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Screening of mixed lactic acid bacteria starter and its effects on the quality and flavor compounds of fermented *Lentinus edodes*

Yuanyang NIE[1], Yajuan JIA[1], Xiunan ZHANG[1], Sen LU[1], Bo LI[1*]

Abstract

The lactic acid bacteria (LAB) with excellent fermented performance were screened for *Lentinus edodes* fermentation by measuring the growth status and acid producing capacity of different LAB in *L. edodes*, and the optimal mixed ratio of LAB was selected for the *L. edodes* fermentation through the mixed fermented test. Factors affecting the quality of fermented *L. edodes* were optimized by single factor experiment and response surface experiment. The results showed that when the mixed ratio of LAB was *Lactobacillus delbrueckii* subsp. *bulgaricus: Lactiplantibacillus plantarum: Lacticaseibacillus rhamnous* = 3:1:2, fermented *L. edodes* had the best fermented quality. The optimized process parameters of mixed LAB fermented *L. edodes* were as follows: fermentation temperature of 37 °C, salt content of 1%, and inoculation amount of 2.7%. Under this process, the total acid and sensory score of fermented *L. edodes* were 0.88 g/100 g and 81.7 points, respectively. Compared with the unfermented *L. edodes*, the contents of acids and ketones in the fermented *L. edodes* increased by 2.16% and 17.8%, while the contents of alcohols, aldehydes and phenols were relatively reduced by 3.66%, 7.42%, and 3.87%, respectively. This study provides a theoretical basis for the development of LAB fermented food of *L. edodes*.

Keywords: *Lentinus edodes*; lactic acid bacteria; fermentation; flavor substances; response surface methodology.

Practical Application: Providing a new way for the deep processing of *Lentinus edodes*.

1 Introduction

Lactic acid bacteria (LAB) is a kind of microorganism that ferments carbohydrate metabolism to produce lactic acid (Wang et al., 2021), and it is also one of the earliest microorganisms used in food processing and preservation (Şanlier et al., 2019). LAB are not only mainly used in the fermentation of dairy products, but also commonly used in the fermentation of fruits and vegetables, including the genus *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Weissella*, and *Pediococcus*, etc. (Wang & Shao, 2018). Cui et al. (2019) studied the effects of different LAB on fermented fruit and vegetable juice, and the results showed that different LAB have different metabolic characteristics and flavor substances, but they all play an important role in the formation of flavor. Duhan et al. (2013) showed that bacteriocin produced by LAB metabolism can effectively inhibit the growth of pathogenic bacteria and improve the food safety. These researches showed that LAB fermentation can obviously improve the flavor, nutrition, and safety of foods. In recent years, with the enhancement of public health awareness, using LAB fermentation to improve the nutrition and quality characteristics of food raw materials has attracted more and more attention.

Lentinus edodes is a kind of edible mushroom with high nutritional value, which contains a variety of nutritional active ingredients, such as polysaccharide, protein, phenolic substances, ergosterol, dietary fiber, etc. (Ke & Chen, 2016; Gaitán-Hernández et al., 2019; Zhu et al., 2019), and has a variety of physiological functions such as anti-tumor, anti-aging, anti-oxidation, immunoregulation, liver protection, etc. (Grotto et al., 2016; Nisar et al., 2017; Li et al., 2019). *L. edodes* is the second most widely consumed edible mushroom in the worldwide (Chen et al., 2021), which is gradually loved by more and more people in recent years because of the unique taste and flavor, especially in East Asian countries. In 2020, the total output of *L. edodes* in China is 11.88 million tons, being the largest yield variety of mushroom (China Edible Fungi Association, 2022). However, there are few reports on the fermentation technology of *L. edodes* with mixed LAB and the development of fermented food. In this study, the LAB species with good fermentation performance were screened by measuring the acid production of different LAB in *L. edodes* culture medium, and then the suitable ratio of mixed LAB starter and fermentation process were determined, and the changes of flavor substances of *L. edodes* before and after fermentation were also compared. The results of this study will be helpful to provide theoretical basis for the development and application of LAB in the fermented food of *L. edodes*.

2 Materials and methods

2.1 Materials and reagents

Fresh *L. edodes*, salt, glucose, skim milk powder were purchased from the local supermarket in Xinxiang City, Henan Province, China. Lyophilized strains of *Lactobacillus delbrueckii* subsp.

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[1] *Henan Institute of Science and Technology, School of Food Science, Xinxiang, Henan, P. R. China*
*Corresponding author: libo@hist.edu.cn

bulgaricus (Lb), *Streptococcus thermophilus* (St), *Lacticaseibacillus rhamnosus* (Lr), and *Lacticaseibacillus casei* (Lc) were purchased from Guangdong Microbial Culture Preservation Center (Guangzhou, China). Lyophilized strains of *Lactiplantibacillus plantarum* (Lp) and *Limosilactobacillus fermentum* (Lf) were provided by Zhengzhou Hehe Bioengineering Technology Co., LTD (Zhengzhou, China). All six LAB are on the list of bacteria that can be used in food released by the China National Center for Food Safety Risk Assessment. MRS and M17 culture medium were purchased from Guangdong Huankai Microbial Technology Co., LTD (Guangzhou, China). Sodium hydroxide, potassium hydrogen phthalate, phenolphthalein, anhydrous ethanol and formaldehyde were purchased from Tianjin Kemio Chemical Reagent Co., LTD (Tianjin, China).

