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Effect of Chitosan Coating Enriched with Thyme Oil on Postharvest Quality and Shelf Life of Shiitake Mushroom (*Lentinus edodes*)

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ABSTRACT: The effect of chitosan-oil coating on the postharvest quality and shelf life of shiitake (*Lentinus edodes*) mushrooms stored at 4 ± 1 °C for 16 days was investigated. Mushroom weight loss, firmness, total phenolics, ascorbic acid, malondialdehyde (MDA), electrolyte leakage rate, and microbial and sensory quality were measured. The results indicate that treatment with chitosan-oil coating maintained tissue firmness, inhibited increase of respiration rate, and reduced microorganism counts, such as yeasts and molds and *pseudomonad*, compared to control treatment. The efficiency was better than that of thyme oil treatment or chitosan coating. Furthermore, shiitake mushrooms treated with chitosan-oil coating also exhibited significantly higher levels of total phenolics, flavonoids, as well as individual phenolic compounds than control. Sensory evaluation proved the efficacy of chitosan-oil coating by maintaining the overall quality of shiitake mushroom during the storage period. Our study suggests that chitosan-oil coating might be a promising candidate for maintaining shiitake mushroom quality and extending their postharvest life.

KEYWORDS: *chitosan, thyme oil, shiitake mushroom, postharvest quality, shelf life*

INTRODUCTION

Recently, application of edible coatings is promising to improve the quality and extend the shelf life of lightly processed produce,[1] because they act as barriers to water loss and gas exchange, creating a micromodified atmosphere around products, and they can serve as carriers for other GRAS compounds.[2] Chitosan, which is mainly made from crustacean shells, is the second most abundant natural polymer in nature after cellulose. Due to its nontoxic nature, antibacterial and antioxidative activity, film-forming property, biocompatibility, and biodegradability, chitosan has attracted much attention as a natural food additive. It has been used to maintain the quality of postharvest fruits and vegetables such as citrus,[3] peach, pear, and kiwi fruit,[4] tomatoes,[5] apples,[6] and longan fruit.[7] Shiitake (*Lentinula edodes*) mushrooms are highly perishable and tend to lose quality right after harvest. Their shelf life is short because of their high respiration rate and high tendency to turn brown and because they have no physical protection to avoid water loss or microbial attack.[8] Bacteria, molds, enzymatic activity, and biochemical changes can cause spoilage during storage. Gram-negative micro-organisms, such as *Pseudomonas tolaasii* and *Pseudomonas fluorescens*, and yeasts, such as *Candida sake*, have been associated with mushroom spoilage.[9] The short shelf life of mushroom is an impediment to the distribution and marketing of the fresh product.

Development of natural preservative coatings with antimicrobial agents is increasing in interest due to the safety aspects of chemical additives.[10] Several studies have shown that incorporation of essential oils (EOs) into chitosan films or coatings may not only enhance the film's antimicrobial and antioxidant properties but also reduce water vapor permeability and slow lipid oxidation of the product on which the film is applied.[11,12] Among the EOs, thyme oil has increasingly gained the interest of researchers and food processors as a potential natural antimicrobial and antioxidant agent. Thyme contains high concentrations of phenolic compounds, including carvacrol, thymol, *p*-cymene, and *γ*-terpinene.[13] The antioxidant and antimicrobial effect of thyme oil has been studied in various commercial food products such as vegetables[14] and seafood.[15] The investigation of Xing et al.[16] concluded that chitosan coating enriched with cinnamon oil used on sweet pepper samples maintained good quality characteristics and extended shelf life.

However, to the best of our knowledge, the use of chitosan as natural antimicrobial agent, either individually or in combination with EOs, including thyme oil, has not been studied to date, in fresh shiitake mushrooms. Thus, the objective of the present study was to determine the effect of chitosan and thyme oil, applied individually and/or in combination, on the microbiological, functional components, and sensory parameters of shiitake mushrooms, during refrigerated storage.

MATERIALS AND METHODS

Preparation of Coating Solutions and Samples. The method to prepare the coating solutions was developed by Xing et al.[16] Pure thyme oil of the genus *Thymus vulgaris* was purchased from International Flavors and Fragrances Inc. (Shanghai, China). The oil consisted of the following (major components): thymol, 57.7%; *p*-cymene, 18.7%; carvacrol, 2.8% (Manufacturer's data). Chitosan (deacetylated ≥95%, and viscosity ≤30 mPa s) was purchased from Zhejiang Xuefeng Calcium Carbonate Co., Ltd. (Zhejiang, China). The chitosan solution (2.0%) containing 1% acetic acid as a medium to dissolve chitosan and glycerol plasticizer (0.75%) was stirred with a magnetic stirrer at room temperature for 1 h to achieve complete dispersion. Then the thyme oil, mixed with Tween 80 (0.2%), to help distribute and completely incorporate the thyme oil, was added to the chitosan solution and then

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stirred using a magnetic stirrer for 30 min. The final coating forming solution consisted of 2% chitosan, 1% acetic acid, 0.75% glycerol, 0.2% Tween 80, and 1.5% thyme oil. The final solution was homogenized under aseptic conditions at 10,000*g* for 1 min.

Shiitake mushrooms used in this study were harvested from a local farm in Hangzhou, China. The mushrooms were transported to the laboratory within 1 h after picking and then stored in darkness at 4 ± 1 °C and 90% relative humidity (RH) for 12 h. Mushrooms were divided into four samples of 60 each. Four different treatments were used: (1) control; (2) chitosan coating; (3) thyme oil treatment (1.5%); and (4) chitosan + thyme oil (1.5%) coating. Mushrooms were dipped into the solution for 30 s. Samples dipped in distilled water were used as control. Treated samples were kept over a plastic sieve for 50 min, and a fan generating low-speed air was used to hasten drying. Then a piece of tissue paper was used to absorb excess solution from the surface. The treated samples were placed and sealed in 18 cm × 20 cm bags of low density polyethylene (PE) (0.04 mm thickness), the PE gas transmission rates were 1078×10^{-18} mol m^{-1} s^{-1} Pa^{-1} for O_2, 4134×10^{-18} mol m^{-1} s^{-1} Pa^{-1} for CO_2 (both at 20 °C and 100% RH), and 2.8×10^{-5} to 6.5×10^{-5} g m^{-2} s^{-1} for H_2O (at 37 °C and 90% RH). They were then stored for 16 days at 4 ± 1 °C and 95% relative humidity (RH). Fifteen replicates were included in each treatment group, and subsequently, every 4 days, three replicates from each treatment group were analyzed.

