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Evaluation of phenolics biotransformation and health promoting properties of blueberry juice following lactic acid bacteria fermentation

Jun WANG[1], Bo-Cheng WEI[1], Bo WEI[1], Hao-Yue YU[1], Kiran THAKUR[2], Chu-Yan WANG[1]*, Zhao-Jun WEI[2,3]*

Abstract

Lactic acid bacteria are widely used as probiotics in food fermentation and exhibit various health-promoting properties. This study describes the effects of lactic acid bacteria (LAB) on the phenolic profiles, antioxidant activities, and enzyme inhibition prosperities of blueberry juice. Three different strains (*Lactobacillus acidophilus* JYLA-16, *Lactobacillus plantarum* JYLP-375, and *Lactobacillus rhamnosus* JYLR-005) were selected to ferment the blueberry juice at 37 °C for 48 h. After fermentation, the content of total phenolics and flavonoids increased. Meanwhile, the UPLC–LTQ–MS/MS system conducted the biotransformation of phenols during fermentation. LAB fermentation induced an upgraded trend of syringic acid, ferulic acid, erucic acid, rutin, catechin, epicatechin, and isoorientin. Pearson's correlation analysis revealed that phenolics' metabolism probably contributed to enhancing antioxidant activities. LAB also improved active ingredient retention and antioxidant capacities after *in vitro* simulated digestion. Finally, we investigated the α-glucosidase inhibitory and α-amylase inhibition of the fermented juice and found that LAB fermentation could improve the inhibition of these enzymes. This study indicated that LAB fermentation could be an efficient strategy for enhancing functional activities in blueberry juice by bio-transforming the phenolics.

Keywords: blueberry; lactic acid bacteria; phenolics; biotransformation; functional properties.

Practical Application: Lactic acid bacteria fermentation can increase blueberry juice's antioxidant activities and health-benefits properties. The application of lactic acid bacteria could help develop a new type of probiotic blueberry juice that can furnish potential value to human health.

1 Introduction

There is unprecedented interest in keeping a balanced and life-like diet, particularly those containing herbal and healthy foods. Hence, food industries attach great importance to producing foods with health-promoting benefits, including berries (Pap et al., 2021). Blueberries were popularized as the 'human healthy food' due to their abundant anthocyanins, flavonoids, and phenolic acids (Kalt et al., 2020). The benefits of blueberries, including their antioxidant, anticancer, and cardiovascular protective properties, have been proven (Silva et al., 2020). However, fresh blueberries are climacteric fruits with juicy and thin peels, making them difficult to transport and store (Zhang et al., 2021). Thus, novel processing technologies are necessary to broaden the value of blueberries.

Recently, plant-based fermented foods have aroused considerable interest due to their probiotic functions (Gustaw et al., 2021). Especially vegans and high cholesterol-risk individuals are increasingly interested in these products. Lactic acid bacteria (LAB) are a series of microorganisms with various health-promoting abilities. Previous study has shown that the fermentation of fruit juices by LAB can help improve or retain the functional properties of the substrate (Inayah et al., 2022). Berries are enriched in sugars presenting the prerequisites for the LAB boom and metabolizing. Emerging research has focused on the fermentation of berries using LABs. Kwaw et al. (2018) utilized Lactobacillus strains fermenting mulberry juice and demonstrated that LABs fermentation promoted the biotransformation of phenolics and significantly increased the content of cyanidin-3-O-rutinoside and quercetin. Gao et al. (2022) fermented blueberry puree using *Streptococcus lactis* and found that the bioavailability of phenolics during the fermentation process was elevated. Liu et al. (2021) administered the goji juice fermented by *Lactobacillus plantarum*, *Lactobacillus reuteri*, and *Streptococcus thermophilus*. Moreover, he found that goji berry juice has more vital anti-ulcerative colitis abilities through fermentation. Thus, LAB fermentation can enhance the bioavailability of polyphenols in berries and benefit human beings.

In this study, we chose three different LAB strains (*Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus rhamnosus*) to investigate the biotransformation of phenols, flavonoids, anthocyanins, and antioxidant capacities during blueberry juice fermentation. We also explored the relationship between individual phenolic compounds and antioxidant abilities. Furthermore, we measured the individual phenolic compounds and antioxidant abilities *in vitro*. In general, this research aimed

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[1]*School of Biological, Food and Environment, Hefei University, Hefei, China*
[2]*School of Food and Biological Engineering, Hefei University of Technology, Hefei, China*
[3]*School of Biological Science and Engineering, North Minzu University, Yinchuan, China*
*Corresponding author: wangchuyan19@163.com; zjwei@hfut.edu.cn

to develop a fermented beverage with unique nutritional benefits and health-promoting qualities.

2 Materials and methods

2.1 Chemicals and regents

Analytical standards of chlorogenic acid, neo-chlorogenic acid, gallic acid, proto-catechuic acid, caffeic acid, erucic acid, quercetin, rutin, orientin, isoorientin, catechin, epicatechin, cyanidin-3-O-rutinoside, cyanidin-3-O- glucoside, and cyanidin-3-O-galactoside were purchased from Solarbio Life Science (Beijing, China). Other chemicals, such as MRS media, Folin phenol, $FeCl_3$, etc., were obtained from Sinopharm Chemical Reagent (Shanghai, China).

