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Postharvest Preservation of *Flammulina velutipes* with Isoamyl Isothiocyanate

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Abstract: *Flammulina velutipes* was treated with 10, 50, and 250 μL L^{-1} isoamyl isothiocyanate (IAITC), and effects on quality preservation were assessed. IAITC displayed positive effects during the storage of *F. velutipes*: (i) maintained tissue integrity, reduced weight loss rates; (ii) reduced browning degree by inhibiting the activities of browning-related enzymes; (iii) enhanced activities of two antioxidant enzymes (superoxide dismutase and catalase) and increased ascorbic acid content; (iv) ameliorated microbial spoilage by inhibiting bacterial growth, especially *Serratia*, a major bacterial community on the surface of *F. velutipes*; (v) enhanced chitin content and thereby maintained structural integrity; and (vi) maintained levels of Asp, Glu, linoleic acid, and polyunsaturated fatty acids. In conclusion, IAITC reduced catalase activity and total phenolic content in *F. velutipes* during storage. The most suitable concentration of IAITC in the preservation of *F. velutipes* was 50 μL L^{-1}.

Keywords: *Flammulina velutipes*; isoamyl isothiocyanate; microbiological flora; enzymatic browning; nutrients

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1. Introduction

Flammulina velutipes, with its high nutrient content and organoleptic and medicinal properties, is the fourth most highly consumed mushroom species worldwide [1] and China has become the largest producer of *F. velutipes* [2]. However, *F. velutipes* also poses a great challenge while increasing the income of mushroom growers: it is a very perishable food product. Because of the thin epidermis of its fruiting bodies, *F. velutipes* is prone to spoilage by microorganisms and loss of moisture. Increased respiration rate of the fruiting bodies after harvest leads to browning, cap opening, stipe elongation, decreasing firmness, and nutrient content loss in *F. velutipes* [3]. Thereby, the quality of appearance and nutrition for *F. velutipes* decrease rapidly within a few days. A proper approach to extend postharvest quality of *F. velutipes* is required.

Postharvest preservation techniques, including physical and chemical methods, play an important role in maintaining postharvest quality and extending the shelf life of *F. velutipes*. Low-temperature preservation is the most common way to store mushrooms, but it needs to be combined with other methods because of incomplete bacterial inhibition [4]. Nanopackaging and plasma treatment are also effective for postharvest preservation [5,6], but high costs of equipment requirements and production are major chal-

lenges. Spraying of an antibacterial agent and 1-methylcyclopropene (1-MCP) is the main chemical method, which is a recent advance in postharvest quality preservation of the golden needle mushroom (*F. velutiper*). However, sprayed mushrooms will have higher moisture content on their surface, which makes microbial attack easier [7] and may reduce consumer's acceptance even if they are safe. Therefore, increasing concerns with food safety and environmental protection have also stimulated interest in novel preservative for *F. velutipes* preservation.

Isothiocyanates (ITCs) are plant essential oils (EOs) that are of interest in food preservation because of their natural, biodegradable, relatively safe, and antimicrobial properties [8,9]. Various ITCs have been explored concerning their effect on preserving postharvest product quality. Allyl isothiocyanate (AITC) displays biocidal activity against pathogenic microorganisms [10], and helps maintain weight and enhances antioxidant properties of mulberry (*Morus alba*) fruit [11]. AITC treatment also maintains a lower decay rate in blackberry (*Rubus* spp.) caused by *Botrytis cinerea* [12]. Benzyl isothiocyanate (BITC) inhibits growth of *B. cinerea* and postpones decay of postharvest strawberry [13]. ITCs are promising safe agents owing to their natural properties, antimicrobial activity, and health benefit. However, the application of ITCs for postharvest mushroom preservation has not been systematically investigated. Isoamyl isothiocyanate (IAITC) is one of isothiocyanates (ITCs) derived from Brassicaceae plants, including *Armoracia lapathifolia*, *Brassica juncea*, and others. Based on the antimicrobial effect of IAITC against microorganisms [14], the effects of IAITC on postharvest preservation of *F. velutipe*s during storage for 8 d at 6 °C and 90% relative humidity was analyzed.

2. Materials and Methods

2.1. Reagents

Isoamyl isothiocyanate (IAITC) was sourced from 3B Scientific Co. (Wuhan, China). Glutaraldehyde, catechol, guaiacol, L-dopa, pyrogallol, Folin–Ciocalteu reagent, gallic acid, trichloroacetic acid (TCA), and thiobarbituric acid (TBA) were from Sigma-Aldrich (St. Louis, MO, USA). Other reagents were of analytical grade.

2.2. Sample Preparation

Fresh *F. velutipe*s mushrooms were from Xiangyunxinglong mushroom plant (Beijing, 40° N, 116° E) and transported to the Laboratory of Medical and Edible Fungi in China Agricultural University within 1 h. Mushrooms free from physical damage were selected by bundle weight at 400 ± 20 g and length at 15.5 ± 0.5 cm.

2.3. Treatment Preparation

Three doses of IAITC (80, 400, 2000 μL) were spotted onto gauze pads laid at the bottom of 8 L polystyrene containers with a lid. Three bundles of mushroom (~1200 g) were placed in the containers, which were kept at 25 °C for 15 min to let IAITC volatilize. Thus, the concentrations of IAITC in the containers were 10, 50, and 250 μL L^{-1}, and they were termed groups I, II, and III, respectively. Each group contained 4 containers and opened one container to sampling every other day. The opened container would be abandoned after sampling because of the change in IAITC concentration. Blank controls (termed group CK) were treated with deionized water rather than IAITC solution. All containers were stored at 6 °C and 90% relative humidity for eight days.

At each time of sampling, one container was opened randomly at day 2, 4, 6, and 8 and 150 g mushroom was taken from three bundles of *F. velutipes* as three replicates. The weight loss rate, browning degree, appearance, microstructure, and microbiological analysis were analyzed immediately after harvesting and sampling. Other samples were stored at −80 °C for further analysis.

2.4. CO_2 Concentration

CO_2 concentration in containers was determined by gas chromatography (model TP-2060, Beifentianpu, China) every two hours until 172 h and expressed as percentage.

2.5. Weight Loss and Browning Degree

Mushrooms were weighed before and after the storage period, and the weight loss rate was calculated by using Formula (1):

$$\text{Weight loss rate (\%)} = (W_0 - W_t)/W_t \times 100\% \quad (1)$$

where W_0 is initial weight and W_t is weight after storage.

Browning degree was determined by spectrophotometry [15]. Mushrooms (2 g) were ground with 10 mL phosphate buffer (0.1 M, pH 6.8) and centrifuged at 13,000× *g* for 20 min. Absorbance of the supernatant at wavelength 420 nm (abbreviated A_{420}) was measured, and the browning degree was calculated by using Formula (2):

$$\text{Browning degree} = (A_{420} \times 10)/\text{fresh weight} \quad (2)$$

2.6. Histological Microstructure

2.6.1. Histological Section

For light microscopic observation, tissue pieces (~10 × 5 × 2 mm) were collected from the stipe surface and fixed with formaldehyde/acetic acid/ethanol fixative (FAA). Fixed tissues were dehydrated by ethanol series (65, 75, 85, 95, 100% *v*/*v*), immersed in paraffin for 40 min, and embedded using a YB-6LF system (Yaguang Co.; Hubei, China). Thin sections (2.5 μm) were cut by rotary microtome (model HM 340E, Thermo Scientific; Hennigsdorf, Germany), stained with Safranin O/Fast Green [16], and observed with light microscope (model Axio Vert A1, Zeiss; Oberkochen, Germany) at ×400 magnification.

2.6.2. Scanning Electron Microscopy (SEM)

Tissues were collected from the stipe, immersed in 2.5 % (*v*/*v*) glutaraldehyde for 3 h, washed for 15 min with 0.1 mL phosphate buffer (pH 7.0) 3×, and dehydrated by ethanol series (30, 50, 70, 80, 90, 100% *v*/*v*). Thick slices (0.5 mm) were cut, coated with a gold layer by carbon coater (model IB-3, Eiko; Tokyo, Japan), and observed by SEM (model S-3400N, Hitachi; Tokyo, Japan) at ×500 magnification [3].

2.7. Chitin Content

A lyophilized mushroom sample (3 g) was ground, mixed with 90 mL NaOH (1 M), stirred at 80 °C for 2 h, and the alkali-insoluble residue (AIR) was washed with deionized water and centrifuged (8000× *g*, 10 min). The wash/centrifuge cycle was repeated several times until reaching neutral pH, and AIR was lyophilized. To isolate chitin, 1 g AIR was added with 100 mL of 2% (*v*/*v*) acetic acid, stirred at 95 °C for 6 h, centrifuged as above, washed with deionized water until reaching neutral pH, and lyophilized. Chitin content was determined by weighing [17].

2.8. Enzyme Activities

Mushroom samples (2 g) were ground with 20 mL phosphate buffer (50 mM, pH 7.0), centrifuged (13,000× *g*, 4 °C, 20 min), and the supernatant collected as crude enzyme extract for analysis of polyphenol oxidase (PPO), tyrosinase (TYR), peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD).

