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RESEARCH ARTICLE

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Influence of Storage Temperature on Levels of Bioactive Compounds in Shiitake Mushrooms (*Lentinula edodes*)

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ABSTRACT

Shiitake mushroom (*Lentinula edodes*) hold high nutritional and medicinal value as they contain an abundance of health-promoting compounds. However, the effect of long-term postharvest storage on the variation in the levels of health-promoting compounds has not been extensively studied. In this study, we investigated the changes in the levels of phenolic compounds, antioxidants, eritadenine, and ergothioneine in shiitake mushrooms stored at three different temperatures (1, 3, and 5 °C) for 4 weeks. Compared to mushrooms stored at lower temperatures, those stored at 5 °C exhibited a higher level of total phenolics in their pileus after 2 weeks of storage; however, storage at 5 °C also increased the deterioration of the fruiting body of these mushrooms. In mushrooms stored at all temperatures, the eritadenine content in the pilei tended to increase up to 2 weeks of storage. In contrast, the ergothioneine content in the pileus decreased during storage, with a significantly lower level detected in mushrooms stored at 5 °C for 4 weeks. Together, these results suggest that the mechanisms underlying the accumulation of phenolics and eritadenine may be related to mushroom deterioration during storage. Our findings indicate that the levels of health-promoting compounds in shiitake mushrooms are influenced by storage temperature, suggesting the potential to control adjustments of specific bioactive compounds by regulating storage conditions.

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1. Introduction

Shiitake (*Lentinula edodes*) is a widely consumed edible mushroom that is cultivated extensively in Korea, Japan, and China, and its production and use have increased in recent years [1,2]. It is nutrient-rich and contains a wide variety of minerals, vitamins, and bioactive compounds that can promote the maintenance of good health and prevent disease [3]. After harvesting, shiitake mushrooms rapidly soften and brown on their surfaces during storage [4], limiting their shelf life. Storage at low temperatures under high humidity can extend the postharvest freshness of mushrooms, prolonging the storage period. Gholami et al. [5] reported that white mushrooms packaged in a modified atmosphere and stored at 4 °C remained fresh for up to 22 d after packaging. Similarly, Azevedo et al. [6] demonstrated that fresh weight loss in oyster mushrooms can be minimized by storage at 2 °C under vapor-saturated conditions. Singh et al. [7] recommended maintaining a low and consistent temperature (above 0 °C) to maximize the longevity of fresh mushrooms. Although low-temperature storage is an effective method for preserving fresh mushrooms, even small variations in low temperature can cause imperceptible quality changes, such as fluctuations in the levels of bioactive compounds. In this study, we explored alterations in several bioactive compounds and antioxidant activity within shiitake mushrooms (*L. edodes* cv. Chungheung 1ho) during storage to elucidate the effect of low-temperature conditions on bioactive compounds in shiitake mushrooms.

2. Materials and methods

2.1. Mushroom material and storage conditions

Freshly harvested shiitake mushrooms (*Lentinula edodes* cv. Chungheung 1ho) were purchased from a mushroom farm in Cheongyang-gun, Chungcheongnam-do, South Korea, and transported to a low-temperature storage facility at the National Institute of Forest Science (Suwon, Gyeonggi-do, South Korea). The mushrooms were packed in polyethylene terephthalate (PET) boxes (320–380 g per box), and stored in plastic containers (6 PET boxes

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per container). Containers were covered with porous, high-density polyethylene film and stored at 1, 3, and 5 °C, respectively, under a relative humidity of approximately 90% for the duration of the experiment.

2.2. Sample preparation

Mushrooms were sliced and dried at 60 °C in an oven (OF-02GW; Jeio Tech. Co., LTD, Daejeon, South Korea) for 2 d. The dried mushrooms were ground to a fine powder using an IKA Multidrive Basic (IKA Korea Ltd., Seoul, South Korea), which was used for subsequent experiments.