2.2 Bacterial strains

Three commercial strains, namely *Lactobacillus acidophilus* JYLA-16 (La 16), *Lactobacillus plantarum* JYLP-375 (Lp 375), and *Lactobacillus rhamnosus* JYLR-005 (Lr 005) were purchased from Zhongke Jia-yi Bioengineering Company (Shandong, China). All stock LABs were stored in lyophilized type at −20 °C. For activation, these LAB strains were propagated twice in the MRS broth at 37 °C to obtain a final concentration of 9.00 Log CFU/mL.

2.3 Blueberry juice fermentation

The blueberry juice was obtained from the wortact self-owned farm (Jiang Su, China), which has organic blueberry orchards. The blueberry juice was extracted from these organic blueberries and industrially pastured. The blueberry juice attributes were as follows: energy of 213 kcal/100 mL, 4% of carbohydrates, 0% of proteins, and 0% of fats. For fermentation, 1% (v/v) of the activated lactic acid bacteria were individually added to the blueberry juice and incubated for 48 h at 37 °C. The unfermented blueberry juice was used as the control. Each fermentation experiment was conducted in triplicate.

2.4 Determination of active substances

Determination of total phenolics

The total phenolic content (TPC) was determined as described by Santos et al. (2021). Firstly, 0.1 mL of diluted juice (dilution factor of 10) was prepared, then 0.5 mL of Folin-Ciocalteu reagent (10%, w/v) was added and allowed to react for 5 min. Following that, 1.5 mL of the Na_2CO_3 solution (20%, w/v) was added, and the mixture reacted at 75 °C for 10 min. The absorbance was measured at 765 nm, and gallic acid was used as the standard. The TPC was expressed as gallic acid equivalent (GAE) in mg/L of the sample.

Determination of total flavonoids

The total flavonoid concentration (TFC) was determined as reported by Shang et al. (2022). Briefly, 1 mL of diluted sample (dilution factor was 10) was added to 0.4 mL $NaNO_2$ (1.25 mg/mL), vortexed, and stood for 6 min. Then, 0.8 mL of $AlCl_3$ (12.5 mg/mL) was added and reacted for 6 min, again. Subsequently, 5 mL of 16 mg/mL of NaOH and 2.8 mL of ddH_2O were added to react for 15 min. The absorbance was read at 510 nm, and rutin was used as the standard. The TFC was expressed as rutin equivalent in mg/L of the sample.

Determination of total anthocyanins

The total anthocyanin content (TAC) was assessed by the differential pH method based on the description of Hardinasinta et al. (2021) . Before the determination, the potassium chloride buffer (pH 1.0, 2.5 mmol/L) and the sodium acetate buffer (pH 4.5, 0.4 mol/L) were prepared. After that, 1 mL of diluted sample (dilution factor was 10) was added to a 9 mL buffer solution with pH 1.0 and 4.5, respectively. Then the absorbance was determined at 520 nm and 700 nm. The TAC was expressed as cyanidin-3-glucoside equivalent (C3GE) in mg/L of the sample.

Characterization of polyphenolic profile

The analysis of the polyphenolic compounds (phenolic acids, flavonoids, and anthocyanins) was performed on a UPLC–LTQ–MS/MS system (Thermo, Waltham, MA, USA). Briefly, 1 mL of each sample was centrifuged at 12,000 rpm for 10 min. After centrifugation, the supernatant was taken, passed through a 0.22 μm filter membrane, and the supernatant was transferred into UPLC-MS/MS for analysis. The chromatographic separation was performed on a Hypercarb column (2.1 × 100 mm, 5 μm, Thermo, USA). The mobile phase used for separation consisted of phase A (pure water) and phase B (acetonitrile). The gradient elution program was as follows: 0 min, 95% A; 0.5–5 min, decreased to 5% A, 5–5.1 min, increased to 95% A, and then held till 6 min. The column was maintained at 40 °C with a flow rate of 0.25 mL/min, and the injection volume was 5 μL. The mass spectrometer was supplied with a HESI II probe in negative ion mode, with the following source decisive: capillary temperature, 275 °C; source heated temperature, 250 °C; sheath gas flow rate, 20; auxiliary gas flow rate, 5; sweep gas flow rate, 0; S-lens RF level, 67.54%; scan range, 60–900 m/z.

2.5 Determination of antioxidant activity

DPPH radical scavenging assay

With slight modifications, the DPPH radical scavenging was investigated using the approach provided by Tan et al. (2022). The 2 mL sample was mixed with 3 mL of 0.1 mmol/L DPPH solution and incubated in the dark for 30 minutes before being measured at 517 nm.

ABTS radical scavenging assay

A modification of the method developed by Wu et al. (2022) was used to conduct the ABTS radical scavenging. Firstly, the 7.4 mmol/L ABTS+ stock solution was first mixed with the 2.6 mmol/L $K_2S_2O_8$ solutions in a 1:1 (v:v) ratio and incubated in the dark. Then the mixture was diluted with 0.1 mol/L phosphate buffer (pH 7.4), resulting in the $ABTS^+$ working solution with the absorption of 0.70 ± 0.02 at 734 nm. For determination, 0.2 mL of the sample was reacted with 0.8 mL of the ABTS working solution for 6 min, and the absorbance was measured at 734 nm.

Reducing power capacity

Firstly, 1 mL of the sample was mixed with 1 mL of potassium ferricyanide (1%, w/v) and 1 mL of 0.2 mol/L phosphate buffer (pH 7.4) and incubated to react for 20 min in the dark. Then, 1 mL of $FeCl_3$ solution (10%, w/v) was added, vortexed, and centrifuged at 3000 rpm for 2 min. After centrifugation, 2.5 mL supernatant was reacted with 1.2 mL of potassium ferricyanide (1%, w/v) and 2.5 mL of distilled H_2O. The absorbance read at 700 nm.