Texture Measurement and Weight Loss Analysis. A penetration test was performed on the Shiitake mushroom cap using a TA.XT Express-v3.1 texture analyzer (Stable Micro Systems, U.K.), 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) 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.

Package Atmosphere Composition. O_2 and CO_2 concentrations in packages were evaluated by using a SCY-2A O_2 and CO_2 analyzer (Xinrui Instrument Co., Shanghai, China). Gas samples were taken from the bags with a 20 mL syringe.

Analysis of Functional Components. To prepare the mushroom extract, 5 g samples from each replicate were extracted twice with 10 mL of precooled 80% methanol and then centrifuged at 18,000*g* for 20 min (4 °C). The supernatant was combined and the final volume made to 25 mL for analysis of functional components. Total phenolics and flavonoids were extracted and determined according to the methods of Singleton and Rossi[17] and Zhishen et al.,[18] respectively. The total phenolic contents were expressed as gallic acid equivalents, in mg/100 g fresh sample. The total flavonoid contents were then expressed as rutin equivalents, in mg/100 g fresh sample. The determination of total ascorbic acid was carried out as described by Hanson et al.[19] 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 color under acidic conditions, the $ABTS^{\bullet+}$ free-radical-scavenging activity was assayed with a method described by Goh et al.[20] with slight modifications.

Analysis of Phenolic Compounds. The supernatants from the extracts described above were concentrated to dryness using a rotavapor in a water bath at 35 °C, dissolved in 5 mL of methanol, and filtered through a 0.20 μm disposable LC filter disk prior to HPLC analysis. The phenolic extracts were analyzed using a Aglient 1100 system controller connected to a photodiode array detector which was set to a scanning range from 240 to 450 nm with a 2.4 nm resolution. Reverse-phase chromatographic analysis was carried out under isocratic conditions using a C-18 reverse phase column (250 mm × 4.6 mm i.d., particle size 5 μm, Agilent Zorbax Eclipse XDB-C18) at 25 °C. The sample volume injection was 10 μL. A solvent system consisting of 0.1% acetic acid in water (solvent A) and acetonitrile (solvent B) was used with the following gradient: starting with 100% A and installing a gradient to obtain 40% B at 4 min, 60% B at 15 min, and 5% B at 18 min, according to the method previously described with slight modifications.[21] The solvent flow rate was 1 mL/min. Flavonoids, such as catechin, naringin, and myricetin, and phenolic compounds, such as gallic acid, *p*-hydroxybenzoic acid, and protocatechuic acid, were identified on the basis of the retention times of standard materials, and the quantification was achieved by the absorbance recorded in the chromatograms relative to external standards, at 280 nm. All standard calibration curves showed high degrees of linearity ($r^2 > 0.99$). The contents of phenolic compounds were expressed as mg/100 g FW.

Malondialdehyde (MDA) Content and Electrolyte Leakage Rate. MDA content was determined as the representative of the level of lipid peroxidation products in mushroom caps.[22] One gram of shiitake mushroom was ground in 0.25% 2-thiobarbituric acid (TBA) in 10% TCA using a mortar and pestle. After heating at 95 °C for 30 min, the mixture was quickly cooled in an ice bath and centrifuged at 10,000*g* for 10 min. The absorbance of the supernatant was read at 532 nm and corrected for unspecific turbidity by subtracting the absorbance of the same sample at 600 nm. The blank was 0.25% TBA in 10% TCA. The amount of MDA was calculated with an extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nmol/g fresh weight.

Electrolyte leakage rate was measured essentially as described by Xing et al.[23] with slight modifications. Mushroom fruit bodies (5 g) were cut into four pieces, leaving the pileus intact, and washed three times with deionized water to remove surface-adhered electrolytes. After drying with filter paper, suspending in 40 mL of deionized water in a 100 mL beaker, and shaking at 25 °C on a rotary shaker for 30 min, electrical conductivity was measured immediately (P_0) and again after 10 min (P_1). Samples were then boiled for 10 min and cooled to room temperature, and a final conductivity measurement (P_2) was taken. The relative electrolyte leakage rate (RELT) was calculated according to the following equation: $(P_1 - P_0)/(P_2 - P_0)$ and expressed as a percentage.

Microbiological Analysis. All samples were analyzed for the mesophilic, psychrophilic, pseudomonad, and yeasts and molds bacteria counts. Twenty-five grams of mushrooms were removed aseptically from each pack and diluted with 225 mL of 0.1% peptone water. The samples were homogenized 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 molds were estimated on potato dextrose agar (PDA; Merck), and incubation conditions were 28 ± 1 °C for 5–7 days.

Sensory Evaluation. The sensory attributes that characterized mushroom deterioration were determined. These attributes were the following: off-odor, gill color, gill uniformity, cap surface uniformity, and presence of dark zones on the cap.[24] Samples were evaluated by a sensory panel of ten trained assessors. Mushrooms were served in closed, odorless plastic containers at room temperature. After opening polyethylene bags, mushrooms were placed in plastic containers and evaluations were performed within 2 h in order to avoid loss of off-odors. 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 color descriptor, for which the anchors were "white" and "brown".

