# Optimization of Lactic Acid Bacteria Fermentation and Antioxidant Activity of Lycium Ruthenicum Murr. Juice

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**Abstract: Lactic acid bacterial fermentation was applied to Lycium ruthenicum Murr. (LRM) in order to enhance and optimize the fermentation process while systematically characterizing its antioxidant properties. The key parameters such as the ratio of solid to liquid, inoculum size, fermentation temperature and time were refined through single-factor trials in conjunction with response surface methodology optimization. The optimized process parameters were applied to comprehensively assess the sensory quality, viable cell count, and antioxidant capacity of the fermented Lycium ruthenicum Murr. juice (LRMJ). The results showed that the optimal fermentation parameters were inoculum size 3.5%, the ratio of solid to liquid 15:1, fermentation temperature 37℃, and fermentation time 4 h. Under these conditions, the sensory score and viable count of fermented beverage of LRM reached the ideal level. Findings from the DPPH and ABTS$^+$ radical scavenging tests revealed that the fermented beverage exhibited considerable antioxidant potential.**

**Keywords: Lycium Ruthenicum Murr; Lactobacillus Beverage; Technology Optimization; Oxidation Resistance**

## 1. Introduction

Improvements in living standards have led to greater public concern regarding the quality and health-related attributes of food [1]. As an ancient food processing method, fermentation technology still occupies an important position in the modern food industry [2,3]. Lactic acid bacteria are widely utilized in the food industry due to their well-documented capacity to enhance sensory characteristics and improve nutritional value [4,5]. The application of fermentation technology in LRMJ can not only improve its nutritional value, but also endow it with unique flavor and taste [6,7]. The purpose of this study was to improve the quality of LRMJ by optimizing the fermentation process of lactic acid bacteria, and to study its antioxidant activity to furnish a theoretical framework and technological resources for facilitating the industrial manufacture of LRMJ.

LRM is rich in polysaccharides, anthocyanins, anthocyanins, amino acids and dietary fibers [8], and has many physiological functions such as antioxidant and hypoglycemic [9]. However, LRM is not suitable for storage and transportation, which seriously limits its industrial development. As a biotransformation process, lactic acid bacteria fermentation can improve the antioxidant activity of LRMJ, and improve its taste and flavor.

Lactic acid bacteria (LAB) are widely distributed in nature, including more than 40 genera and more than 300 species, including cocci and bacilli. Lactobacillus, Leuconostoc, and Bifidobacterium [10] are commonly used in the food industry. Its fermentation is divided into homogeneous type (mainly producing lactic acid) and heterogeneous type (producing lactic acid, acetic acid, etc.) [11]. Lactic acid bacteria are used in dairy products, meat products, fruit and vegetable fermentation and silage [12]. Because of the health risks of dairy products, plant matrix (such as fruit juice) has become a new type of fermentation carrier [13] because of its rich nutrition, polysaccharides, flavonoids and other ingredients. Metabolites of lactic acid bacteria have multiple biological activities such as regulating intestinal flora [14], reducing cholesterol [15], anti-oxidation [16] and bacteriostasis [17].

In order to improve the sensory evaluation and viable count of LRMJ, the fermentation process of LRMJ was optimized by single factor experiment and response surface method [18,19]. At the same time, the antioxidant activity of fermented LRMJ was analyzed to evaluate its health value [7,20]. The findings indicated that optimization of the fermentation process markedly enhanced the quality and nutritional value of LRMJ, thereby offering a theoretical

foundation and technical guidance for its industrial-scale production [2,21].

## 2. Materials and Methods

### 2.1 Materials and Reagents

In this study, the materials and reagents used include: Lycium ruthenicum Murr. whole fruit Freeze-Dried powder (LRMP), Lactobacillus plantarum GH-8 and Lactobacillus plantarum GH-6 were provided by Shanghai Ganhong Biomedical Technology Co., Ltd, and Lactobacillus rhamnosus S60, Lactobacillus fermentum B2271, Lactobacillus plantarum S10 and Lactobacillus paracasei S40 were provided by the lactic acid bacteria culture preservation room of Shanghai Business School.

The Leici pHS-3E laboratory pH meter manufactured by Hangzhou Kexiao Chemical Instrument Equipment Co., Ltd.; the water-proof constant temperature incubator manufactured by Shanghai Shenan Medical Instrument Factory; the centrifuge manufactured by Shanghai Anting Scientific Instrument Factory; Constant temperature water bath manufactured by Shanghai Heng Scientific Instrument Co., Ltd.; Vertical autoclave made by Shanghai Boxun Industrial Co., Ltd.

