Preservation Effect of Ginger Essential Oil Solution on Pre-prepared *Lentinus edodes* Dishes

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Abstract [**Objectives**] To introduce a ginger essential oil solution possessing a dual "antibacterial-antioxidant" protective effect, and investigate its preservation effect on fresh-cut pre-prepared *Lentinus edodes*. [**Methods**] Single-factor experiments combined with response surface methodology were conducted to screen the optimal concentration of ginger essential oil, using sensory score, total phenol content, and polyphenol oxidase (PPO) activity as evaluation indicators. A verification test was carried out to compare the preservation effects of the optimal concentration treatment group and the control group under the storage condition at 25 ℃. [**Results**] Response surface analysis determined that the optimal concentration of ginger essential oil was 0.3%. The verification test showed that under the storage condition at 25 ℃, the 0.3% ginger essential oil treatment group extended the shelf life by 4 d compared with the control group. Compared with the control group, the treatment group exhibited a 23.4% increase in total phenol content and a 59.0% reduction in PPO activity, and its sensory quality was significantly superior. [**Conclusions**] The 0.3% ginger essential oil solution effectively maintained the sensory quality of fresh-cut *L. edodes*, increased the content of endogenous antioxidants, and inhibited the activity of browning-related enzymes, thereby extending the shelf life by 4 d. This study provides a new method for the green preservation of edible fungi.

Key words Ginger essential oil, Fresh-cut *Lentinus edodes*, Preservation studies, Antioxidant, Prepared dishes

0 Introduction

Lentinus edodes is a medicinal and edible fungus of the Pleurotaceae family in Basidiomycetes, which possesses rich nutritional value and health benefits[1]. Fresh-cut pre-prepared *L. edodes* dishes refer to a new product in which fresh *L. edodes* are sliced and immediately covered with plastic wrap for direct placement on shelves for pre-sale, falling within the category of raw pre-prepared dishes[2]. However, after slicing, the cell structure of *L. edodes* is damaged, making it susceptible to enzymatic browning and microbial spoilage[3-4]. At room temperature, the shelf life is only 3 d, which limits its commercial circulation. Existing preservation technologies have limitations such as low-temperature refrigeration with high energy consumption, modified atmosphere packaging requiring complex operations (precise regulation of O_2/CO_2 ratios), and chemical preservatives raising safety concerns[5].

Studies have shown that plant essential oils, as natural plant-based additives, generally possess good *in-vitro* antibacterial activity and antioxidant capacity[6]. Our team previously screened 37 plant essential oils and found that ginger essential oil exhibited relatively significant inhibitory effects against fungi ($P < 0.01$). Li *et al.*[7] reported that essential oils show good inhibitory effects on spoilage fungi of *L. edodes in vitro*, and can influence the post-harvest physiology and quality of *L. edodes*. Therefore, leveraging the antibacterial and antioxidant properties of ginger essential oil, a preservation solution (referred to as ginger essential oil solution) was developed using ginger essential oil as the main raw material. Preliminary studies have confirmed that this preservation solution can effectively extend the storage life of fresh-cut pre-prepared *L. edodes* dishes, and is characterized by good safety, non-toxicity, high volatility, and low residue.

This paper mainly reports the preparation of the preservation solution, the screening of the appropriate concentration range of ginger essential oil in the preservation solution by the single-factor method, and the investigation of the interactive effects of ginger essential oil concentration, storage temperature, and storage time on fresh-cut *L. edodes* through response surface methodology. The optimal concentration of ginger essential oil in the preservation solution was thereby verified and determined. The verification indicators mainly included sensory score, total phenol content, and polyphenol oxidase activities. This study will provide a reference for the development of a novel "natural antibacterial-antioxidant dual-function preservative", and also offer a new solution for the green preservation of edible fungi.

