

Article

Optimization of Blue Honeysuckle Co-Fermentation with *Lactobacillus rhamnosus* and *Saccharomyces cerevisiae* to Enhance Total Phenolic Content and Antioxidant Activity

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Abstract

Blue honeysuckle (*Lonicera caerulea* L.) is a cold-adapted berry rich in bioactive polyphenols. The association of phenolic compounds with the plant cell-wall matrix may limit their extractability. To promote polyphenol release and enhance antioxidant properties, a blue honeysuckle matrix was co-fermented with *Lactobacillus rhamnosus* 6224 and *Saccharomyces cerevisiae* W5. Process parameters were optimized using response surface methodology, with total phenolic content (TPC) as the response variable. Under the optimized conditions, TPC reached 1374.79 mg/100 g. Compared with the unfermented control, DPPH radical, ABTS radical, H₂O₂, and hydroxyl radical scavenging activities increased by 4.27, 15.10, 12.89, and 12.63 percentage points, respectively. LC-MS profiling showed that co-fermentation markedly altered the phenolic profile of the blue honeysuckle matrix and increased the relative abundances of several putatively annotated phenolic compounds, including quercetin, protocatechuic acid, catechin, and caffeic acid. These findings indicate that the optimized co-fermentation process increased TPC, altered phenolic composition, and enhanced *in vitro* antioxidant activity, offering a potential approach to the value-added processing of blue honeysuckle.

Keywords: co-fermentation; blue honeysuckle; antioxidant activity; polyphenols; process optimization



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1. Introduction

Berries are valued for their nutritional composition and potential health benefits [1]. However, their high moisture content, soft texture, and susceptibility to mechanical damage limit their shelf life and commercial utilization. Therefore, exploring green processing methods to fully exploit their functional potential has become an important research direction to support improvements in both quality and production efficiency within the berry industry [2].

Blue honeysuckle (*Lonicera caerulea* L.) is a wild berry species native to cold temperate regions [3]. In China, it occurs mainly in the northeast, north, and northwest, with abundant wild populations in the Greater and Lesser Khingan Mountains and the Changbai

Mountains [4]. It exhibits remarkable antioxidant activity, largely owing to its abundant polyphenolic compounds, including anthocyanins, flavonols, and phenolic acids [5]. A polyphenol content of up to 1142 mg/100 g has been reported, corresponding to 3.46 and 4.80 times that of blueberries and strawberries, respectively [6]. Polyphenols are widely distributed in plants and have been studied for their antioxidant, anti-inflammatory, and antimicrobial activities, as well as their potential protective effects against type 2 diabetes mellitus [7,8]. However, their functional properties depend in part on their chemical forms and accessibility within the plant matrix. Many polyphenols occur in bound forms, which can hinder their release and reduce the extractable phenolic fraction [9]. Processing strategies that promote the liberation of bound polyphenols and increase the abundance of readily extractable phenolic compounds may therefore help enhance the functional value of berry products.

Microbial fermentation, a green and efficient bioprocessing strategy, has been widely applied in fruit and vegetable processing [10]. By facilitating the release and transformation of bound phenolic fractions, it has been reported to increase TPC and the abundance of extractable phenolic compounds, with corresponding changes in antioxidant properties [3,11]. Lactic acid bacteria (LAB) and yeasts are commonly used starter cultures owing to their safety, metabolic diversity, and long history of application in food fermentation. Studies have reported increases in polyphenol content, changes in phenolic composition, and enhanced antioxidant activity following fermentation of various plant matrices [12]. For example, co-fermentation of chickpea milk with *Lactobacillus fermentum* GD01 and *Lactobacillus plantarum* BHP03 increased TPC and altered distinct phenolic fractions, and these changes were strongly correlated with improved antioxidant capacity [13]. Similarly, fermentation of apple juice and berry beverages with LAB and other beneficial microorganisms can also enhance antioxidant capacity and promote phenolic transformation [14,15]. Collectively, these findings suggest that microbial fermentation represents a viable strategy for improving the functional properties of polyphenol-rich plant materials.

Optimization of fermentation process parameters is important for increasing phenolic content and enhancing the functional properties of fermented products. Response surface methodology (RSM) enables the optimization of complex fermentation systems by evaluating both individual effects and interactions among multiple variables. Previous studies have shown that optimized fermentation conditions can increase the abundance of aglycone polyphenols, including quercetin, kaempferol, and gossypetin, and raise TPC [16]. Process optimization has also been reported to enhance antioxidant capacity [17], improve polyphenol extraction, and reduce losses of bioactive compounds during processing [18].

Previous studies have demonstrated the considerable potential of microbial fermentation to promote the release and transformation of plant polyphenols and to enhance their associated functional properties. However, the effects of specific LAB-yeast combinations and key process parameters on phenolic transformation and antioxidant properties require further investigation in blue honeysuckle. Based on preliminary strain-screening results from our research group, *Lactobacillus rhamnosus* 6224 and *Saccharomyces cerevisiae* W5, both of which exhibited favorable growth and adaptability in the blue honeysuckle matrix, were selected to establish a co-culture fermentation system. In addition, because phenolic compounds may be associated with or constrained by the plant cell-wall matrix, cellulase and pectinase were selected as pretreatment enzymes to improve phenolic accessibility by acting on different cell-wall polysaccharide components [19]. Key fermentation conditions were then optimized using RSM, with TPC as the response variable. Subsequently, LC-MS profiling was performed to characterize changes in the phenolic profile before and after fermentation, and *in vitro* antioxidant assays were used to assess changes in antioxidant activity. Associations between phenolic profiles and antioxidant indices were then examined.

This study aimed to establish an optimized LAB-yeast co-fermentation process for blue honeysuckle, thereby providing a basis for its value-added processing and the development of fermented berry products.

2. Materials and Methods

2.1. Microbial Culture Conditions

L. rhamnosus 6224 and *S. cerevisiae* W5 were provided by the Key Laboratory of Microbiology, Heilongjiang University. To prepare the inocula, *L. rhamnosus* 6224 was cultured in de Man, Rogosa, and Sharpe (MRS) medium (Beijing Auboxing Biotechnology Co., Ltd., Beijing, China) at 37 °C for 24 h with shaking at 140 rpm. *S. cerevisiae* W5 was cultured in yeast extract–peptone–dextrose (YPD) medium (Beijing Auboxing Biotechnology Co., Ltd., Beijing, China) at 30 °C for 48 h at the same shaking speed. Before inoculation, the viable cell concentration of each culture was adjusted to 1×10^8 CFU/mL.

2.2. Preparation and Fermentation of the Blue Honeysuckle Matrix

Fresh blue honeysuckle fruits were mixed with deionized water at the predetermined solid-to-liquid ratio and homogenized to obtain a uniform blue honeysuckle matrix. Before enzymatic hydrolysis, the matrix pH was initially adjusted to 5.0 using food-grade sodium bicarbonate. The pH was then adjusted to the value specified in each experimental design. Enzymatic hydrolysis was performed using cellulase (400 U/mg) and pectinase (50,000 U/g), both purchased from Shanghai Yuan Ye Bio-Technology Co., Ltd. (Shanghai, China).

The enzyme-treated matrix was heat-treated at 108 °C for 20 min and then cooled to room temperature. The heat-treated matrix was subsequently divided into two portions, which were incubated in parallel under identical conditions. One portion was left uninoculated as a time-matched control and designated unfermented blue honeysuckle (UBH). The other was inoculated with *L. rhamnosus* 6224 and *S. cerevisiae* W5 according to the experimental design and designated fermented blue honeysuckle (FBH). Both UBH and FBH were subjected to the same thermal pretreatment and subsequent processing conditions, with inoculation being the only difference between the two groups. After incubation, the samples were centrifuged at 6000 rpm for 10 min, and the supernatants were collected for analysis.

2.3. Determination of TPC

TPC was measured using the Folin–Ciocalteu method with minor modifications based on the procedure reported by Zheng et al. [20]. For the assay, an aliquot (0.2 mL) of each sample was mixed with 0.5 mL of Folin–Ciocalteu reagent and allowed to react for 3 min. Subsequently, 1.5 mL of 10% Na₂CO₃ solution was added, and the mixture was brought to the desired volume with distilled water. Following a 20 min incubation at room temperature in the dark, the absorbance of the mixture was recorded at 765 nm using a VersaMax microplate reader (Molecular Devices, San Jose, CA, USA). Gallic acid was used to construct the calibration curve. All reagents mentioned above were obtained from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). TPC was expressed as milligrams of gallic acid equivalents (GAE) per 100 g of initial fresh blue honeysuckle fruit (mg/100 g) and calculated as follows:

$$TPC(\text{mg}/100\text{ g}) = \left(\frac{C \times V \times n}{m} \right) \times 100$$

where C is the GAE concentration in the sample solution (mg/mL); V is the sample solution volume (mL); n is the sample dilution factor; and m is the initial mass of fresh blue honeysuckle fruit (g).