### 2.2 Process Flow

Centrifugal filtration: Add LMRP into 500 mL of water, subject to ultrasonic treatment for 20 minutes, and collect the supernatant after centrifugation. Sterilization and cooling: put the sealed fermentation liquid in a water bath at 90℃, 10 min for sterilization, and cool it to room temperature for later use.

Lactobacillus rhamnosus S60, Lactobacillus plantarum GH-8, Lactobacillus fermentum B2271, Lactobacillus plantarum GH-6, Lactobacillus paracasei S40, Lactobacillus plantarum S10 (referred to as S60, GH-8, B2271, GH-6, S40, S10). Streaks were made on MRS solid medium under a sterile operation environment and incubated anaerobically at 37℃ for 48 h. Single colonies were selected for reactivation for one generation, and then single colonies were selected again and inoculated into MRS liquid medium (5 mL/15 mL), and incubated at 37℃ for 16 h to obtain primary seed solution. Then 0.2 mL was pipetted into a new MRS liquid medium (30 mL/50 mL) and cultured at 37℃ for 16 h to obtain the secondary seed solution. Centrifuge for 15 min at 5000 × g and 4℃, discard the supernatant, wash the thalli twice with sterile saline, suspend the thalli in sterile saline, and adjust the viable count to$10^8$ CFU/mL to obtain the fermentation seed solution.

### 2.3 Screening of Strains

The six selected strains were firstly activated according to the method in 2.2.1, and then inoculated into LRMJ without additional nutrients at the inoculum size of $10^8$ CFU/mL. After anaerobic fermentation at 37℃ for 24 h, that sample was refrigerated at 4℃ for 2 h to terminate the fermentation. Subsequently, the number of viable bacteria in the sample was determined, and a strain with optimal growth performance in LRMJ was identified for downstream experimental work.

### 2.4 Single Factor Condition Optimization

2.4.1 Optimization of solid-liquid ratio

In this study, the effects of different solid-liquid ratios (1:5, 1:10, 1:20, 1:30, 1:40, g/mL) on the fermentation were investigated at a constant temperature of 37℃. $10^8$ CFU/mL was inoculated into LRMJ without additional nutrients for anaerobic fermentation at 37℃ for 24 h, and then placed in a refrigerator at 4℃ for 2 h. Subsequently, the pH value of the fermentation broth and the number of viable bacteria were determined, and the sensory evaluation was performed according to Table 2.3. The optimal ratio of feed to liquid was determined by the number of viable bacteria and sensory score.

2.4.2 Optimization of strain inoculation amount

Under the constant temperature of 37℃, the juice samples with the solid-liquid ratio of 1:20 were selected and divided into five groups, and the strains were inoculated at the volume ratios of 1%, 2%, 3%, 4% and 5%, respectively. After fermentation for 4 h, the pH value and the number of viable bacteria were detected, and the sensory evaluation was carried out according to Table 2.3. The number of viable bacteria and sensory score were used as evaluation criteria to determine the optimal inoculum size.

2.4.3 Optimization of fermentation temperature

In this experiment, the fruit juice samples were fermented for 4 h at five different temperatures (31℃, 34℃, 37℃, 40℃, 43℃) with the solid-liquid ratio of 1:20 and the fixed inoculum of 3%. After that, the pH value of the fermentation broth and the number of viable

bacteria were measured, and the sensory score was determined according to Table 2.3. Based on the results of viable counts and sensory scores, the optimum fermentation temperature was determined.

2.4.4 Optimization of fermentation time

In the experiment, the juice samples were fermented at 37℃ for 0, 4, 8, 12, 20 and 24 h with the ratio of solid to liquid of 1:20 and the inoculum size of 3%. The pH value and viable count of the fermentation broth at each time point were determined, and the sensory evaluation was performed according to Table 2. The optimal fermentation time was determined by comparing the number of viable cells and sensory scores.

**2.5 Response Surface Experiment**

Based on the optimal fermentation time, solid-liquid ratio, inoculum size and fermentation temperature obtained from the single factor experiment, the corresponding condition range was set in this study, and the factor levels of Box-Behnken experimental design were determined accordingly (see Table 1 for details). Response surface methodology (RSM) was used to optimize the fermentation conditions based on sensory evaluation.

**Table 1. Response Surface Box-Behnken Design Experiment Factor Level**

| Level | Factor | | | |
|---|---|---|---|---|
| | A (time/h) | B [Solid-liquid ratio/(g/mL)] | C (Inoculum/%) | D (Fermentation temperature/℃) |
| -1 | 2 | 10 | 2 | 34 |
| 0 | 4 | 20 | 3 | 37 |
| 1 | 6 | 30 | 4 | 40 |

**2.6 Sensory Evaluation**

According to the sensory evaluation standard of National Standard Beverage for food safety (GB/T 7101-2022), an evaluation team composed of 10 trained sensory evaluators was established in this study. The team comprehensively evaluated the four dimensions of color, aroma, taste and organizational structure of the product, and calculated the average score of each dimension as the final sensory score of the product. See Table 2 for detailed rules of sensory evaluation.