1 Materials and methods

1.1 Materials and reagents The materials used included fresh *L. edodes* [Guoxu Dayangchu (Hebei) Prepared Ingredients Co., Ltd.], ginger essential oil (Ji'an Xusheng Spice Oil

Received: March 17, 2026 Accepted: June 28, 2026
Supported by Innovation Team for Edible Fungi Preservation and Processing of Hebei Modern Agricultural Industry Technology System (HBCT2023090208); Special Fund for Basic Scientific Research in Colleges and Universities of Hebei Province (JYT202503).
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Co., Ltd., Jiangxi, purity > 99.9%), plant total phenol detection kit, PPO detection kit (Nanjing Jiancheng Bioengineering Institute), *etc.* The reagents, Tween, monoglycerides, and other food-grade emulsifiers, were all of food grade.

1.2 Instruments and equipment TGL-20M benchtop high-speed refrigerated centrifuge (Hunan Xiangyi Laboratory Instrument Development Co., Ltd.); 722G visible spectrophotometer (Shanghai Yidian Analytical Instrument Co., Ltd.); SQP analytical balance (Sartorius Scientific Instruments Co., Ltd.); 85-2AS digital display constant temperature magnetic stirrer (Tuohe Electromechanical Technology Co., Ltd.); KQ-300DE digital ultrasonic cleaner (Kun Shan Ultrasonic Instruments Co., Ltd).

1.3 Experimental methods

1.3.1 Preparation of ginger essential oil solution. Preparation method for 0.1% ginger essential oil solution (100 mL): 99.40 g of water was first weighed, and 0.50 g of food-grade emulsifiers such as Tween and monoglycerides were added. The mixture was stirred at 50 ℃ at a speed of 1 000 r/min for 15 min, and 0.10 g of ginger essential oil was precisely added. Next, the mixture was then stirred at 75 ℃ for 15 min, cooled to 55 ℃, and stirred at 10 000 r/min for 10 min. At last, the mixture was cooled to obtain the solution. The preparation methods for 0.2%, 0.25%, 0.3%, and 0.4% ginger essential oil solutions were the same as above.

1.3.2 Sample preparation and grouping. Preparation of fresh-cut mushroom prepared dishes: Fresh *L. edodes* that were mature, uniform in size, and free from pests and diseases, and mechanical damage were selected and sliced into approximately 2 – 3 mm thin slices, which were then placed on plates. The slices were sprayed with ginger essential oil solutions at different concentrations (0.1%, 0.2%, 0.25%, 0.3%, and 0.4%), covered with plastic wrap, and stored at room temperature for observation (this condition simulated a supermarket shelf environment). Samples sprayed with the same volume of distilled water under the same conditions were used as the control group. Samples from the five treatment groups and the control group were taken on days 1, 3, 6, and 9 for sensory evaluation, determination of total phenolic content, and measurement of PPO activity. Three replicates were set for each group, and the results were analyzed by analysis of variance (ANOVA) for multiple group comparisons.

1.4 Evaluation indicators

1.4.1 Sensory evaluation. As storage time increases, the color, texture, and off-odor intensity of fresh-cut pre-prepared *L. edodes* dishes will also change. Therefore, sensory quality is an important indicator for evaluating the quality of pre-prepared *L. edodes* dishes. The sensory scoring criteria are shown in Table 1.

Table 1 Sensory evaluation table for fresh-cut *Lentinus edodes*

Level	Score//points	Color	Texture change	Odor intensity
1	80 – 100	Milky yellow, good brightness, uniform color	Plump and elastic, with smooth surface	No off-odor, strong mushroom aroma
2	60 – 80	Milky yellow, reduced brightness, slight browning	Slightly elastic, slightly sticky, with speckles	No off-odor, weak mushroom aroma
3	40 – 60	Reddish brown, poor brightness, dull color, deepened browning	Softened, loss of elasticity, slightly sticky	Slightly sour odor
4	< 40	Dark brown, no brightness, severe browning	Mushy, fragile, decayed	Sour and putrid odor, pungent

1.4.2 Determination of total phenol content. Total phenols are important nutrients in *L. edodes* and serve as endogenous antioxidants. An increase in their content can delay oxidative browning of *L. edodes*[8]. The determination of total phenol content was carried out with reference to the experimental method of Gao *et al.*[9], in combination with the plant total phenol kit method.