2.3. Measurement of total flavonoid and total phenolic content

To extract flavonoids, 0.2 g of the dried shiitake powder was homogenized in 20 mL 80% acetone prior to filtering through Hyundai Micro Filter paper No. 51 (Hyundai Micro Co., Ltd., Seoul, South Korea). The total flavonoid content of the filtered extract was measured using a previously described method [8]. Briefly, 1 mL of the extract was mixed with 4 mL distilled water and 0.3 mL 5% $NaNO_2$ in clean tubes, then incubated for 5 min at 25 °C. Next, 0.3 mL 10% $AlCl_3$ was added to each tube. The tubes were vortexed and incubated for 6 min at 25 °C. Finally, 2.4 mL 1 M NaOH and 2.4 mL distilled water were added to each tube. The tubes were vortexed a second time and the absorbance of the final solution was measured at 510 nm using a spectrophotometer (Epoch 2; Agilent Technologies, Santa Clara, CA). The total flavonoid content was quantified from the absorbance values using a calibration curve constructed with catechin standards (Sigma-Aldrich, St. Louis, MO).

To extract phenolic compounds, 0.2 g of the dried shiitake powder was homogenized in 20 mL acidic MeOH:HCl (99:1, v/v). The mixture was centrifuged at 3000 rpm for 20 min at 10 °C. The total phenolic content of the supernatant was measured using the Folin–Ciocalteu reagent method [9]. In a clean tube, 0.1 mL each of the supernatant, MeOH:HCl, and the Ciocalteu reagent were mixed together. The mixture was incubated for 6 min in the dark at 25 °C, after which 0.7 mL 20% Na_2CO_3 was added. The mixture was then vortexed and incubated for 60 min in the dark at 25 °C. Finally, the mixture was centrifuged at 13,500 rpm for 3 min at 4 °C. The absorbance of the supernatant was measured at 735 nm using a spectrophotometer (Epoch 2). A gallic acid standard (Sigma-Aldrich) curve was used to calculate the total phenolic content.

2.4. Measurement of antioxidant activity

To extract antioxidants, 0.2 g of the dried shiitake powder was homogenized in 20 mL MeOH. The homogenized mixture was filtered through Hyundai Micro Filter paper No. 51 (Hyundai Micro Co., Ltd.), and the flow-through (final extract) was used to measure antioxidant activity. The 2, 2′-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity was measured as described by Stoilova et al. [10] with some modifications. The final extract (1 mL) was added to an equal volume of 0.3 mM DPPH. The mixture was incubated at 25 °C for 30 min. After incubation, the absorbance of the mixture was measured at 517 nm using a spectrophotometer (Epoch 2) and the absorbance value was converted to DPPH scavenging activity (%).

The 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging activity was determined according to the method described by Biglari et al. [11], with some modifications. ABTS (7 mL) was dissolved in potassium persulfate (2.45 mM) and incubated in the dark at 25 °C for 16 h. The ABTS solution was diluted in 70% ethanol to obtain an absorbance of approximately 1.5 at 734 nm. The final extract (200 µL) was added to the diluted ABTS solution (1 mL) and the reaction mixture was incubated at 25 °C for 6 min. Immediately after the reaction, the absorbance of the mixture was measured at 734 nm using a spectrophotometer (Epoch 2), and the absorbance value was converted to ABTS scavenging activity (%).

2.5. Determination of eritadenine content

To extract eritadenine, 0.2 g of the dried shiitake powder was homogenized in 20 mL distilled water. The mixture was filtered using Hyundai Micro Filter paper No. 51 (Hyundai Micro Co., Ltd.), and the flow-through was passed through a syringe filter (0.45 µm pore size) prior to analysis. Ultra-high performance liquid chromatography (UHPLC) analysis of each sample was performed using a Ultimate 3000 UHPLC system (Thermo Fisher Scientific, Waltham, MA) equipped with a Luna®C18 (150 × 3.0 mm, 3.0 µm) column (Phenomenex, Torrance, CA). Conditions were as follows: temperature, 40 °C; flow-rate, 0.3 mL min^{-1}; UV detection: 260 nm. The initial mobile phase consisted of 0.1% trifluoroacetic acid (TFA) in water and 0.1% TFA in acetonitrile (ACN) at a ratio of 100:0 (v/v), and was changed linearly to a ratio of 92:8 over 8 min. The amount of eritadenine in the extract was quantified using a calibration curve constructed using an authentic eritadenine standard (Santa Cruz Biotechnology, Inc., Dallas, TX).