Statistical Analysis. Experiments were performed using a completely randomized design, and each was composed of three replicates. Data were subjected to one-way analysis of variance (ANOVA). Mean separations were performed by Tukey's multiple range test (DPS version 6.55). Differences at $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Effect of Chitosan Coating Enriched with Thyme Oil on Texture and Weight Loss. Shiitake mushroom suffers a rapid loss of firmness during ripening which contributes greatly to its short postharvest life and susceptibility to fungal contamination. Figure 1A shows that the control mushrooms had the fastest softening rate, losing about 35.2% of their firmness in about 16 days. The firmness of mushrooms treated with chitosan coating, oil solution, or chitosan-oil coating also decreased, but to a lesser extent. Chitosan coating combined with oil treatment led to a significantly higher firmness than that in the control sample, due to a synergistic effect of thyme oil and the coating. There were no significant variations between the chitosan-coated and oil-treated samples. Lin et al.[25] have reported the chitosan coating could decrease the reduction in the firmness and the TSS and TA contents of 'Yali' pears. Furthermore, Rojas-Graü et al.[26] reported that the use of alginate- or gellan-edible coatings on fresh-cut apples was effective in controlling moisture loss, in preventing the loss of turgor, and in reducing fruit softening. For mushrooms, softening can occur because of the degradation of cell walls by bacterial enzymes and increased activity of endogenous autolysins.[27] 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 control samples but was inhibited by chitosan-oil coating treatment.

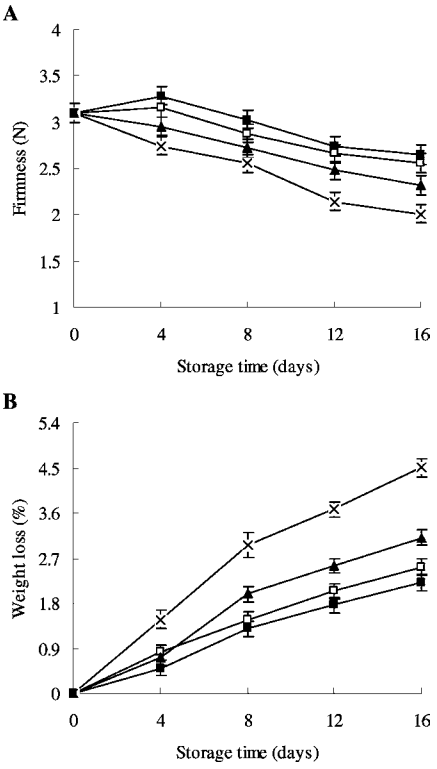

Figure 1. Changes in firmness (A) and weight loss (B) of shiitake mushrooms treated with control (×), chitosan coating (□), 1.5% thyme oil (▲), and chitosan coating + 1.5% thyme oil (■) stored at 4 °C for 16 days. Each data point is the mean of three replicate samples. Vertical bars represent standard deviation of means.

The weight loss throughout the storage time in the control and coated mushrooms is shown in Figure 1B. The highest weight loss was observed in the control samples; it reached 4.51% at the end of storage. From the viewpoint of Bico et al.,[28] a more than 4−6% (of the total fresh weight) weight loss was accompanied by visible wilting or wrinkling of the surface of the fruits and vegetables. For all of the coated mushrooms, the weight loss was less than 4%, which indicates that the coated mushroom maintained freshness during storage. On day 8, the chitosan-oil-coated or chitosan-coated mushroom showed the lowest weight loss (1.29% or 1.48%, respectively), followed by oil-treated samples (1.99%). For up to 16 days of storage, the weight loss in the chitosan-oil-coated sample was slightly lower than that in the other coated mushrooms. Nevertheless, there was no significance difference between the chitosan-oil-coated and chitosan-coated samples ($P > 0.05$). In this study, the chitosan-oil coating significantly reduced the weight loss of mushrooms as compared with control treatment and, therefore, delayed mushroom shriveling and quality deterioration. Similar superior effects of the edible coating treatments in weight loss have been observed in other fruits.[25,26] This was ascribed to the fact that the edible coating formed on the fruit surface can retain the fluid and delay the migration of moisture from the fruit into the environment, and decrease respiration consumption. In this study, the relatively low weight loss in the chitosan-oil-coated mushroom could be due to the synergistic effect of chitosan coating combined with thyme oil treatment.

Effect of Chitosan Coating Enriched with Thyme Oil on Gas Composition. The internal CO_2 concentrations increased in all the treatments during storage (Table 1). A rapid accumulation of CO_2 levels within the control mushrooms occurred during the first 2 days of storage, which is higher than those for other coated samples, indicating that control samples have a higher rate of respiration. A gradual decrease was observed in all treatments during the subsequent storage period until levels of 5.0% and 3.0% of CO_2, obtained with control and chitosan-oil coating treatment, respectively, after 12 days of storage. The control mushrooms showed lower internal O_2 levels than the coated mushrooms during cold storage. After 16 days of storage, the highest internal O_2 contents were recorded for the chitosan-oil, followed by those for chitosan coated mushrooms. The decreased levels of CO_2 and increased levels of O_2 in the chitosan-oil-coated mushrooms during storage suggest that the coating exerted a barrier to the CO_2 and O_2 exchange. The reduced rate of respiration in the chitosan-oil-coated mushrooms might be correlated with a delayed senescence and a reduced susceptibility to decay.

Effect of Chitosan Coating Enriched With Thyme Oil on Functional Components. In this study, total phenolics levels declined in all treatments during the 16 days of storage (Figure 2A). However, chitosan-oil coating was more effective in delaying decrease of phenolics than control. Mushrooms coated with chitosan-oil presented higher level of total phenolics, compared with the cases of chitosan coating or oil treatment. The lowest phenolics content was found in mushrooms from control treatment. Campaniello et al.[29] reported that the use of a chitosan coating could decrease the loss of phenolic compounds and the occurrence of browning of the fruits. In the present study, the chitosan coating, coupled with the thyme oil treatment, synergistically caused a higher retention of the total phenolic content.