**Table 2. Sensory Evaluation Standard of LMRJ Fermented by Lactobacillus Plantarum GH-6**

| Scoring items and scores | Scoring criteria | Rating interval |
|---|---|---|
| | Uniform color, purplish red | 20~11 |
| Color attributes | Uniform color and luster, light red | 10~6 |
| | Uniform color, dark red | 5~0 |
| | Uniform, no sediment, no stratification | 20~11 |
| Textural properties | There is a small amount of sediment without obvious stratification. | 10~6 |
| | There is a large amount of sediment without stratification. | 5~0 |
| | Rich fruit aroma, elegant and harmonious fermentation aroma, no smell | 20~11 |
| Odor profile | Fruity and fermented flavor is strong, slightly different smell. | 10~6 |
| | Fruity flavor is light, with cooking flavor and obvious abnormal smell. | 5~0 |
| | Moderate sweet and sour flavor, pure and rich fruit flavor, no bitter, astringent and other odors | 20~11 |
| Flavor characteristics | The flavor is sour or sweet, the fruit flavor is not pure enough, slightly bitter, astringent and other odors. | 10~6 |
| | Poor flavor, too sour or too sweet, fruity, bitter, astringent and other odors | 5~0 |
| | I prefer it | 20~11 |
| Overall acceptability | Average preference | 10~6 |
| | Don't like | 5~0 |

**2.7 Determination of Oxidation Resistance**

2.7.1 DPPH radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined according to Brand-Williams et al [22], with minor modifications. The assay was initiated by mixing 2.0 mL of sample extract or Trolox standard solution with 2.0 mL of freshly prepared 0.1 mmol/L DPPH ethanol solution.

Following vortex mixing, the samples were incubated for 30 min at ambient temperature under dark conditions, after which their absorbance was determined at 517 nm with a UV–Vis spectrophotometer.

The scavenging activity (%) was calculated as:

[ $(A_0-A_S)/A_0$] × 100%, where $A_S$ is absorbance of the sample solution and $A_0$ is absorbance of the blank (distilled water). The DPPH radical scavenging ability of the samples was expressed as the median inhibitory concentration ($IC_{50}$), which was obtained by standard curve calculation.

2.7.2 Determination of ABTS + scavenging ability

The determination method was based on the literature [23] and slightly improved. ABTS + radicals were produced by combining equal volumes of 7 mmol/L ABTS solution and 140 mmol/L potassium persulfate, followed by incubation in the dark at ambient temperature for 12–16 h.

The mixture was diluted with phosphate-buffered saline (PBS, pH 7.4) to an absorbance of 0.70 ± 0.02 at 734 nm. A mixture of 2.0 mL of sample solution or Trolox standard with 2.0 mL of $ABTS^{+}$·working solution was incubated for 5 min at room temperature in the dark, after which the absorbance was measured at 734 nm.

Activity was expressed as % inhibition, μmol TE/g, and $IC_{50}$ as described above.

## 3. Results and Analysis

### 3.1 Strain Screening Results

Six lactic acid bacterial strains were subjected to fermentation in LRMJ without supplemental nutrients, with the objective of identifying the most suitable candidate for subsequent fermentation processes. While metabolic activity—such as lactic acid production—and preliminary sensory impact are often considered in strain selection, the primary criterion adopted in this screening phase was viable cell count. This approach was chosen because high viability not only reflects strong adaptability to the substrate but also serves as a robust proxy for overall metabolic activity, including acid production and potential probiotic functionality. Moreover, in the initial screening stage, viable count provides a rapid, reproducible, and quantitative measure to compare strain performance under uniform conditions.

As shown in Figure 1, all six strains were capable of growing in LRMJ, though significant differences in growth performance were observed ($p < 0.05$). Except for Lactobacillus plantarum S10, the viable counts of the other five strains reached 108 CFU/mL. Notably, only Lactobacillus plantarum GH-6 exhibited a viable count exceeding 109 CFU/mL, indicating superior proliferation and adaptability in the polyphenol-rich LRMJ matrix.

Although other factors such as organic acid profile and sensory characteristics are relevant for end-product quality, the complex nature of LRMJ and the focus on initial technological feasibility justified the use of viable cell count as the decisive screening metric. Lactobacillus plantarum GH-6 was therefore selected for all subsequent optimization experiments due to its outstanding growth performance, which suggests a high potential for efficient fermentation and metabolic functionality in this unique substrate [24].