1.4.3 Determination of PPO activity. The PPO value of fresh-cut *L. edodes* increases with prolonged storage time. A higher PPO value indicates a faster browning rate of *L. edodes*, as PPO is the primary enzyme responsible for browning in *L. edodes*. Under oxygen catalysis, it rapidly combines with substrates to accelerate tissue browning[10]. The determination of PPO activity was carried out with reference to the experimental method of Huang *et al.*[11], in combination with the plant PPO kit method.

1.5 Response surface design Based on the single-factor experiments, the Box-Behnken design was adopted for experimental design. The concentration of ginger essential oil solution, storage temperature, and storage time were selected as independent variables, and sensory score, total phenol content, and PPO activity were used as response values. A three-factor three-level response surface design table was constructed (Table 2). The results were analyzed and optimized using Design-Expert 8.0.6.1 software.

Table 2 Levels of factors in the response surface design

Level	Concentration A//%	Storage temperature B//℃	Storage time C//d
–1	0.1	4	1
0	0.2	15	3
1	0.3	25	5

1.6 Verification test To evaluate the preservation effect of the ginger essential oil solution at the optimal concentration determined by response surface optimization, and to determine the maximum storage period of fresh-cut pre-prepared *L. edodes* dishes, the verification test was conducted at room temperature (25 ℃). Fresh-cut *L. edodes* slices treated with the optimal concentration of ginger essential oil solution were used as the treatment group, while untreated slices under the same conditions served as the control group. Samples were taken on days 1, 3, 5, and 7 for sensory evaluation, determination of total phenol content, and measurement of PPO activity. Five replicates were set for each group.

1.7 Data processing and analysis Experimental data were

processed and analyzed using SPSS 20.0, Design-Expert 8.0.6, and GraphPad Prism software.

2 Results and analysis

2.1 Results of single-factor experiments Samples of pre-prepared *L. edodes* dishes treated with different concentrations of ginger essential oil solution were taken on days 0, 3, 6, and 9. After analysis of variance (ANOVA) among multiple groups, the results of sensory scores, total phenol content, and PPO activity were obtained as follows.

2.1.1 Sensory scores. As shown in Fig. 1A, on day 0, no significant differences were observed in sensory scores between the treatment groups and the control group ($P > 0.05$). With prolonged storage time, the sensory scores of both the treatment groups and the control group gradually decreased on days 3, 6, and 9. On day 3, the scores of the 0.1% and 0.2% treatment groups were higher than that of the control group ($P < 0.05$). On day 6, the sensory scores of the 0.1%, 0.2%, 0.25%, and 0.3% treatment groups were 77, 73.3, 68, and 65.3 points, respectively, while the control group scored 59.7 points, showing significant differences ($P < 0.05$). On day 9, the score of the 0.1% treatment group was 72.7 points, whereas the control group scored 38.3 points ($P < 0.01$). It indicated that the treatment groups within the concentration range of 0.1% – 0.3% exhibited relatively ideal preservation effects on the sensory quality (color, texture, and odor) of fresh-cut *L. edodes*, although the maximum storage period did not exceed 9 d. In contrast, the 0.4% treatment group showed no significant difference compared with the control group ($P > 0.05$), suggesting that the preservation effect did not continue to increase with the concentration increasing.

2.1.2 Total phenol content. As shown in Fig. 1B, on days 0 and 3, there were no significant differences in total phenol content between the treatment groups and the control group ($P > 0.05$). On day 6, the total phenol contents of the 0.1%, 0.2%, and 0.3% treatment groups were significantly higher than that of the control group ($P < 0.05$). On day 9, the total phenol contents of the 0.2%, 0.25%, and 0.3% treatment groups remained significantly higher than that of the control group ($P < 0.05$). Among these, the 0.3% treatment group showed the most significant effect ($P < 0.01$), with a total phenol content of 7.84 μmol/g on day 6 (compared with 4.40 μmol/g in the control group), and 11.23 μmol/g on day 9 (compared with 6.65 μmol/g in the control group). Therefore, treatment groups with concentrations of 0.1% – 0.3% could stimulate the increase of total phenol content, an endogenous antioxidant, in *L. edodes*.