2.6. Determination of ergothioneine content

To extract ergothioneine, 0.2 g of the dried shiitake powder was homogenized in 20 mL boiling distilled water. The mixture was filtered through Hyundai Micro filter paper No. 51 (Hyundai Micro Co., Ltd.), and the flow-through was passed through a syringe filter (0.45 µm pore size) prior to UHPLC analysis. UHPLC was performed using a Ultimate 3000 UHPLC system (Thermo Fisher Scientific) equipped with an ACQUITY UPLC® BEH HILIC (2.1 × 150 mm; 1.7 µm) column (Waters, Milford, MA). Conditions were as follows: temperature, 35 °C; flow-rate, 0.2 mL min^{-1}; UV detection: 254 nm. The mobile phase was 0.1% formic acid in water and ACN (20:80; v/v). The amount of ergothioneine in the extract was quantified using a calibration curve constructed using an authentic ergothioneine standard (Merck, Darmstadt, Germany).

2.7. Statistical analysis

All data are presented as mean ± standard deviation (*SD*). A one-way analysis of variance (ANOVA) with Tukey's honest significant difference test was used to assess the statistical significance of differences between groups using GraphPad Prism 9 (GraphPad Software, Boston, MA).

3. Results and discussion

Freshly harvested shiitake mushrooms were packaged and stored at 1, 3, and 5 °C, respectively, at a relative humidity of approximately 90%, for 4 weeks. The total flavonoid content of both the pilei and stipes increased over 4 weeks, irrespective of the storage temperature (Figure 1). Following 1 week of storage, the total flavonoid content of the pilei of mushrooms stored at 3 °C was significantly higher than that of those stored at the other temperatures. However, after 2 weeks, their flavonoid content became comparable to that of mushrooms stored at the other temperatures. In contrast, the total flavonoid content of stipes only exhibited a significant difference between storage temperatures after 4 weeks, and was highest in mushrooms stored at 3 °C. Among the pilei of mushrooms stored at different temperatures, the highest total phenolic content was observed in that of those stored at 5 °C for 2 weeks; however, their content decreased after 3 weeks. The total phenolic content in the pilei of mushrooms stored at 1 and 3 °C, respectively, increased gradually during the storage period, with that of those stored at 3 °C exhibiting a significantly higher total phenolic content than the pilei of mushrooms stored at 5 °C after 4 weeks. The total phenolic content in the stipes of mushrooms stored at 5 °C increased during the first 2 weeks of storage, and reached significantly higher levels than that of those stored at other temperatures. However, it decreased from 3 weeks onwards, and at 4 weeks, there were no significant differences in the level of total phenolic compounds in the stipes of mushrooms stored at different temperatures. During storage, changes in the 2,2′-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging activities showed similar trends in both the pileus and stipe. Both DPPH and ABTS increased until 3 weeks of storage, followed by a decline at 4 weeks. Notably, in mushroom pilei, the level of antioxidant activity increased as storage temperatures decreased. Phenolics and their derivatives, such as flavonoids, are synthesized from phenylalanine *via* the phenylpropanoid pathway [12,13]. Although phenolic compounds are recognized antioxidants due to their capacity to neutralize free radicals [14], they are closely linked to mushroom browning which is associated with reduced marketability [15]. The increase in phenolic content and activity of polyphenol oxidase significantly contributes to the browning of mushrooms [16,17], during which large amounts of oxidized phenolic derivatives accumulate in fruit tissues [18]. The activity of the enzymes involved in these reactions is downregulated at low temperatures [19]. In *Pleurotus tuoliensis* mushrooms, higher accumulation of total phenolics was observed mushrooms stored at 25 °C than in those stored at 4 °C [20]. Additionally, higher levels of lipid peroxidation were detected at 25 °C [20]. Lipid peroxidation compromises the integrity of mushroom cellular membranes, causing contact between phenolic compounds and enzymes such as polyphenol oxidase, thereby activating the browning process [17]. In this study, browning was particularly prominent in the pilei of mushrooms stored at 5 °C (Figure 2). Both the total flavonoid and total phenolic content of mushrooms gradually increased during the storage period, irrespective of temperature. The highest levels of flavonoids and phenolics were observed in mushrooms stored at 3 °C and 5 °C, respectively (Figure 1). Therefore, it is reasonable to speculate that the accumulation of antioxidants, such as flavonoids and phenolics, is stimulated by disruption of cellular membrane integrity caused by lipid peroxidation in order to recover oxidative damage. Furthermore, the increased activity of polyphenol oxidase in mushrooms stored at 5 °C compared to that in those stored at lower temperatures may have contributed to the more prominent browning of these mushrooms. Although the highest amount of fresh weight loss was observed in mushrooms stored