2.2 Preparation of LAB starter

Lyophilized *Streptococci* and *Lactobacilli* strains were dissolved in appropriate sterile water and resuscitated on the surface of M17 and MRS solid medium, respectively. After 48 h of constant temperature culture at 37 °C, it was transferred to M17 or MRS liquid medium. After 48 h of secondary activation culture, it was transferred to 10% skim milk medium at 37 °C for 48 h to form LAB seed starter. *Streptococci* were counted on M17 agar, and *Lactobacilli* were counted on MRS agar (Abdesslem et al., 2020).

2.3 Technological process of L. edodes fermented by LAB

Fresh *L. edodes* were cleaned and drained, and then cut into pieces of 0.5 cm × 0.5 cm × 0.5 cm. 100 mL distilled water was added into 100 g mushroom pieces according to the ratio of material to liquid 1:1, and then 2% (based on the total mass of mushroom and water) of salt and glucose were added, respectively. The culture medium were sterilized at 115 °C for 10 min, and then cooled for later use. 3% (based on the total mass of medium) of LAB seed starter were inoculate into the medium by sterile operation (with 10^7 CFU/g of viable LAB), and then sealed and fermented at 37 °C for 48 h. With no raw mushrooms taste and pH 3.5 of the fermented broth, the fermentation could be ended.

2.4 Determination of total acid and sensory score of fermented L. edodes

Twice mass of distilled water were added into the fermented *L. edodes*, homogenized by a homogenizer (model JYL-C16D, Joyoung, China). The total acid content was determined referring to Lee & Yoo (2017) with slightly modified. The sensory evaluation of fermented *L. edodes* was carried out by 15 professionally trained personnel, and the color, taste, smell, tissue state, and soup color of fermented *L. edodes* were scored. The sensory scoring criteria are shown in Table 1.

Table 1. The sensory scoring criteria of fermented *L. edodes*.

Items	Scoring standards	Sensory score
Color (20 points)	Even color and luster	14-20
	Uniform color and low gloss	8-13
	Dark color and no gloss	≤ 7
Taste (20 points)	Soft acidity, pure, no strange taste	14-20
	Partial acid or no sour taste	8-13
	Strange or peculiar smell	≤ 7
Smell (20 points)	Normal fermentation aroma, no peculiar smell	14-20
	With a lighter fermentation aroma	8-13
	No fermented mushroom flavor	≤ 7
Tissue state (20 points)	Tightly organized and chewy	14-20
	The tissue is loose and chewy	8-13
	Soft collapse of tissue, over-fermentation	≤ 7
Soup color (20 points)	The soup is milky white, not cloudy	14-20
	The soup is slightly yellowish and slightly cloudy	8-13
	The soup is cloudy	≤ 7

2.5 Screening of mixed LAB starter for L. edodes

In order to screen out good LAB strains suitable for the fermentation of *L. edodes*, the growth status and acid production capacity of six LAB strains in *L. edodes* culture medium were determined. In *L. edodes* culture medium, 3% (total mass of feed and liquid) LAB seed starter was added (with 10^7 CFU/g of viable LAB), and the samples were fermented at 37 °C for 48 h. The total acid and OD_{600nm} of the fermented broth were determined every 8 h. According to the fermentation performance, three LAB strains were screened for mixed fermentation test, which were used as mixed LAB starter to ferment *L. edodes* by different inoculation mass ratio (1:1:1, 1:2:3, 1:3:2, 2:1:3, 2:3:1, 3:1:2, 3:2:1). The total acid of fermented *L. edodes* were determined every 8 h.

2.6 Processing conditions optimization of L. edodes fermented by mixed LAB starter

Single factor experiments

The fermentation temperature, salt content, and inoculation amount of were optimized by single factor experiment according to the basic technological conditions of fermented *L. edodes*. After fermentation, total acid and sensory score of fermented *L. edodes* were determined. The fermentation temperature were set as 33 °C, 35 °C, 37 °C, 39 °C, and 41 °C, respectively. The salt contents were set as 0%, 0.5%, 1%, 1.5%, and 2.0%, respectively. The inoculation amounts were set as 2.0%, 2.5%, 3.0, 3.5%, and 4.0%, respectively.

Response surface experiments

According to the single factor test results, Box-Benhken design (BBD) was applied to optimize fermentation temperature (*A*), salt content (*B*), and inoculation amount (*C*) by using software Design-Expert (Trial Version 8.0.6, Stat-Ease Inc., Minneapolis, MN, USA). Each factor was designed with three levels, and the total acid and sensory score of fermented *L. edodes* were used as response values to determine the optimal fermentation conditions. Factors and levels design are shown in Table 2.

Table 2. The factors and levels of response surface test.

Levels	Factors		
	Fermentation temperature (°C) (*A*)	Salt content (%) (*B*)	Inoculation amount (%) (*C*)
-1	33	0.5	2.0
0	35	1.0	2.5
1	37	1.5	3.0

2.7 Determination of flavor substances in fermented L. edodes

Headspace solid phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) were used for the determination, and the method of Choi et al. (2019) was refered.

2.8 Statistical analysis

All experiments were repeated three times, and the results were expressed as mean ± standard deviation. SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis of the experimental data. LSD and Dunnett's T3 method were used for multiple comparison analysis of significant differences. $P<0.05$ was considered as statistically significant. The Box-Behnken response surface optimization test was designed and analyzed by Design-Expert 8.0.6 software (Stat-Ease, Inc., MN, USA).

