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Research Article

Innovation Paradigm in Food Preservation: Hypobaric Treatment Effectively Extends Shelf-Life and Inhibits Browning of *Tricholoma matsutake*

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Abstract: The high-value *Tricholoma matsutake* mushroom suffers from severe postharvest deterioration. This study evaluated the efficacy of hypobaric storage (618 Pa, 2 °C, 92% Relative Humidity (RH)) in extending its shelf-life, focusing on the effects of mushroom size (Grades 1-4) and packaging (with or without air-conditioning bags). Whole mushrooms were stored for 28 days, with quality assessments every 7 days. Key quantitative results demonstrated that hypobaric storage effectively limited weight loss to ≤ 9.8% and maintained the browning index below 40% for most groups after 21 days. Unpackaged mushrooms showed the best performance in mitigating weight loss and browning, indicating a paradigm shift away from reliance on physical packaging under controlled hypobaric environments. Larger mushrooms exhibited higher deterioration rates. Hypobaric storage successfully extended the shelf-life to 21 days. This study validates hypobaric storage as a potent, packaging-optimized strategy for extending the marketable life of high-value *T. matsutake*, with direct implications for reducing postharvest loss and improving supply chain flexibility.

***Keywords*:** hypobaric storage, *Tricholoma matsutake*, postharvest quality, shelf-life, browning inhibition

1. Introduction

Tricholoma matsutake, renowned as the "king of fungi," is a globally prized wild edible mushroom with significant economic and nutritional value, attributed to its unique aroma and bioactive compounds associated with health benefits such as immune enhancement and anti-aging properties [1-2]. However, its postharvest potential is severely limited by extreme perishability. Characterized by tender tissue, high moisture content (~ 90%), and the absence of a protective cuticle, harvested matsutake exhibits intense respiration and transpiration rates (Figure 1), leading to rapid water loss, metabolic disruption, and quality deterioration [3-5]. This physiological vulnerability is further exacerbated by enzymatic browning mediated by Polyphenol Oxidase (PPO) and susceptibility to microbial decay, collectively causing a swift decline in sensory and nutritional quality shortly after harvest [6-7]. Consequently, the short shelf-life poses major challenges for long-distance transportation and value preservation within the cold chain, necessitating advanced postharvest technologies.

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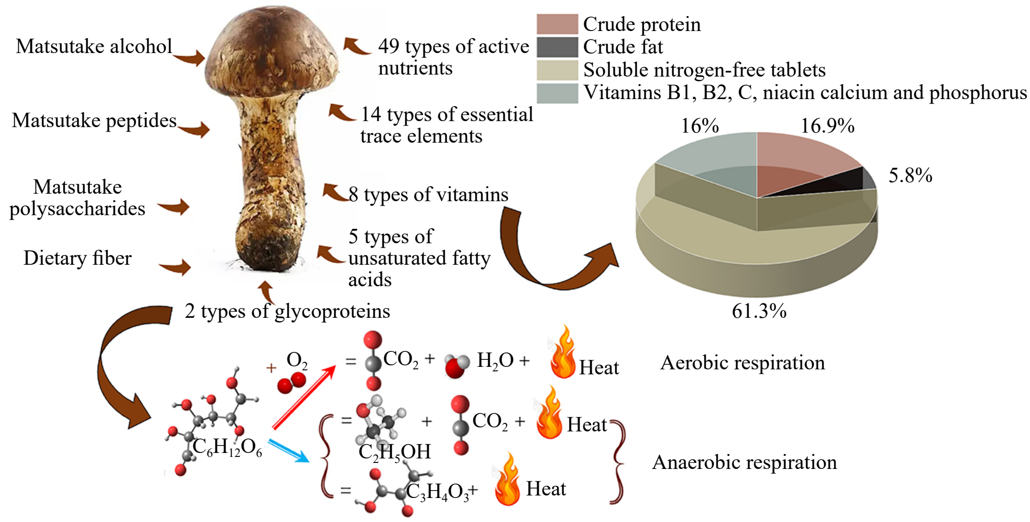

Figure 1. Diagram of nutrient partitioning and Schematic representation based on respiration models in *Tricholoma matsutake* [6, 8]

To address these challenges, various preservation techniques have been explored. Conventional methods such as low-temperature storage, Modified Atmosphere Packaging (MAP), and chemical treatments offer partial solutions but are often inadequate, presenting risks of anaerobic respiration, off-flavors, or raising safety and environmental concerns [9-10]. Research specific to matsutake has applied these technologies with varying success. For instance, studies have optimized MAP materials to extend shelf-life and developed predictive models for quality change [7-8], while antibacterial coatings have been used to improve microbial quality during cold storage [10]. However, these approaches frequently fail to fully address the core physiological drivers of deterioration—intense respiration and water loss—that are critical for this commodity. More recent approaches, like ozone treatment, also face limitations regarding uniform application and potential impacts on the delicate, characteristic aroma of matsutake [11-12]. Therefore, a critical need remains for technologies capable of directly and effectively suppressing metabolism while maintaining optimal humidity.