2.6 Simulated digestion in vitro

The simulated digestion of blueberry juice proceeded on three levels: (i) the simulated saliva digestion, (ii) the simulated gastric digestion, and (iii) the simulated intestinal digestion.

Briefly, simulated saliva fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) was generated according to the methods reported by Wang et al. (2022a). The electrolyte stock solutions including KH_2PO_4 (0.5 mol/L), KCl (0.5 mol/L), $NaHCO_3$ (1 mol/L), NaCl (2 mol/L), $MgCl_2(H_2O)_6$ (0.15 mol/L), and $(NH_4)_2CO_3$ (0.5 mol/L) was used to prepare the simulated digestion fluids. SSF was prepared by adding 500 μL of the $CaCl_2(H_2O)_2$ (44.1 g/L), α-amylase into 99 mL of the stock solutions, and its pH value was adjusted to 7 with 1 mol/L HCl. SGF was prepared by adding 500 μL of the $CaCl_2(H_2O)_2$ (44.1 g/L) and pepsin into 98.5 mL of the stock solutions, and its pH value was adjusted to 3 with 6 mol/L HCl. SIF was prepared by adding 200 μL of the $CaCl_2(H_2O)_2$ (44.1 g/L), pancreatin, and bile salt into 98.3 mL of the stock solutions, and its pH value was adjusted to 6.8 with 6 mol/L HCl. The fermented or unfermented blueberry juice was added at a ratio of 1:10 (v:v) to the SSF, SGF, and SIF and then shaken at 37 °C for 2 h at 100 rpm/min. After simulated digestion, the samples' TPC, TFC, TAC, and antioxidant activities were determined.

2.7 α-Glucosidase inhibition assay

The assessment of -glucosidase inhibition was conducted utilizing a protocol reported by Dang et al. (2018). Phosphate buffer PBS (0.1 mol/L, PH 6.8) was used to prepare α-glucosidase (0.1 U/mL) and pNPG solution (1.5 mmol/L). Firstly, 100 μL of the sample was mixed with 200 μL of α-glucosidase and incubated at 37 °C for 10 min. After adding 200 L of p-nitrophenyl-glucopyranoside (pNPG), the mixture performed a 20 min reaction at 37 °C. The absorbance was then measured at 405 nm after adding 2 mL of the 1 mol/L solution of Na_2CO_3.

2.8 α-Amylase inhibition assay

Firstly, 500 μL of the samples were mixed with 0.2 U/mL of α-amylase at a volume ratio of 1:1 and incubated at 37 °C for 10 min. Subsequently, 500 μL of 1% soluble starch (dissolved in PBS buffer (0.1 mol/L, pH 6.8)) was added and reacted at 37 °C for 10 min. Then, 1 mL of the DNS (3, 5-dinitrosalicylic acid) solution was added to the mixture for the color reaction until the boiling water bath for 5 min. After the reaction, ddH_2O was added to 15 mL, and the absorbance was measured at 540 nm.

2.9 Statistical analysis

Three replicates of each experiment were performed, and the results were presented as mean ± standard deviation (SD). The analysis of variance (ANOVA) and multiple analysis (Duncan's multiple range test) was carried out by IBM SPSS 20.0. The correlation analysis was conducted using Pearson's correlation model and the principal component analysis (PCA) in Origin 2021. $p < 0.05$ was defined as statistically significant.

3 Results and discussion

3.1 Total phenolics, total flavonoids and total anthocyanins

Phenolic performed as one of the most critical factors of blueberries are closely related to their health benefit properties. Hence, it is essential to explore the impact of LAB fermentation on the TPC of blueberry juice. In this study, the TPC in the blueberry juice before fermentation was 2133.99 ± 34.39 GAE mg/L, while the TPC in all the fermented samples was significantly enhanced (Table 1). For example, the TPC in La 16, Lp 375, and Lr 005 fermented samples increased by 20.49%, 37.46%, and 12.46%, respectively. Similar results can be found in the lactic acid fermentation of kiwifruit juice (Wang et al., 2022b), ginkgo kernel juice (Wang et al., 2019) and mulberry juice (Kwaw et al., 2018). Flavonoids are a group of polyphenolic compounds and have favorable biochemical effects on multiple diseases (Shen et al., 2022). As depicted in Table 2, the TFC in the blueberry juice increased from 251.26 ± 4.05 Rutin mg/mL to 345.39 ± 24.22 Rutin mg/mL, ascribed to the enzymes' function to decompose complex polyphenols into simpler flavanols during fermentation. This phenomenon was in line with the results in the jujube puree fermented by *Streptococcus thermophilus* (Li et al., 2022). Anthocyanins are flavonoids that are distinguished by their cyan-red hue. They are significant to the food industry as natural alternatives to synthetic coloring agents and their beneficial properties (Herrera-Balandrano et al., 2021). After fermentation, there was a noticeable decrease in anthocyanin content, from 137.90 ± 3.10 C3GE mg/mL to 109.03 ± 15.80 C3GE mg/mL. Destruction and adsorption by microbial cells are responsible for the disappearance of anthocyanins. Previous research also found that after acetic acid fermentation, the total anthocyanins decreased in the strawberries (Hornedo-Ortega et al., 2017).