2.8.1. PPO Activity

The crude enzyme solution of 0.5 mL was mixed with 2 mL phosphate buffer (50 mM; pH 7.0) and 1 mL catechol (50 mM). One unit of PPO activity was defined as the

amount of enzyme resulting in A_{410} increase rate of 0.001/min. PPO activity was expressed as U mg^{-1} protein [18].

2.8.2. TYR Activity

L-dopa was used as substrate, and dopachrome formation was monitored at 475 nm [19]. L-dopa solution (0.1 % (*w*/*v*)) of 3 mL prepared in phosphate buffer (0.1 M, pH 6.8) was mixed with crude enzyme of 1 mL, and A_{475} measured immediately (one time/min for 3 min). One unit of TYR activity was defined as the amount of enzyme resulting in A_{475} increase rate of 0.001/min. TYR activity was expressed as U mg^{-1} protein.

2.8.3. POD Activity

Assay solution (0.2 mL crude enzyme, 1 mL phosphate buffer (50 mM, pH 7.0), 0.8 mL guaiacol (0.2%, *v*/*v*), 1 mL H_2O_2 (0.3%, *v*/*v*)) was incubated at 35 °C for 3 min, and A_{470} was measured at 30 s intervals for 2 min. One unit of POD activity was defined as the amount of enzyme resulting in A_{470} increase rate of 0.01/min. POD activity was expressed as U mg^{-1} protein [18].

2.8.4. CAT Activity

The crude enzyme solution of 0.5 mL was mixed with 1 mL phosphate buffer (50 mM, pH 7.0) and 0.3 mL H_2O_2 (0.2 %, *v*/*v*). A_{240} was measured at 30 s intervals for 2 min. One unit of CAT activity was defined as the amount of enzyme resulting in A_{240} decrease rate of 0.1/min. CAT activity was expressed as U mg^{-1} protein [18].

2.8.5. SOD Activity

This assay was based on inhibition of pyrogallol autoxidation [20]. A mushroom sample (2 g) was ground with 20 mL phosphate buffer (50 mM, pH 7.8), centrifuged (13,000× *g*, 4 °C, 20 min), and the supernatant was collected as crude enzyme extract. Extract samples were combined with 1.4 mL deionized water and 0.1 mL pyrogallol solution (6 mM) in 1.5 mL phosphate buffer as above, and A_{420} was measured at 30 s intervals for 5 min. Pyrogallol autoxidation rate was assayed based on the slope of the linear portion of the $\triangle A_{420}$ plot, and expressed as $\Delta A'_{420}$ min^{-1}.

Inhibition rate of pyrogallol autoxidation by SOD was determined based on pyrogallol autoxidation rate as above. For this method, 0.1 mL crude enzyme and 0.1 mL pyrogallol solution (6 mM) were added to 2.8 mL phosphate buffer, as described above, and the results were expressed as $\Delta A'_{420}$ min^{-1}. One unit of SOD activity was defined as the amount of enzyme resulting in 50% reduction of pyrogallol autoxidation, and calculated by using Formula (3):

$$\text{SOD activity(U mg}^{-1}\text{ protein)} = [(\Delta A_{420}\ \text{min}^{-1} - \Delta A'_{420}\ \text{min}^{-1}) \times 2/\Delta A_{420}\ \text{min}^{-1}] \times (V_1/V_2) \quad (3)$$

where V_1 and V_2 is the volume of reaction mixture and crude enzyme, respectively.

2.8.6. Succinic Dehydrogenase (SDH) Activity

SDH activity was determined using commercial kits (BOXBIO; Beijing, China) as per manufacturer's instructions, and expressed as U mg^{-1} protein.

2.9. Microbiological Analysis

2.9.1. Total Colony Count

To determine total colony count, a 2 g mushroom sample was washed with 20 mL sterile 0.9% NaCl solution, diluted to 10^{-1}–10^{-9}, and 0.2 mL aliquots were placed in LB medium and cultured for 24 h at 37 °C. Total colony count was expressed as log CFU g^{-1} [21].

2.9.2. Deoxyribonucleic Acid (DNA) Extraction and Sequencing Library Construction

Bacterial composition analysis was performed for selected mushrooms from the various storage period groups. Total bacterial genomic DNA was extracted using a Fast DNA Spin Kit for Soil (MP Biomedicals; Santa Ana, CA, USA) as per manufacturer's instructions. The V3–V4 region of 16S rRNA gene was amplified with primer pairs:

Forward primers: 338F (5′-ACTCCTACGGGAGGCAGCAG-3′);

Reverse primers: 806R (5′-GGACTACHVGGGTWTCTAAT-3′).

The polymerase chain reaction (PCR) program in PCR thermocycler (model GeneAmp 9700, ABI; Culver City, CA, USA) was run with the reaction mixture containing 2× Pro Taq (Sangon Biotech, Beijing, China), 0.8 μL of each primer (5 μM), 10 ng μL^{-1} template DNA, and ddH_2O to final volume 20 μL. PCR amplification cycling conditions were as follows: initial denaturation at 95 °C for 3 min, 27 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, then single extension at 72 °C for 10 min, and finally at 10 °C. Illumina MiSeq sequencing of 16S rRNAs from the samples was performed by Majorbio Technology (Shanghai, China). High-throughput sequencing was performed by 2 × 300 bp paired-end method on the MiSeq platform.

Operational taxonomic units (OTUs) were clustered at 97% sequence similarity level by UPARSE method. The Mothur software program (https://mothur.org/wiki/download_mothur, accessed on 19 December 2021.) was used for taxonomic assignments at 70% confidence level based on the Ribosomal Database Project (RDP) database. Bioinformatic analysis was performed using the Majorbio Cloud platform (https://cloud.majorbio.com, accessed on 5 May 2022.). Relative abundance of each bacterial genus (variables clustering on Y-axis) within each sample (X-axis) was expressed by a heat-map plot. Members of a given community responsible for differences between communities were identified on the platform.

2.10. Contents of Total Phenolics and Ascorbic Acid

Mushroom samples (2 g) were homogenized with 15 mL ethanol (80%, *v*/*v*), centrifuged (13,000× g, 20 min), and the supernatant double-diluted with deionized water. The diluted supernatant (1 mL) was mixed with 0.5 mL Folin–Ciocalteu reagent (1 M) and 1.5 mL sodium carbonate solution (10%, *w*/*v*), kept in the dark for 30 min, and A_{760} was measured. Phenolic content was expressed as mg gallic acid equivalent (GAE) g^{-1} dry weight (DW) [22]. Ascorbic acid was quantified using a commercial kit (Leagene; Beijing, China) as per manufacturer's instructions.

2.11. Malondialdehyde (MDA) Content

Mushroom samples (2 g) were ground with 10 mL TCA (10 %, *w*/*v*), centrifuged (2000× *g*, 4 °C, 10 min), and the MDA extract collected. MDA extract (2 mL) was combined with 2 mL TBA (0.6 *w*/*v*), boiled for 15 min, cooled quickly, centrifuged as above, and the supernatant collected. Absorbance of supernatant was measured at wavelengths 450, 532, and 600 nm [18], and the MDA content was calculated by using Formulas (4) and (5):

$$c = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450} \quad (4)$$

$$\text{MDA (nmol g}^{-1}) = (c \times v_t)/(v_s \times \text{fresh weight}) \quad (5)$$

where c is the MDA concentration in the reaction mixture, v_t is the volume of extract solution, and v_s is the volume of extract solution used in the experiment.

2.12. Nutritional Content

Mushroom samples were lyophilized and ground to powder (60 mesh). Amino acid composition and fatty acid composition were determined according to Chinese National Standards GB/T18246-2019, GB 5009.168-2016, and GB/T6432-2018, respectively.

2.13. Statistical Analysis

All experiments were performed in triplicate; results were analyzed by one-way analysis of variance with Duncan's multiple range test using Prism software 8.0; data are presented as mean ± SD. Differences between means with $p < 0.05$ were considered significant.

3. Results

3.1. CO_2 Concentration

CO_2 concentration in all groups increased rapidly in the first 6 h of storage (Figure A1, available in Appendix A), which indicted that mushroom has high respiratory rate. After 12 h, all groups show relatively flat trends in rising CO_2 concentration until the end of measurement. Group CK had a CO_2 concentration of 20.30% at 8 d, whereas IAITC-treated groups ranged from 20.68–21.23%. No significance observed in the CO_2 concentration between IAITC-treated samples and CK.

3.2. Weight Loss

Weight loss is mainly due to water transpiration and respiration metabolism in mushroom [23]. Weight loss increased for each group during storage. Weight loss was the highest for group CK during days 1–4, and remained higher than that in IAITC-treated groups II and III at day 8 (the end of storage) (Figure 1A). Weight loss in CK, I, II, and III was 4.41%, 4.54%, 3.90%, and 4.09% at day 8, respectively. It was significant that group II was lower than the others ($p < 0.05$). Mushrooms treated with 50–250 μL L^{-1} IAITC showed smaller weight loss.