2.1.3 PPO activity. As shown in Fig. 1C, from day 0 to day 3, there were no significant differences in PPO activity between the treatment groups and the control group ($P > 0.05$), although PPO values in all groups increased significantly. By day 6, the PPO activity in all five treatment groups was significantly lower than that in the control group ($P < 0.05$), with values of 34.4, 34.9, 41.55, 45.5, and 50.7 U/g, respectively, compared with 56.9 U/g in the control group. On day 9, the PPO activity in all five treatment groups increased but remained significantly lower than that in the control group ($P < 0.05$). These results indicated that, under the same storage time, the treatment groups effectively inhibited the browning rate of sliced *L. edodes*.

Based on the above single-factor experiments, it was found that ginger essential oil solution at concentrations of 0.1% – 0.3% had significant effects on the sensory maintenance, total phenol content improvement, and PPO activity inhibition in fresh-cut *L. edodes* compared with the control group ($P < 0.05$). To further precisely determine the optimal concentration of ginger essential oil in the emulsion, storage temperature and storage time were introduced as additional factors, and a three-factor three-level response surface test was carried out.

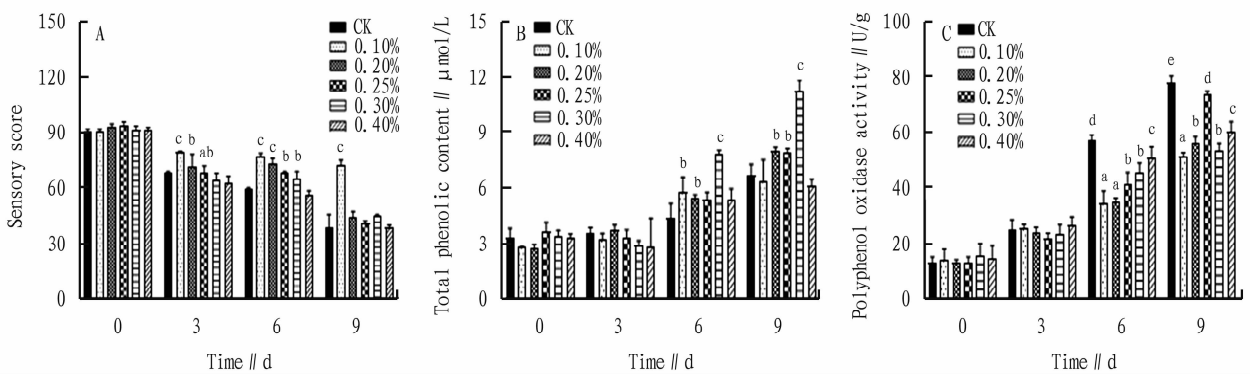

Fig. 1 Single-factor experiments

2.2 Results and analysis of response surface test A Box-Behnken design with three factors and three levels was employed. The experimental design and results are shown in Table 3. Multiple quadratic regression fitting was performed using Design-Expert 8.0.6.1 software, obtaining the regression equations (1) – (3) for sensory score (R1), total phenol content (R2), and PPO activity (R3). The analysis of variance (ANOVA) for the three response values is shown in Table 4 – Table 6.

$$R1 = 75.80 - 4.13A - 4.50B - 12.63C + 1.00AB - 0.75AC - 1.00BC - 0.77A^2 - 0.025B^2 + 3.23C^2 \quad (1)$$

$$R2 = 4.51 - 0.23A + 0.53B - 0.59C + 0.53AB - 0.89AC + 0.39BC - 0.79A^2 - 0.37B^2 + 1.10C^2 \quad (2)$$

$$R3 = 18.00 + 1.75A + 7.33B + 7.58C + 4.17AB + 1.00AC + 3.50BC - 1.50A^2 + 1.33B^2 - 0.50C^2 \quad (3)$$