3 Results and discussion

3.1 Acid production capacity and growth curve of different LAB fermented in L. edodes

By measuring the growth of LAB in *L. edodes* culture medium, it can not only reflect the adaptability of LAB to *L. edodes* fermentation environment (Seo et al., 2021), but also serve as the basis for analyzing the fermentation performance of LAB. As shown in Figure 1a, the total acid gradually increased during the fermentation process of LAB, which of the value was about 0.7 g/100 g at the fermentation end-point. After 48 h of culture, Lp, Lb and Lf grew well in the medium of *L. edodes*, which quickly entered the logarithmic growth phase after 8 h of culture, and the $OD_{600\ nm}$ values were 1.454, 1.435, and 1.245, respectively (Figure 1b). Strain Lr had a slower speed of growth, with the $OD_{600\ nm}$ value of 1.186 after 8 h of culture. On the other hand, Strain St grew more slowly and had a relatively long lag period. Therefore, Lp, Lb, Lf and Lr were selected as the good strains of fermented *L. edodes*.

3.2 Effects of LAB with different mixed proportions on acid production capacity of L. edodes

Mixed fermentation can obtain better quality of products, and adapt to more complex environmental changes with higher stability (Park et al., 2019; Luo et al., 2020; Rothstein et al., 2020). According to the Figure 1c, it can be seen that with the fermentation, the total acid increased gradually, which was in accord with the result of Dan et al. (2019). This might be due to the LAB fermentation of carbohydrate metabolism to produce lactic acid and carbon dioxide, including the glycolysis and pentose phosphate pathway, the CO_2 in the water to form carbonic acid, aldehyde oxidized into acid, which reduces the pH (Martinussen et al., 2013; Liang et al., 2020). The total

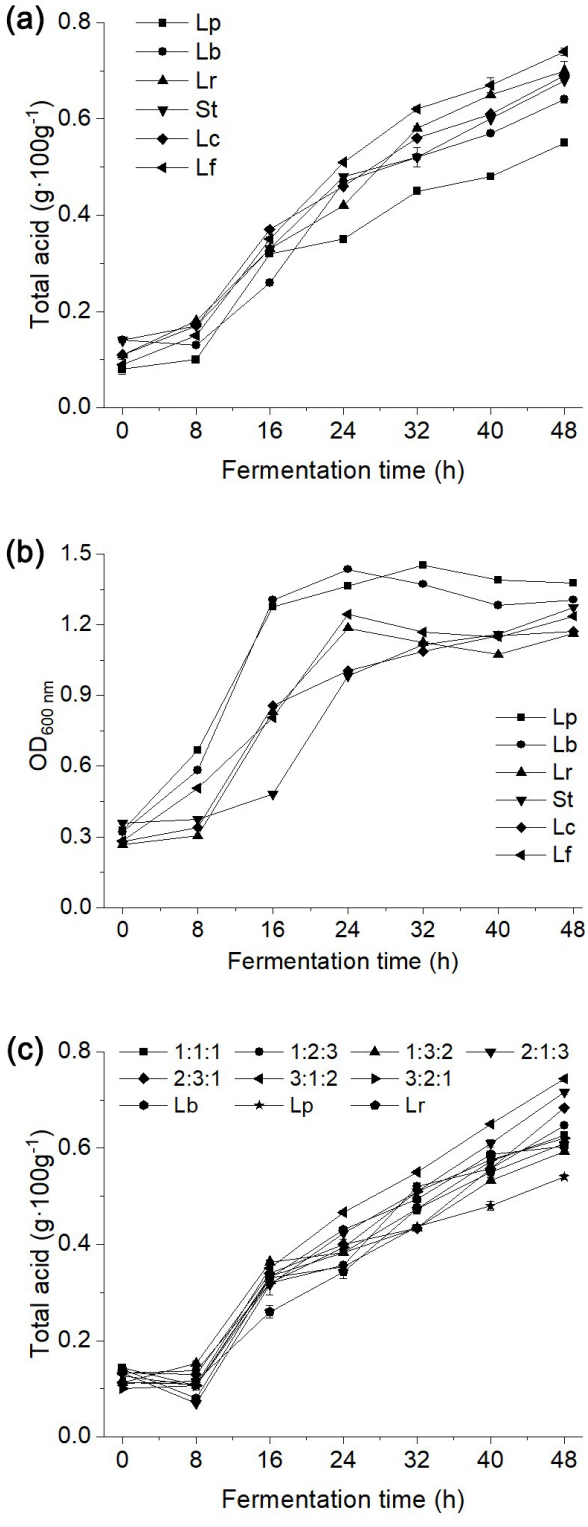

Figure 1. The total acid (a) and growth curve (b) of different LAB fermented in *L. edodes*. (c) Effects of LAB with different mixed proportions on total acid of fermented *L. edodes*.

acid basically increased with the progress of fermentation, but the acid production of LAB with the mixed ratio of 1:1:1 and 1:2:3 suddenly decreased and then gradually increased, which might be caused by the consumption of part of organic acids by LAB in the fermentation process. As shown in Figure 1c, the total acid of the mixed LAB (ratio of Lb: Lp: Lr = 3:1:2) fermentation was the highest at the end of fermentation, which was 0.74 g/100 g. The number of viable LAB of *L. edodes* fermented by mixed LAB starter was up to 10^{10} CFU/g, and therefore, the ratio of Lb: Lp: Lr = 3:1:2 was selected as the mixed LAB starter for the optimization test of optimal fermentation conditions.