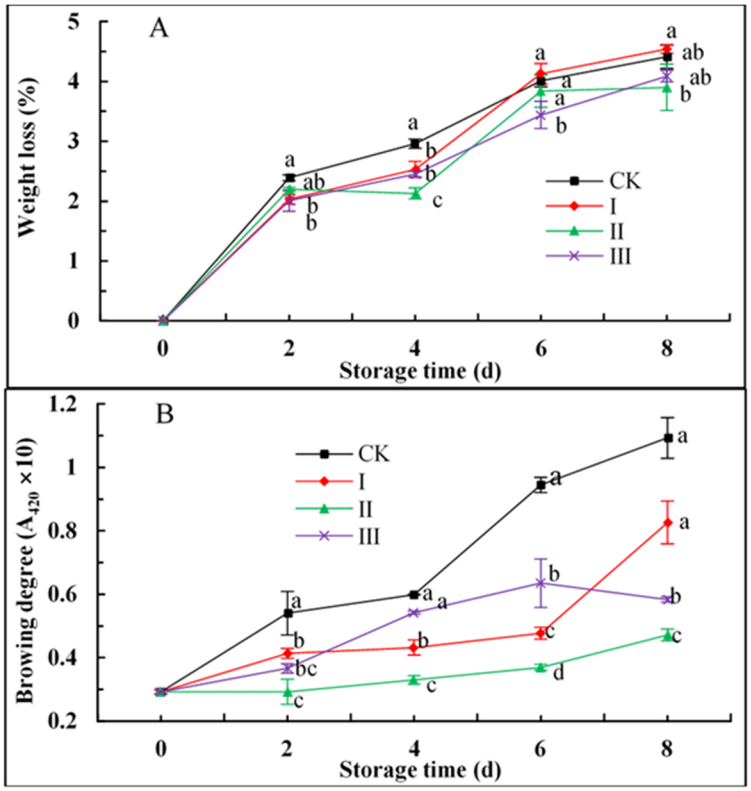


Figure 1. Weight loss (**A**) and browning degree (**B**) in *F. velutipes* stored at 6 °C for the indicated periods following treatment with 10 (group I), 50 (group II), or 250 (group III) μL L^{-1} IAITC, or with distilled water (group CK). Error bars indicate SD calculated from three replicate experiments, and differing letters indicate significant ($p < 0.05$) differences between groups for a given storage period.

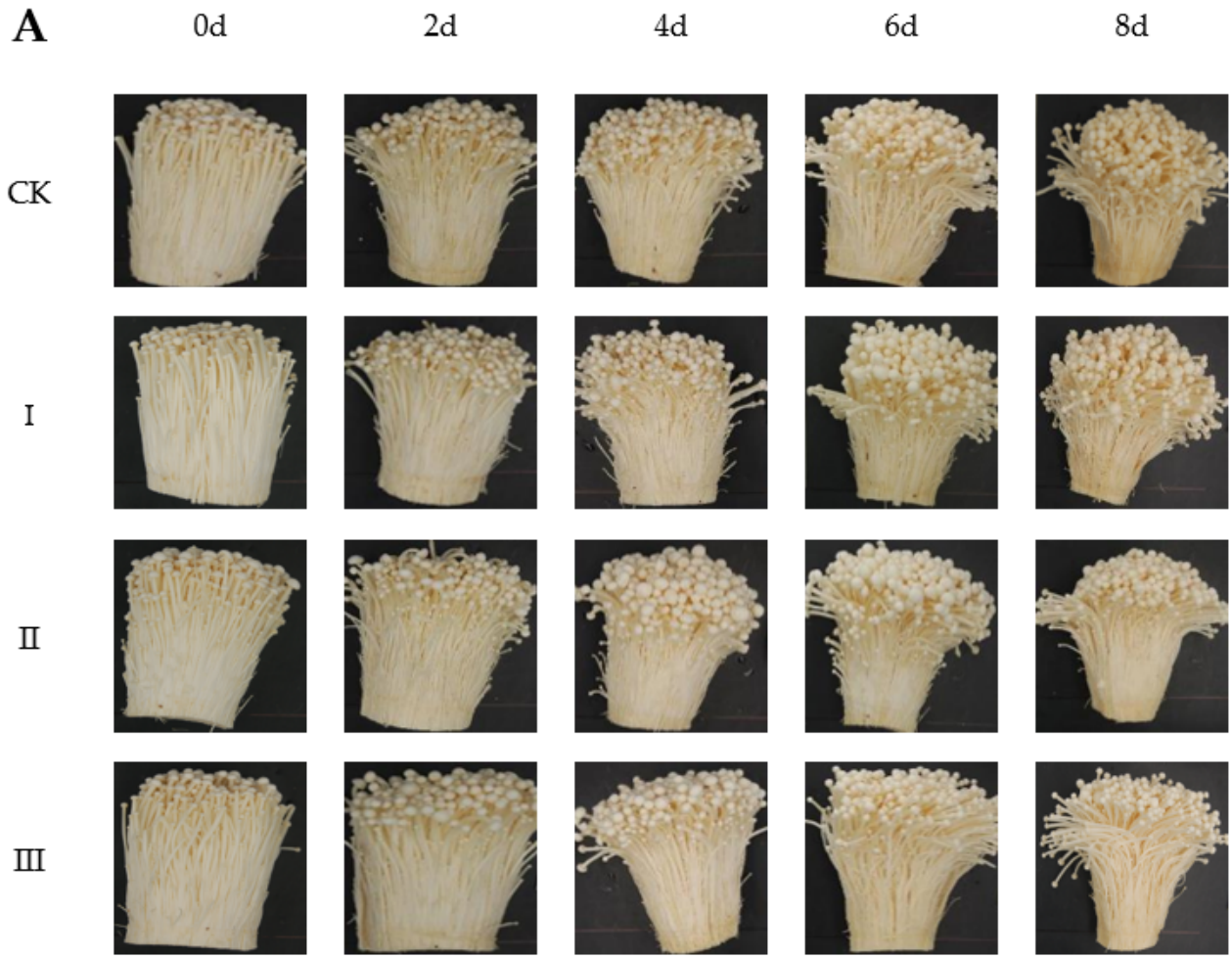
3.3. Browning Degree

Browning degree increased slowly during storage days 1–4 for all groups (Figure 1B), while it increased most rapidly during days 4–8. The browning degree in CK was the highest, followed (in order) by I, III, and II. IAITC inhibited browning in *F. velutipes* effectively and 50 μL L^{-1} (group II) was the optimal concentration.

3.4. Appearance and Microstructure Observation

As storage continued, mushrooms displayed increased degrees of browning, stipe elongation, cap opening, and wilting (Figure 2A). On day 8, CK had notably stronger browning, longer stipes, reduced firmness, and more diffused fruiting bodies compared to day 0, which were milder in the IAITC-treated groups. Group II treated with 50 μL L^{-1} maintained the best quality, including white color and neat appearance.

Stipe tissue microstructure reflects changes in external appearance as described above. Examination by optical microscopy showed that stipe tissues became sparser generally as storage time increased, but it was denser in II and III than in CK and I (Figure 2B). More detailed information from SEM (Figure 2C) revealed that stipe tissue was plump and dense on day 0. The tissue began to loosen (day 4) and collapse (day 8) in CK, while remaining relatively intact in II and III.