Among emerging physical technologies, hypobaric (low-pressure) storage has garnered significant attention for its potential to extend postharvest life [13-15]. The principle involves storing produce in a sub-atmospheric environment with continuous humidified air exchange, which reduces the partial pressure of oxygen, thereby suppressing respiration rates and the action of ethylene, a key ripening and senescence hormone [16-18]. Compelling evidence from commodities such as green asparagus [19], blueberries [20], strawberries [21], and Chinese bayberry [22] has demonstrated that hypobaric conditions can effectively delay ripening, reduce weight loss, and preserve nutritional quality more effectively than conventional cold storage alone. Despite these documented successes across diverse produce, the application of hypobaric storage specifically for preserving fresh, whole *T. matsutake* remains scarce and underexplored. A notable gap exists in systematic studies investigating the interaction between *continuous* hypobaric conditions and key intrinsic/commercial factors such as mushroom size (grade) and packaging configuration. This lack of knowledge hinders the development of optimized, practical protocols for industrial cold-chain integration.

Therefore, this study was designed to address these research gaps by systematically evaluating the efficacy of continuous hypobaric storage for whole *T. matsutake*, with a specific focus on the interplay between mushroom size and packaging method. We hypothesize that storage under optimized continuous hypobaric conditions (approximately 618 Pa, 2 °C, 92% Relative Humidity (RH)) will significantly inhibit respiration and transpiration, thereby effectively delaying weight loss, enzymatic browning, and sensory deterioration, and that these effects will be modulated by specimen size and the presence or absence of packaging. The selection of these specific parameters was based on preliminary respiration-suppression trials with matsutake and is supported by their established efficacy in preserving other high-respiration produce; similar low-pressure ranges (500-700 Pa) combined with near-freezing temperatures and high humidity have been shown to optimally retard metabolic activity while minimizing moisture loss [19, 20, 22]. Specifically, this research aims to: (1) quantify and compare the weight loss rates of different size grades of packaged and unpackaged mushrooms under hypobaric storage over 28 days; (2) assess the progression of surface browning to determine the impact of size and direct humidity exposure on PPO-mediated discoloration; and (3) evaluate the sensory quality throughout storage to establish correlations between physiological changes and organoleptic acceptability. By resolving these questions, this work seeks to verify the practical feasibility of hypobaric technology as a robust method for extending the shelf-life and enhancing the commercial value of fresh matsutake.

2. Materials and experimental methods

2.1 *Materials and experimental device*

2.1.1 *Materials*

Biological Material: *Tricholoma matsutake* mushrooms were harvested from Yajiang County, Ganzi Tibetan Autonomous Prefecture, Sichuan Province, China. On the day of the experiment, fruiting bodies with uniform maturity, absence of mechanical damage, no signs of pathogenic infection, and tightly packed morphology were selected and transported to the laboratory on the same day post-harvest.

Packaging Materials: Polyethylene baskets and air-conditioning bags (a type of packaging bag with gas-regulating functions). The air-conditioning bags were made of Low-Density Polyethylene (LDPE) and featured micro-perforations with an approximate diameter of 5-10 μm. According to the manufacturer's specifications, the Oxygen Transmission Rate (OTR) of these bags is in the range of 3,000-4,000 $cm^3/m^2 \cdot day$ at 23 °C and 0% RH. While the internal O_2/CO_2 concentrations within individual bags were not monitored continuously during storage, the operational mode of the hypobaric system ("continuous pumping, continuous humidification, continuous air change") ensured frequent air exchange throughout the entire chamber, thereby preventing the buildup of undesirable gas atmospheres around the packaged samples.

2.1.2 *Experimental setup*

The core equipment used in this study was a hypobaric storage system, the schematic diagram of which is illustrated in Figure 2.

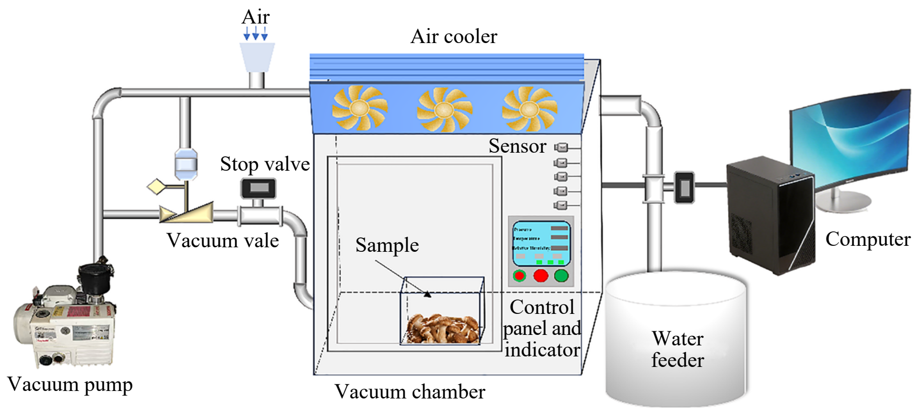

Figure 2. Schematic diagram of the hypobaric treatment system
(Schematic Description: The system primarily consists of a vacuum chamber (storage room), a vacuum pump (for continuous air extraction), a humidifier (for introducing saturated humid air into the chamber), pressure sensors, and a temperature & humidity control system. The entire system operates via "continuous pumping and continuous humidification" to maintain low pressure while preventing sample dehydration)

1) Core Equipment: Hypobaric Refrigerator

Model: JYL0.85A; Pressure Control Accuracy: 0.1 Pa; Hypobaric Technology Type: Continuous pumping type. Its operational mode was "continuous pumping, continuous humidification, continuous air change," ensuring the consistent maintenance of a stable, low-pressure, high-humidity environment.

2) Auxiliary Equipment:

Multifunctional Ozone Generator: Model FG-520 (used for environmental disinfection to prevent microbial contamination).

