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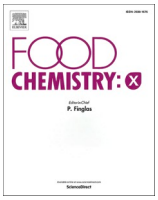

Blue honeysuckle fermentation with *Lacticaseibacillus rhamnosus* L08 improves its biological activity, sensory and flavor characteristics, and storage stability

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ABSTRACT

The objective of this study was to investigate the potential of *Lacticaseibacillus rhamnosus* L08 (*L. rhamnosus* L08) to enhance the functionality, improve the taste, and explore efficient storage methods of blue honeysuckle juice (BHJ). The fermentation process resulted in an increase in the levels of polyphenols, flavonoids, and anthocyanins in blue honeysuckle juice, which was attributed to the action of β-glucosidase on specific phenolic compounds, namely Cyanidin-3-Glucoside and Quinic acid. The increase in phenolic content resulted in an enhancement of the antioxidant capacity of BHJ. The fermentation processed, utilizing *L. rhamnosus L08*, not only enhanced the flavor and taste of BHJ, but also mitigated its bitter aftertaste while minimizing the loss of bioactive components during storage. In conclusion, this study demonstrated a potential avenue for enhancing the commercial value and dietary significance of this lesser-known superfruit, with fermented BHJ emerging as a promising innovation in the field of functional foods.

1. Introduction

The blue honeysuckle (*Lonicera caerulea* L.), a fruit that exemplifies the principles of "medicine and food homology," is regarded as the "third generation king of small berries." It is a natural source of phenolic compounds, which exhibit remarkable antioxidant, anti-inflammatory, and antimicrobial activities (Zhang, Liu, Liu, Chen, & Chen, 2023). Zhang et al. (2023) conducted measurements on 20 varieties of blue honeysuckle and found that the total phenolic content ranged from 107.97 to 8179.26 mg gallic acid equivalent/ 100 g dry weight (DW). The total flavonoid content ranged from 614.73 to 9117.55 mg catechin equivalent / 100 g DW. The anthocyanin content was found to range between 400.10 and 457.50 mg cyanidin-3-glucoside equivalent/ 100 g DW. Developed by Northeast Agricultural University, "Lanjingling" is one of the first registered varieties of blue honeysuckle in China, with a significantly higher content of bioactive compounds than other varieties (Zhang, Ma, et al., 2023). Blue honeysuckle has rich nutritional value and active ingredients, but its bitter taste and difficult storage are the main reasons limiting its development. Therefore, it is important to explore technologies to enhance its flavor, protect its bioactive compounds and extend its shelf life.

Lactic acid bacteria (LAB) are widely used in food production for their probiotic functions. The fermentation process carried out by LAB not only adds a fermented flavor to foods, enhancing their color, aroma and flavor, but also extends their shelf life and improves the content of bioactive compounds (Chen, Xie, He, Sun, & Bai, 2023; Guan, Liu, Li, Wang, & Zhang, 2022). Zhang et al. (2022) used *Lactiplantibacillus plantarum* to ferment blueberry juice, resulting in a significant enhancement in its antioxidant capacity after fermentation. The alteration in phenolic content during LAB fermentation is likely due to the production of hydrolytic enzymes that convert complex phenolic compounds into simpler ones (Kwaw et al., 2018). β-glucosidase is capable of catalyzing the hydrolysis of covalent bonds between phenolic compounds and the cell wall matrix, thereby releasing phenolic substances

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from plant tissues (Lai et al., 2023). *Lacticaseibacillus rhamnosus* L08 (*L. rhamnosus* L08) exhibits significant β-glucosidase activity during fermentation, which accounts for its excellent applications in plant-based fermentation. In a previous study, we demonstrated that the fermentation of *L. rhamnosus* L08 can enhance the biological activity of phenolic compounds in apple pomace, resulting in a significant improvement in antioxidant capacity through biotransformation of the phenolic compounds (Liu et al., 2021). The fermentation of pea protein by *L. rhamnosus* L08 resulted in a change in flavor characteristics and the reduction of unpleasant odors, including nonanal and octanal (Pei et al., 2022).

This study selected *L. rhamnosus* L08 as the strain for fermenting BHJ. At different times during the fermentation process, the viable count, pH and total acidity of the fermented BHJ were monitored separately, while the total phenolic content, total flavonoid content and total anthocyanin content of the fermented BHJ were also determined. Liquid chromatography-mass spectrometry (LC-MS) was employed to determine the relative content of phenolic compounds, and a time-dependent analysis of their changes was conducted. The antioxidant capacity of fermented BHJ was comprehensively evaluated by measuring its ability to inhibit $O_2\cdot^-$, $OH\cdot^-$, and $ABTS^+$. A comprehensive analysis of the quality of the fermented BHJ was conducted by combining color analysis, electronic tongue, electronic nose, volatile compounds, and sensory evaluation. Finally, changes in basic properties and the content of phenolic compounds in the fermented BHJ during storage were measured. This provides a research foundation for the development of delicious probiotic functional beverages made from blue honeysuckle, contributing to the upgrading of agricultural product quality.

2. Materials and methods

2.1. Preparation of blue honeysuckle juice (BHJ)

The blue honeysuckle (variety: Lanjingling, purchased from Yichun City, Heilongjiang Province, China) was processed in a juicer (MJ-WJE2802D, Midea, China) along with distilled water in a ratio of 1:1.5. Following the removal of the filter residue, the clarified juice was obtained. Subsequently, 2% glucose was added to the clarified BHJ and mixed uniformly. The pH (LE438 pH meter, Mettler Toledo, China) of the juice was adjusted to 6.0 ± 0.1 using $NaHCO_3$. The BHJ was sterilized (HVE-50 High-Pressure Steam Sterilization Pot, Hirayama, Japan) at 95 °C for 15 min and cooled down to 4 °C for storage. All chemicals in this study were analytically pure and supplied by Sinopharm Chemical Reagent Co. Ltd. (China) or Shanghai Macklin Biochemical Technology Co., Ltd. (China).

2.2. Preparation of strains

L. rhamnosus L08 was preserved at the Food Science College of Northeast Agricultural University. The strain was initially inoculated into a sterile MRS medium and incubated at 37 °C for 12 h (DHP-9272 Electro-heating Standing-temperature Sultivator, Shanghai Science Instrument, China). Afterward, the culture was reinoculated into fresh sterile MRS culture medium with a ratio of 3% and incubated at 37 °C for 12 h again. The twice-activated strain was employed for the fermentation of BHJ (Zhang et al., 2022).

2.3. Fermentation of BHJ

The bacterial sludge was subjected to two washes with 0.01 M sterile phosphate buffer solution (pH 7.4) and then added to the sterilized BHJ, resulting in an initial viable cell count of approximately 1.0×10^8 CFU/mL. The BHJ was incubated at 37 °C for 24 h, and samples were taken at 0 h, 4 h, 8 h, 12 h, 16 h, 20 h, and 24 h for analysis.

2.4. Analysis of changes in microorganisms and phytochemicals

2.4.1. Analysis of viable count, pH, and total acidity

The viable count, pH, and total acidity of the fermented BHJ were measured at 4 h intervals. Following gradient dilution, the fermented BHJ was spread onto MRS agar plates, which were then incubated at 37 °C for 48 h. The pH of the fermented BHJ was directly measured using a LE438 pH meter (Mettler Toledo, China). Furthermore, total acidity was quantified by titration with a 0.1 M NaOH solution, expressed as g/L of lactic acid (Zhang et al., 2022).