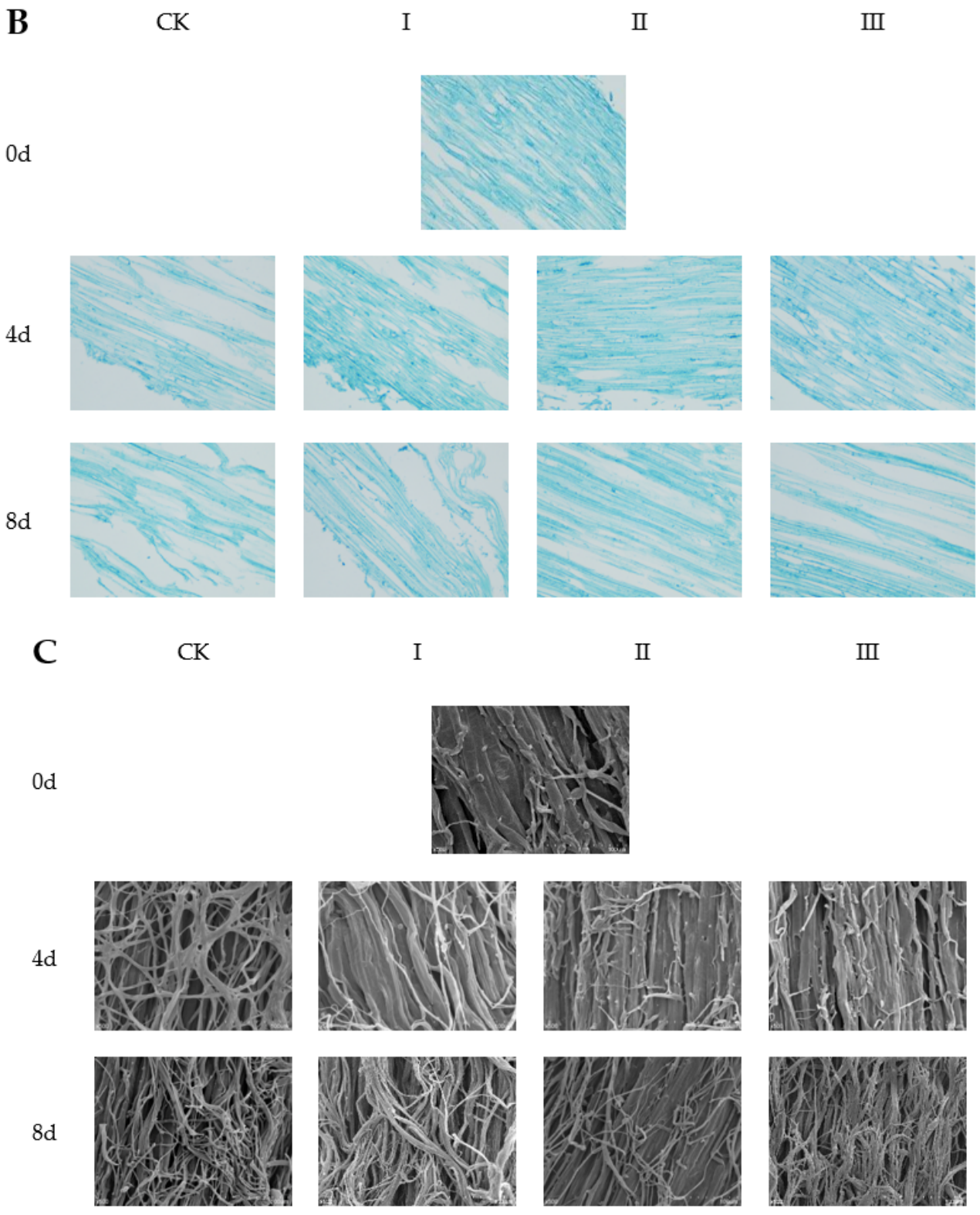


Figure 2. Gross appearance (**A**) and microstructure of *F. velutipes* with different treatment and storage time as viewed by optical microscopy (**B**) and by SEM (**C**).

3.5. Chitin Content

In all groups, the chitin content was positively correlated with storage time (Figure A2, available in Appendix A; R^2 was 0.9489 in Group CK). Chitin content for CK was significantly lower than that for the IAITC-treated groups on day 2. On day 4, there was

significant difference between group III and the other groups. It subsequently increased for all groups, and differences among them were not significant ($p > 0.05$). On day 8, chitin contents were 15.33, 15.67, 15.17, and 15.78 mg g^{-1} fresh weight for CK, I, II, and III, respectively.

3.6. PPO and TYR Activities

During storage days 1–4, PPO activity was significantly higher for CK than that for the IAITC-treated groups (Figure 3A). The activities increased for all groups during days 4–8 and did not differ significantly among them at day 8 ($p > 0.05$).

During the initial 2 days of storage, TYR activity for CK, I, and II was lower than that for III (Figure 3B). TYR activity for CK and I was significantly higher than that for II and III on days 4 and 6, and had the maximal values for CK and I on day 4. The findings indicated that 50 μL L^{-1} IAITC (group II) notably reduced TYR activity during days 1–6, and 250 μL L^{-1} IAITC (group III) had the greatest inhibitory effect over the entire 8 d period.

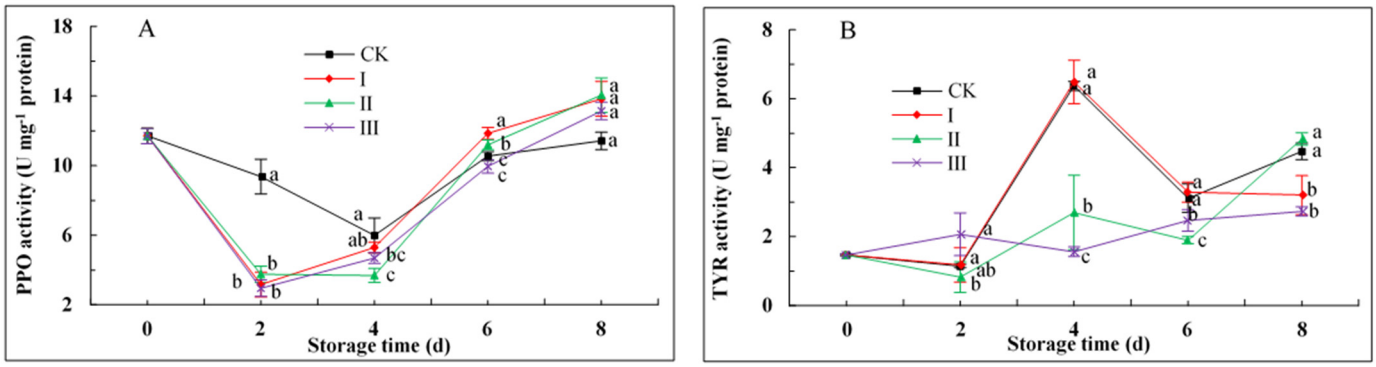


Figure 3. PPO (**A**) and TYR (**B**) activities in *F. velutipes* stored at 6 °C for the indicated periods following treatment with 10 (group I), 50 (group II), or 250 (group III) μL L^{-1} IAITC, or with distilled water (group CK). Error bars indicate SD calculated from three replicate experiments, and differing letters indicate significant ($p < 0.05$) differences between groups for a given storage period.

3.7. SOD, POD, and CAT Activities

SOD activity in all groups remained steady in the first 2 days of storage, and then increased greatly after the second day. At the same time, the differences among groups began to appear. Group CK was significantly lower than that treated with IAITC. At the end of storage, the SOD activities in group CK, I, II, and III were 28.86, 40.18, 43.86, and 53.30 U mg^{-1} protein, respectively.

POD activity in all groups was highest on day 2, and it subsequently declined rapidly (Figure 4B). The activity in all groups treated with IAITC except I was higher than that in CK. There was a significant difference between group II and CK during storage.

CAT activity in groups treated with IAITC was lower than CK throughout the storage period (Figure 4C). The activity in group III was significantly lower than that in the other groups, while II and CK were very close during days 6 to 8.

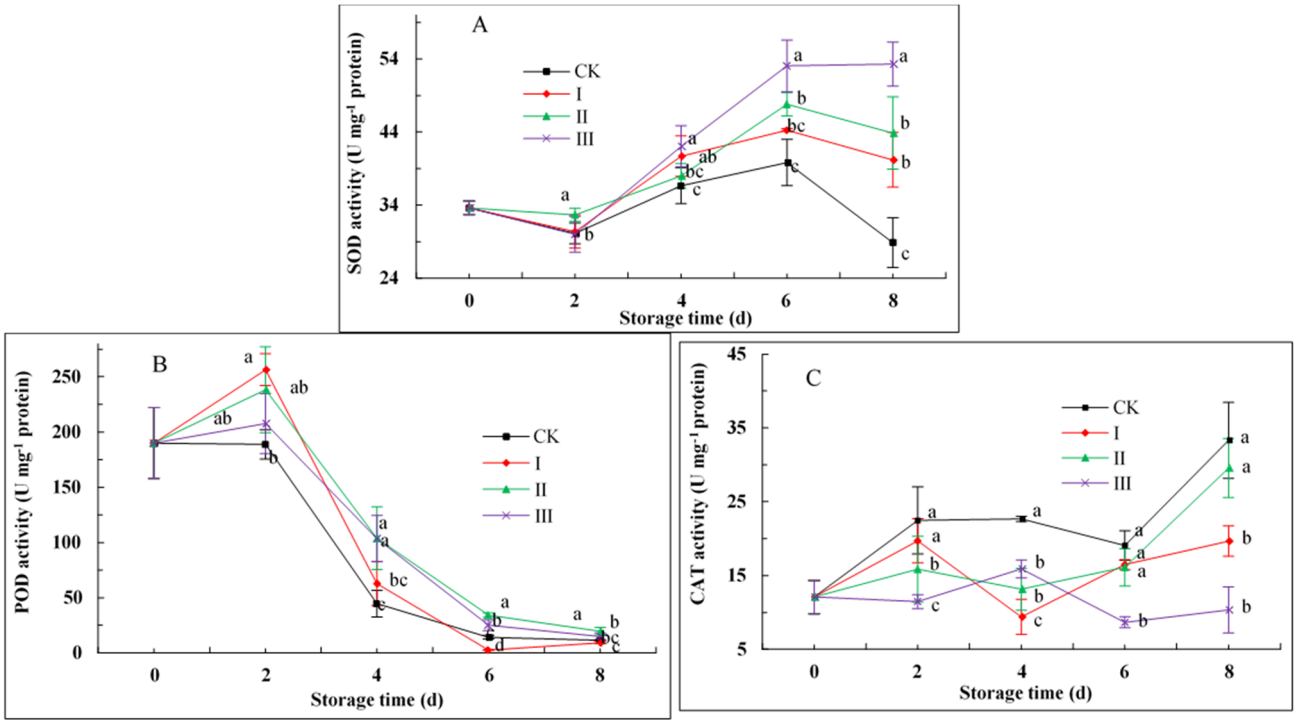


Figure 4. SOD (**A**), POD (**B**), and CAT (**C**) activities in *F. velutipes* stored at 6 °C for the indicated periods following treatment with 10 (group I), 50 (group II), or 250 (group III) μL L^{-1} IAITC, or with distilled water (group CK). Error bars indicate SD calculated from three replicate experiments, and differing letters indicate significant ($p < 0.05$) differences between groups for a given storage period.