Electronic Balance: Zhejiang Sartorius System, No. 00000539, weighing accuracy of ± 0.01 g.

The system maintained the set parameters with high stability. The Relative Humidity (RH) was controlled at 92% on average. While minor, transient fluctuations (e.g., briefly approaching saturation during humidification cycles) were recorded, the environment consistently returned to the target setpoint, ensuring overall stability.

2.2 *Experimental methods*

2.2.1 *Material pre-processing*

1) Grading: Mushrooms were graded according to length (Table 1):

Table 1. The grade of mushrooms in this experiment

Grade	Grade 1 (A1)	Grade 2 (A2)	Grade 3 (A3)	Grade 4 (A4)
Length	> 10 cm	9-10 cm	8-9 cm	7-8 cm

2) Grouping and Packaging:

Fresh matsutake from grades 1 to 4 were placed into individual polyethylene baskets equipped with air-conditioning bags, designated as groups A1 to A4, respectively. Each group consisted of 10 mushroom fruiting bodies, constituting the biological replicates ($n = 10$).

Additional Grade 3 fresh matsutake were placed into polyethylene baskets without air-conditioning bags, designated as group A5, also with 10 biological replicates.

3) Storage Parameter Settings: All groups (A1-A5) were stored in the hypobaric refrigerator. The key parameters were set as follows:

Pressure: A target pressure of 600 Pa was set. During continuous operation, the system stabilized at 618 Pa, and this stabilized value is reported throughout the results.

Temperature: 2 °C.

Relative Humidity: 92%.

4) Measurement Intervals: All indicators were measured once every seven days. Each measurement was performed in triplicate, and the average value was calculated.

2.2.2 *Measurement 1: weight loss determination*

Gravimetric method was used to directly measure the mass loss of matsutake during storage, primarily caused by respiration and transpiration. Weight loss rate is a key physical indicator of preservation efficacy. The Procedures were:

1) The initial mass (m_0) of each mushroom sample was recorded.

2) The mass at the n-th measurement (m_n) was recorded.

The weight loss rate (μ) was calculated using the following equation:

$$\mu\ (\%) = [(m_0 - m_n)/m_0] \times 100 \tag{1}$$

where μ is the weight loss rate (%), m_0 is the initial mass of the matsutake (g), and m_n is the mass at the n-th measurement (g).

2.2.3 *Measurement 2: browning index determination*

Browning progression on the surface, cap, and cut sections of the mushrooms was quantified using a standardized visual assessment protocol. This method was employed because the complex morphology and natural color variation of whole *T. matsutake* fruiting bodies make instrumental colorimetry less representative of overall quality; visual assessment is an established and accepted method in postharvest mycology for evaluating macroscopic deterioration.

1) Assessment Procedure: Evaluations were conducted by two trained panelists under consistent, uniform Light-Emitting Diode (LED) lighting conditions to minimize subjective bias. Browning severity for each experimental group was graded according to the scale in Table 2.

Table 2. The browning scale

Grade	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Browning area	0	< 20%	20% ≤ - < 40%	40% ≤ - < 60%	60% ≤ - < 80%	80% ≤

2) Calculation: The browning index was calculated using Equation (2):

$$\text{Browning Index} = [\Sigma\,(p_2 \times s_2)/(p_1 \times S_1)] \times 100 \quad (2)$$

where p_1 is the highest browning grade (5), p_2 is the browning grade, S_1 is the total number of mushroom samples assessed, and s_2 is the number of samples at a specific browning grade.

2.2.4 *Measurement 3: sensory evaluation*

Given the preliminary and exploratory nature of this sensory assessment, and in consideration of the panel size, the results are intended to provide indicative trends that complement the physicochemical data rather than definitive conclusions. A trained panel evaluated key sensory attributes such as color, odor, hardness, and tissue structure to comprehensively assess the commercial value and acceptability of the mushrooms. The Procedure was:

1) Panel: Comprised six members (including 2 collectors, 2 chefs, and 2 consumers), who conducted independent assessments. Due to the limited panel size, statistical analyses beyond descriptive statistics (mean ± standard deviation) were not applied; the focus was on identifying clear directional trends in quality deterioration across treatments and over time.

2) Evaluation Standard: A standardized form using a 4-point scale (1-4) for various attributes, resulting in a total possible score of 16. Detailed criteria are provided in Table 3.

Table 3. Sensory evaluation standards for matsutake

Score range	Luster	Hardness	Tissue structure	Odor
13-16	Stalks and caps are glossy brown, no browning	Cap is elastic and the stalk is hard	Fine surface, tight structure, no open umbrella, no wrinkles, no curling of slices	Fresh flavor
9-12	Normal color, slight browning	Cap elasticity is good; the hardness of the stalk is slightly reduced	Reduced fineness, cap shows signs of opening slightly, no curling	Fresh flavor fades, no off-odor
5-8	Dark color, obvious browning	The cap is less elastic; the stipe is softened	No finesse, obvious cap opening, obvious curling, and wrinkles	Fresh odor disappears, slight off-odor
0-4	Grey color, strong browning, moldy appearance	The elasticity of the cap disappeared, stipe strongly softened	Surface dry, cap completely open, heavily curled and visibly wrinkled	Strong off-odor

3) Ethical Statement: Ethical permission for the human sensory study was not required by our institution. The research did not harm volunteers recruited for sensory experiments, and informed consent was obtained from each subject prior to their participation. Matsutake samples were commercially sourced through certified supply chains.