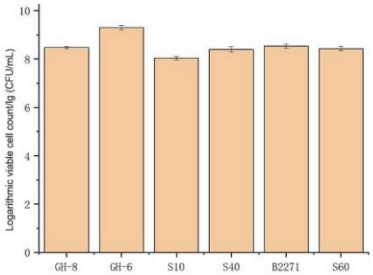
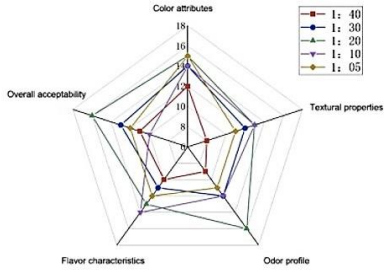



**Figure 1. The Number of Viable Bacteria of LRM Fermented by 6 Strains**

Note: There was a significant difference between the two groups of data marked with different letters ($p < 0.05$, $n = 3$).

### 3.2 Analysis of Single Factor Experiment Results

3.2.1 Optimization of fermentation solid-liquid ratio



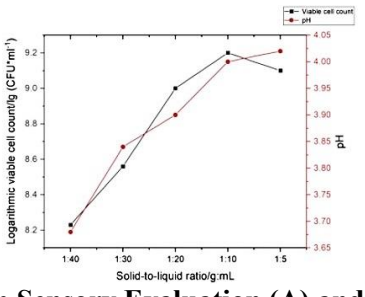




**Figure 2. Effect of Different Solid-Liquid Ratios on Sensory Evaluation (A) and Viable Count (B) of Fermented Lycium Ruthenicum (n = 3)**

Figure 2 illustrates the influence of the solid-to-liquid ratio on the sensory evaluation score and viable cell count of fermented LRMJ. The observation of the chart showed that the sensory score and the number of viable bacteria increased first and then decreased with the increase of the ratio of solid to liquid. When the ratio of solid to liquid was 1:20, the sensory score reached the highest point, and the number of viable bacteria also reached the maximum, which indicated that the flavor and texture of the juice reached the best state, and the growth of lactic acid bacteria was also the best. In the case of low liquid ratio, the increase of juice concentration may inhibit the growth and metabolism of lactic acid bacteria, resulting in a decrease in the number of viable bacteria and a decrease in sensory scores. On the contrary, when the ratio of material to liquid is too high, although the growth environment of lactic acid bacteria becomes diluted, which is conducive to the increase of the number of viable bacteria, the flavor and texture of fruit juice may be damaged by dilution, resulting in a decrease in sensory scores. Therefore, the optimal ratio of material to liquid was 1:20, which not only ensured the full growth of lactic acid bacteria, but also maintained the flavor and texture of LRMJ.

3.2.2.Optimization of inoculum size

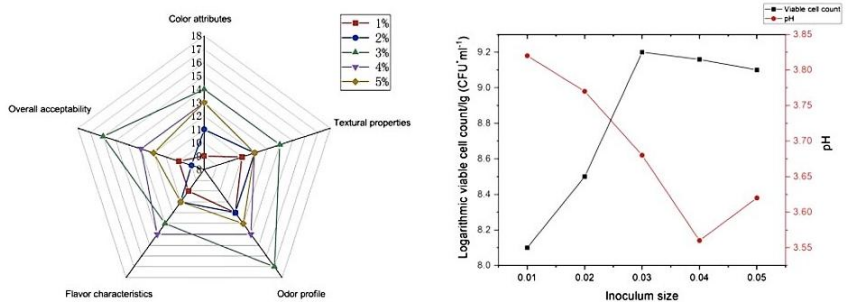



**Figure 3. Effect of Different Inoculation Amount on Sensory Evaluation (A) and Viable Count (B) of Fermented Lycium Ruthenicum Murr. (n = 3)**

Figure 3 shows the effect of different inoculum amount on the sensory evaluation and viable count of fermented LRMJ. The experimental data showed that the increase of inoculation amount was positively correlated with the increase of the number of viable bacteria, indicating that increasing the number of bacteria was conducive to the reproduction of lactic acid bacteria. Nevertheless, the sensory score did not increase monotonically with the increase of inoculation amount, but peaked when the inoculation amount reached 3%, and then fell back. This may mean that although higher inoculum helps the growth of lactic acid bacteria, it may also adversely affect the taste and flavor of juice, resulting in a decline in sensory scores. Therefore, it is most appropriate to set the inoculation amount at 3%, which can ensure the sensory quality of the product while maintaining a high number of viable bacteria. These findings highlight that optimizing the inoculum size is essential for preserving both the flavor and nutritional quality of the product during fermentation.