Table 3 Results of response surface test

Serial Number	Concentration A	Temperature B	Time C	Sensory evaluation//points	Total phenolic content//μmol/g	Polyphenol oxidase//U/g
1	0.2	4	5	75	3.456	14.667
2	0.2	15	3	74	5.046	18.000
3	0.2	15	3	78	4.171	16.000
4	0.1	25	3	76	3.157	18.000
5	0.1	4	3	82	3.548	14.667
6	0.1	15	1	95	4.891	8.000
7	0.2	15	3	75	4.562	17.333
8	0.2	4	1	97	5.593	5.333
9	0.2	15	3	76	4.401	22.000
10	0.3	4	3	72	2.489	9.333
11	0.3	15	5	60	2.972	26.000
12	0.3	15	1	88	5.763	10.000
13	0.1	15	5	70	5.668	20.000
14	0.2	25	1	85	6.247	16.000
15	0.3	25	3	70	4.217	29.333
16	0.2	25	5	59	5.668	39.333
17	0.2	15	3	76	4.355	16.667

Table 4 Analysis of variance for sensory evaluation regression model

Source of variance	Sum of squares	Degree of freedom	Mean squares	*F* value	*P* value	Significance
Model	1 628.29	9	180.99	20.42	0.000 3	**
A – A concentration	136.13	1	136.12	15.36	0.005 8	**
B – B temperature	162.00	1	162.00	18.28	0.003 7	**
C – C time	1 275.12	1	1 275.12	143.85	< 0.000 1	**
AB	4.00	1	4.00	0.45	0.523 3	
AC	2.25	1	2.25	0.25	0.629 9	
BC	4.00	1	4.00	0.45	0.523 3	
A_2	2.53	1	2.53	0.29	0.609 8	
B_2	2.632E – 05	1	2.632E – 05	2.969E – 04	0.986 7	
C_2	43.79	1	43.79	4.94	0.061 6	
Residual	62.05	7	8.86			
Lack of fit	53.25	3	17.75	8.07	0.035 9	*
Pure error	8.80	4	2.20			
Total	1 690.94	16				

NOTE * a significant difference ($P < 0.05$); ** an extremely-significant difference ($P < 0.01$).

Table 5 Analysis of variance for the regression model of total phenol content

Source of variance	Sum of squares	Degree of freedom	Mean squares	*F* value	*P* value	Significance
Model	18.21	9	2.02	11.69	0.001 9	**
A – A concentration	0.42	1	0.42	2.40	0.165 2	
B – B temperature	2.21	1	2.21	12.76	0.009 1	**
C – C time	2.80	1	2.80	16.17	0.005 1	**
AB	1.12	1	1.12	6.49	0.038 3	*
AC	3.18	1	3.18	18.40	0.003 6	**
BC	0.61	1	0.61	3.51	0.103 2	
A^2	2.60	1	2.60	15.03	0.006 1	**
B^2	0.57	1	0.57	3.30	0.112 0	
C^2	5.12	1	5.12	29.58	0.001 0	**
Residual	1.21	7	0.17			
Lack of fit	0.77	3	0.26	2.33	0.215 9	Not significant
Pure error	0.44	4	0.11			
Total	19.42	16				

Table 6 Variance analysis of PPO regression model

Source of variance	Sum of squares	Degree of freedom	Mean squares	*F* value	*P* value	Significance
Model	1 054.42	9	117.16	18.88	0.000 4	**
A – A concentration	24.50	1	24.50	3.95	0.087 3	
B – B temperature	430.21	1	430.21	69.32	< 0.000 1	**
C – C time	460.06	1	460.06	74.13	< 0.000 1	**
AB	69.45	1	69.45	11.19	0.012 3	*
AC	4.00	1	4.00	0.64	0.448 5	
BC	48.99	1	48.99	7.89	0.026 2	*
A^2	9.47	1	9.47	1.53	0.256 5	
B^2	7.48	1	7.48	1.21	0.308 5	
C^2	1.05	1	1.05	0.17	0.692 8	
Residual	43.44	7	6.21			
Lack of fit	21.22	3	7.07	1.27	0.396 6	Not significant
Pure error	22.22	4	5.56			
Total	1 097.87	16				

Regression equations and significance test: The results (Table 4 – Table 6) showed that all three response values, *i.e.*, sensory score (R1), total phenol content (R2), and PPO activity (R3), could be fitted by linear models. The *P*-values of the three response models were all less than 0.01, indicating that the establishment of the three regression equations was statistically significant. Among them, the equations for total phenol content and PPO activity exhibited better fitting (lack-of-fit $P > 0.05$) and could provide relatively accurate predictions.