3.3 Optimization results of single factor test

As shown in Figure 2, fermentation temperature of 35 °C, salt content of 1.0%, and inoculation amount of 2.5% were the optimal value of each factor according to the comprehensive consideration of the total acid and sensory score of fermented *L. edodes*.

3.4 Optimization results of response surface test

According to the BBD, a total of 17 experimental runs were employed and experiments were performed in a randomized order (Hu et al., 2021). All experiments were repeated for three times. Results were shown in Table 3.

The regression equation obtained from the multiple regression fitting analysis is as follows (Equations 1-2):

$$Y1 = 0.96 + 0.099A - 0.046B - 0.015C - 0.020AB + 0.017AC - 0.040BC - 0.17A^2 + 0.003B^2 - 0.058C^2 \quad (1)$$

$$Y2 = 82.80 - 0.32A + 1.23B + 0.100C - 0.050AB + 0.35AC + 0.50BC - 1.50A^2 - 1.60B^2 - 0.90C^2 \quad (2)$$

According to Table 4, for the two response values of total acid and sensory score, the *P* values of regression equation models were both less than 0.01, indicating that the models were extremely significant. All *P* values of the missing items (Lack of Fit) were greater than 0.05, which was not significant, indicating that the model fitting degree was good (Liu & Li, 2021). The correlation coefficients R^2 and R^2_{Adj} in the regression equation models were 0.7542, 0.8916 and 0.9564, 0.9695, respectively, indicating that the models had high reliability, the experimental results were relatively stable, and the equations could better reflect the relationships between independent variables and response values (Demirci et al., 2022). According to the F value, the effect of various factors on total acid value of *L. edodes* fermented by mixed LAB starter was as follows: fermentation temperature > salt content > inoculation amount, and sensory score was as follows: salt content > fermentation temperature > inoculation amount. According to the *P* value, items *A*, *B*, A^2, and C^2 had extremely significant ($P < 0.01$) effects on the total acid, and item *BC* had significant ($P < 0.05$) effects on total acid, while the other items were not significant ($P > 0.05$). The sensory score were extremely significantly ($P < 0.01$) affected by items *B*, *BC*, A^2, B^2, and C^2, and significantly ($P < 0.05$) affected by items *A* and *AC*, while other items were not significant ($P > 0.05$).

The optimal fermentation conditions of *L. edodes* with LAB mixed starter were obtained through response surface optimization with the goal of strong acid production capacity and high sensory score: fermentation temperature of 37 °C, salt content of 1.08%, and inoculation amount of 2.66%. Under these conditions, the predicted total acid and sensory score was 0.86 g/100 g and 81.2 points, respectively. Considering the convenience of practical operation, the optimal technological conditions were set as fermentation temperature of 37 °C, salt content of 1%, inoculation amount of 2.7%. Verification tests were carried out under these technological conditions, and the total acid and sensory score were 0.88 g/100 g and 81.7 points,

Table 3. The response surface experimental design and results.

Test number	Fermentation temperature (°C) (*A*)	Salt content (%) (*B*)	Inoculation amount (%) (*C*)	Total acid (*Y1*) (g/100 g)	Sensory score (*Y2*) (point)
1	1	-1	0	0.90 ± 0.01	78.0 ± 0.1
2	1	0	-1	0.80 ± 0.00	79.8 ± 0.3
3	0	0	0	0.96 ± 0.01	82.5 ± 0.4
4	1	1	0	0.64 ± 0.00	80.4 ± 0.2
5	-1	0	1	0.66 ± 0.00	80.6 ± 0.1
6	0	0	0	0.82 ± 0.01	82.5 ± 0.3
7	0	-1	-1	0.82 ± 0.00	79.5 ± 0.2
8	-1	0	-1	0.96 ± 0.02	81.0 ± 0.2
9	0	0	0	0.59 ± 0.00	83.0 ± 0.3
10	-1	-1	0	0.96 ± 0.01	78.7 ± 0.1
11	0	-1	1	0.96 ± 0.01	78.9 ± 0.1
12	0	1	1	0.97 ± 0.02	82.1 ± 0.3
13	0	1	-1	0.73 ± 0.00	80.7 ± 0.2
14	0	0	0	0.82 ± 0.00	83.0 ± 0.4
15	-1	1	0	0.95 ± 0.01	81.5 ± 0.2
16	1	0	1	0.93 ± 0.01	80.5 ± 0.2
17	0	0	0	0.93 ± 0.01	83.0 ± 0.3