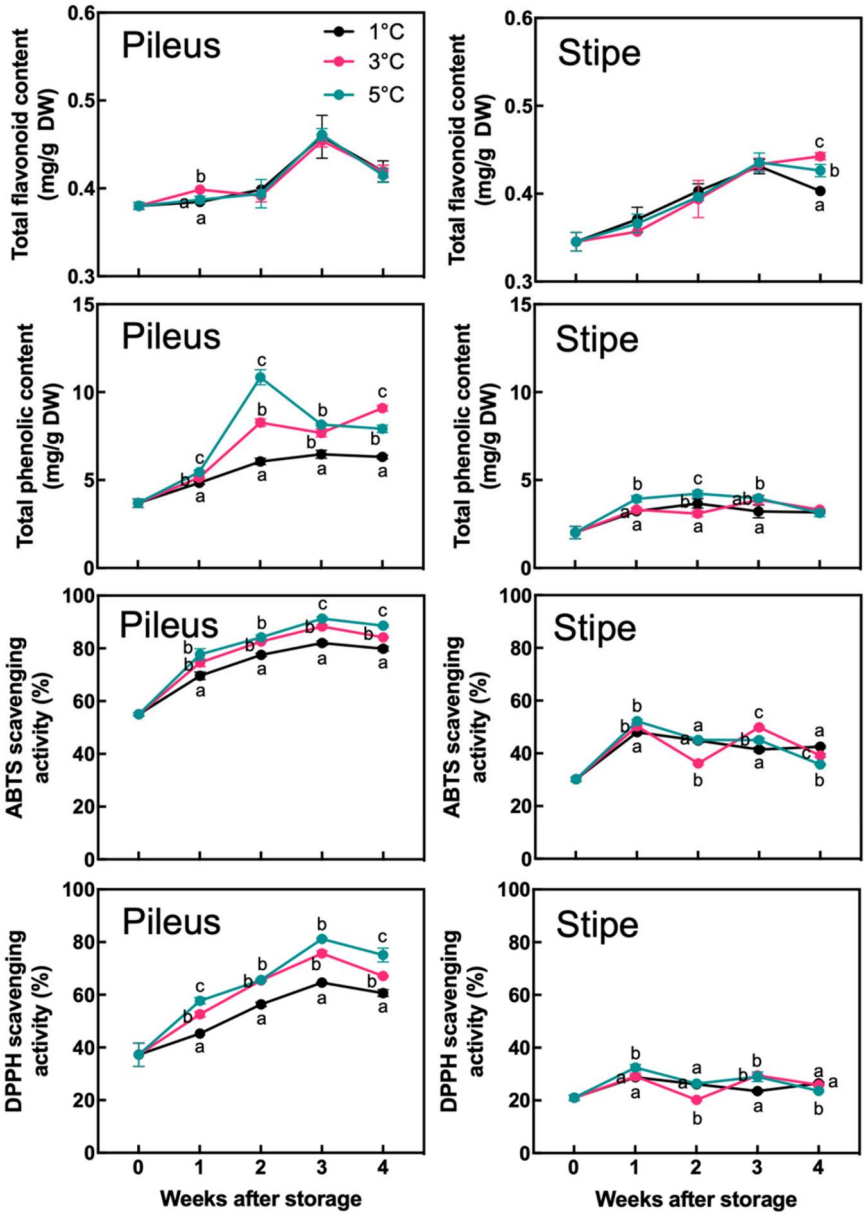

Figure 1. Changes in total flavonoid content, total phenolic content, and antioxidative activity of shiitake mushrooms during storage at 1, 3, and 5 °C, respectively. Different letters indicate significant differences between treatments, calculated using a one-way ANOVA with Tukey's honestly significant difference test ($p < .05$, $n = 3$).

at 5 °C, there was no significant difference in moisture content and firmness between mushrooms stored at different temperatures (Figure S1 in Supporting Information). Mushrooms stored at 1 °C maintained lower respiration rates than those stored at other temperatures. In addition, the fungal score of mushrooms stored at 1 °C increased at a later stage than that of those stored at higher temperatures. Together, these findings support the notion that the postharvest accumulation of flavonoids and phenolics in shiitake mushrooms may correspond to the loss of visible quality during storage.