2.4. Optimization of the Blue Honeysuckle Co-Fermentation Process

RSM uses mathematical models to evaluate relationships between multiple process variables and a response [21]. TPC measured using a standardized Folin–Ciocalteu assay was used as the response variable throughout screening and optimization. In the present study, RSM was applied to elucidate the contributions of individual variables and their interactions, thereby identifying the process conditions associated with the highest TPC [22].

2.4.1. Single-Factor Experiments

Using TPC (mg/100 g) as the response variable, the effects of 12 variables were evaluated in single-factor experiments: enzyme type (cellulase, pectinase, and a combination of cellulase and pectinase), enzyme dosage (0.04, 0.08, 0.16, 0.32, 0.64, and 1.28%), enzyme ratio (1:7, 1:3, 3:5, 1:1, 5:3, 3:1, and 7:1), enzymatic hydrolysis pH (3.5, 4.0, 4.5, 5.0, 5.5, 6.0, and 6.5), enzymatic hydrolysis temperature (35, 40, 45, 50, 55, 60, and 65 °C), enzymatic hydrolysis time (30, 60, 120, 180, 240, and 300 min), solid-to-liquid ratio (1:1, 1:5, 1:10, 1:15, and 1:20 g:mL), inoculum size (1, 2, 3, 4, 5, 8, and 10%, v/v), strain ratio (*L. rhamnosus* 6224: *S. cerevisiae* W5, v/v ; 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, and 1:4), fermentation time (18, 24, 30, 36, 42, 48, 60, and 72 h), fermentation temperature (26, 30, 34, and 38 °C), and shaking speed (0, 60, 120, 140, 160, and 180 rpm).

The initial conditions were as follows: blue honeysuckle fruit was mixed with water at a solid-to-liquid ratio of 1:10 (g:mL) and pretreated with 0.16% of a cellulase–pectinase mixture (1:1) at pH 6.0 and 50 °C for 120 min. Subsequently, the matrix was inoculated with a mixed culture of *L. rhamnosus* 6224 and *S. cerevisiae* W5. Inoculum size was defined as the total volume of the mixed microbial suspension relative to the matrix volume (v/v), whereas strain ratio was defined as the volumetric ratio of the *L. rhamnosus* and *S. cerevisiae* suspensions, each adjusted to 1×10^8 CFU/mL prior to inoculation. The initial strain ratio was 1:1, the total inoculum size was 5% (v/v), and fermentation was performed at 30 °C for 42 h with shaking at 140 rpm.

2.4.2. Screening of Key Factors by Plackett–Burman (PB) Design

Based on the single-factor results, six factors were selected for screening using a PB design in Design-Expert 10: enzyme dosage (X_1), enzyme ratio (X_2), enzymatic hydrolysis pH (X_3), enzymatic hydrolysis temperature (X_4), strain ratio (X_5), and fermentation time (X_6). TPC was used as the response variable. Each factor was evaluated at a low (−1) and a high (+1) level, as shown in Table 1.

Table 1. Selected fermentation variables and their levels in the PB design.

Levels	Factors					
	X_1 Enzyme Dosage/%	X_2 Enzyme Ratio	X_3 Enzymatic Hydrolysis pH	X_4 Enzymatic Hydrolysis Temperature (°C)	X_5 Strain Ratio	X_6 Fermentation Time (h)
−1	0.12	4:3	5.2	52	1:4	44
1	0.20	2:1	5.8	58	1:2	52

2.4.3. Steepest Ascent Design

Factors that significantly affected TPC in the PB design were selected for the steepest ascent experiment. Factor levels were adjusted stepwise toward the region of the highest response to identify an appropriate center point for the subsequent Box–Behnken design (BBD). Factors with positive effects were increased, whereas those with negative effects were decreased.

2.4.4. BBD Experimental Design and Process Optimization

A BBD was used to optimize the blue honeysuckle fermentation process. This design allows a second-order response surface model to be fitted with relatively few experimental runs while avoiding combinations in which all factors are at their extreme levels [23]. A three-factor, three-level design was established using the significant factors identified by PB screening and the response region identified by steepest ascent, with TPC (Y) as the response [24]. The factors and levels are presented in Table 2.

Table 2. Coded factors and corresponding levels in the BBD.

Factors	Number	Levels		
		−1	0	1
Enzymatic hydrolysis pH	X ₃	5.9	6.0	6.1
Enzymatic hydrolysis temperature/°C	X ₄	49	50	51
Fermentation time/h	X ₆	56	58	60

2.4.5. Verification Experiments

The model predictions were evaluated in five independent fermentation runs under the optimized conditions.

2.5. Evaluation of Antioxidant Activity In Vitro

The antioxidant properties of UBH and FBH were evaluated using DPPH radical, ABTS radical, H₂O₂, and hydroxyl radical (·OH) scavenging assays and a reducing power assay. Ascorbic acid (VC) served as the positive control. These assays were performed based on previously reported methods, with slight modifications to suit the characteristics of the sample matrix [25–29]. Further methodological details are provided in Supplementary Text S1. Before analysis, samples were diluted with double-distilled water (ddH₂O) to 10%, 25%, 50%, and 75% (v/v); undiluted samples were designated 100% (v/v). Ascorbic acid solutions were prepared at 0.10, 0.25, 0.50, 0.75, and 1.00 mg/mL. Unless otherwise specified, reactions were performed at room temperature. Absorbance was measured using a VersaMax microplate reader, and scavenging activities and reducing power were calculated using the corresponding equations.

2.6. Profiling of Phenolic Compounds in Blue Honeysuckle

The phenolic profiles of UBH and FBH were analyzed using ultra-performance liquid chromatography coupled to a Q-Exactive HF-X high-resolution mass spectrometer (UPLC–HRMS). Phenolic compounds were putatively annotated by comparing accurate precursor masses and MS/MS fragmentation patterns with entries in the HMDB and METLIN databases. Differences are reported as relative abundances rather than absolute concentrations. Chromatographic and mass spectrometric parameters are provided in Supplementary Text S2.

2.7. Statistical Analyses

Unless otherwise specified, experiments were conducted in triplicate, and results are presented as the mean $\pm$ SD of three independent experiments. Model validation was conducted using five independent fermentation runs. Multi-level single-factor experiments were analyzed by one-way ANOVA followed by Tukey's HSD multiple-comparison test using JMP 13, with $p < 0.05$ considered statistically significant and $p < 0.01$ considered highly significant. Design-Expert 10 was used to design and analyze the PB and BBD experiments. Multivariate statistical analyses were performed using the OmicShare Bioinformatics Cloud Platform (<https://www.omicshare.com>) (accessed on 21 September 2026).

3. Results and Discussion

3.1. Influence of Single Factors on TPC

The effects of cellulase, pectinase, and their combination on TPC are shown in Figure 1a. Enzyme treatment significantly affected TPC, and all enzyme-treated groups had higher values than the untreated control. Cellulase and pectinase alone yielded TPC values of 464.97 ± 1.89 and 462.49 ± 6.42 mg/100 g, respectively, corresponding to 1.16 and 1.15 times the control value. The combined enzyme treatment increased TPC to 486.39 ± 5.84 mg/100 g, corresponding to 1.21 times the control value. Therefore, the combined enzyme treatment was selected for subsequent optimization.