2.4.2. Determination of total phenolic content (TPC)

The method of Do et al. (2014) was referenced and modified to determine the TPC in the fermented BHJ. The diluted sample (0.5 mL) was mixed with 2.0 mL of 0.2 N Folin-Phenol, and the resulting mixture was incubated in the dark for 5 min. Subsequently, 2.0 mL of a 0.7 M Na_2CO_3 solution was added, and the mixture was thoroughly mixed and incubated in the dark for 1 h. Absorbance at 760 nm was measured using a SpectraMax Reg iD3 ELISA reader (Molecular Devices, China) to determine the TPC in the fermented BHJ. The gallic acid (0–60.00 μg/mL) as the standard curve ($y = 0.0049\times + 0.0057$, $R^2 = 0.9947$), the results of TPC were expressed as gallic acid equivalents, and expressed as gallic acid equivalent (GAE) in μg/mL.

2.4.3. Determination of total flavonoid content (TFC)

The method of Do et al. (2014) was referenced and modified to determine the TFC in the fermented BHJ. A 0.5 mL sample was mixed with 0.3 mL of a 0.7 M $NaNO_2$ solution, and the mixture was incubated in the dark for 6 min. Subsequently, 0.3 mL of 0.5 M $Al(NO_3)_3$ solution was added, and the mixture was incubated in the dark for a further 6 min. Finally, 4.0 mL of 1 M NaOH solution was added, and the mixture was incubated in the dark for 15 min. The absorbance was measured at 510 nm using a SpectraMax Reg iD3 ELISA reader (Molecular Devices, China) to determine the TFC in the fermented BHJ. The rutin (0–25.00 μg/mL) as the standard curve ($y = 0.0047\times$ - 0.0036, $R^2 = 0.9953$) and the results of TFC were expressed as rutin equivalents, and expressed as rutin equivalent (RE) in μg/mL.

2.4.4. Determination of total anthocyanin content (TAC)

The method of Zhang et al. (2022) was referenced and modified to determine the TAC in the fermented BHJ. A 1.0 mL sample of the fermentation was taken and diluted it 25 times with 0.2 mM KCl solution at pH 1.0 and 0.2 mM CH_3COONa solution at pH 4.5. After equilibrating for some time, the absorbance was measured at 510 nm and 700 nm using a SpectraMax Reg iD3 ELISA reader (Molecular Devices, China). Substituted the measured values into formulas (1) and (2) successively for calculation (Zhang et al., 2022). The results of TAC were expressed as cyanidin-3-glucoside equivalent, and expressed as cyanidin-3-glucoside equivalent (C3GE) in μg/mL.

$$A = (A_{510} - A_{700})\ pH_{1.0} - (A_{510} - A_{700})\ pH_{4.5} \quad (1)$$

$$Acy = \frac{A \times 449.2 \times n}{26900} \times 10 \quad (2)$$

In the formula: A_{510} represents the absorbance of the sample at a wavelength of 510 nm; A_{700} represents the absorbance of the sample at a wavelength of 700 nm; n is the dilution factor; 449.2 is the molecular weight of cyanidin-3-glucoside; 26,900 is the extinction coefficient of cyanidin-3-glucoside; and Acy represents the content of anthocyanins in μg/mL.

2.4.5. Determination of composition and content of phenolic substances

The method of Zhang, Ma, et al. (2023) was referenced and modified to determine composition and content of phenolic compounds in the fermented BHJ. UPLC I-Class/Xevo G2-XS QTOF type high-performance liquid chromatography-electrospray ionization quadrupole time-of-

flight mass spectrometry (Waters Corporation, America) was combined with the C18 column (Waters Corporation, America) for determination. The mobile phase was 10% acetonitrile, with a flow rate of 0.8 mL/min. The mass spectrometer used positive ionization mode and scanned within a range of 50–2000 *m/z*, using a capillary voltage of 5500 V and a dry gas temperature of 550 °C. The compounds were identified by comparing them with previous literature reports or searching the Pubchem database. The content of various phenolic substances was compared using the area normalization method.

2.5. Determination of antioxidant capacity

The inhibition ability of the sample towards $O_2\cdot^-$ and $OH\cdot^-$ and the Total Antioxidant Capacity(T-AOC) of the sample were determined through ELISA kits (Nanjing Jiancheng Bioengineering Institute, China). Among them, T-AOC was measured using the ABTS method, and the results were represented by Trolox Equivalent Anoxic Capacity (TEAC). All testing procedures were conducted in accordance with the instructions provided by the manufacturer. Finally, a correlation analysis was conducted between the antioxidant activity of the fermented BHJ and its phytochemicals.

2.6. Sensory quality and flavor omics

2.6.1. Determination of color

The color of the fermented BHJ was measured using a ZE6000 portable colorimeter(Nippon Denshoku, Japan). After correcting the whiteboard and blackboard, the color value of the fermented BHJ was measured and recorded. The values of L *, a *, and b * were recorded for this purpose. The calculation method for ΔE was based on the formula (3) (Chen et al., 2023).

$$\Delta E = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]^{1/2} \quad (3)$$

In the formula: ΔL, Δa, and Δb represent the differences between the BHJ after fermentation and the BHJ at 0 h of fermentation, respectively.

2.6.2. Determination of electronic tongue

In the method of Lipkowitz, Ross, Diako, and Smith (2018), the SA402B electronic tongue(Insent, Japan) was used to determine the flavor of fermentation samples. The 10 mL of sample was diluted five-fold and centrifuged (5000 r/min, 10 min) to remove any residual filter material. The filtrate was transferred to a dedicated beaker, an electronic tongue was used for measurement, and the data was recorded.

2.6.3. Determination of electronic nose

Analysis of the odor characteristics of the fermented BHJ used a DM6 electronic nose (BosinTech, China) (Tian et al., 2020). The electronic nose system comprised 10 sensor probes, namely W1C (aromatic), W5S (broad-range), W3C (aromatic), W6S (hydrogen), W5C (arom-aliph), W1S (broad-methane), W1W (sulphur-organic), W2S (broad-alcohol), W2W (sulph-chlor), and W3S (methane-aliph). A sample of 10 mL was taken and placed it in a headspace injection bottle. The electronic nose system parameters were set to a chamber flow rate of 200 mL/min and an injection flow rate of 200 mL/min, and the measurement lasted for 120 s. The sensor array was cleaned with air as the carrier gas to reset the signal response to zero. The volume of the sample injected was 200 μL.

2.6.4. Sensory evaluation

The method of Okokon and Okokon (2019) was referenced and modified as the standard for evaluating the fermented BHJ. The sensory evaluation team consisted of 5 males and 5 females, who evaluated the BHJ at different fermentation times. All evaluators have undergone professional training. All evaluators provided consent to participate in the sensory evaluation and agreed to the use of their information. According to the regulations of our institute, this activity does not require obtaining an ethic code from the institution. The nine items, including color, clarity, sweetness, sourness, astringency, bitterness, fruity aroma, fermentation flavor, and comprehensive score, were evaluated and assigned scores ranging from 1 to 9, representing the intensity from weak to strong (1 point indicating extreme weakness, 9 points indicating extreme strength).

