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Research Article

Effects of lactic acid bacteria fermentation on antioxidant activity and sensory quality of *Rosa sterilis* S D Shi

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Abstract: The objective of this study was to improve the flavor of *Rosa sterilis* S D Shi juice without reducing its antioxidant activity. *Lactobacillus plantarum* BJ-1 and *Lactobacillus brevis* BJ-31, previously isolated and preserved from naturally fermented kimchi, were used to ferment *R. sterilis* S D Shi juice, and the antioxidant activity and organoleptic properties were measured and analyzed to produce *R. sterilis* S D Shi juice with no reduction in antioxidant activity but with improved flavor. The experimental results showed that *L. plantarum* BJ-1 and *L. brevis* BJ-31 significantly increased the content of total phenolics and flavonoids, the scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals by 43.11% and 40.70%, and the scavenging capacity of 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radicals by 40% and 40%, respectively. From the organoleptic point of view, the taste and aroma of the *R. sterilis* S D Shi juice fermented by different lactobacilli gradually improved, and the aromatic amino acids of the fermented BJ-1 juice exceeded those of the non-fermented juice, while the aromatic amino acids and bitter amino acids of the fermented BJ-3 juice exceeded those of the non-fermented juice. *R. sterilis* S D Shi juice was both higher than those of unfermented juice, indicating that fermentation is a good way to improve the taste and flavor of *R. sterilis* S D Shi juice. This indicates that there was a significant improvement in the taste and flavor of the *R. sterilis* S D Shi juice. In this research, adding BJ-1 and BJ-31 respectively to *R. sterilis* S D Shi juice for fermentation, which significantly improved the taste and flavor of the juice, and this research provides a theoretical basis for probiotic fermentation of *R. sterilis* S D Shi juice and development.

Keywords: lactic acid bacteria fermentation; *Rosa sterilis* S D Shi; antioxidant activity; sensory quality; nutritional ingredient

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1 Introduction

Rosa sterilis S D Shi, also known as seedless prickly pear, is a close relative of *Rosa roxburghii* Tratt and belongs to the same genus as Rosaceae. Because its fruits are golden yellow when ripe, the *R. sterilis* S D Shi is a species endemic to Guizhou and is mainly distributed in Anshun, southwestern Guizhou and southern Guizhou, China. The industrial development of *R. sterilis* S D Shi has shown its functional properties, allowing its use as a raw material for several delicious products, such as juice, biscuits, fruit vinegar, etc[1].

The development and utilization of *R. sterilis* S D Shi is mainly based on its functional and nutritional properties, which can enrich human body nutrients and play a role in health care. *R. sterilis* S D Shi fruit contains protein, sugar, vitamins, amino acids, polyphenols, flavonoids, vitamin C, superoxide dismutase (SOD) and various trace elements such as B, Zn, Mo, etc[2-4]. In addition, it also has obvious anti-inflammatory, analgesic, antioxidant, anti-cancer, anti-atherosclerosis, prevention and treatment of diabetes, and immune enhancement effects[5-7].

R. sterilis S D Shi contains about 0.2%−0.6% of tannins, which will react with salivary proteins to produce astringent flavor when drinking[8,9], affecting the sensory quality of the product. In addition, in recent years, the *R. sterilis* S D Shi industry has been rapid development, due to a large number of *R. sterilis* S D Shi fresh fruit concentrated listing, resulted in a sharp decline in price, coupled with the fresh fruit after picking easy to fibrosis, water loss and so on caused by the decline in food quality and commodity value, seriously restricting the development of the industry. Currently on the market of the *R. sterilis* S D Shi beverage products processing mode to add sucrose, sweeteners and other simple sugar-acid ratio adjustment is mainly[10]. The added value of the products is not high and they are not attractive to consumers. Therefore, there is a need to research and develop a new *R. sterilis* S D Shi beverage to improve its taste, while ensuring the added value of its products.

According to the survey, fermentation is a way to preserve *R. sterilis* S D Shi and to increase the added value of *R. sterilis* S D Shi products. Kong *et al.*[11] fermented *R. sterilis* S D Shi fruit pomace as raw material and used prion yeast to produce protein

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feed with reference to protein and amino acid content. The results showed that the protein increased from 7.62 mg/g before fermentation to 17.21 mg/g after fermentation; the total amino acids increased from 4.682 mg/g to 8.732 mg/g, and other nutrients were retained accordingly. In addition, this research can help to solve the shortage of feed resources in China. Lin *et al.*[12] analyzed the changes of flavor substances (amino acids, organic acids and volatile flavor components) of *R. sterilis* S D Shi before and after fermentation treatment. The results showed that the total amino acid content and species of golden prickly pear decreased after fermentation treatment, but the sweet amino acid, bitter amino acid, and aromatic amino acid content/total amino acid increased by 5.78%, 7.96%, and 11.10%, respectively, and the fresh amino acid content decreased by 24.92% after fermentation, and the content and quantity of organic acids increased, in addition, the new organic acids added after fermentation included malic acid, lactic acid and acetic acid were added after fermentation, of which the original citric acid and oxalic acid contents increased by 3 and 7 times, respectively, compared with those before fermentation; The volatile flavor substances changed significantly, of which the relative percentages of alcohols and esters were increased by 23.79% and 32.52%, respectively, while the relative percentages of acids, aldehydes and ketones were decreased by 48.08%, 1.73% and 0.93%, respectively. This research provides data support for the feasibility of utilizing fermentation process in new product development of *R. sterilis* S D Shi, and further analyze the advantages of fermentation process in fruit and vegetable product development from the aspects of functional efficacy and nutrient absorption to provide theoretical support for the development of enzyme industry in the later stage. Wang *et al.*[13] produced *R. sterilis* S D Shi vinegar by alcoholic fermentation and acetic acid fermentation. During the fermentation process, the optimal production conditions of *R. sterilis* S D Shi vinegar were established by orthogonal test using the initial alcohol concentration, fermentation temperature, and the amount of acetic acid inoculation as reference indexes. The results showed that the 8% initial alcohol concentration was 8%, 31 °C fermentation temperature was 31 °C and 0.1% acetic acid inoculum was 0.1%.