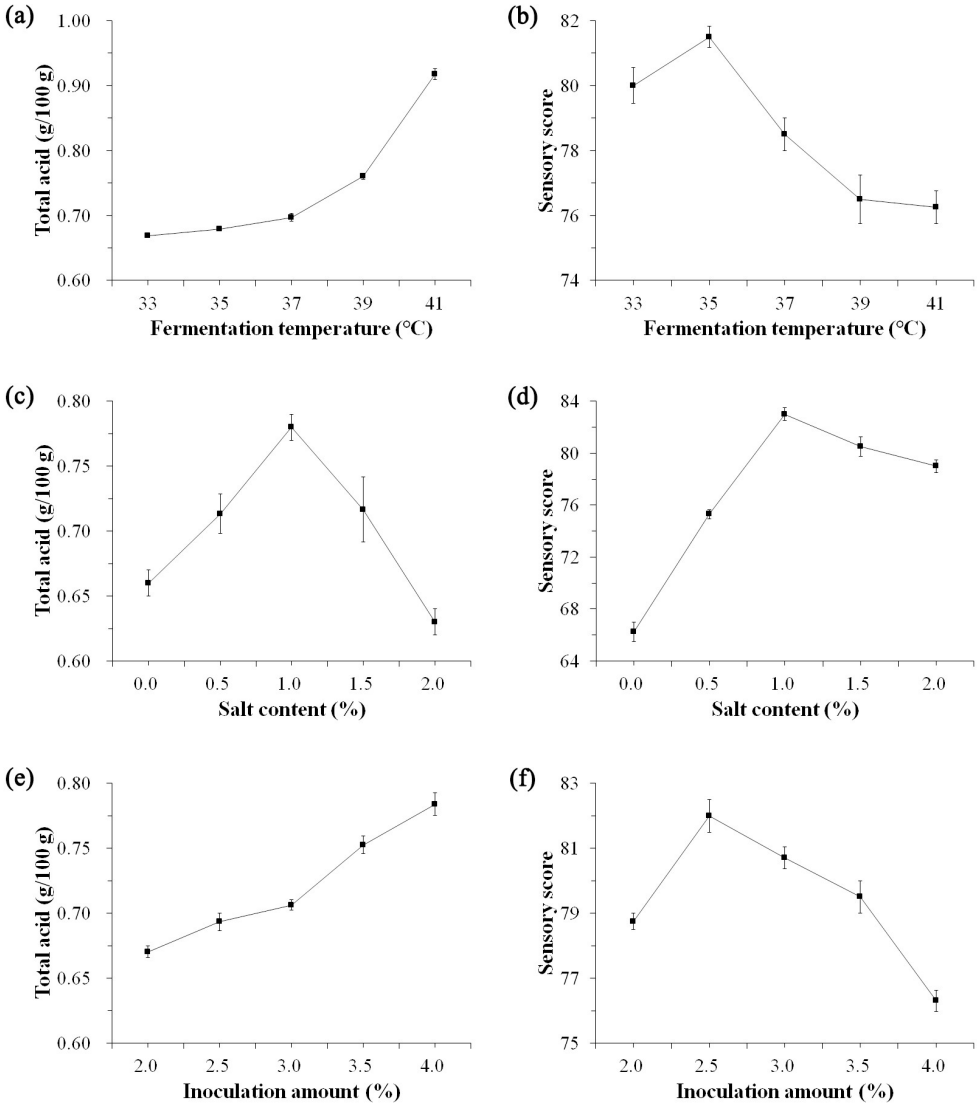

Figure 2. Effects of different fermentation conditions on the quality of fermented *L. edodes*. (a) Effects of different fermentation temperature on the total acid of fermented *L. edodes*. (b) Effects of different fermentation temperature on the sensory score of fermented *L. edodes*. (c) Effects of different salt contents on the total acid of fermented *L. edodes*. (d) Effects of different salt contents on the sensory score of fermented *L. edodes*. (e) Effects of different inoculation amounts on the total acid of fermented *L. edodes*. (f) Effects of different inoculation amounts on the sensory score of fermented *L. edodes*.

respectively. The actual score was close to the predicted score, indicating that the model had high reliability, which can be used to predict the sensory quality of *L. edodes* fermented by the mixed starter.

3.5 Effects of mixed LAB fermentation on the flavor substance of L. edodes

The types and contents of volatile flavor mixeds are important indexes affecting the quality of fermented products (Petersen et al., 2017). *L. edodes* was fermented with the best LAB mixed starter and technological conditions, and the types and contents of volatile flavor compounds in the fermented products were analyzed. Table 5 shows that a total of 31 volatile flavor substances were detected by SPME-GC-MS analysis, including 2 acids, 5 alcohols, 4 ketones, 4 aldehydes, 8 hydrocarbons, 2 lipids, 1 phenolic substance and 5 other substances. The aroma of *L. edodes* was produced by the interaction and balance of various compounds, including sulfur compounds, octa compounds and aldehydes and ketones. The main volatile substances in fresh *L. edodes* were octagonal compounds, such as 1-octene-3-ol, 3-octanol, etc., which usually have fragrance or vegetal aroma. Compared with unfermented *L. edodes*, the contents of volatile flavor components in fermented *L. edodes* were 2.2%, 17.8%, 1.78% and 13.92%, respectively. The contents of acids and ketones in fermented *L. edodes* increased by 2.16% and 17.8%, respectively, while the

Table 4. Analysis of variance for the regression model.