2.3 *Statistical analysis*

Software: Data were analyzed using SPSS 20.0 (IBM, USA).

Data Presentation: For each treatment group (A1-A5), measurements were performed on all 10 biological replicates. Key physicochemical analyses (e.g., weight loss, browning index) were conducted in triplicate (analytical replicates) per biological replicate. Results are expressed as the mean ± standard deviation.

Significance Testing: One-way Analysis of Variance (ANOVA) was performed on data from biological replicates. Differences were considered statistically significant at $p < 0.05$.

The sensory evaluation criteria presented in this table were adapted from established scoring systems used for mushroom quality assessment in previous studies [6, 23], with modifications to align with the specific sensory attributes and commercial grading practices for *Tricholoma matsutake*.

3. Results and discussion

The efficacy of hypobaric storage in preserving the postharvest quality of whole *Tricholoma matsutake* was systematically evaluated over a 28-day period. The analysis focused on three critical parameters: weight loss (a measure of physical integrity and moisture retention), browning index (an indicator of enzymatic spoilage and visual quality), and sensory evaluation (reflecting overall marketability and consumer acceptance). The interplay between mushroom size (grade) and packaging strategy under hypobaric conditions was revealed to be a decisive factor in determining the storage outcome.

3.1 *Weight loss analysis*

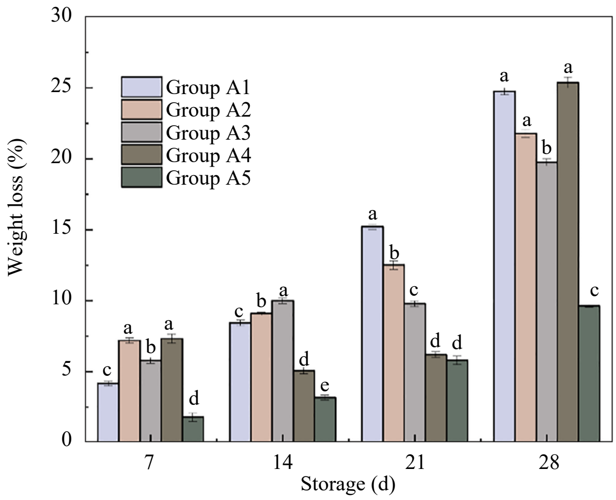

Figure 3. Weight loss of different grades of matsutake versus treatments. Vertical bars represent the Standard Error (SE) (n = 3) of means for three replicates. Different lowercase letters (a, b, c, d, e) indicate differences between days of storage according to the Least Significant Difference (LSD)

Weight loss serves as a primary indicator of postharvest quality deterioration in matsutake, directly resulting from transpiration and respiratory metabolism. Our results demonstrated a progressive increase in weight loss across all experimental groups (A1-A5) throughout the storage period (Figure 3, Table 4), a common trend in postharvest physiology of fresh produce [10]. Weight loss was monitored by measuring each individual mushroom (not on a batch basis) at each time point. To statistically evaluate the effects of storage time, treatment, and their interaction on weight loss, a repeated-measures ANOVA was performed. The analysis confirmed significant main effects of both time and treatment group, as well as a significant time × treatment interaction ($p < 0.01$), supporting the differential weight loss trajectories observed in Figure 3.

Table 4. Effect of treatments on weight loss, browning rate, and sensory scores in matsutake

	7 Days	14 Days	21 Days	28 Days
		Weight loss (%)		
Group A1	4.17 ± 0.17 c	8.43 ± 0.2 c	15.21 ± 0.2 a	24.75 ± 0.25 a
Group A2	7.2 ± 0.2 a	9.1 ± 0.1 b	12.52 ± 0.3 b	21.79 ± 0.29 a
Group A3	5.79 ± 0.2 b	10 ± 0.2 a	9.79 ± 0.2 c	19.75 ± 0.25 b
Group A4	7.32 ± 0.32 a	5.07 ± 0.23 d	6.2 ± 0.2 d	25.37 ± 0.37 a
Group A5	1.79 ± 0.29 d	3.17 ± 0.17 e	5.81 ± 0.3 d	9.63 ± 0.1 c
		Browning rate (%)		
Group A1	7.5 ± 0.5 cd	25 ± 2 b	40 ± 2 b	85 ± 3 a
Group A2	9 ± 1 c	30 ± 3 a	35 ± 3 c	80 ± 2 abc
Group A3	12 ± 1 b	15 ± 3 c	25 ± 2 d	78 ± 3 bc
Group A4	15 ± 3 a	19 ± 2 c	55 ± 4 a	83 ± 3 ab
Group A5	5 ± 0.5 d	10 ± 1 d	20 ± 1 e	75 ± 2.5 c
		Sensory scores		
Group A1	16 ± 0 a	14 ± 0.54 a	10 ± 0.44 ab	7 ± 0.31 ab
Group A2	16 ± 0 a	14 ± 0.45 a	10.2 ± 0.2 a	7.5 ± 0.2 a
Group A3	16 ± 0 a	14 ± 0.32 a	10.4 ± 0.17 a	6.5 ± 0.42 bc
Group A4	16 ± 0 a	14 ± 0.66 a	10.3 ± 0.29 a	7.2 ± 0.2 a
Group A5	16 ± 0 a	12.5 ± 0.38 b	9.5 ± 0.5 b	6 ± 0.29 c

Larger mushrooms (Groups A1 and A2) consistently exhibited higher weight loss rates. By day 21, cumulative loss reached 15.21% and 12.52% for A1 and A2, respectively, compared to only 9.79% and 6.20% for the smaller A3 and A4 groups. This size-dependent effect can be attributed to the larger surface-area-to-volume ratio of bigger specimens. A greater surface area facilitates higher rates of water vapor diffusion into the environment, driven by the vapor pressure gradient between the mushroom's saturated internal tissues and the external storage atmosphere [17].