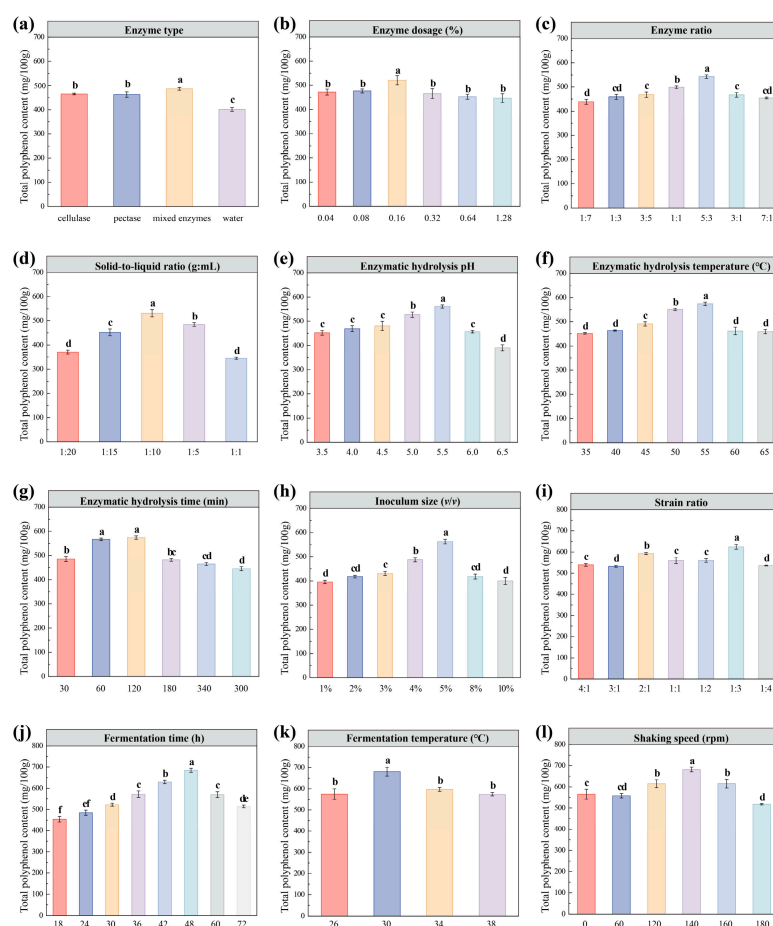


Figure 1. Effects of individual process variables on the total phenolic content of blue honeysuckle: enzyme type (a), enzyme dosage (b), enzyme ratio (c), solid-to-liquid ratio (d), enzymatic hydrolysis pH (e), enzymatic hydrolysis temperature (f), enzymatic hydrolysis time (g), inoculum size (h), strain ratio (i), fermentation time (j), fermentation temperature (k), and shaking speed (l). Different lowercase letters indicate significant differences according to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$).

At suitable levels, cellulase and pectinase can disrupt the plant cell wall matrix, thereby increasing the accessibility of bound polyphenols and contributing to an increase in TPC [30]. As shown in Figure 1b, the combined enzyme dosage significantly affected TPC across the range of 0.04–1.28%. TPC initially increased with increasing enzyme dosage and subsequently decreased at higher levels. The highest TPC (520.58 ± 19.30 mg/100 g) was obtained at an enzyme dosage of 0.16%, which was selected for subsequent experiments.

Because cellulase and pectinase act on different components of the plant cell wall, their relative proportions may influence the efficiency of polyphenol release. The highest TPC (543.24 ± 7.24 mg/100 g) was obtained at a cellulase-to-pectinase ratio of 5:3 (Figure 1c), which was therefore selected for subsequent enzymatic hydrolysis.

The solid-to-liquid ratio can affect matrix consistency, nutrient availability, and osmotic conditions, with consequences for microbial growth and metabolism. As the amount of water per gram of fruit increased, TPC initially increased and then decreased, reaching a maximum of 531.70 ± 15.04 mg/100 g at 1:10 (g:mL) (Figure 1d). At higher fruit loadings, increased matrix viscosity may have limited mass transfer and microbial activity. At greater dilution, lower nutrient availability may have reduced microbial activity and phenolic release or transformation. Thus, the condition corresponding to the maximum TPC was selected for subsequent experiments.

Because cellulase and pectinase exhibit different pH dependencies, identifying an appropriate pH is important for their combined application. TPC initially increased and then decreased as the enzymatic hydrolysis pH increased, reaching a maximum of 560.96 ± 6.34 mg/100 g at pH 5.5 (Figure 1e). Therefore, pH 5.5 was selected for subsequent experiments.

Previous studies have demonstrated that temperature is another critical factor affecting enzyme activity, due to differences in thermal sensitivity among enzymes [31]. TPC initially increased with increasing enzymatic hydrolysis temperature and subsequently decreased (Figure 1f). The maximum TPC (574.14 ± 6.81 mg/100 g) was obtained at 55 °C, which was selected for subsequent screening.

The duration of enzymatic hydrolysis influences the extent of the enzymatic reaction [32]. As shown in Figure 1g, the TPC obtained after 60 min of hydrolysis (566.73 ± 5.15 mg/100 g) did not differ significantly from that obtained after 120 min (574.14 ± 6.81 mg/100 g; $p > 0.05$). Considering processing time, production costs, and energy consumption, a hydrolysis time of 60 min was adopted for subsequent experiments.

Inoculum size is an important factor affecting microbial growth and fermentation performance. As the inoculum size increased, TPC initially increased and then decreased (Figure 1h), reaching a maximum of 562.20 ± 9.11 mg/100 g at 5%. At inoculum sizes below 5%, slower microbial growth and lower biomass may have limited microbial activity and phenolic release or transformation. In contrast, larger inocula may have accelerated nutrient depletion and altered subsequent microbial metabolism, thereby contributing to the decrease in TPC. An inoculum size of 5% was therefore selected for subsequent experiments.

The present study further evaluated the effect of the inoculation ratio of *L. rhamnosus* 6224 and *S. cerevisiae* W5 on TPC, based on the previously established mixed-culture system [33]. As shown in Figure 1i, the highest TPC (624.40 ± 10.77 mg/100 g) was obtained at a strain ratio of 1:3 (*L. rhamnosus* 6224: *S. cerevisiae* W5), which was therefore selected for subsequent experiments. The effect of strain ratio may reflect differences in substrate utilization and metabolic activity between the two microorganisms. LAB-associated acidification and phenolic metabolism, together with yeast carbohydrate utilization and related enzyme-mediated processes, may jointly modify the fermentation environment and influence phenolic transformation [34,35]. However, these strain-specific mechanisms were not directly examined in the present study and require further investigation.

As fermentation proceeded, the TPC increased between 18 and 48 h, reaching a maximum of 684.97 ± 9.33 mg/100 g at 48 h, and then declined (Figure 1j). The relatively small increase between 18 and 24 h may reflect adaptation of the inoculated strains to the matrix. Subsequent increases may be associated with greater microbial activity and phenolic release or transformation. Similar temporal patterns have been reported for LAB-fermented fruit juices, in which TPC peaked at an intermediate stage and declined with prolonged fermentation [16,36]. The later decrease may reflect further microbial transformation of phenolic compounds, rather than a single specific process. Based on the overall TPC response, 48 h was selected as the fermentation time for subsequent screening.

Fermentation temperature affects microbial growth, metabolism, and product formation. TPC increased and then decreased with increasing fermentation temperature, reaching a maximum of 680.43 ± 20.77 mg/100 g at 30 °C (Figure 1k). This pattern may reflect favorable microbial activity at an intermediate temperature, whereas higher temperatures may inhibit microbial physiological activities and promote phenolic oxidation [37]. Accordingly, a fermentation temperature of 30 °C was selected for subsequent experiments.

TPC increased significantly as the shaking speed increased from 0 to 140 rpm, reaching a maximum of 681.67 ± 11.21 mg/100 g, and subsequently decreased at higher speeds (Figure 1l). Moderate shaking may have improved mixing and mass transfer, thereby facilitating microbial–substrate interactions and phenolic release or transformation. At higher shaking speeds, changes in shear conditions and oxygen transfer may have affected microbial activity and phenolic stability; however, the precise mechanism underlying the decrease in TPC remains to be established. Based on these results, a shaking speed of 140 rpm was selected for subsequent experiments.

3.2. Screening Using the PB Design

The PB design is an efficient screening approach for identifying factors that significantly affect a response from a larger set of candidate variables [38]. Based on the single-factor experiments, six factors were evaluated for their effects on TPC (Y) during blue honeysuckle fermentation: combined enzyme dosage (X_1), enzyme ratio (X_2), enzymatic hydrolysis pH (X_3), enzymatic hydrolysis temperature (X_4), strain ratio (X_5), and fermentation time (X_6). The design matrix and corresponding experimental results are summarized in Table 3, and the ANOVA results are presented in Table 4. A regression model was fitted to the experimental data using Design-Expert 10, as expressed by Equation (1):

$$Y = 688.60 - 9.27X_1 - 12.22X_2 + 79.03X_3 - 27.26X_4 + 13.05X_5 + 19.36X_6 \quad (1)$$

Table 3. PB experimental design and results.

Experiment	X_1	X_2	X_3	X_4	X_5	X_6	TPC (mg/100 g)
1	−1	1	1	−1	1	1	847.70 ± 28.78
2	−1	−1	1	−1	1	1	825.45 ± 32.26
3	1	1	−1	−1	−1	1	636.76 ± 16.16
4	−1	−1	−1	−1	−1	−1	633.47 ± 9.60
5	1	−1	1	1	1	−1	752.12 ± 25.40
6	−1	−1	−1	1	−1	1	602.57 ± 23.53
7	−1	1	1	1	−1	−1	719.16 ± 22.83
8	1	−1	1	1	−1	1	750.47 ± 45.13
9	1	1	1	−1	−1	−1	710.92 ± 15.44
10	−1	1	−1	1	1	−1	558.90 ± 23.16
11	1	1	−1	1	1	1	584.85 ± 6.54
12	1	−1	−1	−1	1	−1	640.88 ± 7.03

Table 4. ANOVA for PB experimental results.