2.6.5. Determination of volatile substances

The method of Kuang et al. (2022) was referenced and modified. Volatile substances in the fermented BHJ were detected using Shimadzu nexis GC2030/QP2020 NX gas chromatography–mass spectrometry (Shimadzu, Japan). Divinylbenzene/Carboxyl/Polydimethylsiloxane extraction needles (Zhenzheng, China) were used to adsorb volatile substances, and GC–MS was used for data collection and analysis, with DB-5 chromatographic columns (Shimadzu, Japan). The collected data was compared with the NIST02 MS Library atlas library for retrieval, and the relative content of each volatile component was calculated using the area normalization method. Further detailed information on the compounds can be found in Table 1.

2.7. Determination of basic properties and phytochemicals during storage period

The fermented BHJ was stored at 4 °C for 21 d, and samples were collected for analysis on 0 d, 7 d, 14 d, and 21 d. These samples were analyzed for viable count, pH, total acidity, TPC, TFC, and TAC. The analytical techniques employed were identical to those described in Section 2.4.

2.8. Statistical analysis

A minimum of three independent experiments were performed for each assay, and all values obtained in this study were expressed as the mean ± standard deviation (SD). The data were analyzed using SPSS 25.0 software and Origin 2018. The statistical significance of data comparisons was determined using a one-way analysis of variance (ANOVA), followed by the LSD test and Duncan of $p < 0.05$ were judged to be statistically significant.

3. Results and discussion

3.1. Fermentation of the BHJ by L. rhamnosus L08

The changes in viable count, pH, and total acidity during the fermentation process of the BHJ are shown in Fig. 1 (A). During the initial 0–4 h, there were minor fluctuations in the viable count, pH, and total acidity. From 4 h to 12 h, the rate of change became significantly more pronounced. This acceleration was attributed to the strain entering the logarithmic growth phase, which resulted in a rapid decrease in the pH of the BHJ and a corresponding increase in total acidity. Following a period of 12 h, the strain exhibited a relatively stable phase of growth, with a viable count reaching 9.11 ± 0.02 log CFU/mL by 24 h. Although the rate of change in pH and total acidity slowed down, they continued to accumulate, reaching a pH of 4.54 ± 0.10 and a total acidity of 10.89 ± 0.24 g/L at 24 h. The results indicated that the BHJ can serve as a growth substrate for *L. rhamnosus* L08. The growth of the strains is closely related to the fermentation substrate. The results of Zhang et al. (2022), which are consistent with this conclusion, showed no apparent influence of changes in pH and total acidity during fermentation on juice acceptability.

3.2. Variation of phytochemicals during fermentation

3.2.1. Total polyphenol content

BHJ contains abundant phenolic compounds, which have efficient

Table 1
Basic information of volatile compounds in blue honeysuckle juice at different fermentation times.

Number	Name	PubChem CID	Classification	Formula	MW g/moL	Characteristic(Gemert, 1999)
1	Acetoin	179	Ketones	$C_4H_8O_2$	88.11	Butter
2	3-Methyl-1-butanol	31,260	Alcohols	$C_5H_{12}O$	88.15	Flower, cocoa, malt
3	Hexanal	6184	Aldehydes	$C_6H_{12}O$	100.16	Apples, fresh
4	2-Hexenal	5,281,168	Aldehydes	$C_6H_{10}O$	98.14	Apples
5	3-Hexen-1-ol	5,284,503	Alcohols	$C_6H_{12}O$	100.16	–
6	Formic Acid Hexyl Ester	61,177	Esters	$C_7H_{14}O_2$	130.18	Fruits
7	2-Heptenal, (*Z*)-	5,283,316	Aldehydes	$C_7H_{12}O$	112.17	Fruits, almond
8	Benzaldehyde	240	Aldehydes	C_7H_6O	106.12	Cherries, malt, bitter almonds
9	1-Octen-3-ol	18,827	Alcohols	$C_8H_{16}O$	128.21	Cucumbers, mushrooms, flowers
10	Octanal	454	Aldehydes	$C_8H_{16}O$	128.21	Citrus, green
11	Eucalyptol	2758	Terpenes	$C_{10}H_{18}O$	154.25	Camphor, cool, eucalyptus, mint
12	2-Octenal, (*E*)-	5,283,324	Aldehydes	$C_8H_{14}O$	126.20	Dandelion, fruit, grass, green
13	1-Octanol	957	Alcohols	$C_8H_{18}O$	130.23	Flowers, bitter almonds
14	Linalool	443,158	Terpenes	$C_{10}H_{18}O$	154.25	Coriander, flowers, lavender, lemon, rose
15	Nonanal	31,289	Aldehydes	$C_9H_{18}O$	142.24	Flowers, lemons
16	2-Tridecenal, (E)-	5,283,363	Aldehydes	$C_{13}H_{24}O$	196.33	Fruits
17	Octadecanoic acid	5281	Fatty acids	$C_{18}H_{36}O_2$	284.5	Dairy
18	1-Pentanol	6276	Alcohols	$C_5H_{12}O$	88.15	Vinegar, fruits
19	2-Decenal, (E)-	5,283,345	Aldehydes	$C_{10}H_{18}O$	154.25	Oranges
20	Hexadecane	11,006	Alkanes	$C_{16}H_{34}$	226.44	–
21	2-Octen-1-ol, (E)-	5,318,599	Alcohols	$C_8H_{16}O$	128.21	Soap, plastic
22	2-Nonanone	13,187	Ketones	$C_9H_{18}O$	142.24	Fruits
23	1-Nonanol	8914	Alcohols	$C_9H_{20}O$	144.25	Flowers
24	1-Decanol	8174	Alcohols	$C_{10}H_{22}O$	158.28	–
25	2-Undecanone	8163	Ketones	$C_{11}H_{22}O$	170.29	Oranges
26	2-Tridecanone	11,622	Ketones	$C_{13}H_{26}O$	198.34	Fragrant
27	2-Heptanone	8051	Ketones	$C_7H_{14}O$	114.19	Cheese, fruits, nuts
28	2-Decanone	12,741	Ketones	$C_{10}H_{20}O$	156.26	Fruits
29	Pentadecane	12,391	Alkanes	$C_{15}H_{32}$	212.41	–
30	Nonadecane	12,401	Alkanes	$C_{19}H_{40}$	268.5	–
31	Geraniol	637,566	Terpenes	$C_{10}H_{18}O$	154.25	Geranium, lemon peel, passion fruit, peach, rose

Note: "–" means no detection or no odor description.

antioxidant activity (Zhang et al., 2022). The TPC in the fermented BHJ is shown in Fig. 1 (B). At 4 h, the TPC significantly decreased, possibly due to the utilization of some phenolic compounds during the initial stages of bacterial growth. Subsequently, the TPC exhibited a trend of first increasing and then decreasing. The highest TPC was reached after 16 h of fermentation (2421.63 ± 60.13 μg GAE/mL), which was not significantly different from the TPC at 12 h and 20 h. Compared to the TPC at 0 h, it increased by 14.40%. However, the TPC at 24 h was found to be significantly lower than that at 0 h ($p < 0.05$). Kwaw et al. (2018) suggested that the increase in TPC may be due to the ability of hydrolytic enzymes produced by LAB to hydrolyze complex phenolic compounds into simpler phenolic substances. The decrease in TPC may be due to the interaction between phenolic compounds present in the matrix and macromolecular substances, including polysaccharides and proteins (Escudero-López et al., 2013). Luo et al. (2024) used *Lactobacillus casei* and *Bifidobacterium bifidum* to ferment the BHJ. During the 24 h, the TPC showed a continuous upward trend, which was different from the result of this study. Nevertheless, the findings of Oh, Jeong, Velmurugan, Park, and Jeong (2017) were comparable to those of the present study. Such results may be caused by the difference in the type and generation time of hydrolases due to different strains.