The most striking finding concerning weight loss was the superior performance of the unpackaged group A5. Despite being the same grade (Grade 3) as the packaged group A3, A5 exhibited a significantly lower weight loss (5.81% vs. 9.79% at day 21, $p < 0.05$). This result challenges the conventional wisdom that physical packaging is always necessary to reduce moisture loss. The mechanistic explanation lies in the dynamics of vapor exchange. In group A5, the mushrooms were in direct contact with the high-humidity (92% RH) chamber environment. This setup minimized the vapor pressure deficit, the fundamental driving force for transpiration. Consequently, moisture loss was primarily limited to that sustained by respiratory metabolism. In contrast, for the packaged groups (A1-A4), the air-conditioning bag, while designed to maintain humidity, may have acted as a subtle barrier to the complete and instantaneous equilibration of water vapor around each mushroom. This could create microclimates with marginally lower humidity right at the mushroom surface, sustaining a steeper vapor pressure gradient and thus accelerating weight loss. The weight loss observed under our hypobaric conditions compares favorably with data reported for mushrooms under conventional cold storage. This observation aligns with the mass transport principles discussed by Burg and Davenport [17] for hypobaric systems, where direct exposure to a saturated atmosphere is most effective in curbing water loss.

Notably, the weight loss in all hypobaric-stored matsutake groups was markedly lower than values reported for other mushroom species under conventional cold storage. For instance, Azevedo et al. [23] reported an 8.35% weight loss in oyster mushrooms after just 5 days at 2 °C, and Liu et al. [24] observed a 5.34% loss in shiitake mushrooms after 18 days at 4 °C. Our results, showing losses between 5.81% and 15.21% after 21 days at 2 °C under hypobaric conditions, strongly suggest that hypobaric storage is more effective in mitigating water loss, thereby extending the commercial shelf-life.

The sharp increase in weight loss after day 21 across all groups suggests this time point may represent a critical physiological threshold or 'quality turning point' for *T. matsutake* under these hypobaric conditions, beyond which cellular integrity deteriorates rapidly. This is supported by the concomitant, precipitous decline in sensory scores (Section 3.3). A post hoc exploratory analysis revealed a strong negative linear correlation between cumulative weight loss and sensory scores at day 21 (e.g., $R^2 > 0.82$ for packaged groups A1-A4, exceeding 20%, and A5 reaching 9.63% by day 28), indicating that weight loss is a key predictor of overall quality perception. However, this observational relationship warrants validation and more sophisticated kinetic modeling in future studies to build robust predictive tools for shelf-life.

3.2 *Browning rate*

The browning reported here refers primarily to the enzymatic discoloration mediated by Polyphenol Oxidase (PPO), which is the dominant form of quality loss in the early and middle storage phases. This is distinct from the senescence-related darkening or mycelial growth observed at the stipe base in Section 3.3. Postharvest browning, mediated primarily by PPO, is a major factor that renders matsutake unmarketable. Our data (Figure 4, Table 4) show that browning severity progressed cumulatively in all groups, but the rate and extent were profoundly influenced by the storage conditions.

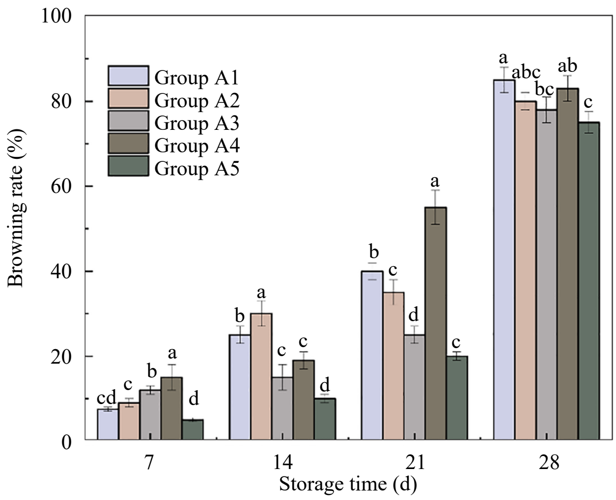

Figure 4. Browning rate of different grades of matsutake versus treatments. Vertical bars represent the Standard Error (SE) ($n = 3$) of means for three replicates. Different lowercase letters (a, b, c, d, e) indicate differences between days of storage according to the LSD

A key finding was that the unpackaged group A5 consistently maintained the lowest browning index throughout the storage period ($p < 0.01$ vs. controls on day 21). During the first 15 days, A5's browning index remained stable within the grade 1-2 range (5% to 20%), while the packaged groups showed more rapid browning progression. This superior anti-browning performance is intrinsically linked to the weight loss data. The direct exposure to the high-humidity environment in the vacuum chamber provided continuous moisture supplementation to the mushroom surface. This effectively counteracted respiratory water loss, helping to maintain cellular turgor and membrane integrity. As a result, the compartmentalization within the cells was preserved, preventing the contact between PPO enzymes and their phenolic substrates, thereby sustaining the phenol-quinone redox homeostasis and suppressing the formation of dark-colored melanin pigments [25-26]. This superior anti-browning performance is intrinsically linked to the mechanisms that suppress enzymatic browning. The direct exposure to high humidity helped maintain cellular integrity, compartmentalizing PPO enzymes from phenolic substrates. Concurrently, the low-oxygen partial pressure in the hypobaric environment directly inhibits the activity of this oxygen-dependent enzyme [27-28].