Table 1. Effect of Chitosan Coating Enriched with Thyme Oil on Gas Composition (%) Changes of Shiitake Mushrooms Stored at 4 °C for 16 days[a]

days at 4 °C	control	chitosan	thyme oil	chitosan + thyme oil
O_2				
0	21	21	21	21
1	13.28 ± 0.16 d	15.36 ± 0.37 b	14.54 ± 0.20 c	16.56 ± 0.24 a
2	14.63 ± 0.18 d	15.90 ± 0.22 b	15.37 ± 0.37 c	17.13 ± 0.30 a
3	14.87 ± 0.27 d	16.46 ± 0.18 b	15.48 ± 0.24 c	17.45 ± 0.18 a
4	15.10 ± 0.28 d	16.78 ± 0.52 b	15.87 ± 0.30 c	17.97 ± 0.23 a
8	14.89 ± 0.17 d	16.58 ± 0.32 b	15.71 ± 0.41 c	17.58 ± 0.22 a
12	14.60 ± 0.38 d	16.95 ± 0.20 b	16.10 ± 0.19 c	17.58 ± 0.30 a
16	15.21 ± 0.25 d	16.75 ± 0.15 b	15.88 ± 0.25 c	17.43 ± 0.18 a
CO_2				
0	0	0	0	0
1	9.45 ± 0.22 a	7.88 ± 0.28 c	7.59 ± 0.27 b	7.23 ± 0.08 d
2	7.73 ± 0.49 a	4.74 ± 0.07 c	5.10 ± 0.38 b	4.02 ± 0.25 d
3	5.76 ± 0.53 a	4.11 ± 0.16 c	4.56 ± 0.57 b	3.41 ± 0.34 d
4	5.23 ± 0.19 a	3.55 ± 0.09 c	4.34 ± 0.33 b	3.38 ± 0.24 d
8	5.35 ± 0.08 a	3.78 ± 0.15 c	4.76 ± 0.20 b	3.35 ± 0.19 d
12	5.15 ± 0.28 a	3.66 ± 0.28 c	4.30 ± 0.21 b	3.20 ± 0.10 d
16	4.98 ± 0.10 a	3.45 ± 0.20 c	4.04 ± 0.36 b	2.89 ± 0.15 d

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

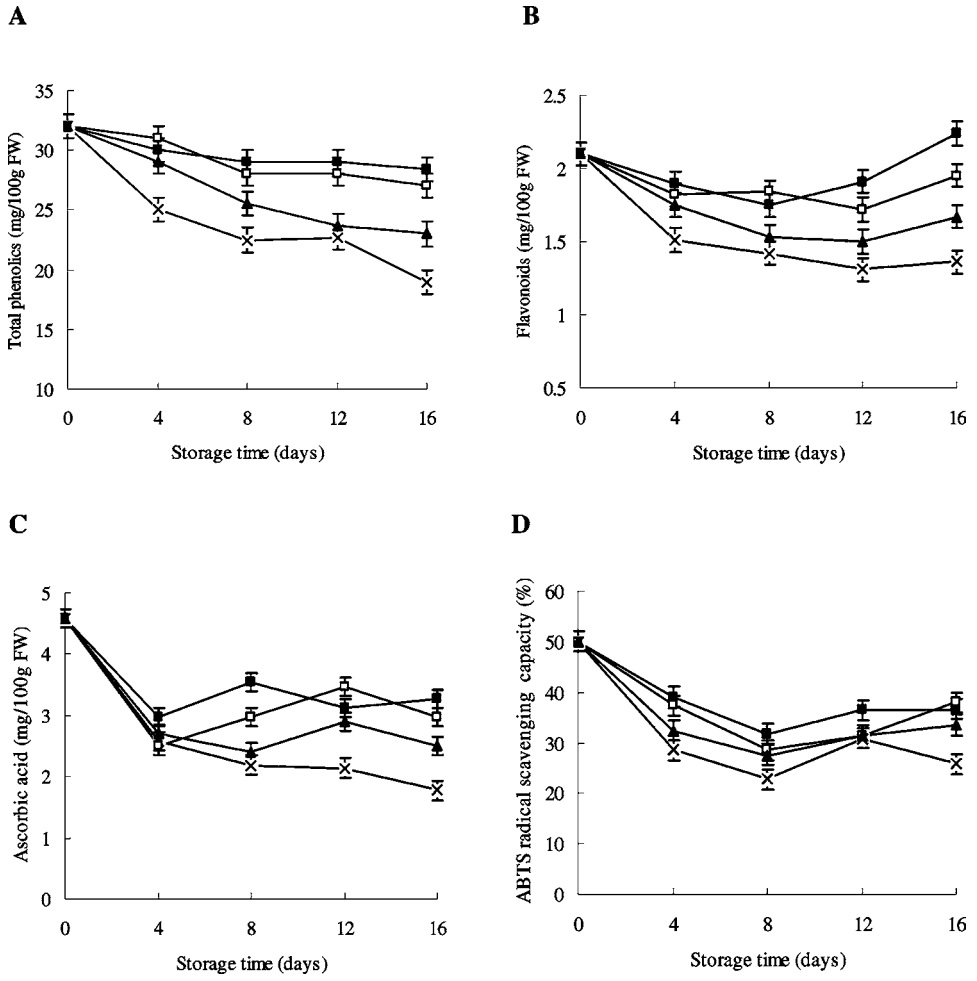

Figure 2. Changes in total phenolics (A), flavonoids (B), ascorbic acid (C), and ABTS radical scavenging capacity (D) of shiitake mushrooms treated with control (×), chitosan coating (□), 1.5% thyme oil (▲), and chitosan coating + 1.5% thyme oil (■) stored at 4 °C for 16 days. Each data point is the mean of three replicate samples. Vertical bars represent standard deviation of means.