3.2 Differences in phenolics profiles after fermentation

A sum of seven phenolic acids, five flavonoids, and three anthocyanins was identified (Table 2). In the unfermented blueberry juice, the principal phenolic acid was syringic acid (37.24 ± 1.28 μg/mL), the dominant flavonoid was quercetin

Table 1. Changes of phytochemicals in blueberry juice after fermentation. Different lowercase letters represent significant differences among same column at *P < 0.05* level.

Sample	TPC (GAE mg/mL)	TFC (Rutin mg/mL)	TAC (C3GE mg/mL)
Control	2133.99 ± 34.39^{d}	251.26 ± 4.05^{c}	137.90 ± 3.10^{a}
La 16	2571.22 ± 18.25^{b}	302.23 ± 1.94^{b}	114.37 ± 14.21^{b}
Lp 375	2933.42 ± 116.08^{a}	345.39 ± 24.22^{a}	110.95 ± 0.64^{b}
Lr 005	2399.99 ± 95.63^{c}	291.46 ± 10.96^{b}	109.03 ± 15.80^{b}

Table 2. The phenolics profile of lactic-acid bacteria fermented blueberry juice. Different lowercase letters represent significant differences among same line at $P < 0.05$ level.

	Concentrations range (μg/mL)			
	Control	La 16	Lp 375	Lr 005
Phenolic acids				
Syringic acid	37.24 ± 1.28^{d}	69.57 ± 3.29^{b}	85.07 ± 1.23^{a}	56.42 ± 2.32^{c}
Ferulic acid	13.35 ± 2.44^{b}	18.98 ± 1.68^{a}	18.79 ± 1.77^{a}	18.33 ± 2.04^{ab}
Chlorogenic acid	4.80 ± 0.10^{a}	1.52 ± 0.10^{b}	1.39 ± 0.28^{b}	1.44 ± 0.34^{b}
Neo-chlorogenic acid	20.46 ± 0.94^{a}	8.98 ± 0.43^{b}	8.68 ± 0.79^{b}	8.77 ± 0.90^{b}
Gallic acid	1.57 ± 0.01^{a}	1.40 ± 0.01^{b}	1.32 ± 0.00^{d}	1.28 ± 0.00^{c}
Protocatechuic acid	0.59 ± 0.03^{b}	0.41 ± 0.01^{c}	0.31 ± 0.10^{d}	0.71 ± 0.01^{a}
Erucic acid	0.92 ± 0.07^{d}	1.71 ± 0.11^{b}	2.08 ± 0.07^{a}	1.39 ± 0.06^{c}
Flavonoids				
Quercetin	3.46 ± 0.20^{a}	2.35 ± 0.25^{b}	2.47 ± 0.27^{b}	2.12 ± 0.22^{b}
Rutin	0.33 ± 0.02^{c}	0.54 ± 0.03^{ab}	0.45 ± 0.09^{a}	0.40 ± 0.07^{bc}
Catechin	0.03 ± 0.02^{b}	0.26 ± 0.06^{a}	0.26 ± 0.03^{a}	0.21 ± 0.07^{a}
Epicatechin	0.61 ± 0.02^{b}	0.80 ± 0.05^{a}	0.78 ± 0.04^{a}	0.74 ± 0.03^{a}
Isoorientin	0.04 ± 0.02^{d}	0.46 ± 0.05^{b}	0.65 ± 0.15^{a}	0.22 ± 0.07^{c}
Anthocyanins				
Cyanidin-3-O-glucoside	1.14 ± 0.01^{a}	0.54 ± 0.04^{b}	0.51 ± 0.06$_{b}$	0.43 ± 0.03^{c}
Cyanidin-3-O-rutinoside	6.30 ± 0.29^{a}	2.82 ± 0.04^{b}	2.98 ± 0.24^{b}	2.99 ± 0.29^{b}
Cyanidin-3-O-galactoside	0.30 ± 0.00^{a}	0.20 ± 0.00^{b}	0.21 ± 0.01^{c}	0.18 ± 0.01^{b}

(3.46 ± 0.20 μg/mL), and the main anthocyanin was cyanidin-3-rutinoside (6.30 ± 0.29 μg/mL). Although this result agrees with the previous studies, some other dominant phenolic compounds are still detected in blueberry-related products. The disparity in the phenolic compounds may be ascribed to different varieties, maturities, and planting conditions for the blueberries (Yu et al., 2020).

After fermentation, individual phenolic compounds were significantly transformed in the blueberry juice. As depicted in Table 2, all detected anthocyanins' content decreased following the determination of total anthocyanins. At the end of fermentation, the content of cyanidin-3- rutinoside decreased from 6.30 ± 0.29 μg/mL to 2.82 ± 0.04 μg/mL, 2.98 ± 0.24 μg/mL, and 2.99 ± 0.29 μg/mL in La 16, Lp 375, and Lr 005 fermented juices, separately. Meanwhile, the content of cyanidin-3-glucoside and cyanidin-3-galactoside decreased from 1.14 ± 0.01 μg/mL to 0.43 ± 0.01 μg/mL, and 0.30 ± 0.00 μg/mL to 0.18 ± 0.01 μg/mL after fermentation. Among the phenolic acids, syringic acid, ferulic acid, and erucic acid exhibited increasing trends, and the content of other phenolic acids decreased. The erucic acid content increased by 85%-126% depending on the microbial strains applied. Moreover, the content of syringic acid under fermentation by La 16, Lp 375, and Lr 005 was increased by 86.8%, 128.4%, and 51.5%, respectively. And the content of ferulic acid fermented by La 16, Lp 375, and Lr 005 was enhanced by 42.17%, 40.75%, and 37.3%, separately. As for the flavonoids, the content of flavonoid monomers increased after fermentation, except for quercetin. Many published literatures also reported similar results in other LAB-fermented fruit products. For example, LAB fermentation increased the rutin content in the jujube juice (Li et al., 2021). In the mulberry juice, the content of rutin, epicatechin, and morin was upregulated after LAB fermentation (Kwaw et al., 2018). In the peach pulp, LAB fermentation increased epicatechin content (Yang et al., 2022). The biotransformation of the phenolic profiles can be related to the phenolic derivation reactions. Previous studies proved that cyanidin-3-glucoside could be transformed into syringic and ferulic acid under LAB fermentation. Additionally, chlorogenic acid can be metabolized into other phenolic compounds by the LABs. In summary, the content of syringic acid, ferulic acid, erucic acid, rutin, catechin, epicatechin, and isoorientin increased after 48 h fermentation. In contrast, the content of chlorogenic acid, neo-chlorogenic acid, gallic acid, quercetin, cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, and cyanidin-3-O-galactoside decreased. Generally, the composition of phenolic compounds is of vital importance for the quality and nutrition of fermented blueberry juice. However, the outcome of the current study could only explain part of the mechanism of phenolic accumulation. Thus, more fundamental studies still need to demonstrate the biotransformation of the individual phenolic compound under LAB fermentation in blueberry juice.

