inhibition of the mushroom browning. In addition, compared to the control samples, the samples exposed to cinnamaldehyde fumigation preserved more AA content (Fig. 3B), involved in inhibiting PPO activity, as high concentrations of AA could inhibit the catalytic action of PPO by decreasing the pH value below the optimum for PPO activity (Sapers, 1993). For PAL, several studies have shown that the accumulation of phenols and anthocyanins paralleled the increase in PAL activity in some fruits (Hiratsuka et al., 2001; Wang et al., 2009). It is possible that essential oils play positive roles in affecting plant secondary metabolites and stimulating biosynthesis of phenolic and anthocyanin compounds by inducing an increased activity of PAL (Jin et al., 2012).

4. Conclusions

Successful inhibition of senescence in cold-stored button mushroom was possible with essential oils fumigation treatments, such as clove, cinnamaldehyde and thyme. Together they kept the sensory characteristics within acceptable limits throughout storage. Moreover, essential oils fumigation not only possessed antimicrobial properties and maintains firmness during storage but also exhibited the capability to increase the total phenolics and ascorbic acid contents. Thus, these natural products have the potential to preserve the quality and safety of button mushroom.

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References

Ajlouni, S.O. (1991). Quality characteristics of two hybrids of the cultivated mushrooms (*Agaricus bisporus*) and the improvement of their shelf life using stripe trimming and gamma irradiation. Ph.D. thesis. The Pennsylvania State University, University Park, PA.

Bagamboula, C. F., Uyttendaele, M., & Debevere, J. (2004). Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri*. *Food Microbiology, 21*, 33–42.

Beelman, R. D., Royse, D., & Chikthimmah, N. (2003). Bioactive components in *Agaricus bisporus* Imbach of nutritional, medicinal, or biological importance. *International Journal of Medicinal Mushrooms, 5*, 321–337.

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.

Chang, S. T. (2005). Witnessing the development of the mushroom industry in China. *Acta Edulis Fungi, 12*(Suppl.), 3–19.

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.

Dorman, H. J. D., & Deans, S. G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology, 88*, 308–316.

Eastwood, D., & Burton, K. (2002). Mushrooms – a matter of choice and spoiling oneself. *Microbiology Today, 29*, 18–19.

Gutiérrez, L., Batlle, R., Sánchez, C., & Nerín, C. (2010). New approach to study the mechanism of antimicrobial protection of an active packaging. *Foodborne Pathogens and Disease, 7*, 1063–1069.

Hanson, P. M., Yang, R. Y., Wu, J., Chen, J. T., Ledesma, D., Tsou, C. S. C., et al. (2004). Variation for antioxidant activity and antioxidants in tomato. *Journal of the American Society for Horticultural Science, 129*, 704–711.

Hiratsuka, S., Onodera, H., Kawai, Y., Kubo, T., Itoh, H., & Wada, R. (2001). Enzyme activity changes during anthocyanin synthesis in 'Olympia' grape berries. *Scientia Horticulturae, 90*, 255–264.

Jiang, T. (2013). Effect of alginate coating on physicochemical and sensory qualities of button mushroom (*Agaricus bisporus*) under high oxygen modified atmosphere. *Postharvest Biology and Technology, 76*, 91–97.

Jin, P., Wu, X., Xu, F., Wang, X. L., Wang, J., & Zheng, Y. H. (2012). Enhancing antioxidant capacity and reducing decay of Chinese bayberries by essential oils. *Journal of Agricultural and Food Chemistry, 60*, 3769–3775.

Koukol, J., & Conn, E. E. (1961). The metabolism of aromatic compounds in higher plants IV. Purification and properties of the phenylalanine deaminase of Hordeum vulgare. *The Journal of Biological Chemistry, 236*, 2692–2698.

López, P., Sanchez, C., Batlle, R., & Nerín, C. (2005). Solid- and vapour-phase antimicrobial activities of six essential oils: susceptibility of selected foodborne bacterial and fungal strains. *Journal of Agricultural and Food Chemistry, 53*, 6939–6946.

López, P., Sanchez, C., Batlle, R., & Nerín, C. (2007). Vapor-phase activities of cinnamon, thyme, and oregano essential oils and key constituents against foodborne microorganisms. *Journal of Agricultural and Food Chemistry, 55*, 4348–4356.

López-Briones, G. L., Varoguaux, P., Chambroy, Y., Bouquant, J., Bureau, G., & Pascat, B. (1992). Storage of common mushroom under controlled atmospheres. *International Journal of Food Science and Technology, 27*, 493–505.

Manso, S., Cacho-Nerin, F., Becerril, R., & Nerín, C. (2013). Combined analytical and microbiological tools to study the effect on *Aspergillus flavus* of cinnamon essential oil contained in food packaging. *Food Control, 30*, 370–378.

Moerschbacher, B. M., Noll, U. M., Flott, B. E., & Reisener, H. J. (1988). Lignin biosynthetic enzyme in stem rust infected resistant and susceptible near-isogenic wheat lines. *Physiological and Molecular Plant Pathology, 33*, 33–46.

Montero-Prado, P., Bentayeb, K., & Nerín, C. (2012). Pattern recognition of peach cultivars (*Prunus persica* L.) from their volatile components. *Food Chemistry, 138*, 724–731.

Palou, E., Lopez-Malo, A., Barbosa-Canovas, G. V., Welti-Chanes, J., & Swanson, G. B. (1999). Polyphenoloxidase activity and colour of blanched and high hydrostatic pressure treated banana puree. *Journal of Food Science, 64*, 42–45.

Pezo, D., Salafranca, J., & Nerín, C. (2007). Development of an automatic multiple dynamic hollow fibre liquid-phase microextraction procedure for specific migration analysis of new active food packagings containing essential oils. *Journal of Chromatography A, 1174*, 85–94.

Ponce, A. G., Valle, C. E., & Roura, S. I. (2004). Natural essential oils as reducing agents of peroxidase activity in leafy vegetables. *LWT – Food Science and Technology, 37*, 199–204.

Rodríguez, A., Nerín, C., & Batlle, R. (2008). New cinnamon-based active paper packaging against *Rhizopus stolonifer* food spoilage. *Journal of Agricultural and Food Chemistry, 56*, 6364–6369.

Sapers, G. M. (1993). Browning of foods: Control by sulfites, antioxidants, and other means. *Food Technology, 47*, 75–84.

Serrano, M., Martínez-Romero, D., Castillo, S., Guillén, F., & Valero, D. (2005). The use of antifungal compounds improves the beneficial effect of MAP in sweet cherry storage. *Innovative Food Science and Emerging Technologies, 6*, 115–123.

Sharma, N., & Tripathi, A. (2006). Fungitoxicity of the essential oil of *Citrus sinensis* on postharvest pathogens. *World Journal of Microbiology and Biotechnology, 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., Castillo, S., Serrano, M., & Valero, D. (2005). Improvement of table grape 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, 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.

Wang, Y. S., Tian, S. P., Xu, Y., Qin, G. Z., & Yao, H. J. (2004). Changes in the activities of pro- and anti-oxidant enzymes in peach fruit inoculated with Cryptococcus laurentii or Penicillium expansum at 0 or 20 °C. *Postharvest Biology and Technology, 34*, 21–28.

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.

Zivanovic, S., Buescher, R. W., & Kim, K. S. (2000). Textural changes in mushroom (*Agaricus bisporus*) associated tissue ultrastructure and composition. *Journal of Food Science, 65*, 1404–1408.