Term	Coefficient Estimate	df	F-Value	p-Value	Significance
Model	—	6	25.14	0.0014	**
Intercept	688.60	1	—	—	—
X ₁	−9.27	1	1.67	0.2530	ns
X ₂	−12.22	1	2.90	0.1493	ns
X ₃	79.03	1	121.24	0.0001	***
X ₄	−27.26	1	14.42	0.0127	*
X ₅	13.05	1	3.30	0.1288	ns
X ₆	19.36	1	7.28	0.0429	*
Residual	—	5	—	—	—
Cor Total	—	11	—	—	—

Note: * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$, and ns indicates no significant difference.

The PB model was significant ($p = 0.0014$), with $R^2 = 0.9679$, adjusted $R^2 = 0.9294$, predicted $R^2 = 0.8152$, and an Adequate Precision value of 14.5208. The predicted R^2 was in reasonable agreement with the adjusted R^2 , and the Adequate Precision value exceeded 4, indicating an adequate signal-to-noise ratio. Among the variables, X₃, X₅, and X₆ exerted positive effects on TPC, whereas X₁, X₂, and X₄ exerted negative effects. As shown in Table 4, X₃, X₄, and X₆ significantly affected TPC ($p < 0.05$), whereas the remaining variables had no significant effects ($p > 0.05$).

3.3. Steepest Ascent Experiment

Based on the PB screening results, a steepest ascent experiment was conducted to approach the region of optimal response. Since X₃ and X₆ showed positive effects, whereas X₄ had a negative effect, enzymatic hydrolysis pH and fermentation time were increased stepwise, while enzymatic hydrolysis temperature was decreased stepwise. The remaining parameters were fixed at the levels selected in the single-factor experiments: a solid-to-liquid ratio of 1:10 (g:mL), a combined enzyme dosage of 0.16% at a cellulase-to-pectinase ratio of 5:3, and an enzymatic hydrolysis time of 60 min. Fermentation was performed at an *L. rhannosus* 6224 to *S. cerevisiae* W5 ratio of 1:3, a total inoculum size of 5% (v/v), 30 °C, and 140 rpm. The experimental settings and corresponding responses are summarized in Table 5. The highest TPC (1294.86 ± 4.68 mg/100 g) was obtained at an enzymatic hydrolysis pH of 6.0, a hydrolysis temperature of 50 °C, and a fermentation time of 58 h, which represented an increase of approximately 85.37% relative to step 0. These conditions were therefore used as the center point for the subsequent BBD optimization.

Table 5. Steepest ascent design and results.

Step	Enzymatic Hydrolysis pH	Enzymatic Hydrolysis Temperature (°C)	Fermentation Time (h)	TPC (mg/100 g)
0	5.5	55	48	698.51 ± 8.77
1	5.6	54	50	721.93 ± 8.56
2	5.7	53	52	782.46 ± 7.95
3	5.8	52	54	927.51 ± 13.61
4	5.9	51	56	1195.08 ± 10.99
5	6.0	50	58	1294.86 ± 4.68
6	6.1	49	60	1220.10 ± 9.60
7	6.2	48	62	1170.25 ± 18.70
8	6.3	47	64	1002.68 ± 7.95

3.4. Response Surface Optimization Using the BBD

3.4.1. Model Fitting and Analysis of Variance

On the basis of the PB design and steepest ascent results, X_3 , X_4 , and X_6 were chosen as the independent variables, with TPC (Y) serving as the response. The experimental TPC values ranged from 998.64 ± 18.92 to 1350.55 ± 16.35 mg/100 g. A quadratic polynomial model was fitted using Design-Expert 10, and the regression equation is given by Equation (2):

$$Y = 1317.49 + 92.70X_3 + 44.40X_4 + 54.68X_6 - 7.67X_3X_4 - 18.63X_3X_6 + 10.96X_4X_6 - 54.89X_3^2 - 72.13X_4^2 - 29.63X_6^2 \quad (2)$$

As shown in Table 6, the fitted quadratic model was highly significant ($p = 0.0002$), with $R^2 = 0.9402$, adjusted $R^2 = 0.8804$, and predicted $R^2 = 0.5225$. The lack-of-fit term was not significant ($p = 0.0637$), and the adequate precision value was 11.6756, exceeding the recommended threshold of 4. These results indicate that the model provided a satisfactory fit to the observed data within the investigated design space, although the relatively low predicted R^2 indicates comparatively limited predictive performance. Among the model terms, X_6^2 was significant ($p < 0.05$), whereas X_3 , X_4 , X_6 , X_3^2 , and X_4^2 were highly significant ($p < 0.01$). None of the interaction terms was statistically significant ($p > 0.05$).

Table 6. Regression model analysis results.

Term	Coefficient Estimate	F-Value	p-Value	Significance
Model	—	15.73	0.0002	**
Intercept	1317.49	—	—	—
X_3	92.70	57.23	0.0001	**
X_4	44.40	13.13	0.0055	**
X_6	54.68	19.91	0.0016	**
X_3X_4	−7.67	0.23	0.6453	ns
X_3X_6	−18.63	1.35	0.2746	ns
X_4X_6	10.96	0.47	0.5107	ns
X_3^2	−54.89	20.05	0.0015	**
X_4^2	−72.13	34.63	0.0002	**
X_6^2	−29.63	5.84	0.0388	*
Lack of fit		6.27	0.0637	ns

Note: * indicates $p < 0.05$, ** indicates $p < 0.01$, and ns indicates no significant difference.

3.4.2. Response Surface and Contour Analysis

Response surface and contour plots were used to visualize the combined effects of enzymatic hydrolysis pH (X_3), enzymatic hydrolysis temperature (X_4), and fermentation time (X_6) on TPC. Surface curvature and contour shape describe the modeled response, whereas the statistical significance of interaction terms was assessed by ANOVA.

The response surface for enzymatic hydrolysis pH and temperature showed pronounced curvature. TPC initially increased and subsequently decreased as pH and temperature increased within the investigated ranges. Although both factors markedly affected the response, their interaction was not statistically significant according to ANOVA (Figure 2a,b).

The response surface for enzymatic hydrolysis pH and fermentation time indicated that TPC was more responsive to changes in pH than to changes in fermentation time within the investigated ranges. At a fixed fermentation time, TPC increased markedly with increasing enzymatic hydrolysis pH, whereas changes in fermentation time produced a comparatively smaller response at a constant pH. Nevertheless, the interaction between these two factors was not statistically significant (Figure 2c,d).

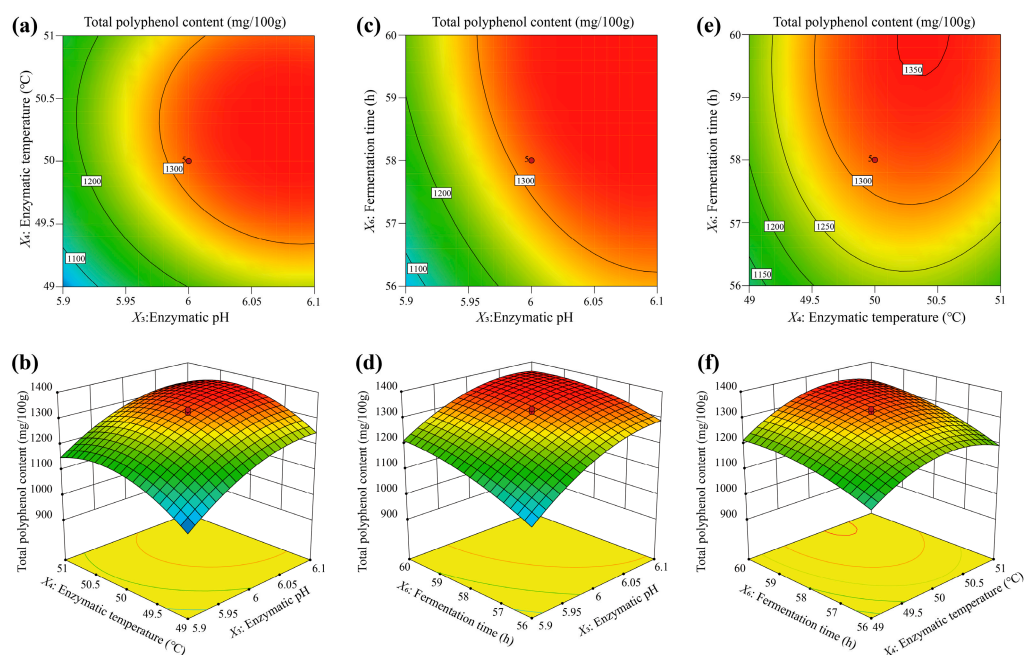


Figure 2. Response surface and contour plots showing the combined effects of enzymatic hydrolysis pH and temperature (a,b), enzymatic hydrolysis pH and fermentation time (c,d), and enzymatic hydrolysis temperature and fermentation time (e,f) on TPC.