3.2.2. Total flavonoid content

Flavonoids are important natural organic compounds widely present in nature, with a wide range of biological activities such as anti-inflammatory, antibacterial, and antioxidant properties. BHJ contains abundant flavonoids (Zhang, Liu, et al., 2023). The TFC in the fermented BHJ is shown in Fig. 1 (C). As the fermentation time increased, the TFC exhibited a trend of first increasing and then decreasing, which is consistent with previous research findings (Xu et al., 2023). The TFC reached its maximum after 12 h of fermentation, increasing from 250.21 ± 12.77 μg RE/mL to 331.77 ± 14.94 μg RE/mL. Compared to the TFC at 0 h, it increased by 32.60%. The decrease in TFC may be caused by changes in the activity of enzymes produced by microbial metabolism, including glycoside hydrolases, glycosyl transferases, tannase, and esterase. In addition, flavonoids may undergo conversion into other metabolites through reactions such as methylation, glycosylation, and flavonoid deglycosylation (He, Zhang, Wang, Wang, & Luo, 2022).

3.2.3. Total anthocyanin content

Anthocyanins, a water-soluble natural pigment in fruits, possess potent antioxidant properties. The TAC of BHJ is higher than that of other juices (Grobelna, Kalisz, & Kieliszek, 2019). The TAC in the fermented BHJ is shown in Fig. 1 (D). The effect of fermentation time on the TAC was relatively small. The TAC was highest (1316.01 ± 31.75 μg C3GE/mL) after 12 h of fermentation and significantly higher than other fermentation times ($p < 0.05$). The TAC after 24 h of fermentation was significantly higher than that after 0 h of fermentation ($p < 0.05$). Luo et al. (2024) also observed a similar trend in the fermentation of BHJ. Specifically, the TAC reached its maximum value after 12 h of fermentation and then subsequently declined. The study by Zhang et al. (2022) also showed that the fermentation of blueberry juice by *Lactobacillus* resulted in an increase in its TAC. The increase in TAC may be attributed to the catalytic action of β-Glucosidase, which hydrolyzes the covalent bonds linking phenolic compounds to the cell wall matrix, thereby releasing anthocyanins from plant tissues (Lai et al., 2023).

3.2.4. Composition of phenolic substances

To further investigate the transformation of phenolic compounds during the fermentation of BHJ by *L. rhamnosus* L08, high-performance liquid chromatography-electrospray ionization quadrupole time-of-flight mass spectrometry was used. This method was used to detect the composition and content of phenolic compounds at different fermentation times, as shown in Fig. 1(E). A total of 20 phenolic compounds were detected, with cyanidin-3-glucoside and quinic acid being the main phenolic compounds in the fermentation broth. These were followed by cyanidin-3,5-diglucoside, cyanidin-3-(6′-coumaroyl)-glucoside, peonidin-3-glucoside, and quercetin. During the fermentation

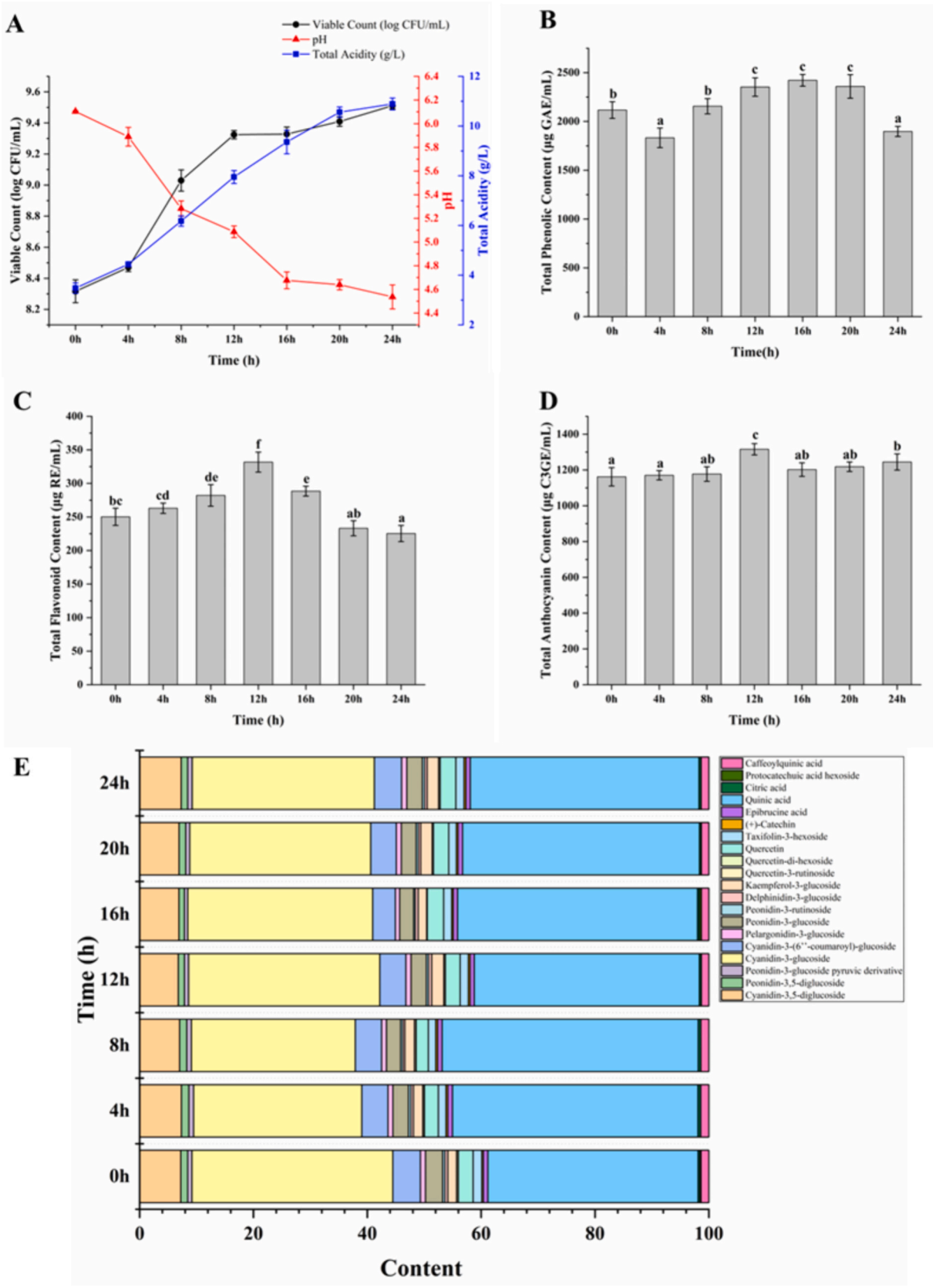

Fig. 1. The basic characteristics and phytochemicals of blue honeysuckle juice during fermentation by *L. rhamnosus* L08. (A) Viable Counts, pH, and Total Acidity. (B) Total Phenolic Content. (C) Total Flavonoid Content. (D) Anthocyanin Content. (E) Composition and content of phenolic substances. Note: Different letters mean significant difference ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