In previous research studies, it was found that with the development of the food industry, lactic acid fermentation is increasingly used in the food industry. It is considered a processing method to maintain and improve the nutritional value and sensory properties of fruits and vegetables. Its use in the food industry can effectively achieve product flavor and taste. and diversification of health effects[14]. Previous research has shown that using lactic acid bacteria to ferment wolfberry juice and blueberry juice can significantly improve the antioxidant capacity of wolfberry juice and blueberry juice through fermentation, and the content of flavonoids, phenols and other compounds were also significantly increased[15, 16]. Ningxia wolfberry and prickly pear were used as raw materials and fermented with *Saccharomyces cerevisiae* BCGQ2107, and the results showed that the fermentation increased the inhibition rate of α-amylase, and at the same time, the fermentation was able to increase its floral, fruity, and alcoholic aromas[17]. Therefore, in the present study, *R. sterilis* S D Shi juice was fermented using *Lactobacillus plantarum* BJ-1 and *Lactobacillus brevis* BJ-31 respectively with the expectation of enhancing the taste of the product without decreasing the antioxidant activity of the product. In this study, antioxidant activity, aroma composition and sensory evaluation were measured and analyzed as physicochemical indicators of the fermentation process.

2 Materials and methods

2.1 Materials

L. plantarum BJ-1 and *L. brevis* BJ-31 were isolated from naturally fermented kimchi, and were stored in the Microbiology Laboratory of the School of Life Sciences of Guizhou University (China) ; *R. sterilis* S D Shi Original juice was a gift from Guizhou Tianci Guibao Food Co., Ltd. (China); Anhydrous ethanol, Sulfosalicylic acid, hydrochloric acid, 2-octanol and sodium chloride were purchased from Chongqing Platinum Strontium Titanium Biotechnology Co., Ltd. (China); Methanol was purchased from Chongqing Chuandong Chemical Co., Ltd. (China); K_2SO_4 was purchased from Xilong Scientific Chemical Industry (China); MRS broth: CM0359, Oxoid Ltd. (China); Automatic amino acid analyzer: L-3000 type, Tianjin Taisi (China); High-performance liquid chromatography: Aglilent 1200Series, Agilent Company (USA); Gas chromatography-mass spectrometry (GC-MS): Agilent 8890-7000D, Agilent Company (USA); Electronic taste sensing system: Shanghai Sunny Hengping Instrument Co., Ltd. (China).

2.2 Methods

2.2.1 *Lactic acid bacteria*

The seed liquids of BJ-1 and BJ-31 were inoculated into MRS broth at an inoculum volume of 3% (*V/V*) respectively. After culturing for 14 h at 37 °C, the seeds were cultured for 4 h. Centrifuged at 8,000 r/min for 10 min at 4 °C, discard the supernatant, washed repeatedly with physiological saline 2–3 times, adjusted the concentration of bacterial suspension to 10^6 CFU/mL, and used it to inoculate into the pomegranate solution.

2.2.2 *Fermentation of R. sterilis S D Shi*

Prepared *R. sterilis* S D Shi Original juice with sterile water in a ratio of 1:3 (*V/V*) and adjusted the pH to 3.5. Transferred the *R. sterilis* S D Shi solution to a 100 mL Erlenmeyer flask and pasteurizing at 71 °C for 10 min[18], after cooling to room temperature, the bacterial suspension of strains BJ-1 and BJ-31 prepared previously were inoculated. During fermentation, used a pH meter to measure the pH value every 24 h until the pH value was stable and terminate the fermentation.

2.2.3 *Determination of total phenols and total flavonoids*

The total phenolic content was determined according to the method of Dewanto *et al.*[18]. Let 0.125 mL of free phenol extract, 0.125 mL of folin phenol reagent and 0.5 mL of distilled water stand for 6 min, then added 1 mL of distilled water and 1.25 mL of 7% 1.25 mL of Na_2CO_3 solution is allowed to react in the dark for 90 min, and then the absorbance value is measured (n = 760 nm). Prepared gallic acid standards with different solubility gradients to prepare a standard curve. The measurement was repeated three times. The flavonoid content was determined according to Hussain *et al.*[19]. Added 400 μL water and 50 μL $NaNO_2$ (50 g/L) to 100 μL sample, after mixing for 6 min, add 50 μL of 100 g/L $Al(NO_3)_3$ solution and let stand for 6 min. Finally, added 200 μL of NaOH at a concentration of 200 g/L and distilled water to obtain a final volume of 1.25 mL. After standing in the dark for

15 min, read the sample absorbance at 510 nm. Total flavonoid content was calculated by a standard curve and expressed as mg equivalents of rutin per milliliter of sample (mg/100 g).

2.2.4 Determination of free radical scavenging rate and SOD activity value

2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging ability was performed using the method described by Feng *et al.*[20]. Prepared two-fold serial dilutions in absolute ethanol (175 μL) using a 96-well titer plate. Subsequently, added dropwise to every other well (final volume 200 μL) 25 μL of the 0.4 mm DPPH methanolic solution just prepared. The trolox concentration used as reference is 0.8–1.25 μg/mL, and a standard curve was established. The mixture was allowed to stand at room temperature for 30 min in the dark. The absorbance was measured at 517 nm using an Infinite 200 reader (Tecan, Mannedorf, Switzerland) and expressed as equivalents of trolox per liter of sample (g/L).

ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) free radical scavenging ability adopted the method of Chang *et al.*[21]. The K_2SO_4 solution was prepared into 2.45 mmol/L and 7.00 mmol/L ABTS stock solution, mixed in equal amounts, and allowed to stand at room temperature in the dark for 12–16 h. Before using, the ABTS solution was diluted with ethanol to $OD_{734\ nm}$ = 0.68–0.72, and mixed with the sample solution at a ratio of 3:1 (*V*/*V*). Let it stand in the dark at room temperature for 10 min. Measured the absorbance at a wavelength of 734 nm (A_i), and setting up a control group at the same time (A_j) and blank group (A_0) was measured three times in parallel. The clearance rate and half maximal inhibitory concentration (IC_{50}) were determined according to the following formula:

$$\text{Clearance}\,(\%) = 1 - \frac{(A_i - A_j)}{A_0} \times 100$$

The SOD measurement method is determined according to the instructions of the SOD kit (Jiancheng, China). Prepared the assay tube and control tube respectively, added all reagents for 0.1 mL respectively, in addition, added 2 mL of sample solution to the assay tube, 2 mL of distilled water to the control tube, vortexed thoroughly, and incubated at 37 °C for 40 min, after that, appended 2 mL of colorant respectively, and leave it at room temperature for 10 min. Used a cuvette with an aperture of 1 cm and adjusted the distilled water to 0, at the wavelength of 550 nm, finally, colorimetry was performed.

2.2.5 Amino acid analysis

The preliminary treatment was based on the sulfosalicylic acid method used by Ge *et al.*[22]. About 100 mg of dry sample was weighed into a centrifuge tube, added 2 mL of 5 mmol/L hydrochloric acid solution and conducted ultrasonic extraction for 30 min. After centrifugation, taken 1 mL of supernatant, added 0.25 mL of 10% sulfosalicylic acid solution, and placed in the refrigerator. Refrigerated at 4 °C for 60 min, and filtered the solution with a 0.45 μm microporous membrane waiting for using.

The determination of amino acids was based on the ninhydrin colorimetric method adopted by Zhou *et al.*[23]. 20 μL of the filtrate was analyzed on an automatic amino acid analyzer. The column temperature was 60 °C and the reactor is 115 °C. The injection rate was 250 μL /min. After the amino acids was separated by the liquid phase ion exchange column, they reacted with ninhydrin. The absorbance of proline and hydroxyproline was measured at a wavelength of 440 nm, and the remained amino acids were measured at a wavelength of 570 nm.

2.2.6 Organic acid analysis

The high-performance liquid chromatography determination was performed using the method adopted by Song *et al.*[17], with slight modifications.

The chromatographic conditions are: chromatographic column Inert Sustain C_{18} column (4.6 mm × 250 mm, 5 μm, C/N5020-07346). Mobile phase: A is methanol, B is 0.01 mol/L potassium dihydrogen phosphate aqueous solution (pH 2.3), *V*(A):*V*(B) is 3:97.

Isocratic elution conditions: total flow rate is 0.7 mL/min; column temperature is 25 °C; injection volume is 10 μL; detection wavelength is 210 nm, measurement time is 30 min.

2.2.7 Volatile component analysis

GC-MS method adopted by Cai *et al.*[24].

Added 1 mL NaCl to the 20 mL headspace bottle, then added 5 mL sample and 10 μL 2-octanol (1,000 μg/L), and performeed adsorption at 40 °C for 30 min.

GC-MS conditions: Using HP-innowax (30 mm × 0.25 mm, 0.25 μm) capillary column, starting temperature 40 °C, holding for 2 min, then increasing to 85 °C with a gradient of 3 °C/min, and then increasing to 1 °C/min to 105 °C, then to 180 °C at 3 °C/min, and finally to 230 °C at 10 °C/min, holding for 5 min, and determining each compound using 2-octanol as the internal standard.

2.2.8 Electronic tongue analysis

The sensory quality was measured with reference to the INSENT SA402B electronic taste sensing system by Bai *et al.*[25]. Diluted the sample to 1:10 (*V*/*V*) with distilled water at room temperature and atmospheric environment, and poured each 30 mL sample into a measuring cup for testing. Between each measurement, rinsed the electrode in buffer (30 mmol/L KCl, 0.3 mmol/L tartaric acid). Each group was repeat four times, and the data from the next three experiments were analyzed.

2.2.9 Sensory taste analysis

Referred to the method of Jia *et al.*[26], 30 graduate and undergraduate students aged 19–26 were selected for sensory evaluation. The samples were poured into transparent glass cups, the color of fermented juice was observed in the well-lighted laboratory, gently shaken the cups and observed the tissue state of the fermented juice, smelled the odor. It is worth noting that the mouth should be rinsed with warm boiled water before each sensory evaluation, spicy and stimulating food was not allowed to be eaten 12 h before the valence, the evaluators did not have any physiological sensory defects, and there was no discussion with each student during the evaluation period, to ensure an objective and true evaluation. Specific evaluation criteria were shown in Table 1.

2.2.10 Statistical analysis

SPSS 19 software was used to process and analyze the experimental data, and the results were expressed in the form of mean ± standard deviation. Duncan's method is used for multiple comparisons of data, with a significance level of 0.05. Used EXCEL to calculate the correlation coefficient and conducted correlation analysis on various indicators. All experiments were performed in triplicate.