3.2.3 Optimization of fermentation temperature

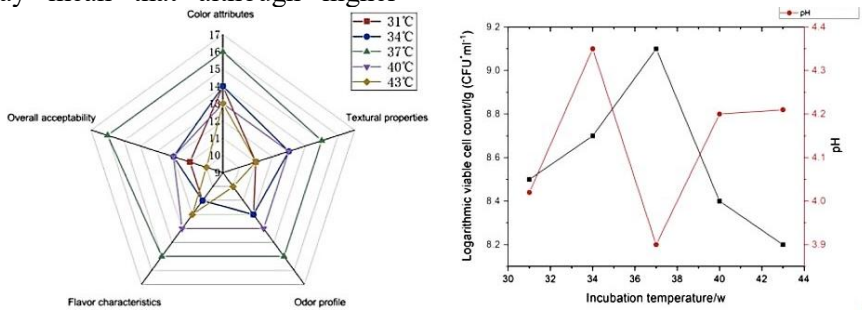



**Figure 4. Effects of Different Fermentation Temperatures on Sensory Evaluation (A) and Viable Count (B) of Fermented Lycium Ruthenicum Murr. (n = 3)**

Figure 4 shows the effect of fermentation temperature on the number of viable bacteria and sensory scores. The results indicated that the viable cell count rose with increasing temperature until reaching a peak, after which it declined, suggesting that the growth rate of lactic acid bacteria is positively influenced by temperature within a specific range, but the

growth of lactic acid bacteria might be inhibited when the temperature exceeded the appropriate range. The change of sensory score was also similar to the number of viable bacteria. When the temperature was 37℃, the sensory score reached the highest, and the number of viable bacteria was also at a high level. This indicated that 37℃ was the ideal temperature for the fermentation of LRMJ, which enhanced lactic acid bacterial development while sustaining the sensory attributes of the juice.

Both excessively low and high temperatures adversely affect fermentation performance; thus, the maintenance of an optimal temperature is critical to ensuring the quality of LRMJ.

3.2.4.Optimization of Fermentation Time

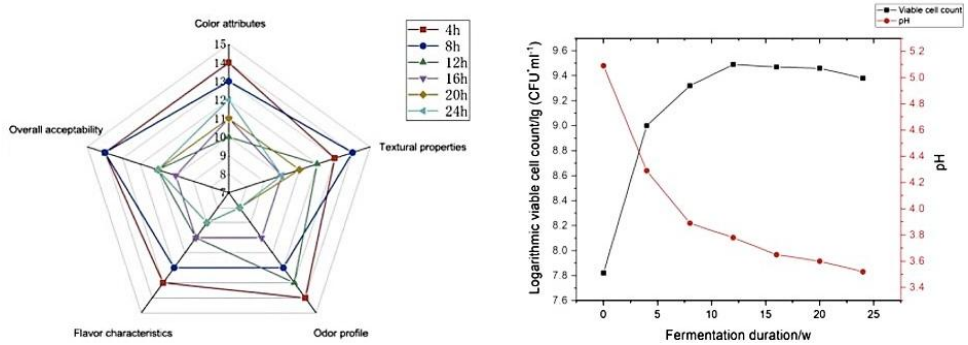



**Figure 5. Effects of Different Fermentation Time on Sensory Evaluation (A) and Viable Count (B) of Fermented Lycium Ruthenicum Murr**

The data in Figure 5 shows that the number of viable bacteria first increases and then decreases when the fermentation time is prolonged, which indicates that the viable cell count increased during the initial phase of fermentation, reaching a maximum at 8 h, after which it gradually declined. This reduction may be attributed to nutrient depletion and accumulation of metabolites that inhibit bacterial growth. Similarly, the sensory score increased initially, peaked at 8 h, and then decreased, suggesting that prolonged fermentation could lead to undesirable changes in flavor and overall acceptability.

Notably, the optimal fermentation time derived from single-factor experiments (8 h) differs from that obtained through RSM optimization (4 h). This discrepancy can be explained by the fundamental differences between the two experimental approaches. In single-factor experiments, each variable is optimized independently while holding other factors constant. Under such conditions, longer fermentation time allowed sufficient metabolic activity and microbial proliferation, thereby enhancing both viability and sensory attributes.

In contrast, RSM accounts for interactions among multiple variables. The shorter optimal time identified by RSM reflects the combined effects of other optimized parameters, particularly higher inoculum size (3.5%) and optimal temperature (37°C), which accelerated microbial metabolism and reduced the time required to achieve peak quality. Additionally, significant interaction terms (e.g., AC and AD) in the RSM model support the notion that fermentation time interacts strongly with inoculum size and temperature, leading to a more efficient process under multi-factor optimized conditions.

Therefore, the REM-derived fermentation time of 4 h is not only statistically validated but also practically advantageous, offering reduced processing time and enhanced efficiency while maintaining high product quality.