PCA was an important dimensionality reduction method used in data processing (Zhang et al., 2022). A biplot of the PCA for the phenolic profiles detected in the blueberry juice after fermentation was exhibited in Figure 1. As shown in Figure 1, two principal components (PCs) were described as PC1 (76.1%) and PC2 (13.1%) for the total variance. The unfermented blueberry juice samples were distributed on the positive side of PC1 and PC2, the La 16 and Lp 375 fermented juice was located on the negative side of PC1 and positive side of PC2, and the Lr 005 fermented juice was situated on the negative side of PC1 and PC2. In detail, La 16 and Lp 375 were highly correlated with primary phenolic acids and flavonoids, such as syringic acid, erucic acid, rutin, and catechin, etc. The unfermented blueberry juice was positively associated with most anthocyanins and some phenolic acids, such as cyanidin-3-glucoside, cyanidin-

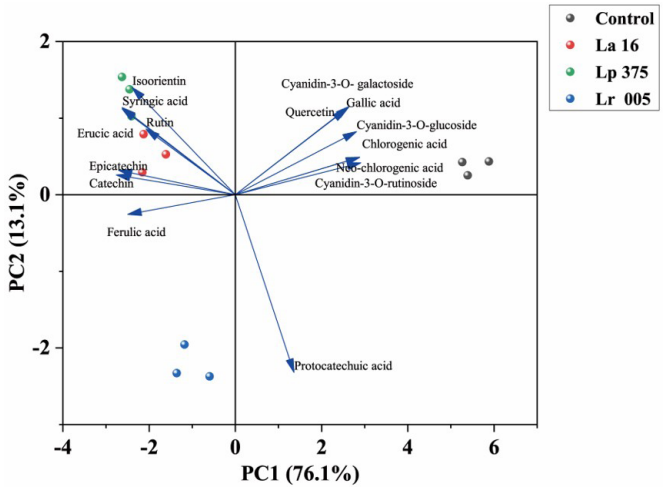

Figure 1. Biplot of the principal component analysis (PCA) of the phenolics profiles detected in the blueberry juice fermented by three lactic acid bacteria (LAB).

3-rutinoside, neo-chlorogenic acid, etc. This result indicated that these three LABs have different capacities for metabolizing phenolic compounds. And La 16 and Lp 375 shared a close correlation in the phenolics profile.

3.3 Effect of lactic acid fermentation on antioxidant activities

The antioxidant activities of phenolic compounds can protect the human body from damage by reactive oxygen species and offer protection against cancers, diabetes, and inflammation (Dutra et al., 2017). The antioxidant activity of blueberry juice fermented by LABs was conducted regarding DPPH and ABTS radical-scavenging activities and reducing power capacity (Table 3).

As can be seen, the DPPH radicals scavenging activity of blueberry juice were significantly increased by all the screened strains. The DPPH radical scavenging capacity of the unfermented sample was 74.96 ± 0.67%. After fermentation, the DPPH radical scavenging capacity of the samples fermented by La 16, Lp 375, and Lr 005 was increased to 90.04 ± 0.53%, 89.76 ± 0.308%, and 89.91 ± 0.25%, respectively (Table 3). The rise of DPPH radical scavenging capacity in the blueberry juice suggested that LAB fermentation positively impacts the phenolic compounds to donate protons (Li et al., 2022). This is supported by the higher significant positive correlation between DPPH% and TFC (R^2 = 0.77) and TPC (R^2 = 0.62). Additionally, there are significant positive correlations between the individual phenolic acid, flavonoid, and DPPH% (syringic acid, R^2 = 0.82; ferulic acid, R^2 = 0.82; erucic acid, R^2 = 0.82; rutin, R^2 = 0.65; catechin, R^2 = 0.90; epicatechin, R^2 = 0.88; isoorientin, R^2 = 0.72), which indicated that some phenolic acids and flavonoids are effective DPPH radical scavengers (Figure 2).

The ABTS assay was conducted to measure the ability of the fermented juice for ABTS radical scavenging by electron transfer (Qiu et al., 2022) . LAB fermentation induced a rise in the ABTS radical scavenging activities, which proved that LAB

Table 3. Changes of antioxidant capacity in blueberry juice after fermentation. Different lowercase letters represent significant differences among same column at $P < 0.05$ level.