Evaluation indicators	Source	Sum of Squares	df	Mean Square	F Value	Prob > F
Total acid (*Y1*)	Model	0.25	9	0.028	40.04	< 0.0001**
	A	0.079	1	0.079	112.04	< 0.0001**
	B	0.017	1	0.017	24.01	0.0018**
	C	1.784×10^{-3}	1	1.784×10^{-3}	2.53	0.1554
	AB	1.526×10^{-3}	1	1.526×10^{-3}	2.17	0.1844
	AC	1.197×10^{-3}	1	1.197×10^{-3}	1.70	0.2334
	BC	6.353×10^{-3}	1	6.353×10^{-3}	9.03	0.0198*
	A^2	0.13	1	0.13	180.90	< 0.0001**
	B^2	3.674×10^{-5}	1	3.674×10^{-5}	0.052	0.8258
	C^2	0.014	1	0.014	20.40	0.0027**
	Residual	4.927×10^{-3}	7	7.039×10^{-4}		
	Lack of Fit	3.870×10^{-3}	3	1.290×10^{-3}	4.88	0.0799
	Pure Error	1.057×10^{-3}	4	2.644×10^{-4}		
	Cor Total	0.26	16			
Sensory score (*Y2*)	Model	40.67	9	4.52	57.52	0.0001**
	A	0.85	1	0.85	10.75	0.0135*
	B	12.00	1	12.00	152.79	0.0001**
	C	0.080	1	0.080	1.02	0.3466
	AB	1.000×10^{-2}	1	1.000×10^{-2}	0.13	0.7318
	AC	0.49	1	0.49	6.24	0.0412*
	BC	1.00	1	1.00	12.73	0.0091**
	A^2	9.47	1	9.47	120.57	0.0001**
	B^2	10.78	1	10.78	137.19	0.0001**
	C^2	3.41	1	3.41	43.41	0.0003**
	Residual	0.55	7	0.079		
	Lack of Fit	0.25	3	0.083	1.11	0.4428
	Pure Error	0.30	4	0.075		
	Cor Total	41.22	16			

*$P < 0.05$ meant significant. **$P < 0.01$ meant highly significant.

Table 5. Analysis results of volatile flavor substances in fermented *L. edodes*.

Classification	Serial number	Name of flavor substance	Retention time (min)	Relative content (%)	
				Fermented *L. edodes*	Unfermented *L. edodes*
Acids	1	Acetic acid	4.75	1.49	—
	2	5-Decen-1-ol, acetate, (E)-	49.01	0.67	—
Alcohols	3	1-Octanol	22.71	0.44	—
	4	1-Nonanol	29.72	0.42	—
	5	Eucalyptol	19.39	1.04	1.70
	6	3-Octanol	17.40	0.84	0.83
	7	1-Octen-3-ol	16.35	—	3.01
Ketones	8	2-Heptanone	10.71	4.67	—
	9	3-Octanone	16.75	1.33	—
	10	2-Nonanone	24.07	10.39	—
	11	2-Undecanone	37.94	1.41	—
Aldehydes	12	Nonanal	24.91	—	5.19
	13	Benzaldehyde	14.81	—	0.40
	14	Octanal	17.86	—	1.00
	15	Decanal	31.98	—	0.83
Hydrocarbons	16	Tridecane	38.37	0.14	0.16
	17	Tetradecane	44.78	0.22	0.29
	18	Pentadecane	50.87	0.17	—
	19	Hexadecane	54.67	0.10	0.13

Table 5. Continued...

Classification	Serial number	Name of flavor substance	Retention time (min)	Relative content (%)	
				Fermented *L. edodes*	Unfermented *L. edodes*
	20	Nonadecane	50.87	—	0.25
	21	p-Xylene	9.46	—	0.49
	22	Benzene, 1,3-dimethyl-	9.32	1.03	—
	23	trans-calamenene	51.86	0.10	—
lipids	24	Sulfurous acid, 2-ethylhexyl hexadecyl ester	29.13	0.34	—
	25	Sulfurous acid, dodecyl 2-ethylhexyl ester	29.15	—	0.53
Phenols	26	Butylated Hydroxytoluene	51.47	10.30	14.17
others	27	1,2,4,5-Tetrathiane	40.25	0.21	0.91
	28	Carbon disulfide	1.91	13.92	9.07
	29	Furan, 2-pentyl-	16.99	0.83	1.53
	30	Lenthionine	54.87	—	0.31
	31	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-	51.87	—	0.20

contents of alcohols, aldehydes and phenols decreased by 3.66%, 7.42% and 3.87%, which was similar to the results of Qi et al. (2021). This might be attribute to the unstable compounds, aldehydes, which are prone to oxidation reactions to generate acids or alcohols (Chen et al., 2020). The results showed that the decrease or disappearance of acids, ketones, alcohols, phenols and aldehydes as well as the balance of these substances had important effects on the flavor formation of fermented *L. edodes*.

4 Conclusions

Through screening single LAB starter and mixed LAB starter of *L. edodes*, it was found that mixed LAB starter (inoculation ratio: *Lactobacillus delbrueckii* subsp. *bulgaricus: Lactiplantibacillus plantarum: Lacticaseibacillus rhamnous* = 3:1:2) had better fermentation performance, which could obviously improve the total acid of fermented *L. edodes* and increase the number of viable LAB. After the optimization of single factor experiment and response surface experiment, the optimal process parameters for mixed LAB fermentation of *L. edodes* were as follows: fermentation temperature of 37 °C, salt content of 1%, and inoculum amount of 2.7%. Under this process, the total acid and sensory score were 0.88 g/100 g and 81.7 points, respectively. The contents of volatile flavor components in fermented *L. edodes* were 2.2%, 17.8%, 1.78%, 13.92% in acids, ketones, hydrocarbons, and other compounds, respectively. Compared with unfermented *L. edodes*, the contents of acids and ketones in fermented *L. edodes* were greatly increased, and the contents of alcohols, aldehydes and phenols were relatively decreased, indicating that the balance of these substances has an important influence on the flavor of fermented *L. edodes*. This study showed that mixed LAB fermentation could improve the flavor, taste and nutritional value of *L. edodes*, which explored a new way for the deep processing of *L. edodes*. In the next step, fermented *L. edodes* will be developed into flavoured ready-to-eat food, and the fermented broth will be developed into LAB powder by spray drying, due to the large number of viable LAB and other nutritional components present in the fermented broth.