3.8. Total Phenolic and Ascorbic Acid Contents

Total phenolic increased in all groups during storage (Figure 5A). On day 2, total phenolic content in group I was significantly lower than the other groups. On day 8, contents in the three IAITC-treated groups were lower than that in CK. Maximal values for all groups were observed on day 6: 2.84, 2.83, 2.66, and 2.51 mg kg^{-1} for CK, I, II, and III, respectively.

Ascorbic acid content varied widely during storage for all groups (Figure 5B). Initial (day 0) content was 3.36 mg kg^{-1}, and the highest values were observed on day 6 for III (7.98 mg kg^{-1}) and II (7.63 mg kg^{-1}). Ascorbic acid in CK was lower than IAITC-treated groups during the first 6 days. The content in group III was significantly higher than others at day 4. Content declined sharply in the three IAITC-treated groups from day 6 to day 8, while it increased slightly in CK.

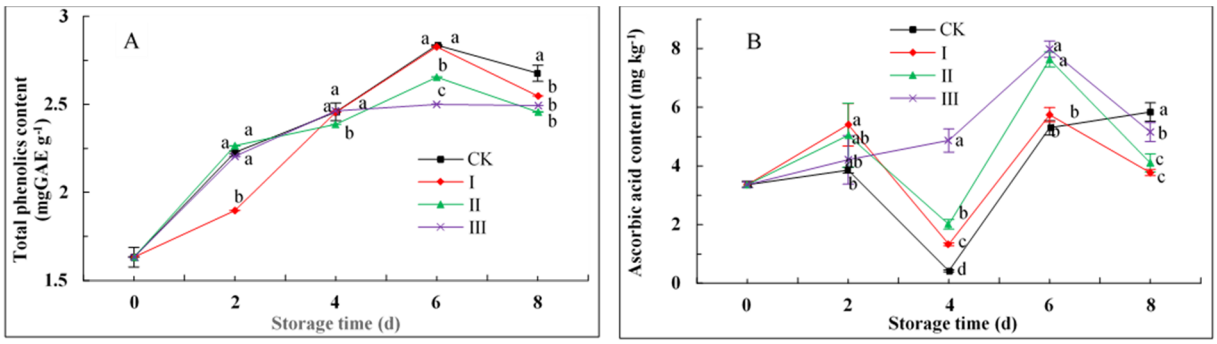


Figure 5. Total phenolic (**A**) and ascorbic acid (**B**) activities in *F. velutipes* stored at 6 °C for the indicated periods following treatment with 10 (group I), 50 (group II), or 250 (group III) μL L^{-1} IAITC, or with distilled water (group CK). Error bars indicate SD calculated from three replicate experiments, and differing letters indicate significant ($p < 0.05$) differences between groups for a given storage period.

3.9. SDH Activity and MDA Content

SDH activity was maximal on day 8 for all groups (Figure 6A). On days 4 and 8, activity for I and II was higher, but for III was lower relative to CK. Activities on day 8 were 64.77, 81.80, 70.55, and 59.32 U mg^{-1} protein for CK, I, II, and III, respectively. Group III was significantly lower than I or II at day 8.

MDA content increased during the 8 days in storage (Figure 6B). At the initial time, the highest MDA content was observed in group I. Subsequently, the content of MDA in group CK was higher than that in IAITC-treated groups until the end of storage. On day 8, the value for CK was 2.29, significantly higher than those for I (1.59 nmol g^{-1}), II (0.96 nmol g^{-1}), and III (1.12 nmol g^{-1}).

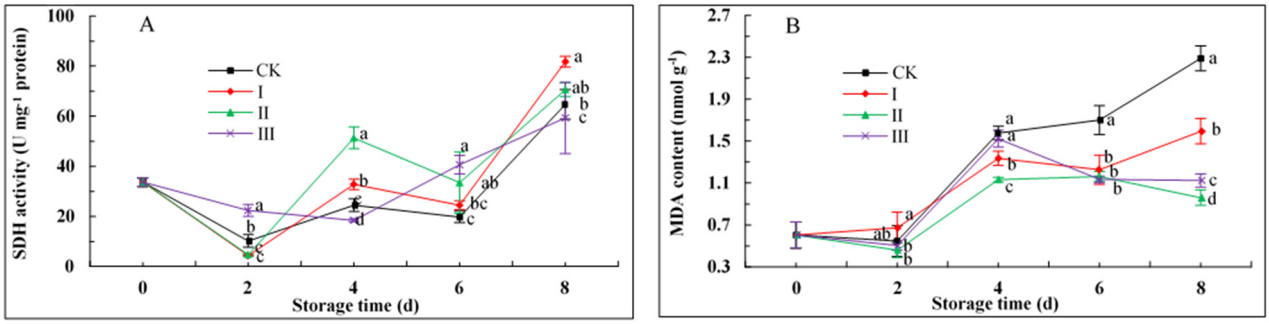


Figure 6. SDH activity (**A**) and MDA content (**B**) activities in *F. velutipes* stored at 6 °C for the indicated periods following treatment with 10 (group I), 50 (group II), or 250 (group III) μL L^{-1} IAITC, or with distilled water (group CK). Error bars indicate SD calculated from three replicate experiments, and differing letters indicate significant ($p < 0.05$) differences between groups for a given storage period.

3.10. Bacterial Colony Counts

In the present study, microbial quantity (total plate count) was 4.36 log CFU g^{-1} at the beginning of storage, increasing steadily as storage time extended (Table 1). The microbial quantity in CK was significantly higher than that in the IAITC-treated groups. Values on day 8 for CK, I, II, and III were 7.34, 6.87, 6.76, and 7.01 log CFU g^{-1}, respectively. IAITC effectively inhibited microbial proliferation, and the optimal inhibitory concentration was 50 μL L^{-1} (group II).

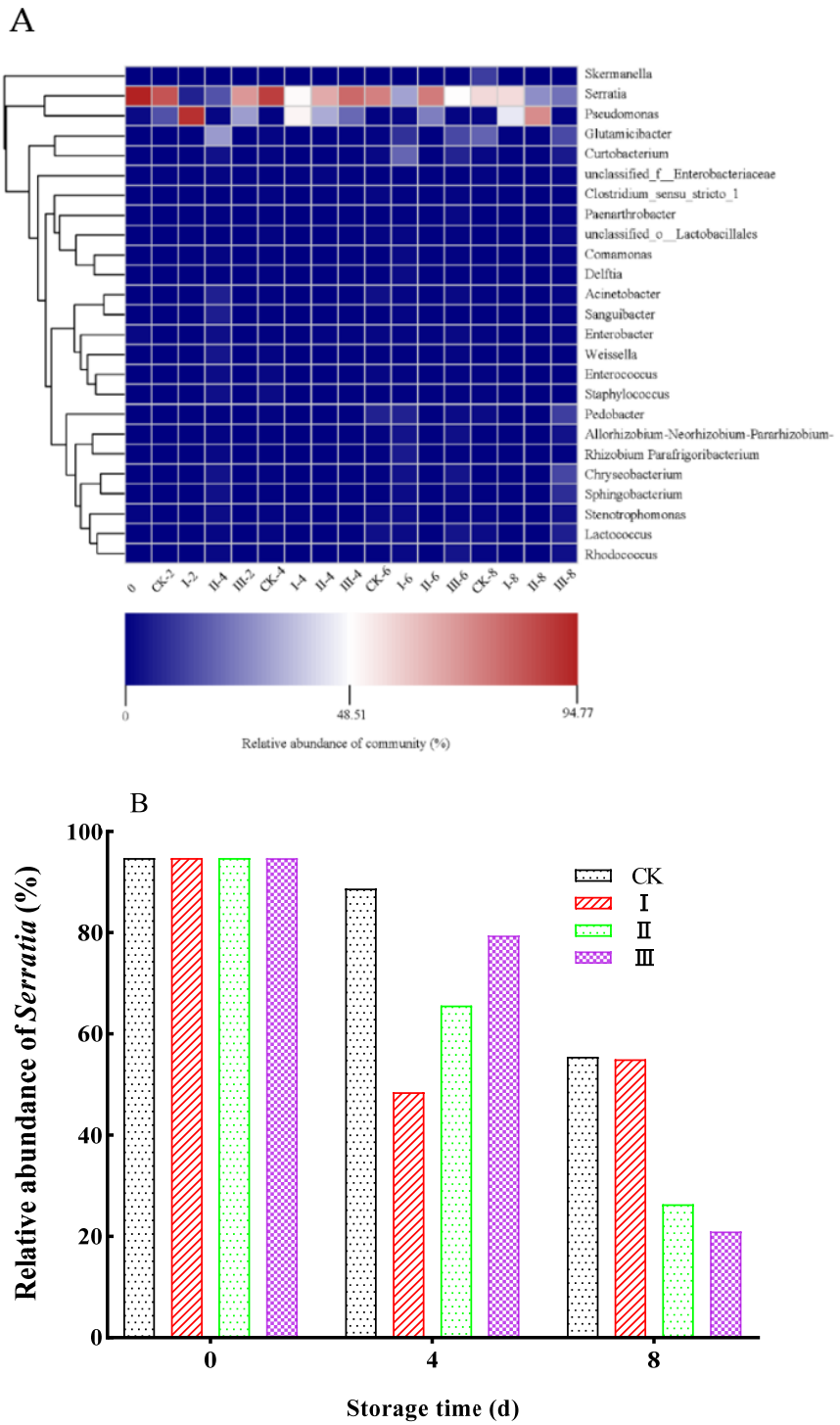
Table 1. Microbial quantities (total plate count; log CFU g^{-1}) in *F. velutipes* stored at 6 °C following treatment with 10 (group I), 50 (group II), or 250 (group III) μL L^{-1} IAITC, or with distilled water (group CK). Differing letters indicate significant ($p < 0.05$) differences between groups for a given storage period.