Table 1 Sensory evaluation standards of *R. sterilis* S D Shi juice fermented by lactic acid bacteria

Items	Scoring standard	Score (out of 100)
Color and luster	Golden normal luster	18–25
	Pale yellow, poor luster	9–17
	Reddish or dark brown, no luster	1–8
Perfume	Strong aroma with fermented fruity flavors	18–25
	Not a strong aroma, too much wine flavor	9–17
	Strange, irritating or malodorous odor	1–8
Taste	Soft taste and harmonization of flavors	18–25
	Medium mouthfeel, slightly bitter	9–17
	Rough texture, excessively acidic and astringent	1–8
Organizational status	Clear and bright	18–25
	Mostly clear with slight flocculation	9–17
	Excessive flocculent, unclarified, with impurities	1–8

3 Results and discussion

3.1 Effect of lactic acid bacteria fermentation on total phenols and flavonoids

Previous studies have shown that that *R. sterilis* S D Shi is rich in phenols and flavonoids and has the ability to scavenge free radicals and resist oxidation[27]. Phenolics and total flavonoids are important active substances in fruits and vegetables, they produce stable intermediates after donating electrons, which can effectively prevent oxidation at the cellular and physiological levels. The comparison of the content of antioxidant active substances in *R. sterilis* S D Shi juice before and after fermentation by BJ-1 and BJ-31 were shown in Fig. 1.

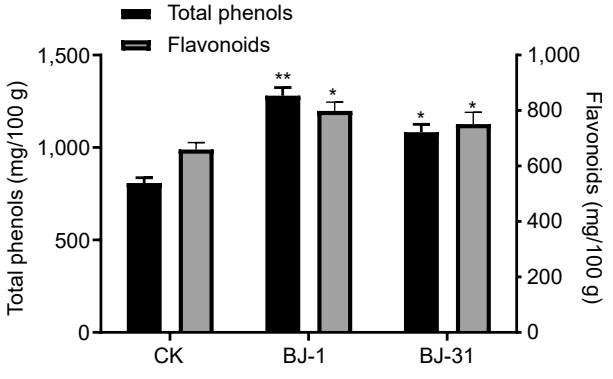

Figure 1 Changes in total phenols and flavonoids of *R. sterilis* S D Shi juice fermented by lactic acid bacteria. The data was shown as the mean value ± standard deviation (n = 3). Compared with CK, * $P < 0.05$, ** $P < 0.01$. CK: non-fermented, BJ-1: fermentated by BJ-1 for 48 h, BJ-31: fermentated by BJ-31 for 48 h. The same below.

The total phenolic content of *R. sterilis* S D Shi fruit juice fermented with BJ-1 and BJ-31 increased by 58.46% and 34.05%, respectively, as compared to the total phenolic content of unfermented juice. In addition, after fermentation with BJ-1 and BJ-31, the total flavonoid content of *R. sterilis* S D Shi fruit uice increased by 19.80% and 13.88%, respectively, with respect to the unfermented juice. *Lactobacillus* fermentation altered the content of antioxidant actives in *R. sterilis* S D Shi juice. The reason for the change in the content of phenolic substances may be that phenolic substances have two forms: free state and bound state. The bound state is divided into insoluble covalently bound phenol and soluble covalently bound phenol[28]. Soluble Covalently bound phenols can be linked to long-chain fatty acids, long-chain alcohols and other substances. During fermentation, lactic acid bacteria produced various enzymes that acted on the substrate, which may lead to the release of soluble bound phenols from *R. sterilis* S D Shi fruit juice, resulted in an increase in total phenol content[29]. The alteration in total flavonoid content following lactic acid bacteria fermentation may be attributed to the release of bound flavonoids into their free form by *Lactobacillus* fermentation, thereby augmenting the overall flavonoid content. This phenomenon could potentially account for the observed increase in total flavonoid content within this study. The specific mechanisms underlying this change encompass a complex interplay of enzyme systems and inherent instability of flavonoids themselves[30].

3.2 Effect of lactic acid bacteria fermentation on antioxidant activity of *R. sterilis* S D Shi juice

The fermentation of cabbage used *Leuconostoc* by Kusznierewicz *et al.*[31] resulted in a 3-fold increase in the total phenolic content compared to pre-fermentation levels. Moreover, the ABTS antioxidant capacity and DPPH free radical scavenging capacity also exhibited a threefold augmentation by following fermentation. Ye *et al.*[32] studied the scavenging ability of DPPH free radicals during apple fermentation. The results showed that the scavenging rate first increased and then decreased, reaching the highest value on the 9th day of fermentation, which was higher than that of unfermented apple juice. Research by Erel[33] showed that the content of phenolics was positively correlated with the scavenging rate of ABTS free radicals.

The impact of lactic acid bacteria fermentation on the antioxidant activity of *R. sterilis* S D Shi juice was investigated by assessing the scavenging rate of DPPH free radicals, ABTS free radicals, and SOD activity. The results were shown in Fig. 2. The *R. sterilis* S D Shi juice was sterilized with Bj-1 and Bj-31, respectively. Compared to the unfermented group, the DPPH free radical clearance rate and ABTS free radical clearance rate were increased by 43.11% and 40.70%, respectively, while the SOD activity increased by 2.47% and 5.03%, respectively. Moreover, in comparison to the ascorbate positive group, both ABTS clearance exceeded 95% and DPPH clearance exceeded 90%, there was a similar effect was observed. Analyzed this phenomenon, the reason may be that DPPH can form stable nitrogen-containing radicals in solution. After the addition of antioxidants, a stable molecular state DPPH was generated through electron or proton transfer mechanisms. *Lactobacillus* fermentation may produce