Browning initiation was observed to begin in the mid-upper stipe region, which possesses higher metabolic activity. The larger-sized specimens in groups A1 and A2, having a proportionally greater biomass of stipe tissue, consequently exhibited accelerated browning kinetics during days 7-21. Interestingly, the smallest mushrooms in group A4, while showing lower overall weight loss, displayed a surprisingly high browning index (55% by day 21). This apparent paradox can be explained by a density effect: in smaller fruiting bodies, the deposition of melanin, once initiated, becomes more concentrated per unit surface area, making the blackening visually more prominent and rapidly pushing the browning index to a higher grade.

The browning behavior observed in our study under hypobaric conditions compares favorably with other preservation methods. Peng et al. [29] reported significant browning (Grade ≥ 3) in *Coprinus comatus* after only 5 days at 4 °C, and *Flammulina velutipes* exhibited severe browning after 15 days at 4 °C [11]. Our hypobaric storage successfully maintained most groups below 40% browning until day 21, demonstrating its potent efficacy in delaying this critical quality defect.

3.3 *Sensory evaluation*

Sensory evaluation provides an indicative, complementary perspective on marketability, integrating color, odor, texture, and aroma to support the instrumental measurements. The sensory scores of all groups followed a parallel decline with storage time (Figure 5, Table 4), mirroring the physiological degradation recorded by instrumental measurements.

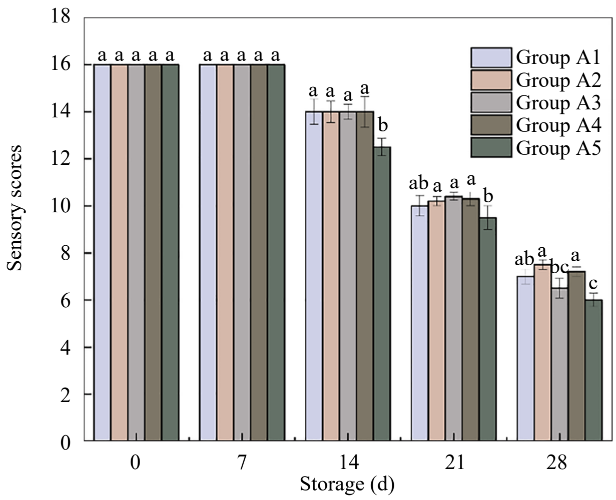

Figure 5. Sensory scores of different grades of matsutake versus treatments. Vertical bars represent the Standard Error (SE) (n = 3) of means for three replicates. Different lowercase letters (a, b, c) indicate differences between days of storage according to the LSD

Progressive senescence manifested during storage, characterized by chromatic deterioration, textural degradation, and mycelial proliferation at stipe bases. On day 14, filamentous mycelial growth manifested exclusively at the stipe bases of unpackaged group A5 specimens. This phenomenon is interpreted not as exogenous microbial contamination—as no foreign mold colonies were observed—but as an intrinsic senescence biomarker. In basidiomycete fungi like *T.*

matsutake, the germination of immature basidiospores or resident hyphae at the stipe base into aerial mycelia under postharvest stress conditions is a documented aspect of continued differentiation and reproductive development [30]. The consistent and localized pattern of this growth across replicates further supports its endogenous origin. Throughout the storage period, no white spots or mold growth occurred on the surface. The low-pressure and low-temperature environment within the vacuum chamber effectively inhibited the life activities of harmful bacteria and fungi. The relative humidity inside the vacuum chamber was unstable, and the relative humidity reached 100% at some times, and the oxygen content inside the vacuum chamber was extremely low, which would accelerate the aging of matsutake (Figure 6). Between days 14 and 21 of storage, fine white spores emerged at the stipe bases across all groups. By day 28, spores developed on most specimens' stipe bases, while no spores were observed on stalks or caps (Figure 7).

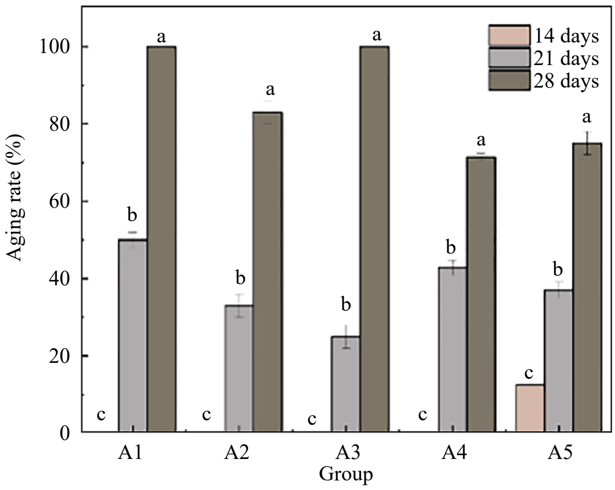

Figure 6. Aging rate of matsutake versus treatments. Vertical bars represent the Standard Error (SE) (n = 3) of means for three replicates. Different lowercase letters (a, b, c) indicate differences between days of storage according to the LSD test, $P < 0.05$