Sample	DPPH %	ABTS %	Reducing power capacity
Control	74.96 ± 0.67^b	94.78 ± 0.43^b	1.22 ± 0.09^b
La 16	90.04 ± 0.53^a	97.62 ± 0.09^a	1.67 ± 0.15^a
Lp 375	89.76 ± 0.30^a	97.08 ± 0.33^a	1.74 ± 0.15^a
Lr 005	89.91 ± 0.25^a	97.13 ± 0.23^a	1.57 ± 0.13^a

could promote the transfer of electrons to scavenge ABTS free radicals in the blueberry juice. The association between the ABTS% and the phenolic compounds could be ascribed to the specific structure of the phenolic hydroxyl groups. The resonance between their aromatic benzene rings and free electron pair on the phenolic oxygen can accelerate the delocalization of the electrons, increasing resistance from the oxygen free radicals (Jia et al., 2022). Additionally, the antioxidant activities can vary due to the disparities in the active components of the products. This can be supported by the different coefficient of association between ABTS% and TFC (R^2 = 0.73) and TPC (R^2 = 0.69). Also, strong correlations were found among the ABTS%, the phenolic acids (syringic acid, R^2 = 0.81; ferulic acid, R^2 = 0.81; erucic acid, R^2 = 0.82), and the flavonoids (rutin, R^2 = 0.74; catechin, R^2=0.89; epicatechin, R^2 = 0.89; isoorientin, R^2 = 0.76). Reducing power is imperative to test the phenolic-reducing properties (Kwaw et al., 2018) . LAB fermentation promoted the reducing power capacity of the juice and reached its maximum (1.74 ± 0.15) in Lp 375 fermented samples. Moreover, the strong correlation coefficients between catechin (R^2 = 0.80), epicatechin (R^2 = 0.83), and reducing power capacity demonstrated that specific flavonoids in the fermented samples have reducing power properties. Generally, our findings align with the results of lactic acid fermented goji berry juice (Wang et al., 2021) and apple juice (Bai et al., 2021), which ascertained increases in antioxidants after fermentation.

3.4 Changes of active substance and antioxidant activity after in vitro- simulated gastrointestinal digestion

The *in vitro*- simulated gastrointestinal digestion assay was conducted to estimate the effect of the gastrointestinal environment on the active substance and antioxidant activity in the blueberry juice. The obtained results are shown in Table 4.

The TPC of fermented blueberry juice during the SSF period all decreased from 2571.22 ± 18.25 GAE mg/mL to 1876.27 ± 62.37 GAE mg/mL, from 2933.42 ± 116.08 GAE mg/mL to 2311.86 ± 307.41 GAE mg/mL, and from 2399.99 ± 95.63 GAE mg/mL to 2078.17 ± 188.42 GAE mg/mL in La 16, Lp 375 and Lr 005 fermented blueberry juice, separately And the TPC of unfermented blueberry juice during the SSF period was decreased from 2133.99 ± 34.39 GAE mg/mL to 1812.17 ± 168.42 (Table 1,4). Thus, LAB fermented blueberry juice has higher retention of TPC than unfermented blueberry juice, with the highest TPC retention in Lp 375 fermented blueberry juice. Similarly, the retention of the TFC was also higher in the LAB fermented

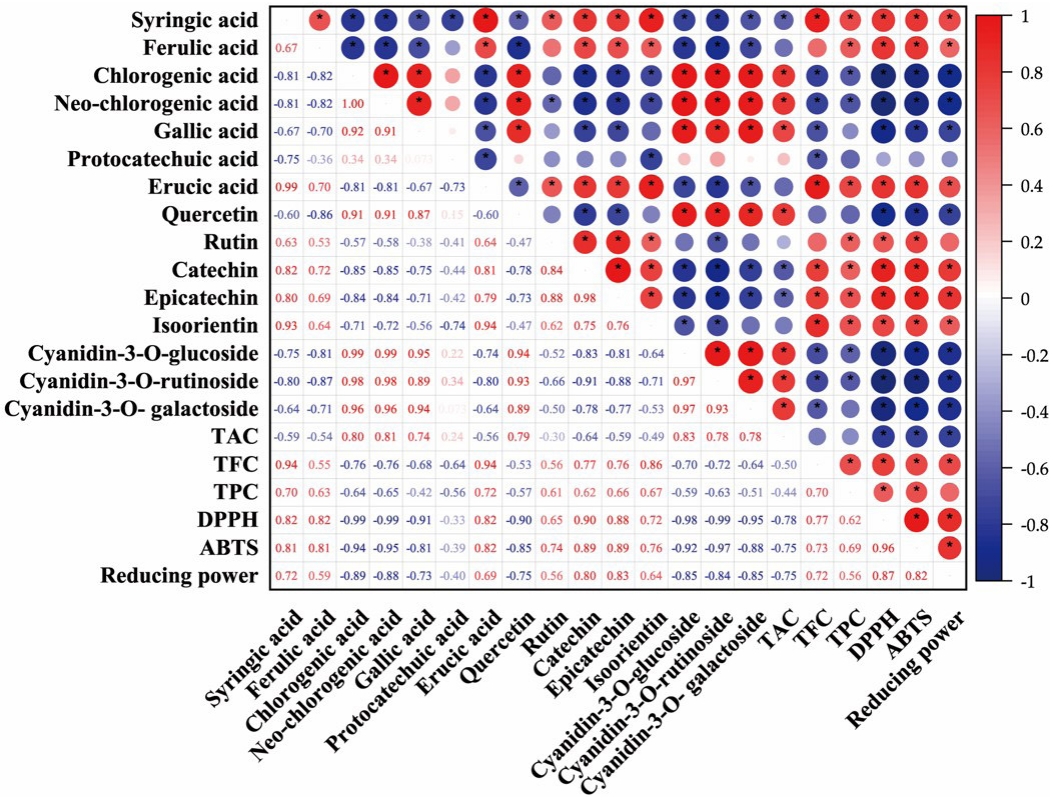

Figure 2. The Pearson's correlation analysis of antioxidant activities and phytochemical concentration of lactic acid bacteria fermented blueberry juice.