certain compounds with proton transfer capabilities, as highlighted by Kedare and Singh[34], which can effectively enhance the clearance rate. In addition, the change in ABTS free radical scavenging ability is mainly related to the change in polyphenol mass concentration. *R. sterilis* S D Shi juice is fermented by lactic acid bacteria, which decomposes the nutrients in *R. sterilis* S D Shi juice, causing the content of phenolic substances to increase, and improving the scavenging rate of DPPH free radicals and ABTS free radicals[35]. SOD can catalyze the decomposition of superoxide radicals into H_2O_2 and O_2, which are non-toxic to the body, by dismutation reaction, therefore reducing the damage of reactive oxygen radicals to cells, which is an important part of the human body's antioxidant system, and protects cells from oxidative damage. Consumption of SOD from food or supplements can increase the level of SOD in the body, improve intracellular antioxidant levels and slow down the aging process[36]. The decrease in SOD activity may be caused by the instability of SOD itself under fermentation temperature conditions, or it may be due to the fact that the growth of lactic acid bacteria is affected by changes in the environment (such as pH value, pressure), which in turn affects the SOD activity[37].

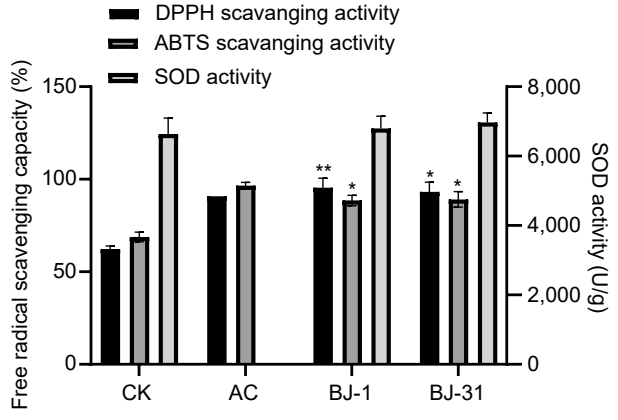

Figure 2 Changes in free radical scavenging capacity and SOD activity of *R. sterilis* S D Shi juice fermented by lactic acid bacteria, AC: ascorbic acid was used as positive control.

In conclusion, the present study demonstrated that fermentation by *Lactobacillus* significantly enhanced the antioxidant activity of *R. sterilis* S D Shi juice, thereby augmenting the value of *R. sterilis* S D Shi products.

3.3 Effect of lactic acid bacteria fermentation on the sensory properties of *R. sterilis* S D Shi juice

3.3.1 Organoleptic evaluation

Organoleptic evaluation is an effective indicator of the fermentation quality. The fermented products were subjected to sensory evaluation by the evaluators in four major aspects, including color, aroma, taste and tissue state, and the results are shown in Fig. 3. In terms of aroma and taste, both BJ-1 and BJ-31 increased dramatically as fermentation proceeded; From the aspect of color, there was a decreasing trend in BJ-1, but BJ-31 did not change much in general; As for the tissue state, it showed a decreasing trend as fermentation proceeded, and then it reached the highest level when the fermentation was carried out at the end. The total

sensory scores of *R. sterilis* S D Shi juice fermented by BJ-1 and BJ-31, respectively, showed an increasing trend and significant changes. From the sensory analysis, the fermentation of *R. sterilis* S

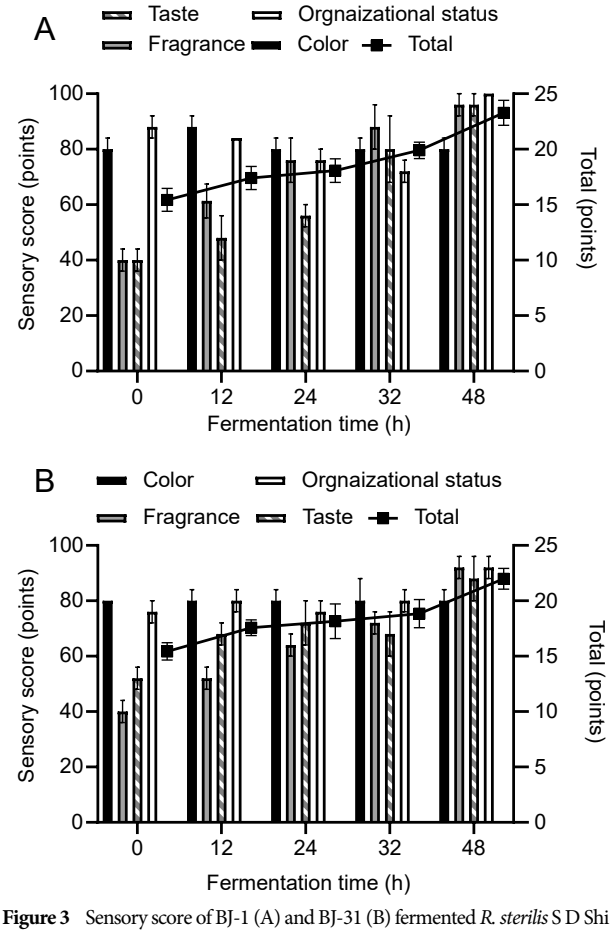

Figure 3 Sensory score of BJ-1 (A) and BJ-31 (B) fermented *R. sterilis* S D Shi juice.

D Shi juice by BJ-1 and BJ-31, respectively, could significantly improve the sour taste of the juice due to the reaction between tannins and saliva, in addition, enhanced the public acceptance of *R. sterilis* S D Shi juice.

3.3.2 Electronic tongue analysis

The electronic tongue is mainly based on taste tissue, cells and receptor unsensory components, including a complete set of taste sensation and transmission mechanisms, simulating human taste and being able to respond more intuitively to the process of stimulating the taste system by taste substances[38], which can well eliminate the subjectivity of sensory evaluation.