Flavonoids act in plants as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellents, and light screening. As shown in Figure 2B, coating treatment and storage time significantly ($P < 0.05$) affected the flavonoid content of

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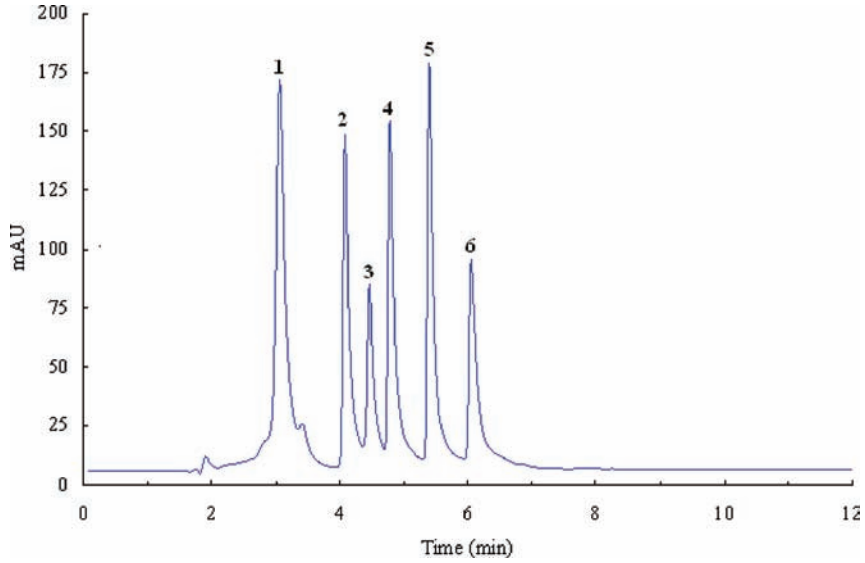

Figure 3. Chromatogram of phenolic compounds analysis by HPLC. Standard mixture of phenolics: 1, gallic acid; 2, protocatechuic acid; 3, catechin; 4, *p*-hydroxybenzoic acid; 5, naringin; 6, myricetin.

shiitake mushrooms during the storage period. The pattern of flavonoids content of control mushrooms during storage at 4 °C tended to decrease, which was the same as that of the phenolic content (Figure 2A). On the other hand, the flavonoid content of chitosan-oil-coated mushrooms increased after 8 days of storage and showed a continuous increase during later storage.

Changes in ascorbic acid content of coated and control shiitake mushrooms during 16 days of storage are shown in Figure 2C. The initial ascorbic acid content of shiitake mushrooms was 4.58 mg/100 g FW. Although ascorbic acid of both coated and control samples decreased throughout storage, the use of chitosan-oil coating significantly reduced the loss of ascorbic acid in mushroom samples. After 16 days of storage, ascorbic acid retention of mushroom treated with oil, chitosan, and chitosan-oil was 2.5, 2.97, and 3.26 mg/100 g FW, respectively, whereas control samples maintained 1.77 mg/100 g FW of initial ascorbic acid content. Since ascorbic acid loss can be greatly favored by the presence of O_2, the incorporation of chitosan to coating formulations may reduce O_2 diffusion, slow down the ripening rate, and consequently better preserve ascorbic acid content and delay senescence of shiitake mushroom. Similar results were obtained by Ayranci and Tunc,[30] who reported that methylcellulose-based edible coating reduced ascorbic acid loss of both button mushrooms and cauliflower. However, It has been suggested that edible coatings containing chitosan promote vitamin C loss by acting as an abiotic elicitor, generating reactive oxygen species (ROS), which are scavenged by antioxidant compounds, such as vitamin C.[31] Chitosan-oil coating could inhibit vitamin C loss, due to the protection effected by antioxidant phenolics in the thyme oil. The addition of essential oils may improve the antioxidant activity of the chitosan coating.[12,32]

Changes during storage in the percentage of ABTS radical inhibition by antioxidants present in shiitake mushrooms are shown in Figure 2D. The control treatment does not seem to substantially contribute to enhancement of the antioxidant capacity of mushrooms. On the other hand, coating treatments maintained the antioxidant capacity of mushrooms, although a decrease was observed during the storage. Mushrooms coated with chitosan-oil presented higher ABTS scavenging ability, compared with chitosan coating or oil treatment. In summary, chitosan-oil coating had a positive effect on antioxidant capacity, mainly influenced by total phenolics and ascorbic acid of shiitake mushrooms.

Effect of Chitosan Coating Enriched with Thyme Oil on Phenolic Compounds. Figure 3 depicts a typical HPLC chromatogram of the flavonoids, such as catechin, naringin, and myricetin, and phenolic compounds, such as gallic acid, *p*-hydroxybenzoic acid, and protocatechuic acid, recorded at 280 nm. In general, all the flavonoids and phenolic compounds decreased gradually during 16 days of storage. However, significantly ($P < 0.05$) higher levels of catechin, gallic acid, and protocatechuic acid were observed in chitosan-oil-coated mushrooms during the whole storage time compared to the case of the control mushroom (Table 2). Protocatechuic acid contents were significantly ($P < 0.05$) higher in chitosan-oil-coated mushroom on the 12th and 16th days compared to the case of the control mushroom. Control treatment induced the loss of certain flavonoids and phenolic compounds in shiitake mushroom in comparison with chitosan-oil coated mushrooms. This phenomenon could be due to the fast oxidation of flavonoids and phenolic compounds on the mushroom surface, directly in contact with the O_2 in the package headspace. Enzymatic oxidation of phenolics via polyphenol oxidase (PPO) has been associated with mushroom browning. Previous research shows a significant positive relationship between total phenolic and antioxidant activity in shiitake mushrooms;[33] thus, the higher radical scavenging activity in chitosan-oil-coated mushrooms in the present study could be mainly attributed to its higher level of total phenolic compounds. The results indicate that thyme oil suppressed spoilage in shiitake mushrooms not only with their antimicrobial properties but also with their promotion of decay resistance in the mushroom tissues through increasing the amounts of phenolic compounds, flavonoids, and antioxidant capacities.