Shiitake mushrooms contain high levels of eritadenine, a health-promoting purine alkaloid that can reduce blood cholesterol levels [21,22]. The eritadenine content in the pilei initially increased within the first 2 weeks of storage, followed by a slight decrease. A significant difference in eritadenine content between mushrooms stored at different temperatures could only be observed at 3 weeks (Figure 3). The eritadenine content in the stipes increased consistently during storage, and a significant difference between mushrooms stored at different temperatures was observed after 2 weeks. Adenine is a precursor of eritadenine biosynthesis in shiitake mushrooms [23]. The addition of hypoxanthine, a purine compound, significantly increases eritadenine production during liquid fermentation of these mushrooms [24], demonstrating the involvement of purine compounds in eritadenine biosynthesis in shiitake mushrooms. Purine compounds also contribute to the umami taste of edible mushrooms [25]. Adenosine monophosphate (AMP), synthesized from adenine or inosine monophosphate, shows a significant

correlation with the umami intensity of *Pleurotus geesteranus*, which increases with an increase in storage temperatures after harvest [26]. Physiological changes in edible mushrooms occur rapidly at ambient temperatures; the degree of senescence is impacted by storage conditions. This may affect the accumulation of purine compounds, which could explain the variation in the eritadenine content of mushrooms under different storage conditions observed in the present study. However, the precise role of eritadenine in harvested shiitake mushrooms remains to be elucidated.

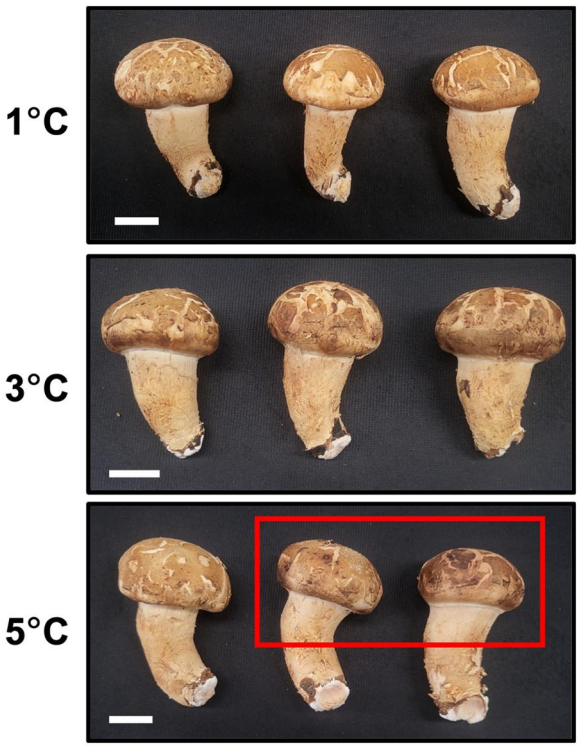

Figure 2. Shiitake mushrooms stored at 1, 3, and 5 °C, respectively, for 4 weeks. Scale bars indicate 2 cm.

Shiitake mushrooms also contain high levels of ergothioneine, a sulfur-containing amino acid with antioxidant activity [27,28]. In this study, the ergothioneine content in the pilei and stipes decreased significantly during the first week of storage (Figure 3). This decrease was the smallest in pilei of mushrooms stored at 1 °C. After the first week, a statistically significant difference between the ergothioneine levels in the stipes of mushrooms stored at different temperatures was observed. However, because the ergothioneine content in the stipes was so much less than that in the pilei, these differences can be considered negligible when the whole fruit is considered. Nevertheless, these results show that storage temperature impacts the ergothioneine content of shiitake mushrooms. The biosynthetic pathway of ergothioneine has been extensively studied in many bacteria and most fungal species. Three amino acids: glutamine, cysteine, and histidine, have been found to be main precursors for ergothioneine production [29,30]. Li et al. [20] proposed that changes in ergothioneine content in *P. tuoliensis* during storage could be attributed to its role in the antioxidant system. This observation is consistent with our findings that mushrooms stored at 5 °C exhibited a notable decline in fruit quality combined with the lowest ergothioneine content among mushrooms stored at