This study utilized an electronic tongue system to assess the impact of lactobacillus fermented *R. sterilis* S D Shi juice fermentation on its sensory attributes, including sourness, bitterness, astringency, astringency aftertaste, bitter aftertaste, umami, saltiness and richness. The results are shown in Fig. 4. The fermentation process conducted by BJ-1 resulted in a significant increase in the sourness and umami taste of *R. sterilis* S D Shi juice, while simultaneously reducing bitterness and astringency. On the other hand, fermentation with BJ-31 led to a decrease in the salty taste of *R. sterilis* S D Shi juice, without causing any notable changes in other taste sensor signals. According to the analysis, the increase in sourness may be attributed to the microbial decomposition of sugar, resulting in a substantial production of lactic acid during fermentation. Furthermore, it is plausible that the metabolic activity of lactic acid bacteria stimulates the bioactive compounds present in *R. sterilis* S D Shi, thereby contributing to an augmentation in umami flavor.

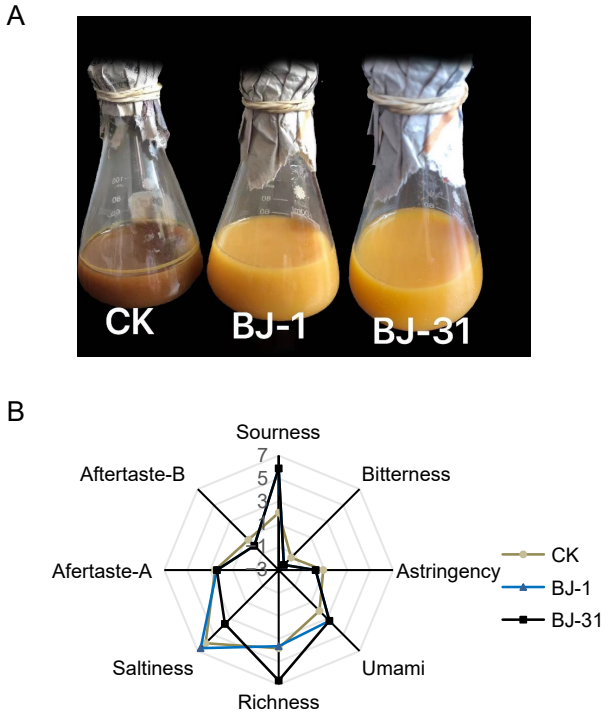

Figure 4 (A) The radar graph for taste responses of *R. sterilis* S D Shi juice fermented by lactic acid bacteria after 48 h of fermentation; (B) Comparison of different fermentation conditions after 48 h of fermentation.

3.4 Effect of lactic acid bacteria fermentation on amino acid content of *R. sterilis* S D Shi juice

Amino acids, as the fundamental building blocks of proteins, play a crucial role in determining the nutritional value and flavor profile of fermented juice. Recent research has demonstrated that lactic acid bacteria fermentation can significantly alter the amino acid composition, thereby influencing the overall quality of the product. According to the literature by Shi *et al.*[39], they used lactic acid bacteria to ferment roses, which has showed that the content of free amino acids increased by 16.46% after fermentation. Wang *et al.*[40] used lactic acid bacteria to ferment *Pueraria lobata* juice and measured the content of essential amino acids before and after fermentation. The results showed that the content of essential amino acids increased by 65.58% compared with unfermented Pueraria lobata juice.

Amino acids are the main contributors to the taste of food, and they can present various flavors such as sour, sweet, bitter, and umami[41,42]. Referred to the classification method of Ardo[41], according to different tastes, amino acids can be divided into sweet amino acids (Ala, His, Pro, Ser, Thr, Gly), umami amino acids (Glu, Lys, Asp), bitter amino acids Amino acids (Arg, Ile, Leu, Val, Trp, Met), sour amino acids (Asp, Gly) and aromatic amino acids (Tyr, Cys, Phe). In addition, lactic acid bacteria were used to ferment mango juice by Shao *et al.*[43] and studied the changes in the taste amino acid composition during the fermentation process, their data showed that the sweet and umami amino acid contents increased by 4.35% and 12.9% after fermentation, respectively. Moreover, bitter amino acids were reduced by 28.57%, which improved the taste of mango puree to a certain extent.

The measurement results of the free amino acid content of *R. sterilis* S D Shi juice before and after fermentation were shown in Table 2. The results showed that a total of 16 amino acids were detected in the *R. sterilis* S D Shi fermentation broth. The free amino acid content in *R. sterilis* S D Shi juice before fermentation was 51.25 mg/mL. The total amino acids in *R. sterilis* S D Shi juice fermented by BJ-1 and BJ-31 were 78.00 and 54.65 mg/mL respectively, increased by 52.2% and 6.22% compared to before fermentation, which indicated that lactic acid bacteria fermentation makes *R. sterilis* S D Shi more nutritious.

Table 2 Changes in Amino acids of *R. sterilis* S D Shi juice fermented by lactic acid bacteria