The coefficients of determination (R^2) for the models of R1, R2, and R3 were 0.963 3, 0.937 6, and 0.960 4, respectively. The adjusted R^2 values (Adj R^2) were 0.916 1, 0.857 5, and 0.857 5, respectively. These results indicated that the measured values showed good fit with the quadratic multiple regression equations established for each factor, and the models could be used for result prediction.

Factor analysis: Based on the *F*-values, the effects of the factors on the three response values (R1-R3) ranked as C > B > A, *i.e.*, storage time > storage temperature > ginger essential oil concentration. The linear terms B and C had significant effects on R1, R2, and R3 ($P < 0.01$), indicating that for fresh-cut *L. edodes*, storage time and temperature were the primary factors affecting slice freshness, and low temperature and shorter storage time played significant roles in maintaining the freshness of *L. edodes* slices. However, the linear term A also had an extremely significant effect on sensory score (R1) ($P < 0.001$), suggesting that the preservation effect of ginger essential oil solution on the sensory quality of sliced *L. edodes* is also worthy of attention (Table 4 – Table 6).

Response surface analysis: As shown in Fig. 2A, the contour plot for the AB interaction was elliptical, indicating a significant interaction effect between temperature and concentration on total phenol content. The highest total phenol content, and thus the best antioxidant capacity and preservation effect, was observed at the red convex point (concentration 0.2%, temperature 15 ℃). Fig. 2B shows that the contour plot for AC interaction was non-elliptical, and therefore, it had no reference significance.

As shown in Fig. 2C, the AB interaction for PPO activity (R3) was significant ($P < 0.05$) (Table 6). With the temperature and concentration increasing, PPO activity also increased, but the upward trend with temperature was steeper than that with concentration, indicating that the effect of temperature elevation on PPO activity was greater than that of concentration change. The AB interaction also revealed that when A = 0.2% and B = 15 ℃, PPO activity was at the central concave point, indicating a relatively optimal preservation control condition.

Fig. 2D shows the BC interaction for PPO activity, indicating that with prolonged storage time and increased temperature, the BC interaction also had a significant effect on PPO activity ($P < 0.05$). PPO activity increased accordingly, which in turn affected the freshness of *L. edodes*.

Prediction: Based on the three multiple regression equations, the optimal storage and preservation conditions for fresh-cut pre-prepared *L. edodes* dishes were predicted through software fitting and optimization as follows: ginger essential oil solution concentration of 0.3%, storage temperature of 25 ℃, and storage time of 3 d. Under these conditions, the predicted sensory score was 67 points, total phenol content was 4.18 μmol/g, and PPO activity was 31.1 U/g.

2.3 Results of verification test To verify the preservation effect and maximum storage period of the 0.3% ginger essential oil solution on fresh-cut pre-prepared *L. edodes* dishes at 25 ℃, a verification test was conducted in comparison with the control group. The results showed that at room temperature, the shelf life of the 0.3% ginger essential oil solution treatment group was 7 d, while that of the control group was only 3 d. Thus, the treatment group extended the shelf life by 4 d compared with the control group (Fig. 3).

After sampling and observation on days 1, 3, 5, and 7, it was found that from day 1 to day 7, the sensory scores of the treatment group were 28.2% higher than those of the control group averagely (Fig. 4A), the total phenol content was on average

23.4% higher (Fig. 4B), and the PPO activity was on average 59.0% lower (Fig. 4C). In specific, on day 7, the treatment group showed a sensory score of 53 points, a total phenol content of 14.3 μmol/g, and a PPO activity of 29.6 U/g, while in the control group, the values were 23 points, 11.4 μmol/g, and 65.7 U/g, respectively. These results indicated that the 0.3% ginger essential oil solution exerted a significant preservation effect on fresh-cut pre-prepared *L. edodes* dishes.