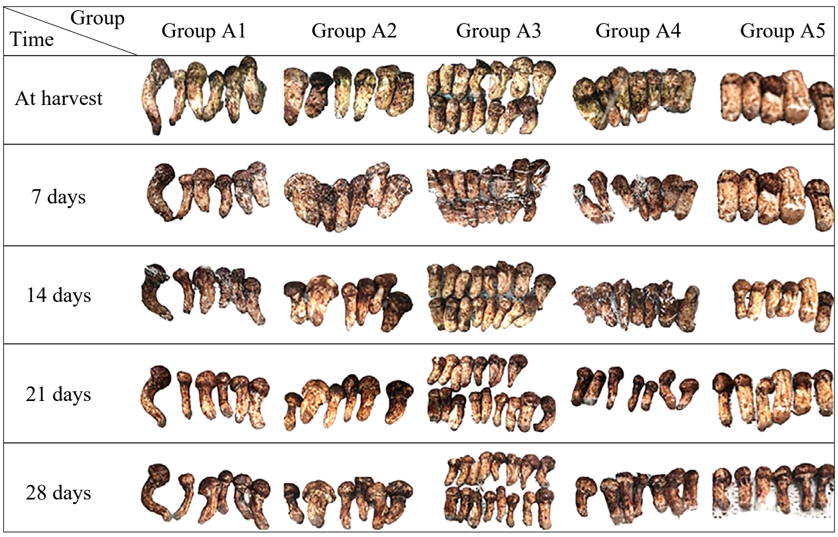

Figure 7. Effect of different treatments on matsutake

During the initial 7 days, all groups maintained excellent sensory scores (16/16), indicating no detectable loss in freshness. By day 14, a critical divergence emerged: group A5, despite its superior performance in weight loss and browning, began to show a significant decline in sensory score (12.5), primarily due to the emergence of filamentous mycelial growth at the stipe base (Figure 7). This phenomenon, absent in other groups at this stage, is not a sign of microbial spoilage but rather an intrinsic senescence biomarker in basidiomycete fungi. Under the high-humidity, low-oxygen stress of the hypobaric environment, the immature basidiospores at the stipe base germinate into aerial mycelia as part of the mushroom's reproductive development [30]. This finding is crucial for practical application, as it indicates that while direct high-humidity exposure is beneficial for retarding water loss and browning, it may inadvertently accelerate certain senescence-related morphological changes in specific mushroom grades.

Between days 14 and 21, all groups exhibited a marked shift in volatile profiles, with the characteristic matsutake fragrance diminishing and musty notes intensifying. This was accompanied by textural softening and shrinkage of stipe diameters, consequences of intensified respiratory water loss, and the reduction in cellular turgor pressure. The hyper-saturated surface condition in unpackaged A5 likely accelerated cuticular degradation, further reducing its sensory score at day 21 compared to A4. By day 28, all groups suffered severe structural collapse and melanization, rendering them commercially worthless (sensory scores ≤ 7.5).

It is noteworthy that no white spots or exogenous mold growth were observed on any specimens throughout the storage period. This suggests that the low-pressure, low-temperature environment within the vacuum chamber was highly effective in suppressing the life activities of harmful bacteria and fungi, a significant advantage over conventional cold storage. Taken together, these exploratory sensory trends indicate that while hypobaric storage effectively preserves initial freshness, the decline in scores after 14 days, particularly in unpackaged samples, underscores the complex interplay between humidity-mediated preservation and senescence progression.

3.4 *Comprehensive discussion and integration of findings*

The present study provides compelling evidence that hypobaric storage at 618 Pa, 2 °C, and 92% RH can effectively extend the shelf-life of whole *Tricholoma matsutake* to 21 days, a significant improvement over the conventional method. The research successfully decouples the effects of specimen size and packaging, revealing nuanced but critical interactions that dictate preservation success.

While direct experimental controls were not included in the present study, our observed weight loss (e.g., ≤ 9.8% after 21 days for unpackaged group A5) under hypobaric storage compares favorably with reported values for mushrooms under conventional cold storage or MAP. For instance, Azevedo et al. [23] reported an 8.35% weight loss in oyster mushrooms after only 5 days at 2 °C, and Liu et al. [24] noted 5.34% loss in shiitake after 18 days at 4 °C. Similarly, Wei et al. [8] observed significant browning and sensory decline in pine mushrooms under MAP within 15 days. These comparisons further underscore the potential of hypobaric conditions to better maintain postharvest quality in highly perishable fungi such as *Tricholoma matsutake*.

The central finding is the dual role of humidity. On one hand, the maintenance of a near-saturated atmosphere is paramount for inhibiting weight loss and enzymatic browning, as unequivocally demonstrated by the superior performance of the unpackaged A5 group in these metrics. This finding complements the work of Wei et al. [8], who emphasized the importance of humidity control in MAP, but our results suggest that a well-managed hypobaric environment can achieve this more effectively without the risk of anoxic conditions. On the other hand, the same high-humidity condition that preserves moisture and color can trigger premature myceliation at the stipe base, negatively impacting sensory perception. This indicates a delicate balance must be struck, potentially suggesting that the optimal humidity level might be dynamic or grade-specific.