The response surface for enzymatic hydrolysis temperature and fermentation time was comparatively flat. At different enzymatic hydrolysis temperatures, TPC generally showed an increase followed by a decrease as fermentation time was extended. Consistent with the ANOVA results, the interaction between enzymatic hydrolysis temperature and fermentation time was not statistically significant (Figure 2e,f).

3.4.3. Model Validation

The model-derived optimal conditions were an enzymatic hydrolysis pH of 6.07, an enzymatic hydrolysis temperature of 50.33 °C, and a fermentation time of 59.53 h, under which the predicted maximum TPC was 1377.80 mg/100 g. For practical operation, these parameters were rounded to pH 6.1, 50.3 °C, and 60 h. Under the adjusted conditions, the experimentally measured TPC was 1374.79 ± 1.66 mg/100 g, compared with the predicted value of 1377.80 mg/100 g, corresponding to a relative error of only 0.22%. These results show that a high TPC was obtained under the selected operating conditions.

3.5. In Vitro Antioxidant Properties of Fermented Blue Honeysuckle

The antioxidant properties of UBH and FBH were evaluated using DPPH radical, ABTS radical, H_2O_2 , and $\cdot\text{OH}$ scavenging assays, as well as a reducing power assay, with VC as the positive control (Figure 3a–e). All measured antioxidant responses generally increased with sample concentration. As fermentation progressed, the antioxidant activity of the blue honeysuckle matrix increased significantly, with the highest values observed at 60 h among the tested fermentation times. At the highest sample concentration tested, the DPPH radical, ABTS radical, H_2O_2 , and $\cdot\text{OH}$ scavenging activities of FBH were $40.59 \pm 0.06\%$, $63.23 \pm 0.77\%$, $60.55 \pm 0.19\%$, and $60.56 \pm 0.17\%$, respectively, while the reducing power was 0.84 ± 0.03 (A_{700}). Relative to UBH, these four scavenging activities increased by 4.27, 15.10, 12.89, and 12.63 percentage points, respectively. For the reducing power assay, FBH exhibited a concentration-dependent increase of 0.303 absorbance units across the tested concentration range. At the tested endpoints, these assay responses were 42.28%, 63.54%, 60.64%, 60.76%, and 85.06% of those measured for the VC control at 1 mg/mL, respectively.

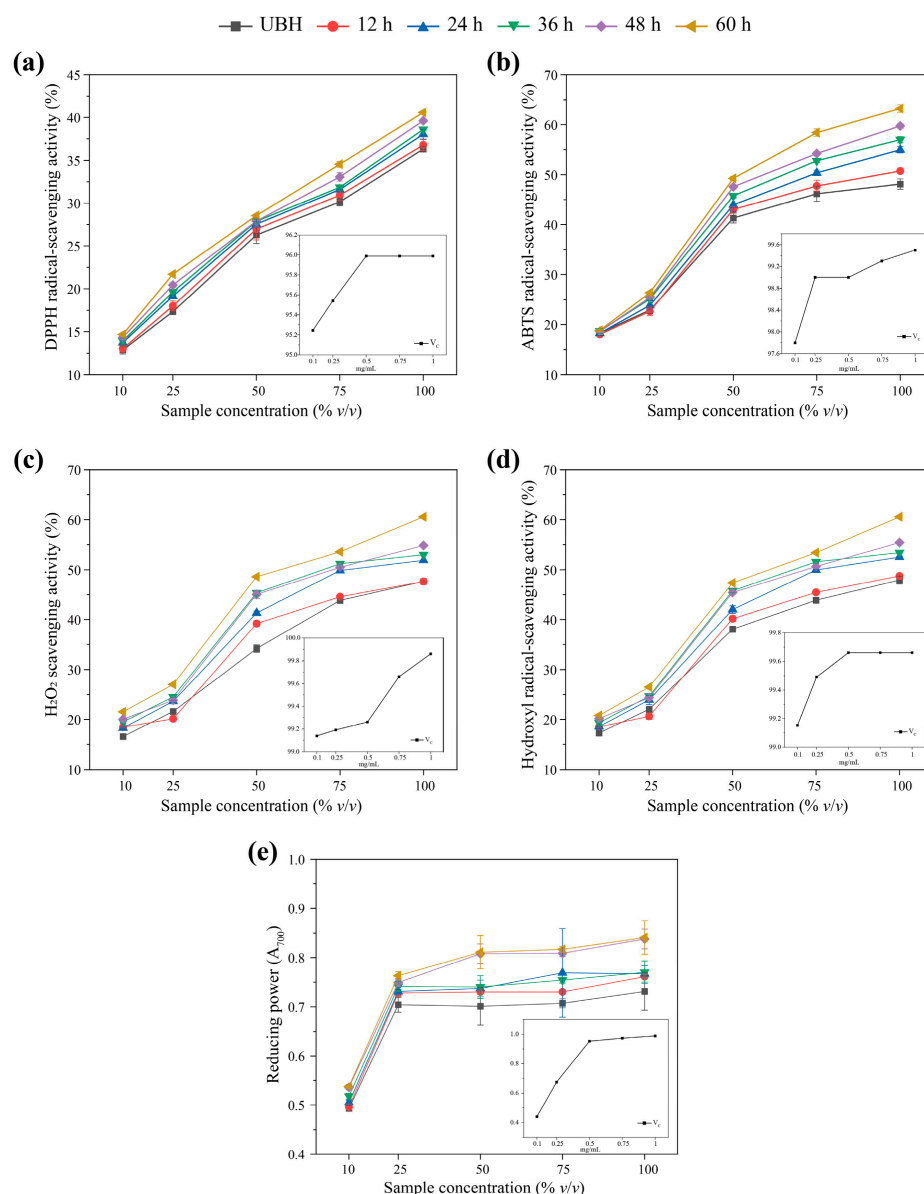


Figure 3. Antioxidant responses of the blue honeysuckle matrix at different sample concentrations and fermentation times: DPPH radical scavenging activity (a), ABTS radical scavenging activity (b), H_2O_2 scavenging activity (c), $\cdot OH$ scavenging activity (d), and reducing power (e).

Notably, the increase in DPPH radical scavenging activity was markedly smaller than those observed for ABTS radical, H_2O_2 , and $\cdot OH$ scavenging activities, indicating that the magnitude of the antioxidant response was assay-dependent. Such variation may reflect differences in the molecular structures of phenolic compounds, the nature of the reactive species involved, and the specific assay conditions employed [39]. Similar assay-dependent antioxidant responses have also been reported in other LAB-fermented plant matrices [36,40]. The enhanced antioxidant capacity may be associated with fermentation-induced changes in phenolic composition and extractability, with the hydrolytic release of bound phenolic compounds representing a possible contributing mechanism. For instance, cashew apple juice fermented with *L. plantarum* TISTR 543 exhibited an approximately 27% increase in antioxidant capacity [41]. Previous studies have suggested that microbial hydrolysis and cell-wall degradation can facilitate the release of antioxidant compounds, while microbial metabolism may also transform these compounds or generate additional antioxidant metabolites [42].

3.6. Polyphenol Composition and Its Correlation with Antioxidant Activity During Fermentation

Differences in phenolic composition between UBH and FBH were characterized using UPLC-Q-Exactive HF-X HRMS. Correlation analysis was further performed to examine the associations between phenolic profiles and antioxidant capacity. PCA revealed clear separation between UBH and FBH, indicating that fermentation led to marked changes in the polyphenolic composition of the blue honeysuckle matrix (Figure 4a). OPLS-DA was applied for exploratory discrimination and feature screening [43]. The model yielded $R^2X = 0.998$, $R^2Y = 1$, and $Q^2 = 0.999$ (Figure 4b). A 200-permutation test gave R^2 and Q^2 intercepts of approximately 0.61 and -0.05 , respectively. Given the limited sample size ($n = 3$ per group), the OPLS-DA results were interpreted primarily as exploratory discrimination and feature screening.