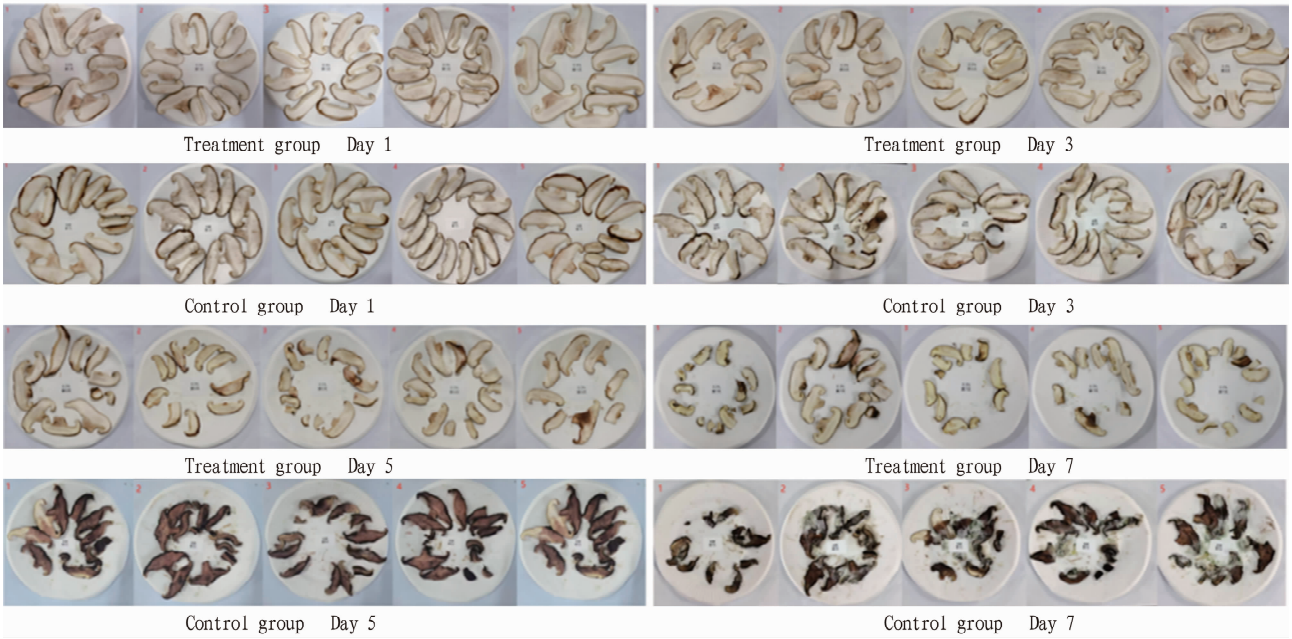

Fig. 2 Response surface plot of the effects of interaction among various factors on total phenol and PPO content