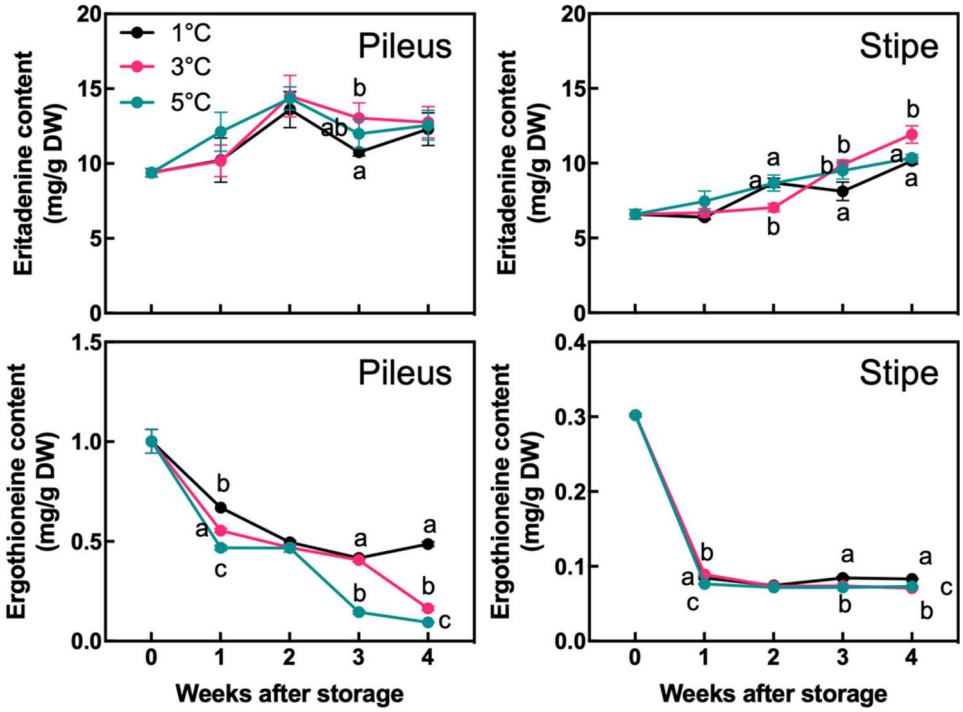

Figure 3. Changes in the eritadenine and ergothioneine contents of shiitake mushrooms during storage at 1, 3, and 5 °C, respectively. Different letters indicate significant differences between treatments, calculated using a one-way ANOVA with Tukey's honestly significant difference test ($p < .05$, $n = 3$).

different temperatures. Although changes in the total free amino acid content of *P. tuoliensis* showed a positive correlation with changes in ergothioneine content during short storage periods (12 d) [20], the exact relationship between the levels of free amino acids and ergothioneine in mushrooms has not been established. Changes in the level of free amino acids during long-term storage remain to be elucidated; however, it is possible that the level and fluctuations of free amino acids in mushrooms may influence ergothioneine content during storage.

The health benefits of shiitake mushrooms, including their antimicrobial, antiviral, anticancer, antidiabetic, antihyperlipidemic, anticholesterol, antioxidant, anti-aging, hepatoprotective, and immunomodulatory properties, have been extensively studied [3]. In the present study, we demonstrated that the levels of health-promoting compounds in shiitake mushrooms were impacted by storage conditions. The storage temperature of edible mushrooms is not only important to prolong their shelf life; it can also be regulated to modulate the levels of specific health-promoting compounds in mushrooms after harvest. Consequently, strategic manipulation of the postharvest environment to enhance specific health-promoting compounds presents a viable strategy to advance the versatile applications of shiitake mushrooms in the health industry.

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Authors' contributions

YK, UL, and HJE conceived and designed the study. YK and HJE performed experiments. YK and HJE analyzed the data and wrote the manuscript. All authors assisted in the interpretation of the results.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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