Table 2. Effect of Chitosan Coating Enriched with Thyme Oil on Phenolic Compounds (mg/100 g FW) Change of Shiitake Mushrooms Stored at 4 °C for 16 days[a]

days at 4 °C	control	chitosan	thyme oil	chitosan + thyme oil
Catechin				
0	3.32 ± 0.12 a	3.30 ± 0.13 a	3.31 ± 0.07 a	3.32 ± 0.10 a
4	3.11 ± 0.14 a	3.17 ± 0.04 a	3.14 ± 0.05 a	3.31 ± 0.06 a
8	2.84 ± 0.05 b	3.03 ± 0.08 ab	3.16 ± 0.05 a	2.95 ± 0.13 ab
12	2.57 ± 0.06 b	3.10 ± 0.12 a	2.94 ± 0.05 a	3.15 ± 0.11 a
16	2.18 ± 0.10 c	3.02 ± 0.03 a	2.66 ± 0.08 b	2.86 ± 0.11 ab
Gallic Acid				
0	10.36 ± 0.32 a	10.22 ± 0.24 a	10.42 ± 0.16 a	10.33 ± 0.14 a
4	7.79 ± 0.37 b	10.15 ± 0.16 a	9.12 ± 0.22 a	9.44 ± 0.25 a
8	6.48 ± 0.26 b	9.33 ± 0.28 a	8.78 ± 0.12 a	9.18 ± 0.36 a
12	6.53 ± 0.24 b	8.78 ± 0.30 a	8.32 ± 0.19 a	8.95 ± 0.21 a
16	5.02 ± 0.25 c	8.04 ± 0.25 ab	7.21 ± 0.14 b	8.45 ± 0.20 a
Naringin				
0	2.17 ± 0.06 a	2.13 ± 0.11 a	2.15 ± 0.14 a	2.18 ± 0.07 a
4	2.23 ± 0.13 a	2.17 ± 0.08 a	2.04 ± 0.10 a	2.26 ± 0.04 a
8	1.92 ± 0.07 b	1.87 ± 0.06 b	2.20 ± 0.12 a	2.04 ± 0.06 ab
12	1.74 ± 0.14 b	2.35 ± 0.15 a	1.84 ± 0.03 b	2.12 ± 0.04 ab
16	1.51 ± 0.08 b	2.01 ± 0.11 a	1.72 ± 0.07 b	2.13 ± 0.06 a
Protocatechuic Acid				
0	14.54 ± 0.37 a	14.50 ± 0.32 a	14.48 ± 0.21 a	14.55 ± 0.19 a
4	12.38 ± 0.24 b	12.46 ± 0.14 b	13.45 ± 0.24 a	13.73 ± 0.21 a
8	10.49 ± 0.36 c	13.10 ± 0.13 a	11.38 ± 0.44 b	13.24 ± 0.35 a
12	9.10 ± 0.40 d	12.63 ± 0.20 b	10.66 ± 0.31 c	13.69 ± 0.23 a
16	8.12 ± 0.24 b	9.57 ± 0.22 b	12.47 ± 0.28 a	13.10 ± 0.16 a
p-Hydroxybenzoic Acid				
0	3.30 ± 0.06 a	3.21 ± 0.14 a	3.35 ± 0.08 a	3.26 ± 0.13 a
4	3.24 ± 0.12 a	3.17 ± 0.07 a	3.14 ± 0.05 a	3.28 ± 0.10 a
8	3.18 ± 0.09 a	3.13 ± 0.10 a	3.11 ± 0.14 a	3.30 ± 0.05 a
12	2.85 ± 0.04 b	3.15 ± 0.12 a	3.23 ± 0.07 a	3.02 ± 0.06 ab
16	2.64 ± 0.13 b	3.02 ± 0.03 a	3.15 ± 0.12 a	2.91 ± 0.13 ab
Myricetin				
0	1.48 ± 0.03 a	1.45 ± 0.07 a	1.44 ± 0.13 a	1.46 ± 0.05 a
4	1.32 ± 0.05 b	1.48 ± 0.11 a	1.36 ± 0.04 b	1.37 ± 0.02 b
8	1.43 ± 0.02 ab	1.24 ± 0.10 b	1.33 ± 0.13 b	1.57 ± 0.04 a
12	1.26 ± 0.03 b	1.27 ± 0.04 b	1.53 ± 0.05 a	1.30 ± 0.12 b
16	1.10 ± 0.05 c	1.21 ± 0.07 b	1.06 ± 0.03 c	1.43 ± 0.08 a

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

Effect of Chitosan Coating Enriched with Thyme Oil on MDA and Relative Conductivity. MDA is widely applied as an index of lipid peroxidation, usually resulting from oxidative stress, which, in turn, causes a reduction in membrane integrity, increases membrane leakage, and enhances cell senescence. As shown in Figure 4A, MDA levels in control samples increased almost 2-fold at the end of storage. Increases were also observed in mushrooms treated with chitosan coating, oil solution, and chitosan-oil coating. However, MDA levels in chitosan-oil-coated samples were relatively lower during this period. When the experiment was terminated, MDA levels in control and chitosan-oil-coated mushrooms were 167.8 and 93.8% higher than initial levels, respectively. Corresponding values for samples treated with chitosan coating and oil solution were 111.5 and 127.8%, respectively.