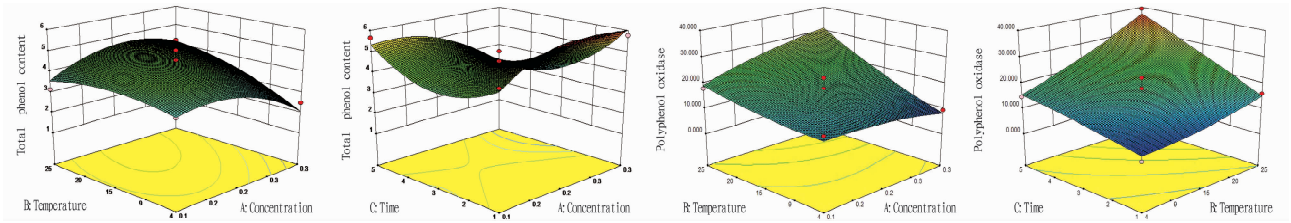

Fig. 3 Verification images of fresh-cut *Lentinus edodes*

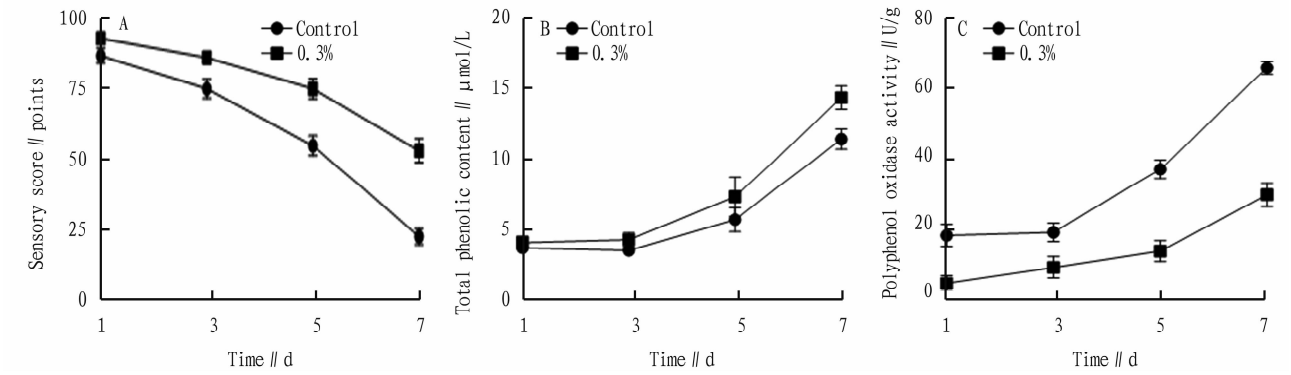

Fig. 4 Comparison of verification indicators for fresh-cut *Lentinus edodes*

3 Conclusion

The above experiments demonstrated that storage time and temperature were the primary factors affecting the freshness of sliced *L. edodes*, and low temperature and short storage time had significant preservation effects on the freshness of sliced *L. edodes*. However, under the same storage conditions (25 ℃), the 0.3% ginger essential oil solution exhibited a relatively good preservation effect on the sensory color, texture, and odor of fresh-cut pre-prepared *L. edodes* dishes, increasing total phenol content by 23.4% and reducing the browning-related PPO activity by 59.0% com-

pared with the control group. This study suggests that ginger essential oil solution can help improve the preservation effect of fresh-cut pre-prepared *L. edodes* dishes, and extend their storage time by 3 – 4 d at 25 ℃.

4 Discussion

The main components of ginger essential oil include various terpenes, alcohols, and aldehydes/ketones, such as shogaol, zingiberol, zingiberene, gingerols, citral, zingiberone shogaols, gingerols, zingiberene, gingerones, and citral, which contribute to the good antibacterial and antioxidant properties of ginger essential oil[12-13]. Its antibacterial mechanism involves altering cell membrane permeability, affecting bacterial energy metabolism, and inhibiting bacterial biofilm formation[14]. In addition, the hydrophobic groups in essential oils can alter the fluidity and permeability of cell membranes, causing ion leakage from cells and ultimately leading to bacterial death[6]. Lu *et al.*[15] confirmed by GC-MS that terpenoids accounted for over 80% of ginger essential oil, which exhibited moderate antibacterial activity against common pathogens such as *Bacillus subtilis* and *Staphylococcus aureus*. Its antibacterial mechanism involved disruption of bacterial cell membrane integrity, leading to leakage of intracellular contents and cell death. This also provides theoretical support for the antibacterial role of ginger essential oil in the preservation of fresh-cut *L. edodes*.

The results of this study showed that ginger essential oil solution could significantly increase total phenolic content and reduce PPO activity in *L. edodes*, which is consistent with the findings of Zhang *et al.*[16], who confirmed that ginger essential oil possesses good antioxidant properties. The application of ginger essential oil solution in preservation also aligns with the conclusions of LU *et al.*[17], who applied ginger essential oil nano-emulsion coatings to preserve young ginger and found that this system effectively inhibited browning-related enzyme activities, increased antioxidant compound content, and reduced microbial diversity and richness, thereby achieving dual preservation effects of antibacterial and antioxidant properties. Therefore, ginger essential oil solution possesses a dual "antibacterial-antioxidant" protective effect, which may provide a reference for the preservation of edible fungi such as *L. edodes*, *Pleurotus ostreatus*, and *Pleurotus eryngii*.

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