### 3.3 Analysis of Response Surface Experimental Results

Through the above single factor experiment, it was found that when the fermentation time was 4 hours, the ratio of solid to liquid was 20:1, the inoculum size was 3%, and the fermentation temperature was 37 ℃, the sensory score was the highest, and the number of viable bacteria met the fermentation requirements. Therefore, with the sensory score as the evaluation standard, the experimental range was set around the condition values obtained by single factor optimization (fermentation time 2 ~ 6 h, solid-liquid ratio 10:1 ~ 30:1, inoculation amount 2% ~ 4%, fermentation temperature 34 ~ 40 ℃), and the response surface optimization experiment was carried out on the four key factors. Based on the Box–Behnken experimental design, a total of 29 experiments were conducted, with the corresponding design parameters and results presented in Table 3.

**Table 3. Box-Behnken Experimental Design and Results**

| Serial number | A Fermentation time | B Solid-liquid ratio | C Inoculum size | D Fermentation temperature | Y Sensory score |
|---|---|---|---|---|---|
| 1 | 0 | 1 | 0 | 1 | 68.3 |
| 2 | 0 | 1 | -1 | 0 | 68.7 |
| 3 | 1 | -1 | 0 | 0 | 68.4 |
| 4 | -1 | 0 | 1 | 0 | 71 |
| 5 | 0 | 0 | 0 | 0 | 75.5 |
| 6 | -1 | 0 | 0 | -1 | 63.5 |
| 7 | 0 | 0 | -1 | 1 | 64 |
| 8 | -1 | 0 | 0 | 1 | 71 |
| 9 | -1 | 1 | 0 | 0 | 70 |
| 10 | 0 | 0 | -1 | -1 | 66.2 |
| 11 | 0 | 0 | 0 | 0 | 77.5 |
| 12 | 0 | 0 | 0 | 0 | 76.8 |
| 13 | 1 | 0 | 0 | 1 | 62 |
| 14 | 1 | 0 | 1 | 0 | 64.5 |
| 15 | 0 | -1 | 0 | 1 | 71.3 |
| 16 | 1 | 0 | -1 | 0 | 65.1 |
| 17 | 0 | 0 | 0 | 0 | 76.5 |
| 18 | 1 | 1 | 0 | 0 | 61.5 |
| 19 | 0 | 1 | 0 | -1 | 67.8 |
| 20 | 0 | 0 | 0 | 0 | 75 |
| 21 | 0 | -1 | 0 | -1 | 68 |
| 22 | 0 | -1 | -1 | 0 | 69.7 |
| 23 | 0 | 1 | 1 | 0 | 70.5 |
| 24 | -1 | 0 | -1 | 0 | 61.5 |
| 25 | 0 | -1 | 1 | 0 | 74.7 |
| 26 | 0 | 0 | 1 | 1 | 70.5 |
| 27 | -1 | -1 | 0 | 0 | 70 |
| 28 | 0 | 0 | 1 | -1 | 68.4 |
| 29 | 1 | 0 | 0 | -1 | 64.4 |

According to the results of central composite design, the model was fitted by Design Expert 13 software, and the multiple quadratic regression equation between sensory score and four experimental factors was obtained:

$Y=76.26-1.76A-1.28B+2.03C+0.73D-1.72AB-2.52AC-2.48AD-0.8BC-0.7BD+1.07CD-6.56A^2-2.06B^2-3.82C^2-5.00D^2$

The data of the response surface model were subjected to analysis of variance, and the results are listed in Table 4. Tabulated results show a p-value of less than 0.0001 for the model, indicating that the multivariate quadratic model possesses statistical significance and an excellent fit quality.

The p value of the lack of fit term is 0.3412, which exceeds the threshold of 0.05, indicating that the abnormal data in the experiment is within the acceptable range, the experimental error is small, and the data is credible. The model exhibited a coefficient of determination ($R^2$) of 0.9663 and an adjusted $R^2$ of 0.9326, suggesting that the four independent variables accounted for 93.26% of the observed experimental variation.

Among the four factors, fermentation temperature (D) had a p-value greater than 0.05, indicating that it had no significant effect on the sensory score, while the other three factors had a p-value less than 0.05, indicating that they had a significant effect on the sensory score. According to the F value of the regression coefficient, it can be judged that the influence degree of the four factors on the sensory score is C > A > B > D, that is, the inoculum size (C) has the greatest influence on the sensory score of the fermented beverage of LRM, followed by the fermentation time (A), then the ratio of material to liquid (B), and finally the fermentation temperature (D). It can be seen from Table 4 that the interaction items AB, AC and AD have significant effects on the sensory score (p <

0.05), which means that there are significant interactions between the fermentation time and the ratio of solid to liquid, inoculum size and fermentation temperature, and these interactions exert substantial influence on the sensory characteristics of lactic acid–fermented LRM beverages.