process, the cyanidin-3-glucoside content underwent a change, first decreasing and then increasing, while quinic acid showed the opposite trend. This turning point occurred precisely at the 8th hour of fermentation. This phenomenon was attributed to the transformation mechanism of phenolic substances triggered by microbial metabolism. A study by Lais Alves Almeida Nascimento et al. (2023) had shown a similar pattern, indicating that approximately 80% of cyanidin-3-glucoside is metabolized during the initial stage of fermentation and that there is a positive correlation between β-glucosidase levels and cyanidin-3-glucoside accumulation. *L. rhamnosus* L08 shows a high capacity to produce β-glucosidase, confirming our hypothesis (Liu et al., 2021). The trajectory of quinic acid changes was consistent with the findings of Kwaw et al. (2018) in their mulberry fermentation study, suggesting that the initial stage of strain metabolism promotes quinic acid accumulation, while its subsequent decreased may be due to its involvement in the esterification process with caffeic acid (Duan et al., 2023). With the extension of fermentation time, the content of various phenolic compounds changed, indicating that under the action of *L. rhamnosus* L08, phenolic substances will undergo transformation in the BHJ.

3.3. Antioxidant capacity

During the fermentation process, compounds in BHJ underwent biotransformation, produced compounds with different antioxidant properties. As shown in Fig. 2 (A), the ability of BHJ to inhibit $O_2 \cdot^-$ was similar when fermented for 8 h and 16 h. At 16 h, the inhibitory capacity was at its maximum (78.47 ± 2.88 U/L), which was an increase of 24.32% compared to 0 h. Subsequently, its inhibitory ability weakened until the fermentation was completed, and the inhibitory ability was similar to 0 h. As shown in Fig. 2 (B), with the extension of fermentation time, the ability of BHJ to inhibit $OH \cdot^-$ gradually increased, and the inhibitory ability basically stabilized after 12 h. The ability to inhibit $OH \cdot^-$ was strongest at 16 h of fermentation (173.60 ± 55.47 U/mL). The result of using Trolox Equivalent to determine the total antioxidant activity of the fermented BHJ is shown in Fig. 2 (C). The T-AOC showed a trend of first increasing and then decreasing with fermentation time, and the highest antioxidant activity (0.84 ± 0.05 mM) was obtained after 12 h of fermentation. Fermentation can affect the antioxidant capacity of the BHJ, which was similar to previous research findings (Wu et al., 2021; Zhang et al., 2022). Wu et al. (2021) suggested that the metabolism of phenolic substances may be a factor affecting the antioxidant capacity of fermentation broth. During the fermentation process of LAB, some bioactive substances are produced, which can also affect the antioxidant capacity of the fermentation broth. These include vitamins, γ- aminobutyric acid, bioactive peptides, and extracellular polysaccharides, among others (Linares et al., 2017).

To further explore the relationship between phytochemicals and antioxidant capacity in fermented BHJ, a Pearson's test was conducted, as shown in Fig. 2(D). In short, the TPC, TFC, and TAC were positively correlated with antioxidant capacity, and most of them were significant ($p < 0.05$). Consequently, the accumulation of phenolic substances during fermentation is the primary factor responsible for the enhanced antioxidant capacity (Wang et al., 2021).

3.4. Sensory quality and flavor omics

3.4.1. Analysis of color

The CIELAB space model was used to describe the color changes of fermented BHJ, as shown in Fig. 3 (A). The parameter L * represents the brightness of the sample surface, and 0–100 represents the brightness

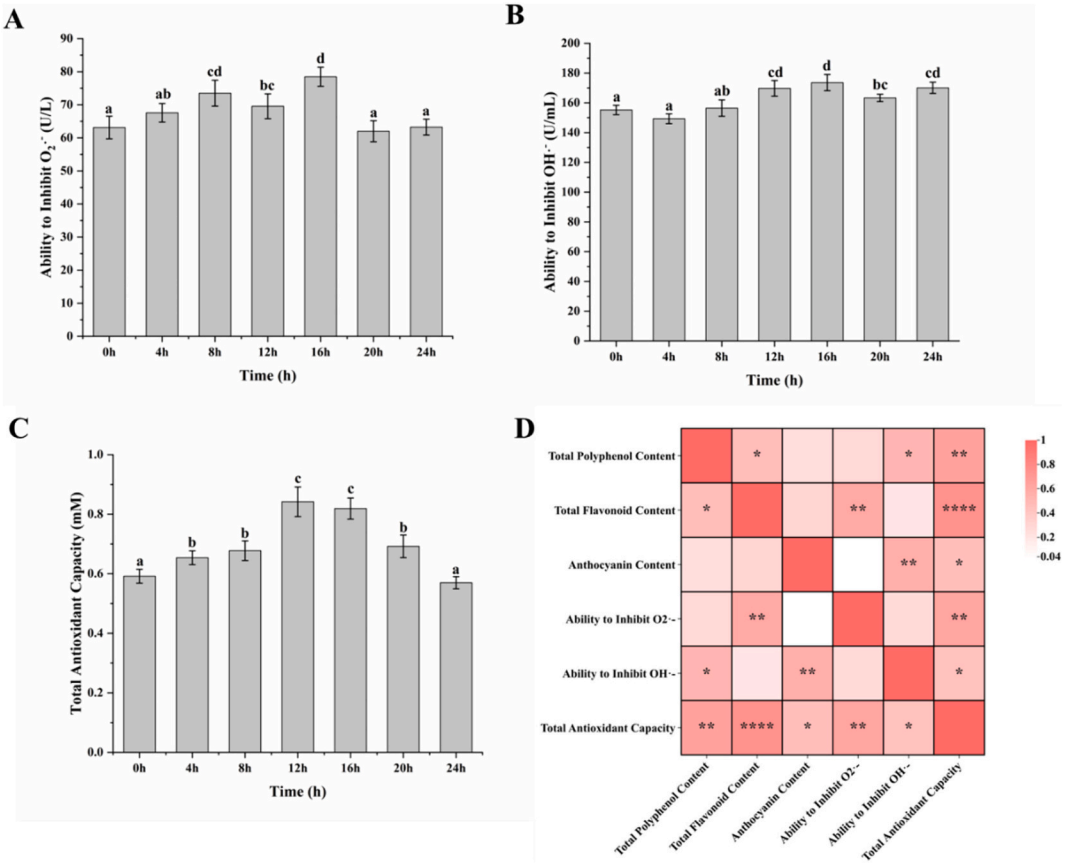

Fig. 2. The antioxidant capacity of blue honeysuckle juice during fermentation by *L. rhamnosus* L08. (A) Ability to Inhibit $O_2 \cdot^-$. (B) Ability to Inhibit $OH \cdot^-$. (C) Total Antioxidant Capacity. (D) Correlation analysis between phenolic content and antioxidant capacity. Note: Different letters and "*" mean significant difference ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