Table 4. The concentration of active ingredient after *in vitro*-simulated digestion. Different lowercase letters represent significant differences among same line at $P < 0.05$ level.

	Period	Control	La 16	Lp 375	Lr 005
TPC (GAE mg/mL)	SSF	1812.17 ± 168.42^{b}	1876.27 ± 62.37^{b}	2311.86 ± 307.41^{a}	2078.17 ± 188.42^{ab}
	SGF	3749.04 ± 135.85^{a}	2932.13 ± 92.26^{c}	3387.08 ± 74.17^{b}	2997.79 ± 202.21^{c}
	SIF	4327.03 ± 119.50^{a}	3549.35 ± 175.15^{b}	3428.26 ± 85.16^{b}	3410.78 ± 273.27^{b}
TFC (Rutin mg/mL)	SSF	550.40 ± 11.93^{b}	1060.00 ± 99.95^{a}	1053.97 ± 140.57^{a}	911.44 ± 111.48^{a}
	SGF	862.56 ± 111.37^{b}	920.07 ± 36.67^{b}	1127.91 ± 63.90^{a}	947.79 ± 103.04^{b}
	SIF	672.80 ± 21.78^{a}	418.69 ± 40.52^{b}	466.61 ± 5.81^{b}	419.37 ± 33.12^{b}
TAC (C3GE mg/ mL)	SSF	2.91 ± 0.05^{c}	7.09 ± 0.90^{a}	5.01 ± 0.66^{b}	5.46 ± 0.85^{b}
	SGF	1.34 ± 0.22^{c}	4.84 ± 0.13^{b}	8.17 ± 0.58^{a}	7.91 ± 0.67^{a}
	SIF	2.22 ± 0.35^{c}	4.84 ± 0.22^{b}	7.90 ± 0.10^{a}	5.02 ± 2.00^{b}
DPPH %	SSF	37.91 ± 2.80^{d}	71.01 ± 0.53^{a}	62.18 ± 2.01^{b}	56.57 ± 3.37^{c}
	SGF	67.18 ± 3.01^{b}	70.29 ± 1.33^{ab}	72.11 ± 2.42^{ab}	73.64 ± 3.53^{a}
	SIF	29.92 ± 2.62^{c}	56.29 ± 1.17^{a}	40.28 ± 2.14^{b}	33.29 ± 4.88^{c}
ABTS %	SSF	91.46 ± 0.42^{b}	94.15 ± 0.58^{a}	94.18 ± 1.42^{a}	93.98 ± 1.24^{a}
	SGF	71.76 ± 4.97^{b}	74.84 ± 2.87^{ab}	81.62 ± 0.35^{a}	81.79 ± 3.38^{a}
	SIF	84.62 ± 0.57^{d}	87.80 ± 1.53^{c}	90.28 ± 1.40^{a}	89.78 ± 1.52^{b}
Reducing power	SSF	1.25 ± 0.01^{b}	1.35 ± 0.07^{a}	1.11 ± 0.06^{c}	1.02 ± 0.03^{c}
	SGF	0.96 ± 0.03^{a}	1.04 ± 0.04^{a}	0.95 ± 0.10^{a}	0.92 ± 0.01^{a}
	SIF	1.52 ± 0.01^{b}	1.67 ± 0.02^{a}	1.54 ± 0.09^{b}	1.42 ± 0.06^{b}

groups compared to the unfermented blueberry juice, with the highest retention in Lp 375 fermented blueberry juice. However, the TPC and TFC of all the samples showed significantly higher concentrations than the corresponding undigested samples in the SSF and SIF periods. The observed discrepancies might be attributed to the interaction and interference of the food matrix and interactions with other dietary components such as fibers, proteins, pH, and enzymes (Mackie et al., 2020). And the decrease of pH during fermentation also enhanced these phenolics and flavonoid stabilities (Pinton et al., 2022). In contrast, the observed variation in TPC and TFC during the *in vitro* simulation of gastrointestinal digestion may be due to the

LAB strains' different rates of cell survival, which are in charge of the higher metabolism and biotransformation of the phenolic acids and flavonoids (Valero-Cases et al., 2017). However, the cell population of the LAB strains during the *in vitro*-simulated digestion was not qualified in the study. Additionally, the TPC and TFC may have been impacted by the chemical configuration of the various bioactive molecules or by interactions between the bioactive compounds and other dietary components (Adebo & Medina-Meza, 2020) . The TAC of all the samples suffered a significant decrease during the *in vitro*-simulated digestion. The substantial reduction in the content of anthocyanins may be due to their conversion to other phenolic compounds (Jiao et al., 2018). Previous studies showed that cyanidin-3-O-glycoside and cyanidin-3-O-rutinoside could transform into 18 phenolics after gastrointestinal digestion (Gonçalves et al., 2021). The cyanidin-3-O-glucoside can convert into protocatechuic acid after the *in vitro*-simulated digestion (Gao et al., 2022).