**Table 4. Analysis of Variance of Response Surface Model**

| Source | Sum of Squares | df | Mean Square | F Value | P value Prob > F |
|---|---|---|---|---|---|
| Model | 584.85 | 14 | 41.77 | 28.67 | < 0.0001 |
| A-Fermentation time | 37.10 | 1 | 37.10 | 25.46 | 0.0002 |
| B-Liquid-material ratio | 19.51 | 1 | 19.51 | 13.39 | 0.0026 |
| C-inoculum size | 49.61 | 1 | 49.61 | 34.04 | < 0.0001 |
| D-fermentation temperature | 6.45 | 1 | 6.45 | 4.43 | 0.0539 |
| AB | 11.90 | 1 | 11.90 | 8.17 | 0.0127 |
| AC | 25.50 | 1 | 25.50 | 17.50 | 0.0009 |
| AD | 24.50 | 1 | 24.50 | 16.81 | 0.0011 |
| BC | 2.56 | 1 | 2.56 | 1.76 | 0.2063 |
| BD | 1.96 | 1 | 1.96 | 1.34 | 0.2656 |
| CD | 4.62 | 1 | 4.62 | 3.17 | 0.0966 |
| A2 | 279.07 | 1 | 279.07 | 191.49 | < 0.0001 |
| B2 | 27.50 | 1 | 27.50 | 18.87 | 0.0007 |
| C2 | 94.74 | 1 | 94.74 | 65.01 | < 0.0001 |
| D2 | 161.95 | 1 | 161.95 | 111.12 | < 0.0001 |
| Residual | 20.40 | 14 | 1.46 | | |
| Lack of Fit | 16.35 | 10 | 1.64 | 1.61 | 0.3412 |
| Pure Error | 4.05 | 4 | 1.01 | | |
| Cor Total | 605.25 | 28 | | | |

Note: $p < 0.01$ indicates extremely significant difference, $p < 0.05$ indicates significant difference, and $p > 0.05$ indicates no significant difference.

The effects of individual factors on the sensory score are visually represented by the response surface and contour plots.

The steeper the response surface and the more compact the contour line in the figure, indicating that the stronger the influence of this factor on the sensory score of LRM lactic acid fermented beverage. Figure 6 shows the response surface and contour plots for the statistically different interaction terms AB, AC, and AD. Observing Fig. 6 (A) and (D), it can be found that the contour lines on the fermentation time axis are denser than the axis of the ratio of material to liquid, and the response surface is steeper, which indicates that the sensory score is more sensitive to the change of the fermentation time. It can be seen from Fig.6 (B) and (E) that when the values of fermentation time and inoculum size are low (-1 to 0), the contour density of inoculum size slightly exceeds the fermentation time, demonstrating that the effect of inoculum size on sensory quality is most evident within this specific range.

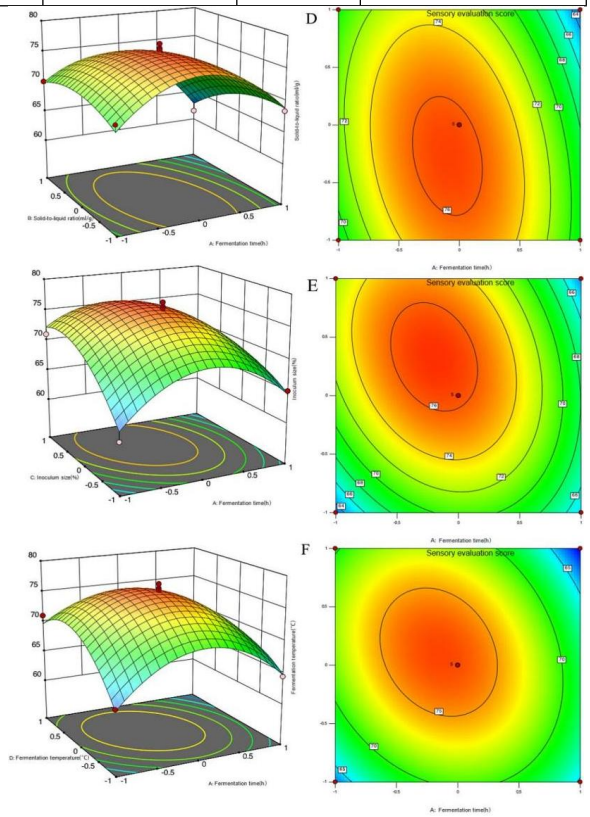



**Figure 6. Response Surface Plots (A-C) and Contour Plots (D-F) between Factors**

However, when the values of fermentation time and inoculum size were higher (0 to 1), the

contour density of fermentation time was slightly higher than that of inoculum size, indicating that the effect of fermentation time on sensory evaluation was particularly pronounced in this range.