Membrane permeability changes during storage were evaluated by determining the intensity of electrolyte leakage. This parameter was included in order to have more information on membrane stability and thereby on the relative ion content in the apoplastic space.[34] Changes in electrolyte leakage of mushrooms are presented in Figure 4B. The initial electrolyte leakage was 1.8% in the shiitake mushrooms. In general, electrolyte leakage was enhanced as storage time increased. The electrolyte leakage contents of chitosan-oil coated samples and control samples were 18.5% and 37.5%, respectively, at the end of storage. The increase of membrane permeability in the control groups was much higher than that in the chitosan-oil-coated mushrooms. These results demonstrated that membrane permeability, as an indicator of membrane integrity, gradually increased during storage. However, chitosan-oil-coated mushrooms had significantly lower MDA contents and relative leakage rate than the control mushrooms (Figure 3), indicating that a higher membrane integrity was maintained.

Effect of Chitosan Coating Enriched with Thyme Oil on Microbiological Quality. As shown in Table 3, chitosan-oil coating and thyme oil treatment were more effective in reducing microbial counts than control. In any of the studied treatments, the psychrophilic bacteria counts increased less than two orders during the entire storage period. All samples had counts below 10^4 cfug^{-1}. This contamination level suggests

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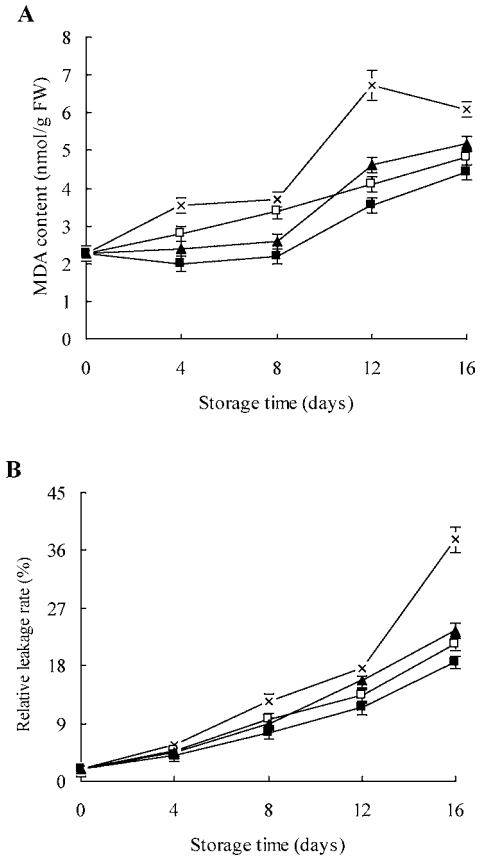

Figure 4. Changes in MDA (A) and relative leakage rate (B) of shiitake mushrooms treated with control (×), chitosan coating (□), 1.5% thyme oil (▲), and chitosan coating + 1.5% thyme oil (■) stored at 4 °C for 16 days. Each data point is the mean of three replicate samples. Vertical bars represent standard deviation of means.

Table 3. Effect of Chitosan Coating Enriched with Thyme Oil on Microbial Counts (log_{10} $cfug^{-1}$) Change of Shiitake Mushrooms Stored at 4 °C for 16 days[a]

days at 4 °C	control	chitosan	thyme oil	chitosan + thyme oil
Mesophilic				
0	3.93 ± 0.12 a	3.96 ± 0.06 a	3.90 ± 0.14 a	3.95 ± 0.13 a
4	4.65 ± 0.08 a	4.44 ± 0.18 b	4.22 ± 0.20 c	4.10 ± 0.07 c
8	5.43 ± 0.22 a	4.55 ± 0.13 b	4.73 ± 0.26 b	4.23 ± 0.11 c
12	5.68 ± 0.05 a	5.10 ± 0.11 c	5.43 ± 0.08 b	4.47 ± 0.25 d
16	6.56 ± 0.13 a	5.28 ± 0.10 c	5.80 ± 0.06 b	4.88 ± 0.16 d
Psychrophilic				
0	1.76 ± 0.04 a	1.74 ± 0.20 a	1.77 ± 0.07 a	1.75 ± 0.16 a
4	2.36 ± 0.26 a	2.16 ± 0.26 b	2.11 ± 0.14 b	2.14 ± 0.13 b
8	2.55 ± 0.28 a	2.35 ± 0.07 b	2.25 ± 0.27 b	2.20 ± 0.04 b
12	2.78 ± 0.10 a	2.80 ± 0.19 a	2.60 ± 0.29 b	2.52 ± 0.26 b
16	3.34 ± 0.16 a	3.23 ± 0.10 a	2.97 ± 0.15 b	2.77 ± 0.15 c
Pseudomonad				
0	5.13 ± 0.11 a	5.18 ± 0.08 a	5.10 ± 0.13 a	5.12 ± 0.12 a
4	6.67 ± 0.04 a	5.82 ± 0.15 b	5.65 ± 0.18 c	5.40 ± 0.30 d
8	6.98 ± 0.07 a	6.42 ± 0.27 b	6.17 ± 0.24 c	5.68 ± 0.25 d
12	7.66 ± 0.13 a	6.78 ± 0.32 b	6.43 ± 0.25 c	5.90 ± 0.28 d
16	8.37 ± 0.18 a	7.44 ± 0.26 b	6.76 ± 0.27 c	6.04 ± 0.16 d
Yeasts and Molds				
0	3.75 ± 0.10 a	3.76 ± 0.11 a	3.76 ± 0.22 a	3.73 ± 0.20 a
4	4.46 ± 0.14 a	4.10 ± 0.17 b	4.21 ± 0.16 b	3.97 ± 0.26 c
8	5.87 ± 0.28 a	4.97 ± 0.08 b	4.53 ± 0.20 c	4.56 ± 0.17 c
12	6.12 ± 0.06 a	5.43 ± 0.20 b	5.04 ± 0.16 c	4.98 ± 0.14 c
16	6.87 ± 0.13 a	5.88 ± 0.26 b	5.37 ± 0.23 c	5.15 ± 0.23 d

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

that shiitake mushrooms under the studied coating conditions did not favor the development of this type of bacteria. Mushrooms from the control treatment exhibited tiny brown spots on day 4 that developed into dark zones, characteristic of *Pseudomonas* spoilage by day 8. Mushrooms were highly decayed at this point, and the end of shelf life was due to microbial spoilage. The chitosan-oil coated samples did not exhibit these characteristics of microbial degradation even on day 12. The organisms usually responsible for spoilage of mushrooms are Gram-negative, psychrotrophic bacteria, particularly belonging to the *Pseudomonasae* family, because of contamination of the product from compost. In our experiment, we found chitosan-oil exhibited superior antimicrobial activity compared to that of chitosan in coating shiitake mushrooms during storage. The synergistic effect may be due to the chitosan coating, which reduced oil vapor diffusion and loss of the thyme oil, rendering it more effective in inhibiting microbial growth and maintaining the keeping quality of mushrooms.