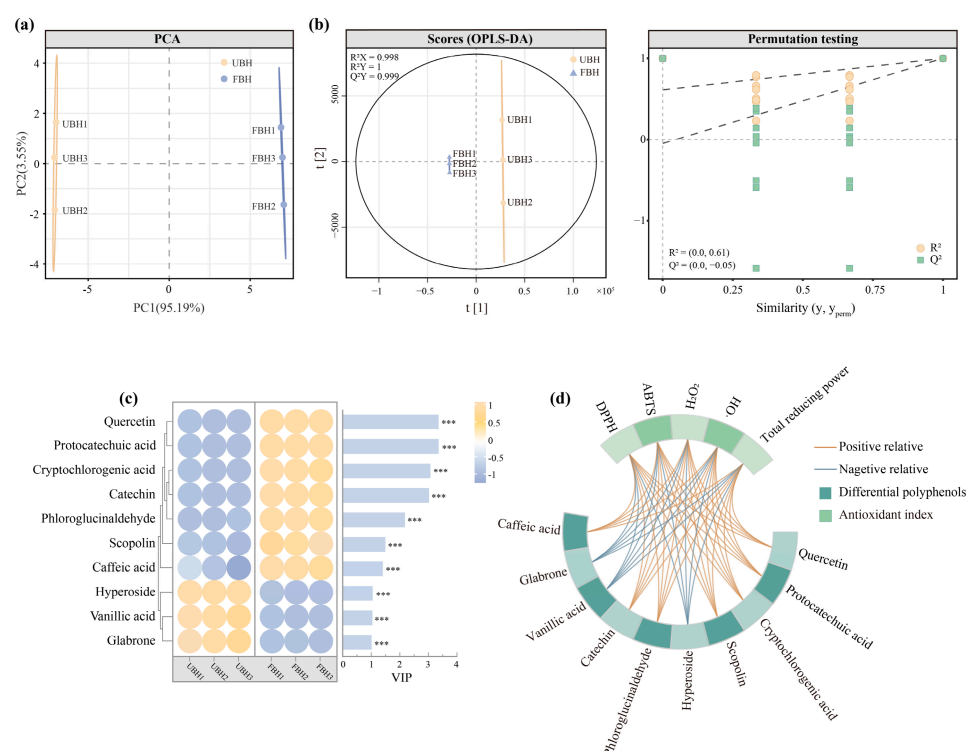


Figure 4. Differences in phenolic profiles between UBH and FBH and their associations with antioxidant activity. PCA (a), OPLS-DA score plot and permutation test (b), clustered heat map and VIP value analysis of differential polyphenols (c), correlation analysis between differential polyphenols and antioxidant indices (d). Note: UBH represents the unfermented blue honeysuckle sample, and FBH represents the fermented blue honeysuckle sample; in panel (c), yellow and blue indicate higher and lower scaled relative abundances, respectively; *** $p < 0.001$.

Overall, 376 phenolic metabolites were putatively annotated across the UBH and FBH samples, and among those meeting the differential screening criteria ($VIP > 1$ and $p < 0.05$), the top 10 differential phenolic metabolites were selected for further analysis and visualization, namely quercetin, protocatechuic acid, cryptochlorogenic acid, scopolin, hyperoside, phloroglucinaldehyde, catechin, vanillic acid, glabrone, and caffeic acid (Figure 4c). Most of these compounds are phenolic acids and flavonoids bearing phenolic hydroxyl groups, which are widely recognized for their antioxidant properties. Related antioxidant effects of phenolic constituents have also been reported in fungal fermentation systems such as *Sanghuangporus vaninii* and in plant-based fermented matrices [44]. Moreover, Tang et al. [45] compared single- and mixed-culture fermentation of litchi pulp using *L. plantarum* ATCC 7469 and *L. rhamnosus* GDMCC 1.1798 and found that co-fermentation promoted

the accumulation of catechin and quercetin, further illustrating the potential of microbial fermentation to modulate phenolic profiles.

The associations between the normalized abundances of differential phenolic features and antioxidant indices were further evaluated by correlation analysis (Figure 4d). Significant associations were observed between antioxidant activity and the phenolic profile of the blue honeysuckle matrix. The relative abundances of quercetin, protocatechuic acid, cryptochlorogenic acid, scopolin, phloroglucinaldehyde, catechin, and caffeic acid were significantly higher in FBH than in UBH and showed positive correlations with all antioxidant indices ($p < 0.05$, $|r| > 0.7$). Conversely, hyperoside, vanillic acid, and glabrone exhibited significantly lower relative abundances in FBH and were negatively correlated with the antioxidant indices ($p < 0.05$, $|r| > 0.7$). Similar associations between phenolic composition and antioxidant properties have also been reported in other fermented plant matrices. For example, fermented guava pulp exhibited significantly higher TPC (701 mg/g) and DPPH radical scavenging activity (81%) than the unfermented counterpart ($p < 0.05$) [46]. Schulz et al. [47] further reported significant positive associations between the bioaccessibility of quercetin and protocatechuic acid and antioxidant activity during *in vitro* gastrointestinal digestion of “Juçara” fruit.

The concurrent changes in TPC, phenolic profiles, and antioxidant indices suggest an association between phenolic remodeling and enhanced antioxidant activity after fermentation. LC-MS profiling complemented the TPC measurements by characterizing changes in individual phenolic features. These associations do not establish causality or quantify the contributions of individual compounds.

Several limitations should be considered when interpreting the findings of this study. The hydrolytic release of bound phenolics may contribute to the changes observed during fermentation; however, direct confirmation of this mechanism requires separate quantification of free and bound phenolic fractions and measurements of relevant enzyme activities. In addition, concurrent changes in multiple phenolic compounds and other fermentation-derived metabolites limit the extent to which the observed antioxidant responses can be attributed to individual compounds. Future studies incorporating fraction-specific phenolic quantification, targeted analysis of key compounds using authentic standards, and enzymatic activity measurements would help clarify the mechanisms underlying phenolic transformations and their contributions to antioxidant activity.

4. Conclusions

The optimized process comprised enzymatic pretreatment at a solid-to-liquid ratio of 1:10 (g:mL), with a combined enzyme dosage of 0.16% at a cellulase-to-pectinase ratio of 5:3, pH 6.1, and 50.3 °C for 60 min, followed by co-fermentation with *L. rhamnosus* 6224 and *S. cerevisiae* W5 at a strain ratio of 1:3 and a total inoculum size of 5% (v/v), at 30 °C and 140 rpm for 60 h. Under these conditions, the TPC of FBH reached 1374.79 mg/100 g, approximately 102% higher than the value obtained under the conditions selected after single-factor screening (681.67 ± 11.21 mg/100 g), further supporting the effectiveness of multifactorial optimization. Co-fermentation significantly enhanced the *in vitro* antioxidant activity of the blue honeysuckle matrix ($p < 0.05$) and was accompanied by marked changes in phenolic composition and the relative abundances of several phenolic compounds. These phenolic changes were significantly associated with the measured antioxidant indices, although the causal contributions of individual compounds were not established. The optimized process offers a potential approach to increasing TPC and enhancing the *in vitro* antioxidant properties of blue honeysuckle products.

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Abbreviations

The following abbreviations are used in this manuscript:

TPC	Total Phenolic Content
DPPH	2,2-Diphenyl-1-picrylhydrazyl
ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
·OH	Hydroxyl Radical
LC-MS	Liquid Chromatography-Mass Spectrometry
LAB	Lactic Acid Bacteria
RSM	Response Surface Methodology
MRS	De Man, Rogosa and Sharpe
YPD	Yeast Extract Peptone Dextrose
UBH	Unfermented Blue Honeysuckle
FBH	Fermented Blue Honeysuckle
PB	Plackett-Burman
BBD	Box-Behnken Design
VC	Ascorbic Acid
ANOVA	Analysis of Variance

References

- Ge, Y.; Ma, Q.Y.; Wang, S.Y.; Mujumdar, A.S.; Huang, X.J.; Xiao, H.W.; Liu, Z.L.; Nie, S.P. Recent advances of pretreatments and drying technologies in processing small berries: A comprehensive review. *Trends Food Sci. Technol.* **2026**, *169*, 105553. [CrossRef]
- Xiao, Z.; Sun, J.; Sun, J.H.; Huo, J.W.; Zhang, Y.; Sui, X.N. Exploring the inhibitory mechanism of blue honeysuckle fruit anthocyanins on α -amylase and α -glucosidase: Enzyme kinetics, multi-spectroscopic analysis, and molecular docking. *Food Biosci.* **2026**, *84*, 109746. [CrossRef]
- Liu, J.Y.; Ruan, W.; Yu, H.; Huo, J.W.; Meng, X.C.; Yue, Y.X. *Lactiplantibacillus plantarum*-mediated bioconversion of blue honeysuckle juice amplifies phenolics to inhibit xanthine oxidase and reshapes volatile metabolome. *Food Chem.* **2025**, *495*, 146350. [CrossRef] [PubMed]
- Sun, J.; Li, D.L.; Huan, W.; Hong, X.Q.; He, S.M.; Huo, J.W.; Jiang, L.Z.; Zhang, Y. Blue honeysuckle seeds and seed oil: Composition, physicochemical properties, fatty acid profile, volatile components, and antioxidant capacity. *Food Chem. X* **2024**, *21*, 101176. [CrossRef] [PubMed]