It can be observed from Figure 6 (C) and (F) that when the values of the fermentation time and the fermentation temperature are high (0 to 1), the contour density of the fermentation time is significantly higher than that of the fermentation temperature, indicating that the influence of the fermentation time on the sensory score is more significant in this range; However, when the values of fermentation time and fermentation temperature were low (-1 to 0), a slightly higher contour density was observed for fermentation temperature compared with fermentation time, implying that temperature exerted a stronger effect on sensory scores in this range

Note: (A) and (D) are interactive graphs of fermentation time and liquid-solid ratio; (B) and (E) are interactive graphs of fermentation time and inoculum size; (C) and (F) are interactive graphs of fermentation time and fermentation temperature.

In this study, Design Expert 13 software was used to optimize the fermentation process parameters by numerical optimization function. Taking the sensory score as the optimization objective, the optimal process parameters calculated by the software were as follows: fermentation time was 3.596 hours, the ratio of solid to liquid was 16.67:1, the inoculum size was 3.394%, and the fermentation temperature was 37.56℃. Under these conditions, the predicted sensory score was 77.12. In order to ensure the practicality of the experiment, we adjusted the optimal process parameters as follows: fermentation time 4 h, solid-liquid ratio 15:1, inoculation amount 3.5%, fermentation temperature 37℃. The results showed that the fermented beverage had a fresh taste, appropriate acidity, bright color and uniform texture, and the final sensory score was 78, which showed a high level of agreement with the predicted value.

### 3.4 Analysis of Antioxidant Activity of LMRJ

3.4.1 DPPH free radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical exhibits a characteristic absorption maximum at 517 nm, which decreases upon reaction with hydrogen- or electron-donating antioxidants [25]. Antioxidant activity is thus quantified via the reduction in absorbance, which correlates with radical neutralization capacity.

In this study, the $IC_{50}$ of LRMJ decreased from 0.1421 μmol/L pre-fermentation to 0.1092 μmol/L post-fermentation, representing a 23.15% improvement in radical scavenging capacity. This enhancement may derive from LAB-mediated hydrolysis of complex phenolics into lower molecular weight compounds with higher antioxidant potential, as well as the cleavage and liberation of phenolic acids bound within the fruit matrix.

3.4.2 ABTS +·free radical scavenging activity

The $ABTS^{+}$ assay detects both hydrogen atom transfer (HAT) and single electron transfer (SET) antioxidant mechanisms [26]. In the present study, fermentation reduced the $IC_{50}$ for $ABTS^{+}$·scavenging from 0.0587 μmol/L to 0.0467 μmol/L, indicating a 20.44% increase in antioxidant capacity. Similar to the DPPH results, this may be attributed to the liberation of phenolic compounds and anthocyanins via microbial enzymatic activity, as well as the generation of bioactive fermentation metabolites such as exopolysaccharides and peptides with inherent antioxidant properties.

## 4. Conclusion

This study demonstrated that controlled fermentation with Lactiplantibacillus plantarum GH-6 significantly enhances the sensory properties and antioxidant activity of Lycium ruthenicum Murr. juice (LRMJ). Using a combination of single-factor and response surface methodology (RSM) optimization, the key process parameters were established as follows: inoculum size 3.5%, solid-to-liquid ratio 15:1 (g/mL), temperature 37°C, and fermentation time 4 h. Under these conditions, the fermented beverage achieved a high sensory score of 78 and a viable count exceeding $10^9$ CFU/mL, along with a notable increase in antioxidant capacity—23.15% for DPPH and 20.44% for $ABTS^{+}$• radical scavenging activities.

The improvement in antioxidant activity is likely attributed to the biotransformation of phenolic compounds, increased liberation of bound antioxidants, and possible production of microbial metabolites such as exopolysaccharides and bioactive peptides during fermentation.

Despite the promising outcomes, several limitations must be addressed for industrial

scalability. The fermentation process relies on strict control of parameters such as temperature and inoculum size, which may pose challenges in large-scale production due to potential heterogeneity in mixing and heat transfer. In addition, the use of pure culture fermentation requires aseptic conditions and may increase operational costs. Further studies are needed to evaluate the stability of antioxidant compounds and probiotic viability during storage, as well as the economic feasibility of scaling up the proposed process. The potential need for additives or processing aids to enhance stability and shelf-life should also be considered.

Future research should focus on the validation of these results in pilot-scale fermenters, detailed profiling of phenolic and anthocyanin transformation using HPLC–MS/MS, in vivo assessment of health benefits, and exploration of mixed-culture fermentation strategies to further improve functionality and reduce production costs.

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