Amino acids (mg/100 g)	CK	BJ-1	BJ-31
Asp	4.85 ± 0.49^{b}	7.45 ± 0.49^{a}	5.95 ± 0.49^{b}
Glu	5.45 ± 0.64^{b}	10.15 ± 0.35^{a}	7.25 ± 1.20^{b}
Lys	4.65 ± 0.07^{b}	8.00 ± 0.42^{a}	5.15 ± 0.35^{b}
Umami-taste	14.95 ± 0.42^{b}	25.60 ± 1.43^{a}	18.35 ± 1.06^{b}
Thr	2.00 ± 0.14^{b}	3.75 ± 0.35^{a}	2.55 ± 0.35^{b}
Ser	2.10 ± 0.14^{b}	3.90 ± 0.42^{a}	2.65 ± 0.78^{ab}
Gly	2.25 ± 0.21^{b}	4.45 ± 0.35^{a}	2.40 ± 0.00^{b}
Ala	3.00 ± 0.28^{c}	5.90 ± 0.28^{a}	3.80 ± 0.14^{b}
Sweet-taste	9.35 ± 0.45^{b}	18.00 ± 0.98^{a}	11.40 ± 0.64^{b}
Tyr	2.55 ± 0.07^{ab}	3.50 ± 0.00^{a}	1.05 ± 1.48^{b}
Phe	1.60 ± 0.14^{b}	3.10 ± 0.14^{a}	1.50 ± 0.14^{b}
Perfume-taste	4.15 ± 0.67^{ab}	6.60 ± 0.28^{a}	2.55 ± 0.32^{b}
Val	2.25 ± 0.21^{b}	3.60 ± 0.28^{a}	2.25 ± 0.21^{b}
Met	1.90 ± 0.42	1.05 ± 1.06	0.65 ± 0.35
Ile	1.65 ± 0.21^{b}	3.35 ± 0.35^{a}	2.25 ± 0.64^{ab}
Leu	3.40 ± 0.00^{b}	6.20 ± 0.57^{a}	3.95 ± 0.35^{b}
His	0.90 ± 0.00^{b}	1.55 ± 0.07^{a}	0.90 ± 0.14^{b}
Arg	2.20 ± 0.14^{b}	4.10 ± 0.14^{a}	1.7 ± 0.00^{c}
Pro	10.50 ± 1.27^{a}	7.95 ± 0.49^{b}	10.65 ± 0.49^{a}
Bitter-taste	22.80 ± 3.28^{b}	27.80± 2.44^{a}	22.35 ± 3.46^{b}
Sum	51.25 ± 2.32^{b}	78.00 ± 2.51^{a}	54.65 ± 2.69^{b}

Note: The data was shown as the mean value ± standard deviation (n = 3). Different letters in same row indicate significant differences at the P < 0.05.

The changes in the content of flavor-producing amino acids before and after fermentation were shown in Fig. 5. The results showed that umami amino acid content in *R. sterilis* S D Shi juice was 14.95 mg/mL before fermentation. After fermentation by BJ-1 and BJ-31, the umami amino acid content increased by 71.23% and 22.74% respectively; The umami amino acid of sweet amino acid content in *R. sterilis* S D Shi juice before fermentation was 9.35 mg/mL, and the content increased by 92.51% and 21.93% respectively after fermentation. The aromatic amino acid content in the *R. sterilis* S D Shi juice before fermentation was 4.15 mg/mL, and after fermentation, the content increased by 92.51% and 21.93% respectively. In addition, after fermentation, the content increased by 59.04% and decreased by 38.5% respectively; Before fermentation, the bitter amino acid content in *R. sterilis* S D Shi juice was 22.8 mg/mL, and after fermentation by BJ-1 and BJ-31, the content increased by 21.93% and decreased by 1.97% respectively. The sour amino acid content in *R. sterilis* S D Shi juice before fermentation was 7.10 mg/mL, and after fermentation, the content increased by 67.61% and 17.61% respectively. The amino acid content of *R. sterilis* S D Shi juice did not change much before and after fermentation by BJ-31. However, fermentation by BJ-1 resulted in a significant increase in the content of umami, sweet, aromatic and sour amino acids in

R. sterilis S D Shi juice, and the bitter amino acid content did not change much.

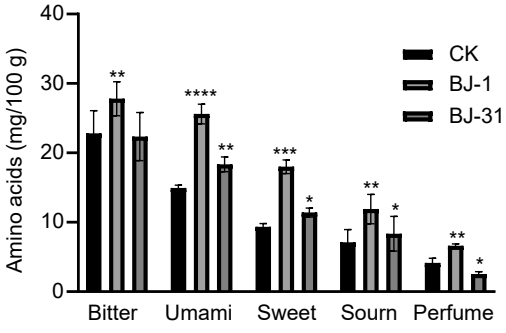

Figure 5 Changes in Amino acids composition & content of *R. sterilis* S D Shi juice fermented by lactic acid bacteria. Compared with CK, *** $P < 0.001$, **** $P < 0.0001$.

The variation in taste of amino acids is likely attributed to the differing hydrophobicity of their side chains. A higher degree of hydrophobicity in the side chain corresponds to a bitter taste, while a lower degree results in a sweeter taste. Amino acids predominantly exhibit a sweet flavor profile[44]. Therefore, our study used BJ-1 to ferment *R. sterilis* S D Shi juice can improve the taste of *R. sterilis* S D Shi juice to a greater extent than *L. brevis* fermentation.

3.5 Effect of lactic acid fermentation on organic acids of *R. sterilis* S D Shi

Organic acid content is one of the indicators of the fermentation end point of lactic acid bacteria fermented fruit and vegetable juice, and has a huge impact on the nutrition and flavor of lactic acid bacteria fermented fruit and vegetable juice products[45]. Previously, Ma[46] used *L. plantarum* to ferment carrot puree found that the content of lactic acid gradually increased and the content of malic acid gradually decreased as the fermentation time increased. Bah *et al.*[47] studied that the rich organic acids in fermented prickly pear juice play an important role in the regulation of human intestinal microecology.

Therefore, this experiment studied the changes in organic acid content in *R. sterilis* S D Shi juice before and after lactic acid bacteria fermentation. The experimental results were shown in Table 3 and Fig. 6. It can be seen from them, the organic acid content of *R. sterilis* S D Shi juice has changed significantly before and after fermentation. After fermentation of *R. sterilis* S D Shi juice by BJ-1 and B-31, the content of ascorbic acid and citric acid decreased, while the content of malic acid and lactic acid decreased, oxalic acid, tartaric acid and succinic acid contents increased.