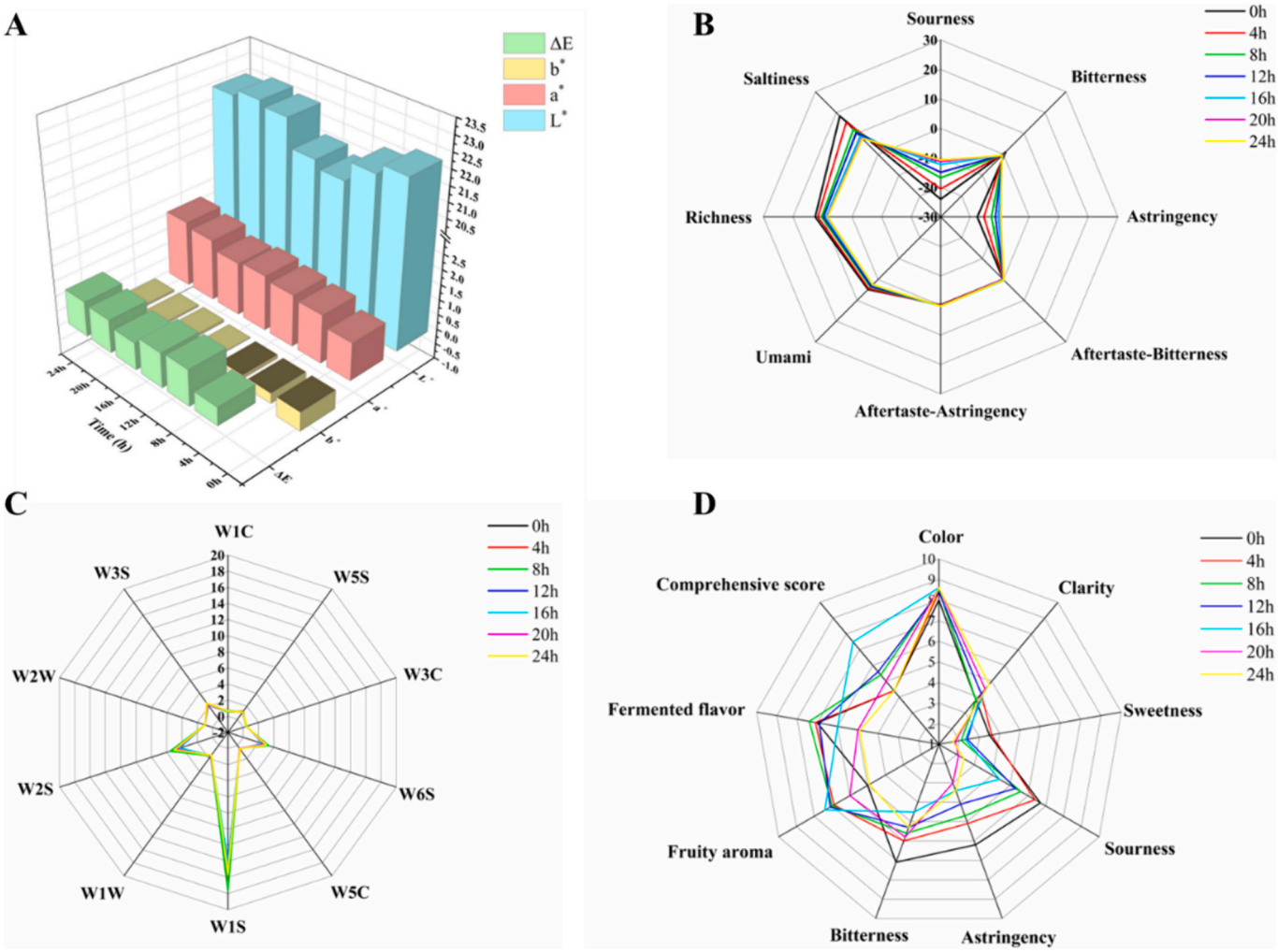

Fig. 3. The sensory characteristics of blue honeysuckle juice during fermentation by *L. rhamnosus* L08. (A) Color Analysis. (B) Analysis of Electronic Tongue. (C) Analysis of Electronic (D) Sensory Evaluation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

from weak to strong. A positive value of the parameter a * is indicative of a red tone, whereas a negative value is indicative of a green tone. A positive value of parameter b * indicates a yellow tone, while a negative value indicates a blue tone. The parameter L * of the sample showed a pattern of initial decline followed by an uptick, which aligns with the findings of Chen et al. (2023). Fermentation caused the sample to change in a redder direction, and the parameter b * gradually increased, with 16 h being the time when the parameters a * and b * of the sample changed. Research had shown that the TAC was positively correlated with the parameters a * and b * values and negatively correlated with the parameter L * value (Suriano, Balconi, Valoti, & Redaelli, 2021). There was a discrepancy between this conclusion and the results of the present study. A study indicated that a decrease in pH may lead to the conversion of anthocyanins into more flavylium cations, subsequently increasing the parameters a* and b* values and decreasing the parameter L* value of the juice (Chen et al., 2023). ΔE reflects the color change of the fermented BHJ. If the value is between 0 and 0.5, it is considered no color difference; if between 0.5 and 1.5, it is considered a slight color difference; if between 1.5 and 3.0, it is considered an obvious color difference; if between 3.0 and 6.0, it is considered a visible color difference (Cserhalmi, Sass-Kiss, Tóth-Markus, & Lechner, 2006). Compared to the BHJ fermented for 0 h, the ΔE values of the fermented BHJ were all within the range of 0.5–1.5, indicating that fermentation alters the color of the BHJ but with relatively minor effects. This difference was mainly attributed to the changes in the values of a* and b*. In conclusion, the color of fermented fruit juice was influenced by various factors, with anthocyanins and pH being the most significant.

3.4.2. *Analysis of electronic tongue*

The electronic tongue can mimic the human tongue to determine the taste information of samples. As shown in Fig. 3(B), the response values for sourness, saltiness, umami, and richness of the BHJ exhibited a gradual decrease as the fermentation time was extended. During fermentation by *L. rhamnosus* L08, a considerable quantity of carbohydrates and a small amount of inorganic salts were consumed, resulting in a reduction of the umami taste of the juice. Additionally, there was a synergistic effect between sourness and saltiness (Hu et al., 2021). Therefore, microbial fermentation can improve the taste of BHJ.

3.4.3. *Analysis of electronic nose*

To analyze the differences in the aroma of fermented BHJ, an electronic nose was used to analyze the samples, and a plot was generated based on the response values collected by the sensors, as shown in Fig. 3 (C). The response rates of sensors W6S, W1S, and W2S were relatively high, respectively identifying hydrogen, broad metal, and broad alcohol, especially W1S. This was due to the lower content of volatile substances representing other odors (Li, Cheng, Yang, Wang, & Lü, 2022). The fermentation time had a relatively small impact on the response values

of each sensor, and the sensors almost overlap with each other, indicating that they had similar odors. In summary, fermentation had a relatively small impact on the odor changes of BHJ.