5. Zhao, X.Y.; Wang, N.; Wang, Y.T.; Liu, K.X.; Xiao, Y.R.; Zhu, X.R.; Gang, H.X.; Huo, J.W.; Qin, D. Integrated physiological, metabolome and transcriptome data to reveal the regulatory effects of citral on postharvest blue honeysuckle fruit quality at different temperatures. *Postharvest Biol. Technol.* **2026**, *234*, 114161. [\[CrossRef\]](#)
6. Luo, J.Y.; Jiang, L.; Liang, S.; Gao, B.; Bao, Y.H.; Huo, J.W.; Jiang, S.L. Fermentation of *Lonicera caerulea* by complex probiotics and its effect on hypolipidemic. *Food Biosci.* **2024**, *57*, 103502. [\[CrossRef\]](#)
7. Ramos, S.; Van Nieuwenhove, C.; Molina, V.; Cortez, C.; Van Nieuwenhove, G.; Isas, A.S.; Mozzi, F.; Mateos, R. Novel lactic fermentation of pomegranate juice by *Lactiplantibacillus plantarum* preserves polyphenolic profile, bioactivity and sensory acceptance. *Innov. Food Sci. Emerg. Technol.* **2026**, *110*, 104507. [\[CrossRef\]](#)
8. Carnauba, R.A.; Sarti, F.M.; Marchioni, D.M.; Lotufo, P.A.; Hassimotto, N.M.A.; Bensenor, I.M.; Lajolo, F.M. Longitudinal associations of total and class-specific polyphenol intake with incident type 2 diabetes and changes in glycemic markers: Findings from the brazilian longitudinal study of adult health (ELSA-Brasil) cohort. *J. Nutr.* **2026**, *156*, 101692. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Cheng, Z.; Bao, Y.W.; Li, Z.Y.; Wang, J.X.; Wang, M.S.; Wang, S.H.; Wang, Y.Y.; Wang, Y.H.; Li, B. *Lonicera caerulea* (Haskap berries): A review of development traceability, functional value, product development status, future opportunities, and challenges. *Crit. Rev. Food Sci.* **2023**, *63*, 8992–9016. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Cheng, Z.P.; Zhang, Y.Z.; Guan, Z.Z.; Liu, R.S.; Lu, Z.M.; Chai, L.J.; Xu, H.Y.; Shi, J.S.; Zhang, X.J.; Xu, Z.H. Lactic acid bacteria-mediated transformation of black tea extract: Insights into metabolite profile and sensory characteristics. *Food Biosci.* **2025**, *66*, 106299. [\[CrossRef\]](#)
11. Isas, A.S.; Balcels, M.F.; Galdeano, C.M.; Palomo, I.; Rodriguez, L.; Fuentes, E.; Pizarro, P.L.; Briz, R.M.; Mozzi, F.; Van Nieuwenhove, C. Fermented pomegranate juice enriched with pomegranate seed oil ameliorates metabolic disorders associated with a high-fat diet in C57BL/6 mice. *Food Chem.* **2025**, *463*, 141434. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Rizwan, A.; Karim, S.; Andrabi, I.L.; Mushtaq, M.; Farooqi, H. Fermentation alters the anticancer properties of dietary polyphenols in pulses. *J. Nutr.* **2026**, *156*, 101254. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Zhang, Z.; Wang, J.L.; Hao, Y.P.; Meng, Y.; Meng, J.; Chen, J.H.; Ding, C.H.; Xi, J. Effects of *Lactobacillus* fermentation on the composition, existing forms, digestive characteristics, and antioxidant activity of polyphenols in three kinds of bean milk. *Food Biosci.* **2025**, *68*, 106482. [\[CrossRef\]](#)
14. Yang, J.; Sun, Y.; Gao, T.; Wu, Y.; Sun, H.; Zhu, Q.; Liu, C.; Zhou, C.; Han, Y.; Tao, Y. Fermentation and storage characteristics of “Fuji” apple juice using *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus plantarum*: Microbial growth, metabolism of bioactives and *in vitro* bioactivities. *Front. Nutr.* **2022**, *9*, 833906, Correction in *Front. Nutr.* **2025**, *12*, 1695110. <https://doi.org/10.3389/fnut.2025.1695110>. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Wu, Y.; Li, S.J.; Tao, Y.; Li, D.D.; Han, Y.B.; Show, P.L.; Wen, G.Z.; Zhou, J.Z. Fermentation of blueberry and blackberry juices using *Lactobacillus plantarum*, *Streptococcus thermophilus* and *Bifidobacterium bifidum*: Growth of probiotics, metabolism of phenolics, antioxidant capacity *in vitro* and sensory evaluation. *Food Chem.* **2021**, *348*, 129083. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Cheng, S.S.; Zheng, Y.P.; Li, H.X.; Jiang, Y.J.; Zhang, Y. Metabolic engineering of blueberry juice by *Lactiplantibacillus plantarum* JM065: Fermentation optimization. *Food Chem.* **2025**, *496*, 146669. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Yu, H.; Xu, X.; Hao, J.X.; Zuo, X.X.; Wang, J.; Zhu, L.Q.; Chen, M.; Lyu, Y.; Yan, Z.H.; Shen, Y.G.; et al. Mixed fermentation of citrus peel pomace with *Trichoderma koningii*, *Aspergillus oryzae* and *Lactobacillus casei*: Process optimization, antioxidant activities and non-targeted metabolomics analysis. *Food Biosci.* **2025**, *66*, 106180. [\[CrossRef\]](#)
18. Xiao, Y.H.; Li, X.Y.; Zhang, H.M.; Zhu, Y.W.; Gao, W.M.; Jiang, S.J.; Ru, G.J.; Sun, A.D. Efficient release and possible mechanism of polyphenols in the pomace of chokeberry (*Aronia melanocarpa* L.) through solid-state fermentation using *Trichoderma viride*. *Food Biosci.* **2025**, *68*, 106783. [\[CrossRef\]](#)
19. Liu, N.; An, X.P.; Wang, Y.; Qi, J.W.; Jia, Y.; Li, X.; Chen, X.N. Polyphenol composition and antioxidant activity of fermentation combined with enzymatic hydrolysis modified *Astragalus membranaceus* stems. *Chem. Biol. Technol. Agric.* **2024**, *11*, 153. [\[CrossRef\]](#)
20. Zheng, Y.T.; Liu, S.; Xie, J.H.; Chen, Y.; Dong, R.H.; Zhang, X.J.; Liu, S.Q.; Xie, J.Y.; Hu, X.B.; Yu, Q. Antioxidant, α -amylase and α -glucosidase inhibitory activities of bound polyphenols extracted from mung bean skin dietary fiber. *LWT-Food Sci. Technol.* **2020**, *132*, 109943. [\[CrossRef\]](#)
21. Li, H.Y.; Liu, Y.G.; Guo, S.Y.; Shi, M.; Qin, S.; Zeng, C.X. Extraction of ursolic acid from apple peel with hydrophobic deep eutectic solvents: Comparison between response surface methodology and artificial neural networks. *Foods* **2023**, *12*, 310. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Kaboutari, P.; Bashiri, H. Modeling and optimization of hydrogen production from ethanol decomposition on Ni(100) catalyst using kinetic Monte Carlo simulation and response surface methodology. *Fuel* **2026**, *420*, 138911. [\[CrossRef\]](#)
23. Wu, W.B.; Zheng, Z.Y.; Wang, B.; Li, J.X.; Cheng, X.J.; Qiu, W.L.; He, S.; Chen, H.Y.; Abdeen, M.A. Study on the optimization of lychee quick-freezing process parameters and damage assessment model based on response surface methodology. *LWT-Food Sci. Technol.* **2025**, *237*, 118741. [\[CrossRef\]](#)