The positive correlation between browning incidence and specimen size underscores the importance of pre-storage grading. Larger, more mature mushrooms are inherently closer to senescence and possess physiological traits (e.g., larger stipe volume, higher metabolic load) that make them more susceptible to quality deterioration, consistent with the maturation indices discussed by Yang et al. [31] and Wang et al. [32]. Therefore, for long-distance transport and extended storage, prioritizing medium and smaller-sized matsutake (Grades 3 & 4) would yield better quality retention.

The expanded discussion reveals a nuanced trade-off between packaged and unpackaged storage under hypobaric conditions, where humidity management emerges as the pivotal factor. Direct exposure to the high-humidity (92% RH) environment, as in unpackaged group A5, minimized the vapor pressure deficit at the mushroom surface, thereby effectively curtailing transpiration-driven weight loss and suppressing enzymatic browning—a finding that challenges the routine use of physical packaging in such controlled atmospheres. However, this same condition of sustained surface moisture appeared to accelerate endogenous senescence processes, manifesting as premature mycelial growth at the stipe base, which negatively impacted sensory scores. Consequently, the superiority of packaging versus no packaging is not absolute but contingent on storage duration and commercial priorities. For short-term storage or when visual quality and weight retention are paramount, unpackaged or minimally obstructive packaging in a precisely humidified hypobaric environment may be optimal. For extended storage, a strategy that perhaps combines initial high humidity to prevent browning with subsequent humidity modulation to deter senescence morphology warrants investigation. This underscores that the design of hypobaric protocols must be factor-specific, moving beyond a binary choice of packaging to integrate dynamic control of environmental variables.

The "continuous pumping, continuous humidification" mode of the hypobaric system used here was instrumental in its success. Unlike static or intermittently vented systems, this approach ensures a constant removal of ethylene and other volatile senescence promoters while steadily replenishing the chamber with fresh, humidified air, thus maintaining a stable, low-O_2, high-H_2O environment that profoundly suppresses metabolic activity [17, 19, 29, 33-34].

The present study demonstrates that hypobaric storage at 618 Pa, 2 °C, and 92% RH can extend the shelf-life of whole *Tricholoma matsutake* to 21 days. The efficacy is co-determined by mushroom size and packaging strategy. Key observational findings include: (1) unpackaged storage resulted in the lowest weight loss and browning index; (2) larger mushrooms exhibited higher deterioration rates; and (3) direct high-humidity exposure, while beneficial for moisture retention, was associated with earlier appearance of stipe-base myceliation, a visual senescence marker.

These observations indicate that humidity management is a pivotal factor. The superior performance of unpackaged mushrooms suggests that minimizing the vapor pressure deficit at the product surface is critical for reducing transpiration. Conversely, the same high-humidity condition may accelerate certain endogenous morphological changes related to senescence. This trade-off underscores that the interaction between hypobaric conditions and product-specific factors is nuanced and not universally linear.

3.5 *Practical implications and limitations*

Practical Implications: This work provides a validated technological framework for the matsutake industry. The findings suggest that for medium-and long-term hypobaric storage, prioritizing medium and smaller mushrooms (Grades 3 & 4) would yield better quality retention. Furthermore, the results challenge the routine use of internal packaging in precisely controlled hypobaric environments, pointing toward the potential of bulk storage with direct humidity control to optimize vapor exchange. This could simplify logistics and reduce packaging costs in applicable cold chains.

Limitations and Future Perspectives: This study has several limitations that delineate directions for future research. First, the evaluation was conducted at a single pressure level; testing a gradient of pressures would help identify an optimal range. Second, the underlying biochemical mechanisms (e.g., PPO activity, phenolic profiles) were not quantified, and the sensory analysis was exploratory. Future work should integrate molecular assays and instrumental analyses (e.g., texture analyzers, electronic nose) to decipher the mechanistic pathways and objectively quantify quality changes. Finally, to address practical risks of bulk storage (e.g., mechanical damage), investigating synergistic strategies such as combining hypobaric storage with antimicrobial edible coatings or intermittent pressure cycles is recommended [35].

4. Conclusion

This study demonstrates that continuous hypobaric storage at 618 Pa, 2 °C, and 92% RH effectively extends the marketable shelf-life of whole *Tricholoma matsutake* to 21 days by significantly delaying weight loss and browning. A key finding is that, contrary to conventional practice, unpackaged storage under these conditions provided superior preservation of physical quality, highlighting the critical importance of direct vapor exchange in a controlled high-humidity environment. Furthermore, mushroom size was established as a decisive factor, with larger specimens exhibiting higher deterioration rates, which underscores the necessity of implementing grade-specific cold chain strategies.

It is important to acknowledge the limitations of this work. The study was conducted at a single pressure level, and did not include microbiological analyses or biochemical quantification of underlying mechanisms (e.g., PPO activity, phenolic compounds). The sensory validation was also exploratory in nature. Future research should therefore focus on multi-pressure optimization, integrate molecular and microbial assessments, and employ more robust sensory methodologies. These steps will further elucidate the mechanisms involved and facilitate the translation of hypobaric technology into a reliable, optimized solution for reducing postharvest losses in the high-value matsutake industry.

Ethical statement

Ethical permission, to conduct a human sensory study, is not a requirement of our institution. Our research institution does not harm volunteers recruited for sensory experiments. The food we purchase is all qualified food from the regular channels of market inspection and quarantine.

Data availability

The datasets supporting the conclusions of this article and the sequencing data correspond with Figures will be made available upon reasonable academic request within the limitations of informed consent by the corresponding author upon acceptance.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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