3.4.4. Sensory evaluation

The sensory evaluation group rated the color, taste, aroma, and overall experience of fermented BHJ, as shown in Fig. 3(D). The sensory group did not significantly perceive the color difference, but the clarity of the juice gradually increased. A reduction in the perceived sweetness, sourness, bitterness, and astringency was observed. As fermentation progressed, the fruity flavor of the juice gradually weakened and the fermented flavor gradually enhanced. The comprehensive score was an evaluation conducted by researchers based on color, taste, and odor. Due to the low sourness and sweetness in the later stage of fermentation, as well as the lack of attractive fruity aroma, the highest score was obtained after 16 h of fermentation. The inconsistency between the sensory scores and the results of the electronic tongue and electronic nose may be due to human subjectivity (Hu et al., 2021).

3.5. Analysis of volatile substances

To identify odor-active compounds at the molecular level, GC–MS technology was used to characterize the odor components. Table 1 shows the basic information and odor characteristics of the detected substances. There were 31 volatile substances detected in total, including 6 kinds of ketones, 8 kinds of alcohols, 9 kinds of aldehydes, 1 kind of esters, 3 kinds of terpene compounds, 1 kind of fatty acid, and 3 kinds of alkanes. Most of these substances exhibit the characteristics of fruit and flower fragrance.

The relative content of volatile substances is shown in Fig. 4(A). With the extension of fermentation time, the relative content of ketones fluctuated, reaching its highest at 12 h (16.24%). It has been demonstrated that ketones can produce unique aromas, including fruity and mushroom-like odors (Hu et al., 2020). Most ketones are produced by the microbial oxidation or decarboxylation of fatty acids. (Wang, Wei, Zhai, Li, & Wang, 2023).

Alcohols are products of *Lactobacillus* metabolism, derived from the thermal oxidation of lipids and the degradation of carbohydrates, and can provide plant and aromatic odors (Guan et al., 2022). The growth and metabolism of microorganisms have led to an increase in the types and contents of alcohol substances. The results of the study indicated that the accumulation of alcohols increased with the extension of fermentation time, which was consistent with the findings of Wang et al. (2023) in the fermentation of blueberry juice. Among the alcohols, 3-Hexen-1-ol and 1-Octanol had relatively high content, mainly exhibiting floral characteristics.

Alcohols react with organic acids to produce esters, which are closely related to fruit aroma and play an indispensable role in the fermentation process of BHJ (Corrëa Lelles Nogueira, Lubachevsky, & Rankin, 2005). Formic Acid Hexyl Ester was the only ester compound detected. As the fermentation time extends, the content of formic acid hexyl ester gradually increased, which may be related to the accumulation of alcohols (Chen et al., 2017).

Appropriate aldehydes can bring pleasant flavors, while excessive aldehydes can bring undesirable flavors (Melgarejo et al., 2011). Most aldehydes were reduced during the fermentation process. For example, the relative content of Hexanal decreased from 8.62% to 3.34%, and the types of aldehydes also decreased relatively. The reduction in aldehyde content may be due to the antibacterial effect of phenolic compounds in BHJ, which inhibits certain metabolic reactions (Perricone, Bevilacqua, Altieri, Sinigaglia, & Corbo, 2015). Consequently, fermentation can enhance the palatability of food by modifying its undesirable flavor.

In this study, three terpenes were detected: Linalool, Eucalyptol, and Geraniol. Linalool possesses a remarkably low sensory threshold yet boasts a high concentration, rendering it a crucial flavor compound in this study. It gives the product floral fragrances such as lavender, lemon,

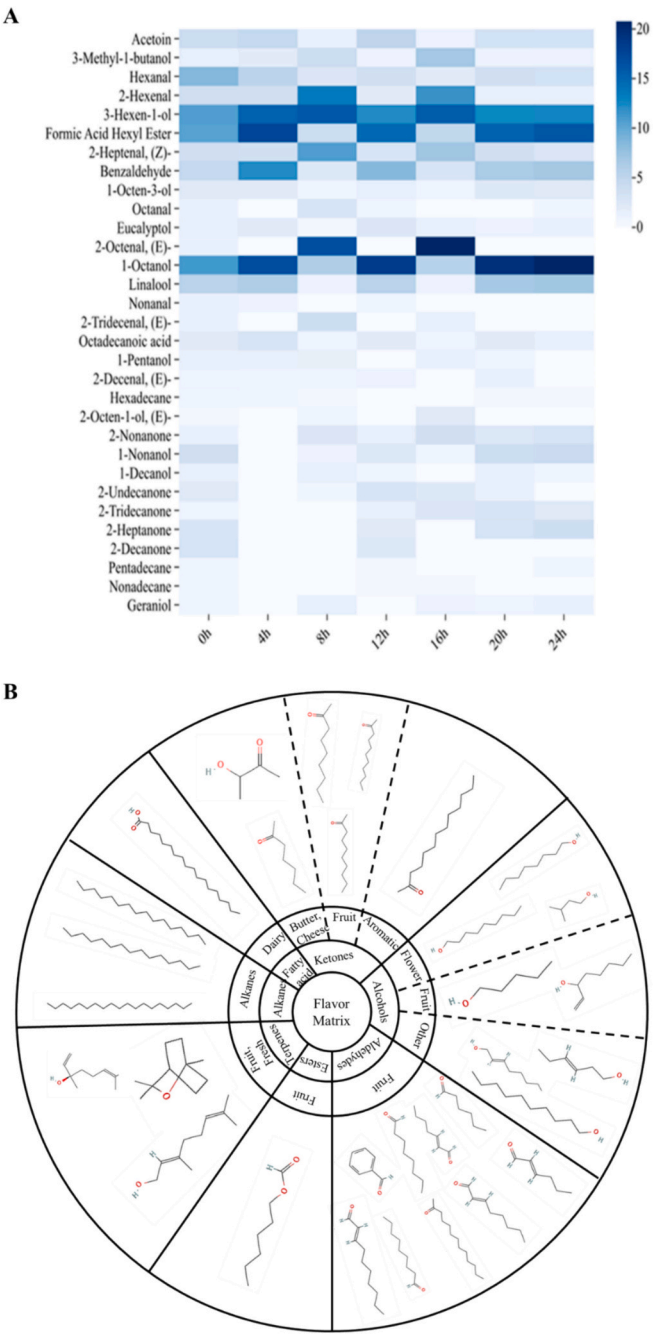

Fig. 4. (A) Composition and content of volatile substances of blue honeysuckle juice during fermentation by *L. rhamnosus* L08. (B) Odor characteristics and structural flavor wheels of aroma components. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and rose. Eucalyptol and Geraniol impart refreshing and fruity characteristics to the product, respectively. During the fermentation process, the accumulation of terpenes occurs due to the acid hydrolysis of glycosides (Liu, Li, Gao, Cheng, & Yuan, 2019).

In addition, this study also detected the presence of a minor quantity of octadecanoic acid and alkanes, which exert a negligible influence on the olfactory perception. Fig. 4(B) demonstrates the structures of different types of compounds with distinct aroma characteristics, indicating that ingredients with similar flavor profiles tend to possess structurally related similarities.