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References

Abdesslem, S. B., Moussa, O. B., Boulares, M., Elbaz, M., Chouaibi, M., Ayachi, S., & Hassouna, M. (2020). Evaluation of the effect of fennel (*Foeniculum vulgare* Mill) essential oil addition on the quality parameters and shelf-life prediction of yoghurt. *International Journal of Dairy Technology*, 73(2), 403-410. http://dx.doi.org/10.1111/1471-0307.12667.

Chen, Y., Xu, H., Ding, S., Zhou, H., Qin, D., Deng, F., & Wang, R. (2020). Changes in volatile compounds of fermented minced pepper during natural and inoculated fermentation process based on headspace-gas chromatography-ion mobility spectrometry. *Food Science & Nutrition*, 8(7), 3362-3379. http://dx.doi.org/10.1002/fsn3.1616. PMid:32724601.

Chen, Z. Q., Gao, H. Y., Wu, W. J., Chen, H. J., Fang, X. J., Han, Y. C., & Mu, H. L. (2021). Effects of fermentation with different microbial species on the umami taste of Shiitake mushroom (*Lentinus edodes*). *Lebensmittel-Wissenschaft + Technologie*, 141, 110889. http://dx.doi.org/10.1016/j.lwt.2021.110889.

China Edible Fungi Association. (2022). Analysis of statistical survey results of edible fungi in China in 2020. *Zhongguo Shiyongjun*, 41(1), 85-91.

Choi, Y.-J., Yong, S., Lee, M. J., Park, S. J., Yun, Y.-R., Park, S.-H., & Lee, M.-A. (2019). Changes in volatile and non-volatile compounds

of model kimchi through fermentation by lactic acid bacteria. *Lebensmittel-Wissenschaft + Technologie*, 105, 118-126. http://dx.doi.org/10.1016/j.lwt.2019.02.001.

Cui, S., Zhao, N., Lu, W., Zhao, F., Zheng, S. Y., Wang, J. W., & Chen, W. (2019). Effect of different *Lactobacillus* species on volatile and nonvolatile flavor compounds in juices fermentation. *Food Science & Nutrition*, 7(7), 2214-2223. http://dx.doi.org/10.1002/fsn3.1010. PMid:31367350.

Dan, T., Ren, W., Liu, Y., Tian, J., Chen, H., Li, T., & Liu, W. (2019). Volatile flavor compounds profile and fermentation characteristics of milk fermented by *Lactobacillus delbrueckii* subsp. bulgaricus. *Frontiers in Microbiology*, 10, 2183. http://dx.doi.org/10.3389/fmicb.2019.02183. PMid:31620117.

Demirci, M., Tomas, M., Tekin-Çakmak, Z. H., & Karasu, S. (2022). *Berberis crataegina* DC. as a novel natural food colorant source: ultrasound-assisted extraction optimization using response surface methodology and thermal stability studies. *Food Science and Technology*, 42, e03421. http://dx.doi.org/10.1590/fst.13421.

Duhan, J. S., Nehra, K., Gahlawat, S. K., Saharan, P., & Surekha, D. (2013). Bacteriocins from lactic acid bacteria. In: R. Salar, S. Gahlawat, P. Siwach & J. Duhan (Eds.), *Biotechnology: prospects and applications*. New Delhi: Springer, pp. 127-141. http://dx.doi.org/10.1007/978-81-322-1683-4_11.

Gaitán-Hernández, R., López-Peña, D., Esqueda, M., & Gutiérrez, A. (2019). Review of bioactive molecules production, biomass, and basidiomata of shiitake culinary-medicinal mushrooms, *Lentinus edodes* (Agaricomycetes). *International Journal of Medicinal Mushrooms*, 21(9), 841-850. http://dx.doi.org/10.1615/IntJMedMushrooms.2019031849. PMid:32450024.

Grotto, D., Bueno, D. C. R., Ramos, G. K. A., Costa, S. R., Spim, S. R. V., & Gerenutti, M. (2016). Assessment of the safety of the shiitake culinary-medicinal mushroom, *Lentinus edodes* (Agaricomycetes), in rats: biochemical, hematological, and antioxidative parameters. *International Journal of Medicinal Mushrooms*, 18(10), 861-870. http://dx.doi.org/10.1615/IntJMedMushrooms.v18.i10.20. PMid:27910754.

Hu, J., Gao, J., Zhao, Z., & Yang, X. (2021). Response surface optimization of polysaccharide extraction from *Galla Chinensis* and determination of its antioxidant activity *in vitro*. *Food Science and Technology*, 41(1), 188-194. http://dx.doi.org/10.1590/fst.38619.

Ke, L., & Chen, H. Y. (2016). Homogenate extraction of crude polysaccharides from *Lentinus edodes* and evaluation of the antioxidant activity. *Food Science and Technology*, 36(3), 533-539. http://dx.doi.org/10.1590/1678-457X.00916.

Lee, H. G., & Yoo, S. R. (2017). Use of laser-etched pouches to control the volume expansion of kimchi packages during distribution: impact of packaging and storage on quality characteristics. *Journal of Food Science*, 82(8), 1876-1884. http://dx.doi.org/10.1111/1750-3841.13798. PMid:28678333.