Effect of Chitosan Coating Enriched with Thyme Oil on Sensory Attributes. Effects of chitosan-oil coatings as natural biopreservatives on the sensory of shiitake mushrooms were investigated. As expected, mushroom off-odor, gill color, gill uniformity, cap uniformity, and dark zones significantly ($P < 0.05$) changed with storage time, supporting the validity of using these parameters as indicators of mushroom deterioration. Average values for the sensory attributes are shown in Table 4. Off-odor intensity significantly increased after 8 days of storage in control samples. The color of mushroom gills gradually became browner and less uniform with time for all the evaluated conditions. The gills of control mushrooms showed a color intensity of 5.35 and uniformity of 5.12 at the 12th day of storage. However, the gills of chitosan-oil coated mushrooms did not reached these intensities even at the end of storage. A better trend was observed for the uniformity of the cap surface and the presence of dark stains on the cap in chitosan-oil coated samples. These results suggest that chitosan-oil coatings were more effective in retarding mushroom sensory deterioration. The browning of mushrooms is attributed to the action of polyphenol oxidase (PPO) enzyme and the action of bacteria and mold on the mushroom tissue. As chitosan-oil coating causes reduction of spoilage organisms, such as *Pseudomonas*, responsible for oxidation of phenolic compounds to form brown-colored melanins, it prevents the formation of brown patches, hence improving the appearance and color.

In summary, our research showed that the senescence inhibition of cold-stored shiitake mushroom by the chitosan-oil coating treatment involved the maintenance of tissue firmness and sensory quality, reduction of microbial counts, increase of phenolics and ascorbic acid contents, and enhancement of antioxidant ability compared with control. Chitosan-oil coated samples also exhibited lower levels of electrolyte leakage and malondialdehyde contents during the storage period. These results suggest that chitosan-oil coating is promising as an edible coating to be used for maintaining shiitake mushroom quality and extending their postharvest life.

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Table 4. Effect of Chitosan Coating Enriched with Thyme Oil on Sensory Attributes' Change of Shiitake Mushrooms Stored at 4 °C for 16 days[a]

days at 4 °C	control	chitosan	thyme oil	chitosan + thyme oil
Off-Odor				
0	0	0	0	0
4	1.77 ± 0.06 a	1.51 ± 0.04 b	1.62 ± 0.10 ab	1.57 ± 0.06 b
8	2.65 ± 0.11 a	2.18 ± 0.07 b	2.32 ± 0.05 b	2.23 ± 0.04 b
12	5.43 ± 0.14 a	4.32 ± 0.16 c	4.68 ± 0.13 b	4.36 ± 0.15 c
16	7.10 ± 0.25 a	5.76 ± 0.19 c	6.22 ± 0.24 b	5.55 ± 0.20 d
Gills Color				
0	0	0	0	0
4	1.31 ± 0.05 a	1.14 ± 0.04 c	1.20 ± 0.02 b	1.12 ± 0.06 c
8	3.12 ± 0.07 a	2.47 ± 0.07 b	2.56 ± 0.16 b	2.25 ± 0.05 c
12	5.35 ± 0.17 a	4.23 ± 0.17 c	4.54 ± 0.14 b	3.64 ± 0.08 d
16	7.76 ± 0.24 a	5.12 ± 0.22 b	4.86 ± 0.18 c	4.64 ± 0.15 d
Gills Uniformity				
0	10	10	10	10
4	8.54 ± 0.15 a	8.10 ± 0.20 b	8.25 ± 0.14 b	8.23 ± 0.12 b
8	6.60 ± 0.14 c	7.53 ± 0.16 b	7.45 ± 0.33 b	7.79 ± 0.15 a
12	5.12 ± 0.08 c	5.75 ± 0.11 b	5.57 ± 0.17 b	6.21 ± 0.05 a
16	4.20 ± 0.26 c	5.12 ± 0.18 b	5.16 ± 0.08 b	5.77 ± 0.18 a
Cap Uniformity				
0	10	10	10	10
4	8.44 ± 0.11 b	8.58 ± 0.13 a	8.62 ± 0.24 a	8.60 ± 0.20 a
8	6.58 ± 0.19 c	7.88 ± 0.27 b	7.85 ± 0.21 b	8.16 ± 0.32 a
12	5.11 ± 0.20 c	7.30 ± 0.14 b	7.16 ± 0.16 b	7.67 ± 0.28 a
16	4.26 ± 0.14 d	6.14 ± 0.08 b	5.88 ± 0.26 c	6.82 ± 0.14 a
Dark Zones				
0	0	0	0	0
4	1.35 ± 0.04 a	0.85 ± 0.01 b	1.12 ± 0.03 a	0.64 ± 0.04 c
8	2.44 ± 0.08 a	1.83 ± 0.10 c	2.24 ± 0.12 b	1.23 ± 0.07 d
12	3.76 ± 0.12 a	2.67 ± 0.13 c	3.47 ± 0.13 b	2.10 ± 0.14 d
16	5.80 ± 0.18 a	3.24 ± 0.07 c	5.15 ± 0.26 b	2.66 ± 0.17 d

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

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