3.6. Basic properties of fermented BHJ during storage and changes in phytochemicals

3.6.1. Analysis of viable count, pH, and total acid

The changes in the viable count, pH, and total acid of the fermented BHJ stored at 4 °C are shown in Fig. 5 (A). The viable count gradually decreased from 9.45 ± 0.02 log CFU/mL to 9.04 ± 0.03 log CFU/mL, the pH decreased from 4.54 ± 0.07 to 4.17 ± 0.04, and the total acid increased from 9.69 ± 0.14 g/L to 10.71 ± 0.09 g/L. This trend of change was similar to the results of the research conducted by Gumus and Demirci (2022). The increase in total acid in the juice can have a negative impact on the viable count. The increase in total acid alters the charge properties of the strain's cell membrane, inhibiting the activity of enzymes produced by the strain and interfering with its normal metabolic activities, significantly affecting the strain's survival rate. The increase in total acid may be due to the production of organic acids as microorganisms utilize carbohydrates in the juice for growth and metabolism.

3.6.2. Analysis of changes in phytochemicals

Fig. 5 (B), (C), and (D) show the TPC, TFC, and TAC of the fermented BHJ during cold storage. During the storage period, both TPC and TAC showed a decreasing trend, but the decrease in TAC was not significant ($p > 0.05$). These results suggested that anthocyanins are relatively stable under acidic conditions (Lai et al., 2023). The TPC decreased from 1908.71 ± 98.50 μg GAE/mL to 1614.83 ± 73.62 μg GAE/mL, representing a reduction of 15.40%. At low temperatures, microbial activity was reduced, and the presence of dissolved oxygen in the sample made oxidation the primary cause for the decrease in TPC (Nematollahi, Sohrabvandi, Mortazavian, & Jazaeri, 2016). There was no significant change in the content of total flavonoids, which was similar to the result of Łopusiewicz et al. (2019).

4. Conclusion

This study developed functional BHJ using *L. rhamnosus* L08 and conducted in-depth research on the dynamic changes that occur during the BHJ fermentation process. The fermentation process resulted in alterations to the content of phenolic substances, with higher concentrations of total phenolics, flavonoids, and anthocyanins being observed following fermentation. At the same time, fermentation also improved the antioxidant properties of BHJ. There was no significant difference in bioactive substances and antioxidant capacity between 12 h and 16 h of fermentation for BHJ. Given that a shorter fermentation time is more energy-efficient, a more reasonable fermentation time for BHJ is believed to be 12 h. *L. rhamnosus* L08 also improved the taste defects of

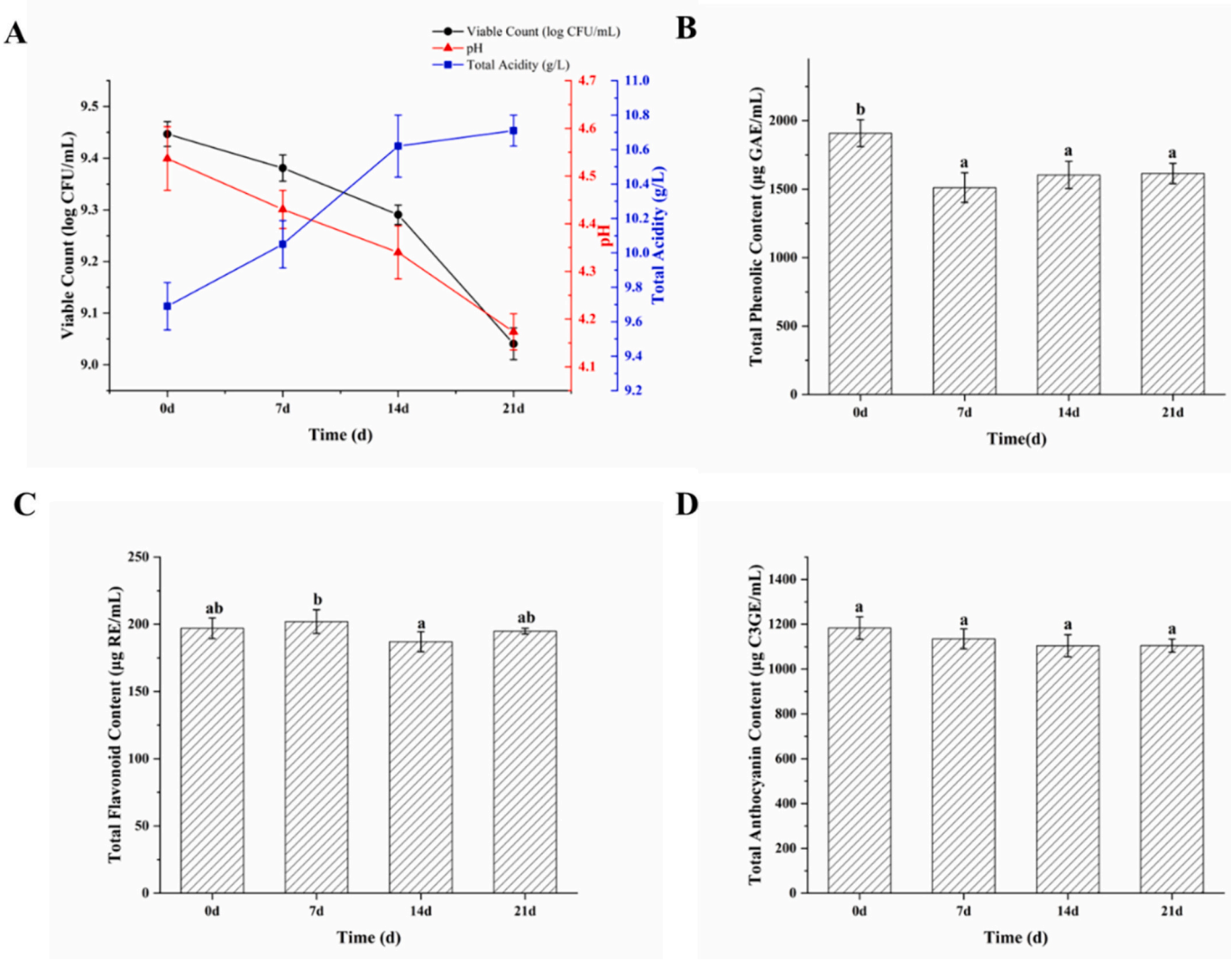

Fig. 5. Basic properties and phytochemicals of fermented blue honeysuckle juice during storage period (A) Viable Counts, pH, and Total Acidity. (B) Total Phenolic Content. (C) Total Flavonoid Content. (D) Total Anthocyanin Content. Note: Different letters mean significant difference ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

BHJ, making it more acceptable, and the loss of bioactive substances was smaller during storage. In summary, both the functionality and taste of BHJ were improved, providing feasibility for the development of blue honeysuckle probiotic functional juice.

CRediT authorship contribution statement

Shengnan Liang: Writing – review & editing, Visualization, Software, Methodology, Data curation, Conceptualization. **Siyang Yu:** Writing – review & editing, Methodology, Data curation. **Yishu Qin:** Investigation. **Honglin Yu:** Investigation. **Zifu Zhao:** Writing – review & editing, Visualization. **Yunhui Xu:** Investigation. **Guofang Zhang:** Investigation. **Chun Li:** Investigation. **Libo Liu:** Investigation. **Peng Du:** Investigation. **Junwei Huo:** Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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