24. Thangadurai, D.; Chippalakatti, P.; Nimbalkar, M.; Sangeetha, J.; Hospet, R.; Cruz-Martins, N. Optimization of extraction process for phenolics and antioxidants from edible algae *Ulva fasciata* using response surface methodology and its nutritional composition. *Innov. Food Sci. Emerg. Technol.* **2025**, *106*, 104295. [[CrossRef](#)]
25. Shen, S.A.; Chen, D.J.; Li, X.; Li, T.; Yuan, M.; Zhou, Y.H.; Ding, C.B. Optimization of extraction process and antioxidant activity of polysaccharides from leaves of *Paris polyphylla*. *Carbohydr. Polym.* **2014**, *104*, 80–86. [[CrossRef](#)] [[PubMed](#)]
26. Oh, B.T.; Jeong, S.Y.; Velmurugan, P.; Park, J.H.; Jeong, D.Y. Probiotic-mediated blueberry (*Vaccinium corymbosum* L.) fruit fermentation to yield functionalized products for augmented antibacterial and antioxidant activity. *J. Biosci. Bioeng.* **2017**, *124*, 542–550. [[CrossRef](#)] [[PubMed](#)]
27. Wang, M.J.; Cheng, Y.L.; Li, X.; Nian, L.Y.; Yuan, B.; Cheng, S.J.; Wang, S.L.; Cao, C.J. Effects of microgels fabricated by microfluidic on the stability, antioxidant, and immunoenhancing activities of aquatic protein. *J. Future Foods* **2025**, *5*, 57–67. [[CrossRef](#)]
28. Chen, Y.X.; Liu, X.Y.; Xiao, Z.; Huang, Y.F.; Liu, B. Antioxidant activities of polysaccharides obtained from *Chlorella pyrenoidosa* via different ethanol concentrations. *Int. J. Biol. Macromol.* **2016**, *91*, 505–509. [[CrossRef](#)] [[PubMed](#)]
29. Liu, Y.; Du, Y.Q.; Wang, J.H.; Zha, X.Q.; Zhang, J.B. Structural analysis and antioxidant activities of polysaccharide isolated from Jinqian mushroom. *Int. J. Biol. Macromol.* **2014**, *64*, 63–68. [[CrossRef](#)] [[PubMed](#)]
30. Núñez-Serrano, A.; García-Reyes, R.B.; Solís-Pereira, S.; García-González, A. Production and immobilization of pectinases from *Penicillium crustosum* in magnetic core-shell nanostructures for juice clarification. *Int. J. Biol. Macromol.* **2024**, *263*, 130268. [[CrossRef](#)] [[PubMed](#)]
31. Goderska, K.; Pena, S.A. An *in vitro* gastrointestinal model to evaluate the tolerance of encapsulated *Lactobacillus* and *Lactococcus* strains with synbiotic containing lactobionic acid via lyophilization technique to harsh gastric conditions during storage time. *Eur. J. Pharm. Biopharm.* **2024**, *197*, 114147. [[CrossRef](#)] [[PubMed](#)]
32. Song, L.; Chen, Y.; Liu, H.P.; Zhang, X.W. Preparation, biological activities, and potential applications of hen egg-derived peptides: A review. *Foods* **2024**, *13*, 885. [[CrossRef](#)] [[PubMed](#)]
33. Pei, F.Y.; Cao, X.B.; Ren, Y.X.; Mao, L.Y.; Liu, Y.Y.; Ge, J.P.; Ping, W.X. Microbial fermentation of blue honeysuckle (*Lonicera caerulea* L.) polyphenol promotes insulin secretion and intervenes in obesity by promoting short-chain fatty acids production. *Food Sci. Hum. Wellness* **2025**, *14*, 9250091. [[CrossRef](#)]
34. Gaur, G.; Gänzle, M.G. Conversion of (poly)phenolic compounds in food fermentations by lactic acid bacteria: Novel insights into metabolic pathways and functional metabolites. *Curr. Res. Food Sci.* **2023**, *6*, 100448. [[CrossRef](#)] [[PubMed](#)]
35. Mirmahdi, R.S.; Mahoozi, T.; Zoghi, A.; Montazeri, N.; Khosravi-Darani, K. The roles of *Saccharomyces cerevisiae* on the bioaccessibility of phenolic compounds. *World J. Microbiol. Biotechnol.* **2024**, *40*, 221. [[CrossRef](#)] [[PubMed](#)]
36. Tong, C.; Chen, X.X.; Deng, R.B.; Gao, H.Y. Dynamic changes in physicochemical characteristics, bioactivity and flavor profile of fermented strawberry juice by *Lactiplantibacillus plantarum*. *Food Chem.* **2025**, *495*, 146388. [[CrossRef](#)] [[PubMed](#)]
37. Xu, Z.T.; Nie, C.L.; Chen, Z.; Liu, B.J. Low-temperature co-fermentation of *Lactobacillus bulgaricus* 134 and *Saccharomyces cerevisiae*: Effects on polyphenols composition, flavor compounds and antioxidant activity of black rice slurry. *Foods* **2026**, *15*, 2036. [[CrossRef](#)] [[PubMed](#)]
38. Shi, B.H.; Xue, B.J.; Tao, Y.J.; Xu, R.; Li, Y.Z.; Han, R.; Wang, R.; Zhu, D.R. Optimization of fermentation conditions and fed-batch production of ectoine by *Halomonas campaniensis* G9-72. *Biochem. Eng. J.* **2026**, *230*, 110123. [[CrossRef](#)]
39. Hou, F.X.; Cai, Y.X.; Wang, J.H. Antioxidant capacity changes and untargeted metabolite profile of broccoli during lactic acid bacteria fermentation. *Fermentation* **2023**, *9*, 474. [[CrossRef](#)]
40. Özdemir, Z.Ö.; Soy, M.; Ataseven, S.; Özer, A.; Elmastaş, M. Comparative antioxidant profiling of phenolic acids and flavonoids: Assay-resolved structure-activity relationships under harmonized *in vitro* conditions. *Molecules* **2026**, *31*, 1478. [[CrossRef](#)] [[PubMed](#)]
41. Kaprasob, R.; Sarkar, D.; Kerdchoechuen, O.; Laohakunjit, N.; Khanongnuch, C.; Shetty, K. Beneficial lactic acid bacteria based bioprocessing of cashew apple juice for targeting antioxidant nutraceutical inhibitors as relevant antidotes to type 2 diabetes. *Process Biochem.* **2019**, *82*, 40–50. [[CrossRef](#)]
42. Hur, S.J.; Lee, S.Y.; Kim, Y.C.; Choi, I.; Kim, G.B. Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chem.* **2014**, *160*, 346–356. [[CrossRef](#)] [[PubMed](#)]
43. Zhang, C.; Wang, C.C.; Wang, H.J.; Ablimit, A.; Sun, Q.; Dong, H.J.; Zhang, B.B.; Hu, W.L.; Liu, C.J.; Wang, C.T. Identification of characteristic volatiles and metabolomic pathways during the fermentation of red grapefruit by *Monascus purpureus* using HS-SPME-GC–MS and metabolomics. *Food Chem.* **2024**, *464*, 141786. [[CrossRef](#)] [[PubMed](#)]
44. Gao, H.; Yin, C.M.; Li, C.; Li, Y.H.; Shi, D.F.; Fan, X.Z.; Yao, F.; Wu, W.J.; Li, J.T. Phenolic profile, antioxidation and anti-proliferation activity of phenolic-rich extracts from *Sanghuangporus vaninii*. *Curr. Res. Food Sci.* **2023**, *6*, 100519. [[CrossRef](#)] [[PubMed](#)]
45. Tang, S.Y.; Luo, N.; Zeng, Q.Z.; Dong, L.H.; Zhang, R.F.; He, S.; Nag, A.; Huang, F.; Su, D.X. Lychee pulp phenolics fermented by mixed lactic acid bacteria strains promote the metabolism of human gut microbiota fermentation *in vitro*. *Food Funct.* **2023**, *14*, 7672–7681. [[CrossRef](#)] [[PubMed](#)]

46. Kaur, H.; Ghosh, M. Probiotic fermentation enhances bioaccessibility of lycopene, polyphenols and antioxidant capacity of guava fruit (*Psidium guajava* L.). *J. Agric. Food Res.* **2023**, *14*, 100704. [[CrossRef](#)]
47. Schulz, M.; Biluca, F.C.; Gonzaga, L.V.; Borges, G.D.C.; Vitali, L.; Micke, G.A.; de Gois, J.S.; de Almeida, T.S.; Borges, D.L.G.; Miller, P.R.M.; et al. Bioaccessibility of bioactive compounds and antioxidant potential of juçara fruits (*Euterpe edulis Martius*) subjected to *in vitro* gastrointestinal digestion. *Food Chem.* **2017**, *228*, 447–454. [[CrossRef](#)] [[PubMed](#)]
48. Zhang, J.; Chen, X.; Cao, J.; Geng, A.; Chu, Q.; Yan, Z.; Zhang, Y.; Liu, H. Metabolomics reveals glycerophospholipids, peptides, and flavonoids contributing to breast meat flavor and benefit properties of Beijing-You chicken. *Foods* **2024**, *13*, 2549. [[CrossRef](#)] [[PubMed](#)]
49. Yang, X.Z.; Xie, S.; Zhang, R.; Zhuo, X.Y.; Tao, S.; Li, Y.H.; Lian, H.; Jin, J.Y.; Hu, S.T.; Gao, Y.N.; et al. High-oxygen pretreatment of whole fruit delays softening of fresh-cut apples: A metabolic perspective associated with cell wall homeostasis. *Food Qual. Saf.* **2026**, *10*, 111–126. [[CrossRef](#)]
50. Wei, Y.Q.; Shi, J.J.; Wang, J.H.; Hu, Z.Y.; Wang, M.; Wang, W.; Cui, X.J. Integrated analysis of metabolome and microbiome in a rat model of perimenopausal syndrome. *mSystems* **2024**, *9*, e00623-24. [[CrossRef](#)] [[PubMed](#)]

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