Li, S., Wang, A., Liu, L., Tian, G., & Xu, F. (2019). Extraction of polysaccharides under vacuum condition from *Lentinus edodes* stipe and their antioxidant activities in vitro. *Food Science and Biotechnology*, 28(3), 759-767. http://dx.doi.org/10.1007/s10068-018-0513-z. PMid:31093433.

Liang, H., He, Z., Wang, X., Song, G., Chen, H., Lin, X., Ji, C., & Zhang, S. (2020). Bacterial profiles and volatile flavor compounds in commercial Suancai with varying salt concentration from northeastern China. *Food Research International*, 137, 109384. http://dx.doi.org/10.1016/j.foodres.2020.109384. PMid:33233086.

Liu, Y., & Li, S. M. (2021). Extraction optimization and antioxidant activity of *Phyllanthus urinaria* polysaccharides. *Food Science and Technology*, 41(Suppl. 1), 91-97. http://dx.doi.org/10.1590/fst.11320.

Luo, Y., Liu, Y., Ren, T., Wang, B., Peng, Y., Zeng, S., & Su, Y. (2020). Sichuan paocai fermented by mixed-starter culture of lactic acid bacteria. *Food Science & Nutrition*, 8(10), 5402-5409. http://dx.doi.org/10.1002/fsn3.1833. PMid:33133542.

Martinussen, J., Solem, C., Holm, A. K., & Jensen, P. R. (2013). Engineering strategies aimed at control of acidification rate of lactic acid bacteria. *Current Opinion in Biotechnology*, 24(2), 124-129. http://dx.doi.org/10.1016/j.copbio.2012.11.009. PMid:23266099.

Nisar, J., Mustafa, I., Anwar, H., Sohail, M. U., Hussain, G., Ullah, M. L., Faisal, M. N., Bukhari, S. A., & Basit, A. (2017). Shiitake culinary-medicinal mushroom, *Lentinus edodes* (Agaricomycetes): a species with antioxidant, immunomodulatory, and hepatoprotective activities in hypercholesterolemic rats. *International Journal of Medicinal Mushrooms*, 19(11), 981-990. http://dx.doi.org/10.1615/IntJMedMushrooms.2017024504. PMid:29345560.

Park, S. E., Seo, S. H., Kim, E. J., Byun, S., Na, C. S., & Son, H. S. (2019). Changes of microbial community and metabolite in kimchi inoculated with different microbial community starters. *Food Chemistry*, 274, 558-565. http://dx.doi.org/10.1016/j.foodchem.2018.09.032. PMid:30372979.

Petersen, K. V., Liu, J., Chen, J., Martinussen, J., Jensen, P. R., & Solem, C. (2017). Metabolic characterization and transformation of the non-dairy *Lactococcus lactis* strain KF147, for production of ethanol from xylose. *Biotechnology Journal*, 12(8), 1700171. http://dx.doi.org/10.1002/biot.201700171. PMid:28418108.

Qi, J., Huang, H., Wang, J., Liu, N., Chen, X., Jiang, T., Xu, H., & Lei, H. (2021). Insights into the improvement of bioactive phytochemicals, antioxidant activities and flavor profiles in Chinese wolfberry juice by select lactic acid bacteria. *Food Bioscience*, 43(1), 101264. http://dx.doi.org/10.1016/j.fbio.2021.101264.

Rothstein, S. M., Sen, S., & Mansell, T. J. (2020). Towards high-throughput genome engineering in lactic acid bacteria. *Current Opinion in Biotechnology*, 61, 181-188. http://dx.doi.org/10.1016/j.copbio.2019.12.015. PMid:31986469.

Şanlier, N., Gökcen, B. B., & Sezgin, A. C. (2019). Health benefits of fermented foods. *Critical Reviews in Food Science and Nutrition*, 59(3), 506-527. http://dx.doi.org/10.1080/10408398.2017.1383355. PMid:28945458.

Seo, H., Bae, J. H., Kim, G., Kim, S. A., Ryu, B. H., & Han, N. S. (2021). Suitability analysis of 17 probiotic type strains of lactic acid bacteria as starter for kimchi fermentation. *Foods*, 10(6), 1435. http://dx.doi.org/10.3390/foods10061435. PMid:34205741.

Wang, Y., Wu, J., Lv, M., Shao, Z., Hungwe, M., Wang, J., Bai, X., Xie, J., Wang, Y., & Geng, W. (2021). Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry. *Frontiers in Bioengineering and Biotechnology*, 9, 612285. http://dx.doi.org/10.3389/fbioe.2021.612285. PMid:34055755.

Wang, Z., & Shao, Y. (2018). Effects of microbial diversity on nitrite concentration in *pao cai*, a naturally fermented cabbage product from China. *Food Microbiology*, 72, 185-192. http://dx.doi.org/10.1016/j.fm.2017.12.003. PMid:29407396.

Zhu, L., Wang, S., Zhang, Z., Zhou, S., Tang, Q., Wu, F., & Zhang, J. S. (2019). Dissolution of bioactive components from dried fruiting bodies of the culinary-medicinal shiitake mushroom, Lentinus edodes (Agaricomycetes), during cleaning, soaking, and cooking. *International Journal of Medicinal Mushrooms*, 21(1), 37-45. http://dx.doi.org/10.1615/IntJMedMushrooms.2018029006. PMid:30806254.