Treatment	Storage Time (d)				
	0	2	4	6	8
CK	4.36 ± 0.04	5.74 ± 0.11 ^{a}	5.57 ± 0.01 ^{a}	6.33 ± 0.06 ^{a}	7.34 ± 0.04 ^{a}
I	4.36 ± 0.04	5.15 ± 0.01 ^{b}	5.48 ± 0.03 ^{b}	6.14 ± 0.03 ^{b}	6.87 ± 0.06 ^{c}
II	4.36 ± 0.04	5.12 ± 0.05 ^{b}	5.41 ± 0.01 ^{c}	6.03 ± 0.03 ^{b}	6.76 ± 0.08 ^{d}
III	4.36 ± 0.04	5.05 ± 0.02 ^{b}	5.34 ± 0.01 ^{d}	6.04 ± 0.04 ^{b}	7.01 ± 0.01 ^{b}

3.11. Bacterial Community Structure

In order to clarify the changes in the relative abundance of bacterial genera, samples from day 0, 4, and 8 were selected for analysis of the bacterial community structure (Figure 7A). A heat map revealed that there were two main bacteria on the surface of *F. velutipes*. On day 0, the predominant genus was *Serratia* (relative abundance 94.77 %). Along with the extension in storage time, relative abundance of *Serratia* declined (Figure

7B). On the other hand, *Pseudomonas* grew rapidly, and became the predominant bacterial community member on day 4 in I, and on day 8 in II (Figure 7C).



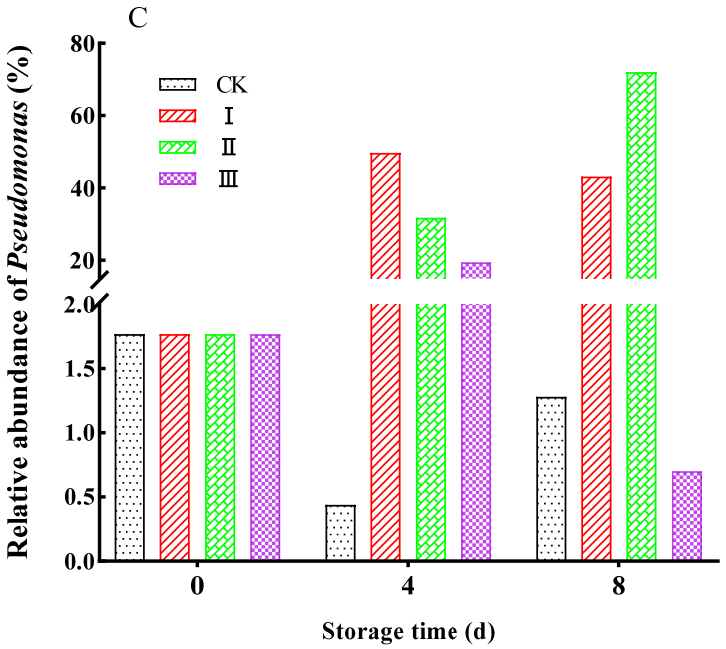


Figure 7. Relative abundances of various bacterial genera (**A**), *Serratia* (**B**), and *Pseudomonas* (**C**) in postharvest *F. velutipes* for the four experimental groups (10 (group I), 50 (group II), or 250 (group III) μL L^{-1} IAITC) at storage times 0, 4, and 8 days.

3.12. Amino Acid and Fatty Acid Contents

The amino acid and fatty acid contents in samples before and after storage were detected. Mushrooms are good sources of amino acids. Eighteen amino acids in *F. velutipes* were categorized on the basis of taste characteristics as MSG-like, sweet, bitter, or tasteless [24] (Table 2). In order to clarify the effect of IAITC on the quality of *F. velutipes*, the changes in amino acid content before and after storage were detected. On day 0, total amino acid content was 112.11 mg g^{-1} DW. Total content on day 8 was much higher for all groups, particularly II and III. Asp and Glu are MSG-like amino acids that contribute to mushroom flavor profiles [25]. The IAITC-treated groups had increased contents of Asp, Glu, Ser, Met, and Lys on day 8. Thus, IAITC could maintain the flavor of *F. velutipes* to a certain extent.

Fourteen fatty acids were analyzed and categorized based on the degree of unsaturation, as saturated, monounsaturated, or polyunsaturated fatty acids (termed SFAs, MUFAs, or PUFAs, respectively). The most abundant components of these three categories were palmitic acid, oleic acid, and linoleic acid, respectively (Table 3). Total fatty acid content increased during storage (day 8 vs. day 0) for all groups, and the increase was greater for I and II than for CK.

The content of MSG-like amino acids for the IAITC-treated groups and that of PUFA except III was higher than CK on day 8.

Table 2. Amino acids in *F. velutipes* treated with 10 (group I), 50 (group II), or 250 (group III) μL L^{-1} IAITC when storage time was 0 and 8 days. Differing letters indicate significant ($p < 0.05$) differences between groups for a given storage period.

Amino Acid (mg g^{-1})		0 d	8 d			
			CK	I	II	III
MSG-like	Aspartic acid	10.85 ± 0.06	11.59 ± 0.09 ^{b}	11.67 ± 0.21 ^{b}	11.71 ± 0.18 ^{b}	12.85 ± 0.02 ^{a}
	Glutamic acid	23.67 ± 1.18	34.04 ± 0.56 ^{b}	34.97 ± 0.67 ^{b}	40.48 ± 0.67 ^{a}	39.72 ± 0.05 ^{a}
	Total	**33.76**	**45.63**	**46.64**	**52.19**	**52.57**
Sweet	Alanine	6.63 ± 0.83	10.46 ± 0.02 ^{a}	9.50 ± 0.18 ^{b}	10.34 ± 0.17 ^{a}	9.06 ± 0.10 ^{c}