It demonstrated that the fermented blueberry juice exhibited higher retentions of DPPH% and ABTS% compared to the unfermented samples (Table 4). The DPPH% values of La 16, Lp 375, and Lr 005 fermented juices were more elevated than the unfermented samples in the SSF, SGF, and SIF digestions. In the SSF and SIF phrases, the highest DPPH% was obtained in the La 16 fermented juice, while in the SGF condition, the highest DPPH% was obtained in the Lp 375 fermented juice. The highest ABTS% in the SSF and SIF phrases was the Lp 375 fermented juice. La 16 showed higher reducing power capacity under *in vitro* digestion process compared to the unfermented samples. Many studies have revealed a substantial association between phenolic content and antioxidant activity, which is confirmed by the results of this research. The varieties of antioxidant activities during different digestion phrases may be ascribed to the pH variation during the digestion procedure. Previous studies confirmed that the modifications of pH would induce the change in the structure of phenolic compounds, thus affecting their antioxidant capacities (Song et al., 2022) . Therefore, the various pH conditions utilized in the different *in vitro* digesting processes will impact the assessment of the antioxidant activities.

3.5 Enzyme inhibition ability before and after fermentation

α-Glucosidase is a kind of starch-digesting enzyme to hydrolyze oligosaccharides to glucose through α-1, 4 glycosidic interactions. Thus, by inhibiting α-glucosidase activity, the active substance eases the rate of glucose release and regulates postprandial blood glucose, thereby improving insulin sensitivity.

As shown in Figure 3, the inhibition of α-glucosidase in the blueberry juice was 55.94%. The inhibition of α-glucosidase was significantly increased after fermenting, with 79.77% in La 16, 63.82% in Lp 375, and 75.69% in Lr 005. α-Amylase is a hydrolase enzyme similar to α-glucosidase, which can metabolize carbohydrates into polymers composed of glucose units and regulate the blood sugar in human bodies (Gong et al., 2020). The inhibition of α-amylase was 83.53% in the blueberry juice. After fermentation, the inhibition of α-glucosidase activity in the blueberry juice fermented by La 16, Lp 375, and Lr 005 was significantly enhanced to 92.62%, 96.60%, and 90.66%, respectively (Figure 2). The increased enzyme inhibition ability

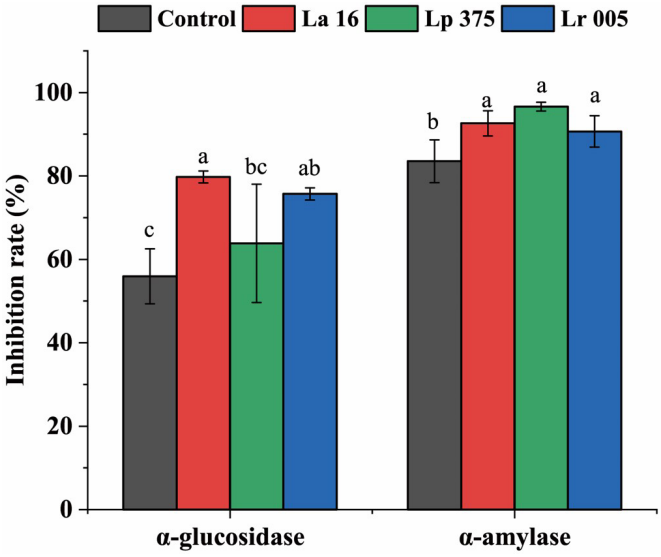

Figure 3. The α-glucosidase and α-amylase inhibition ability of lactic acid bacteria fermented blueberry juice.

after fermentation was ascribed to increased active substances such as phenolic acids and flavonoids and the biotransformation of these active substances by the LABs. It has been proved that polyphenols could bind to the active site of α-glucosidase and α-amylase by hydrophobic interactions, competitively inhibiting the catalytic activity of these enzymes (Tiji et al., 2021). The difference in the enzyme inhibition abilities in these different LAB fermented juices may be due to the distinction in the phenols and the diverse phenols metabolism of these strains.

Blueberries seem to be a suitable substrate for fermentation and produce functional beverages of enhanced nutritional value. Moreover, due to the difficulties of preserving fresh, the practical application of LAB could be an excellent way to improve or retain the functional properties of the blueberries. LAB fermentation can enhance the bioavailability of polyphenols and increase antioxidant activities and health-benefits properties in blueberry juice. Thus, LAB fermentation provides novel methods for the bioprocessing of blueberries, potentially expanding the use of health-promoting blueberries in the food industry. The combination of LAB and blueberry juice lays the foundation to develop a novel probiotic plant-based fermentation product with potential health benefits.

4 Conclusions

This study showed that the fermentation of blueberry juice by La 16, Lp 375, and Lr 005 certainly affects the phenolic constitutes of the blueberry juice. The fermenting approach increased total phenolics and total flavonoids contents substantially. After fermentation, radical-scavenging activities of DPPH and ABTS increased significantly, whereas the power capacity of blueberry juice decreased. And fermented blueberry juice had more retention of functional components and antioxidant capacities after *in vitro* simulated digestion. Moreover, LAB fermentation enhanced the inhibition of α-glucosidase and α-amylase in blueberry juice. All in all, LAB fermentation can increase the functional properties of blueberry juice, which

provides feasibility to developing an available beverage in which the lactic acid bacteria combined with blueberry.

Conflict of interest

The authors declared no conflict of interest.

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