	Glycine	5.36 ± 1.03	6.98 ± 0.03 ^{b}	6.91 ± 0.12 ^{b}	7.04 ± 0.12 ^{b}	7.25 ± 0.01 ^{a}
	Serine	6.00 ± 0.76	6.80 ± 0.05 ^{c}	6.82 ± 0.12 ^{c}	7.02 ± 0.11 ^{b}	7.29 ± 0.02 ^{a}
	Threonine	5.84 ± 0.43	7.43 ± 0.08 ^{a}	7.19 ± 0.13 ^{b}	7.36 ± 0.12 ^{ab}	7.39 ± 0.02 ^{a}
	Total	**23.83**	**31.67**	**30.42**	**31.76**	**30.99**
Bitter	Arginine	5.38 ± 0.74	7.10 ± 0.20 ^{b}	6.99 ± 0.09 ^{b}	7.37 ± 0.12 ^{a}	7.35 ± 0.02 ^{a}
	Histidine	3.14 ± 1.75	4.17 ± 0.06 ^{a}	4.10 ± 0.05 ^{ab}	4.19 ± 0.09 ^{a}	4.03 ± 0.02 ^{b}
	Leucine	7.87 ± 0.64	9.34 ± 0.05 ^{a}	8.79 ± 0.23 ^{b}	8.67 ± 0.21 ^{b}	8.92 ± 0.08 ^{b}
	Phenylalanine	5.23 ± 0.76	7.12 ± 0.04 ^{a}	6.89 ± 0.12 ^{b}	6.90 ± 0.14 ^{b}	7.15 ± 0.12 ^{a}
	Methionine	1.69 ± 0.04	1.82 ± 0.08 ^{a}	1.89 ± 0.05 ^{a}	1.88 ± 0.03 ^{a}	1.91 ± 0.07 ^{a}
	Cysteine	1.11 ± 0.04	1.92 ± 0.02 ^{a}	1.82 ± 0.02 ^{b}	1.80 ± 0.03 ^{b}	1.90 ± 0.04 ^{a}
	Valine	5.94 ± 0.59	7.81 ± 0.05 ^{a}	7.27 ± 0.17 ^{b}	7.31 ± 0.14 ^{b}	7.28 ± 0.02 ^{b}
	Total	**30.36**	**39.28**	**37.75**	**38.12**	**38.54**
Tasteless	Isoleucine	4.61 ± 0.03	5.54 ± 0.04 ^{a}	5.14 ± 0.20 ^{b}	5.07 ± 0.18 ^{b}	5.19 ± 0.02 ^{b}
	Lysine	7.63 ± 0.02	10.22 ± 0.07 ^{b}	10.33 ± 0.19 ^{b}	10.60 ± 0.18 ^{a}	10.82 ± 0.07 ^{a}
	Proline	5.12 ± 2.64	5.41 ± 0.11 ^{b}	5.44 ± 0.14 ^{ab}	5.23 ± 0.14 ^{ab}	5.54 ± 0.07 ^{a}
	Tryptophan	2.22 ± 0.28	2.42 ± 0.01 ^{c}	2.40 ± 0.01 ^{d}	2.47 ± 0.01 ^{b}	2.53 ± 0.01 ^{a}
	Tyrosine	4.58 ± 1.20	5.99 ± 0.11 ^{a}	5.87 ± 0.13 ^{a}	5.98 ± 0.17 ^{a}	6.02 ± 0.04 ^{a}
	Total	**24.16**	**29.58**	**29.14**	**29.35**	**30.1**
Total amino acids		**112.11**	**145.3**	**143.12**	**150.51**	**151.20**

Table 3. Fatty acids in *F. velutipes* treated with 10 (group I), 50 (group II), or 250 (group III) μL L^{-1} IAITC when storage time was 0 and 8 days. Differing letters indicate significant ($p < 0.05$) differences between groups for a given storage period. n.d.: not detected.

Fatty Acids (mg g^{-1})		**0 d**	**8 d**			
			CK	**I**	**II**	**III**
Saturated fatty acids (SFA)	Lauric acid C12:0	0.16 ± 0.01	0.30 ± 0.02 ^{b}	0.36 ± 0.01 ^{a}	0.27 ± 0.01 ^{c}	0.27 ± 0.02 ^{c}
	Myristic acid C14:0	0.32 ± 0.03	0.38 ± 0.02 ^{b}	0.47 ± 0.01 ^{a}	0.38 ± 0.02 ^{b}	0.38 ± 0.02 ^{b}
	Pentadecanoic acid C15:0	0.09 ± 0.01	0.22 ± 0.01 ^{b}	0.24 ± 0.0 ^{b}	0.29 ± 0.02 ^{a}	0.23 ± 0.01 ^{b}
	Palmitic acid C16:0	2.76 ± 0.21	2.61 ± 0.15 ^{b}	3.05 ± 0.04 ^{a}	2.72 ± 0.16 ^{b}	2.49 ± 0.13 ^{b}
	Heptadecanoic acid C17:0	n.d.	n.d.	n.d.	0.03 ± 0.01 ^{a}	0.02 ± 0.01 ^{b}
	Stearic acid C18:0	0.22 ± 0.02	0.20 ± 0.01 ^{c}	0.25 ± 0.01 ^{a}	0.23 ± 0.01 ^{b}	0.19 ± 0.01 ^{c}
	Arachidic acid C20:0	n.d.	n.d.	n.d.	n.d.	n.d.
	Heneicosanoic acid C21:0	n.d.	0.20 ± 0.00 ^{a}	0.20 ± 0.00 ^{a}	0.20 ± 0.00 ^{a}	0.20 ± 0.00 ^{a}
	Behenic acid C22:0	0.10 ± 0.01	0.90 ± 0.01 ^{b}	0.11 ± 0.00 ^{a}	0.11 ± 0.00 ^{a}	0.09 ± 0.01 ^{b}
	Lignoceric acid C24:0	n.d.	n.d.	n.d.	n.d.	n.d.
	Total	**3.65**	**3.82**	**4.50**	**4.01**	**3.69**
Monounsaturated fatty acids (MUFA)	Palmitoleic acid C16:1	0.18 ± 0.02	0.33 ± 0.02 ^{a}	0.30 ± 0.01 ^{b}	0.30 ± 0.02 ^{b}	0.24 ± 0.01 ^{c}
	Oleic acid C18:1n9c	0.78 ± 0.06	0.95 ± 0.07 ^{b}	1.05 ± 0.02 ^{a}	0.95 ± 0.06 ^{b}	0.80 ± 0.03 ^{c}
	cis-11-Eicosenoic acid C20:1	n.d.	n.d.	n.d.	n.d.	n.d.
	Erucic acid C22:1n9	n.d.	0.04 ± 0.00 ^{d}	0.06 ± 0.01 ^{b}	0.07 ± 0.01 ^{a}	0.05 ± 0.01 ^{c}
	Nervonic acid C24:1	n.d.	0.20 ± 0.02 ^{ab}	0.25 ± 0.01 ^{a}	0.24 ± 0.03 ^{a}	0.13 ± 0.10 ^{c}
	Total	**0.96**	**1.53**	**1.66**	**1.55**	**1.27**
Polyunsaturated fatty acids (PUFA)	Linoleic acid C18:2n6c	8.34 ± 0.34	11.18 ± 0.74 ^{b}	12.66 ± 0.17 ^{a}	12.15 ± 0.77 ^{ab}	11.13 ± 0.44 ^{b}
	α-linoleic acid C18:3n3	4.53 ± 0.41	5.17 ± 0.34 ^{b}	5.63 ± 0.09 ^{a}	4.90 ± 0.29 ^{bc}	4.46 ± 0.17 ^{c}
	cis-11,14,17-Eicosatrienoic acid C20:3n3	n.d.	n.d.	n.d.	n.d.	n.d.
	Total	**13.83**	**16.35**	**18.32**	**17.05**	**15.59**
Total fatty acids		**18.44**	**21.69**	**24.48**	**22.67**	**20.55**

4. Discussion

The respiration rate for *F. velutipes* is high, and the thin epidermis cannot prevent transpiration effectively during postharvest, which results in weight loss and tissue fragility. Water loss in mushrooms accelerates senescence and cap opening by reducing the cohesive force between water and proteins [3]. Water loss also leads to widening of intercellular space and loss of tissue turgidity. In the present study, there were some changes in the appearance of *F. velutipe*s, including cap opening and stipe elongation and bending. The underlying changes in mushroom appearance were revealed by microstructure observation, which revealed tissue breakage and shrinkage increasing with lengthening storage time. These changes were caused by water loss rather than respiration metabolism, and IAITC fumigation could mitigate the water loss from *F. velutipe*s during storage.

Chitin, the major component of fungal cell walls, plays an essential role in maintaining the structure. The content of chitin was associated with mushroom toughness during postharvest. Along with the increase in chitin content, covalent bonds between chitin and R-glucan formatted and enhanced the rigidity of the hyphal cell wall [26]. Group CK had relatively lower chitin content than others. As a result, higher chitin content in groups treated with IAITC could be responsible for maintaining the integrity of cell walls and increasing the texture toughening. In the present study, the increase in chitin may be the reason why IAITC fumigation maintained *F. velutipe*s morphology.

After harvest, sensory attributes of *F. velutipes* odor deteriorated rapidly [27,28]. Browning, which reduced commercial value and consumer acceptability greatly, is a common phenomenon in *F. velutipes* and other mushrooms [28–31]. In this study, browning degree was measured by spectrophotometry for mirroring the visual change in the sensory attributes of *F. velutipes*. IAITC treatment reduced the degree of browning significantly in *F. velutipe*s. The major causative factors in this process are enzymatic reactions, physical damage, and microbial infestation [29]. PPO promotes browning by catalyzing oxidation of polyphenols to melanin [31]. TYR, also called catecholase or diphenol oxidase, is a member of the PPO family [32]. As a mono- and di-phenolase, TYR catalyzes hydroxylation of monophenols (e.g., L-tyrosine) to 3,4-dihydroxyphenylalanine (L-dopa), and oxidizes L-dopa to dopaquinone in the first two rate-limiting steps of melanin biosynthesis, respectively [18,33]. The findings indicated that 50 μL L^{-1} IAITC (group II) notably reduced PPO and TYR activity during days 1–4, which relieved the degree of browning by reducing oxidation of phenols.

During maturation/aging processes of mushrooms, oxidative damage by ROS causes damage to membrane and alters cell structure and functions [34]. ROS-scavenging enzymes (SOD, POD, and CAT) help to reduce oxidative damage [18,22]. Ascorbic acid (vitamin C) is another powerful antioxidant that inhibits ROS damage and delays senescence [34,35]. IAITC treatment increased the activity of SOD and slowed the decline in POD/CAT activity. Fumigation with IAITC essentially enhanced ascorbic acid content in *F. velutipes* through day 6. These contributed to lower degrees of ROS damage and cell membrane peroxidation. Although phenolic compounds, another important type of antioxidant, scavenge free radicals and suppress lipid peroxidation in mushrooms [21,22], the accumulation of phenolic in *F. velutipes* was suppressed by IAITC. The results were in agreement with previous findings regarding the effects of ITCs on quality preservation of mulberries [11] and strawberries [13]. ITCs did not contribute to the maintenance of phenolic content. MDA is a product of lipid peroxidation and serves as an indicator of cell membrane damage in postharvest mushrooms [18,22]. In this study, IAITC suppressed the accumulation of MDA content in mushrooms, which illustrated that IAITC reduced the damage to cell membrane by ROS. It indicates that IAITC mitigated cell membrane damage in *F. velutipe*s. This could be attributed to the increase/maintenance of SOD and POD/CAT activities and ascorbic acid content under IAITC treatment. Considering the content of chitin, it is speculated that IAITC maintains the structural integrity of the cells.

Intact cells have higher vitality. Control of ripening and aging processes in mushrooms depends on energy metabolism, and tricarboxylic acid (TCA) cycle plays an essential role in ATP production and intracellular energy metabolism. In postharvest mushrooms, the level of energy metabolism is reflected by the activity level of SDH, a rate-limiting enzyme in the TCA cycle [30]. High ITC concentration inhibited biophysical activity by suppressing SDH activity and energy metabolism in *Aspergillus niger* ("black mold" fungus) [14]. In the present study, 250 μL L^{-1} IAITC (group III) had similar effects. As a result, IAITC concentrations 10 and 50 μL L^{-1} (groups I and II) enhanced postharvest SDH activity and ATP production by delaying senescence.

Deterioration of mushroom quality is typically caused by microorganisms (microbes) presenting in culture medium, packaging, or storage environments [28]. *Serratia*, an anaerobic genus, is a frequent cause of "blown pack" spoilage in foods such as vacuum-packaged meats [36]. *Pseudomonas* frequently contributes to quality deterioration of mushrooms by accelerating browning and softening processes [37–39]. In this study, the increasing concentration of IAITC reduced growth of *Serratia*, but not *Pseudomonas* (Figure 4C). *Serratia* growth was also inhibited by three aliphatic ITCs (AITC, methyl ITC, and propyl ITC). Cluster analysis revealed similar antibacterial activities of these ITCs against various genera [10]. Color, firmness, antioxidant bioactivity, and odor in postharvest mushrooms are all negatively affected by microbial spoilage [28,37,39]. IAITC has the potential to delay quality deterioration of mushrooms during storage through its antibacterial activity.

Asp and Glu are MSG-like amino acids that contribute to mushroom flavor profiles [25]. The IAITC-treated groups had increased contents of Asp, Glu, Ser, Met, and Lys on day 8. The major fatty acid components were unsaturated types, particularly PUFAs, as observed previously in other mushroom species. The most abundant mushroom PUFAs in general are linoleic acid and α-linoleic acid, which are essential fatty acids that cannot be synthesized by the human body and therefore have important nutritional value [40]. During the present study, linoleic acid was the predominant fatty acid, followed by α-linoleic acid. Linoleic acid content increased in all groups during storage, and was significantly higher on day 8 in groups I and II compared to CK. We analyzed amino acid and PUFA levels because these are important nutritional components of mushrooms. Asp and Glu, MSG-like amino acids contributing to "umami" taste in *F. velutipes*, were accumulated in the IAITC-treated groups. In many edible mushroom species, linoleic acid is a precursor of 1-octen-3-ol, an aromatic compound that contributes to specific flavors [41]. IAITC treatment evidently helped maintain aroma profiles, and promoted accumulation of PUFAs, in I and II postharvest.

5. Conclusions

To summarize, IAITC is capable in controlling postharvest decay in *F. velutipes*. Inhibition of tyrosinase (TYR) activity by IAITC treatment effectively promoted antioxidant bioactivity and suppressed the browning process. On the other hand, the treatment with IAITC inhibited the expansion of bacterial population on the *F. velutipes* surface, thus alleviating bacterial infection. It is helpful in preserving the nutritional contents in mushroom and maintaining higher levels of amino acids and fatty acids. Furthermore, IAITC could reduce the damage to cells. IAITC treatment (50–250 μL L^{-1}) maintained the tissue structure in *F. velutipe*s by increasing the chitin content and relieving the weight loss by reducing the transpiration rate. The overall effect of 50 μL L^{-1} IAITC was the best. These results imply that IAITC application may provide a new insight for postharvest preservation in mushroom or other agricultural products.

Author Contributions: P.Z.: Data curation, Formal analysis, Investigation, Methodology, Resources, and Writing—original draft. P.W.: Data curation, Formal analysis, Investigation, Methodology, Resources, Writing—original draft, and Writing—review and editing. Q.T.: Data curation, Formal analysis, Investigation, Methodology, Resources, and Validation. T.C.: Data curation, For-

mal analysis, Investigation, Methodology, and Resources. G.T.: Data curation, Formal analysis, and Writing—review and editing. C.Y.: Data curation, Formal analysis, and Funding acquisition. N.Y.: Formal analysis and Writing—review and editing. Q.L.: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing—original draft, and Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The authors are not authorized to release data.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

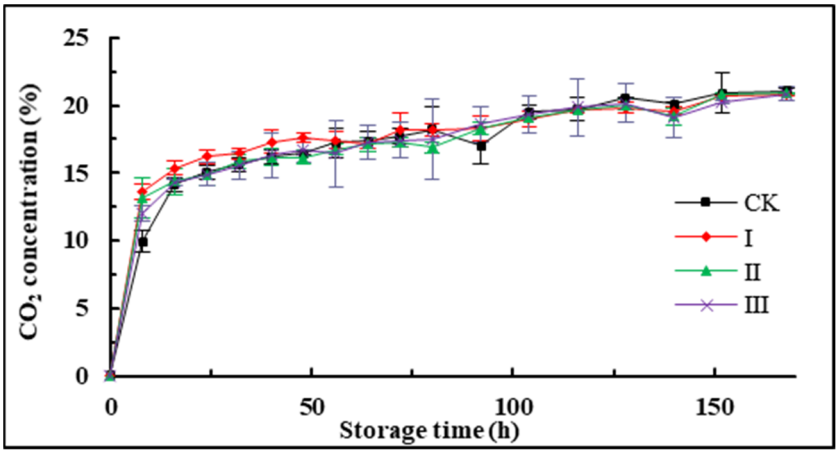

Figure A1. CO_2 concentration in containers stored at 6 °C for the indicated periods following treatment with 10 (group I), 50 (group II), or 250 (group III) μL L^{-1} IAITC, or with distilled water (group CK). Error bars indicate SD calculated from three replicate experiments, and differing letters indicate significant ($p < 0.05$) differences between groups for a given storage period.

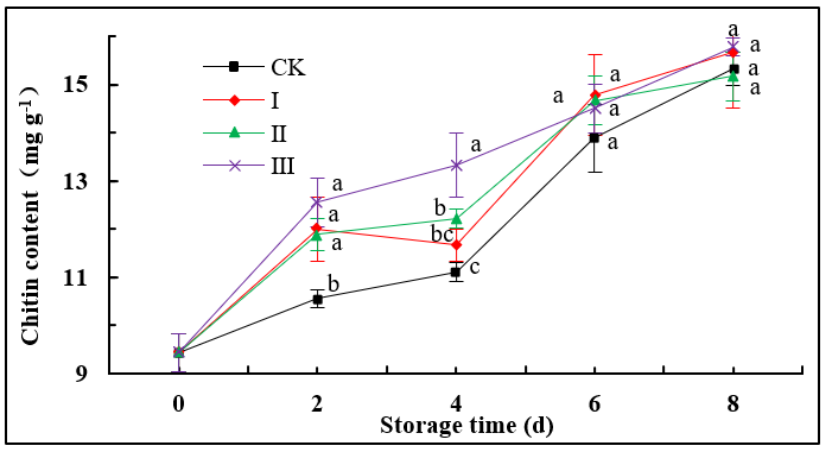

Figure A2. Chitin content in *F. velutipes* stored at 6 °C for the indicated periods following treatment with 10 (group I), 50 (group II), or 250 (group III) μL L^{-1} IAITC, or with distilled water (group CK). Error bars indicate SD calculated from three replicate experiments, and differing letters indicate significant ($p < 0.05$) differences between groups for a given storage period.

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