The reason for the above phenomenon may be that citric acid is

Table 3 Changes in organic acid content of *R. sterilis* S D Shi juice fermented by lactic acid bacteria

Organic acid (mg/kg)	CK	BJ-1	BJ-31
Ascorbic acid	738.20 ± 26.16^{a}	581.10 ± 35.40^{b}	546.80 ± 39.44^{b}
Malic acid	481.51 ± 2.81^{c}	654.32 ± 7.28^{a}	610.17 ± 2.26^{b}
Citric acid	455.91 ± 7.66^{a}	273.10 ± 1.32^{b}	233.52 ± 1.36^{c}
Lactic acid	342.07 ± 8.78^{b}	350.59 ± 14.55^{b}	455.95 ± 17.20^{a}
Tartaric acid	171.92 ± 7.64^{c}	278.32 ± 6.14^{a}	211.11 ± 5.21^{b}
Suiccinic acid	61.17 ± 2.79^{c}	91.40 ± 3.43^{b}	102.38 ± 3.42^{a}
Oxalic acid	37.09 ± 4.28^{c}	51.48 ± 0.50^{b}	63.79 ± 1.86^{a}

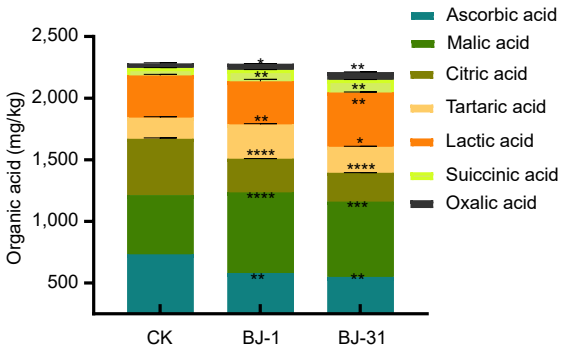

Figure 6 Changes in organic acid composition and content of *R. sterilis* S D Shi juice fermented by lactic acid bacteria.

an intermediate substance in the tricarboxylic acid metabolic cycle and is consumed in large quantities during the metabolism of lactic acid bacteria, producing some intermediate products, such as pyruvic acid and succinic acid, while ascorbic acid may be oxidized due to instability. Therefore, it is not easy to accumulate during the fermentation process [48,49]. In addition, malic acid and lactic acid have a buffering effect, and integrate and coordinate the sugar, amino acids and aroma components in the *R. sterilis* S D Shi juice, making it has a soft sour taste and a mellow taste. From a flavor perspective, the organic acids in the fermented *R. sterilis* S D Shi juice are Mainly malic acid, combining with the assistance of other types of organic acids, and extending the taste persistence of *R. sterilis* S D Shi juice. The taste saturation is soft and fresh, reflecting fullness, refreshing, long-lasting and layered feeling.

3.6 Effect of lactic acid bacteria fermentation on volatile aroma components of *R. sterilis* S D Shi

Previous studies have demonstrated that non-volatile components contribute to fundamental taste qualities, such as sweetness, sourness, bitterness, and saltiness. However, volatile compounds present at lower concentrations also exert a significant influence on the overall flavor profile of food due to their lower threshold characteristics, ultimately impacting product quality. Ricci *et al.*[50] employed lactic acid bacteria for cherry juice fermentation and observed an increase in the levels of volatile compounds including terpenes, ketones, alcohols, and acids compared to pre-fermentation. The research results of Di Cagno *et al.*[51] showed that volatile compounds such as alcohols, ketones, alkenes, terpenes, etc. The concentration of certain aldehydes with odor significantly decreased, while the levels of these compounds increased in pomegranate juice fermented by lactic acid bacteria. Numerous studies have demonstrated that lactic acid bacteria fermentation enhances the volatility of juice. Regarding the types and contents of substances, significant alterations were observed in the main volatile aroma components of *R. sterilis* S D Shi juice after undergoing fermentation by lactic acid bacteria (Table 3).

The effect of lactic acid bacteria fermentation on the volatile components in *R. sterilis* S D Shi juice is shown in Table 4. The results revealed significant alterations in the contents of alcohols, esters, aldehydes, ketones and terpenes after fermentation by BJ-1. Specifically, the relative content percentages of alcohols, esters and terpenes increased by 0.76, 1.06 and 5.43 times respectively; whereas those of aldehydes and ketones decreased by 0.31 and 0.35 times respectively with no significant changes observed in acids or other components. Similarly, fermentation by BJ-31 resulted in notable modifications in the levels of alcohols, esters,

Table 4 Changes in Volatile compounds identified of *R. sterilis* S D Shi juice fermented by lactic acid bacteria

Compounds	Molecular formula	Retention time (min)	Area (%)		
			CK	BJ-1	BJ-31
		Alcohols			
Ethamol	C_2H_6O	6.130	ND	0.418 ± 0.021^{a}	0.283 ± 0.014^{b}
1-Butanol	C_4H_9O	9.244	0.486 ± 0.014^{ab}	0.471 ± 0.025^{b}	0.524 ± 0.021^{a}
6-Nonen-1-ol	$C_9H_{18}O$	13.710	0.429 ± 0.022^{b}	0.279 ± 0.018^{c}	0.532 ± 0.038^{a}
2-Heptanol	$C_7H_{14}O$	14.929	1.059 ± 0.034^{c}	1.234 ± 0.043^{b}	1.446 ± 0.060^{a}
3-Buten-2-ol	$C_{13}H_{22}O$	35.214	2.115 ± 0.013^{b}	2.720 ± 0.410^{a}	2.392 ± 0.101^{ab}
Benzyl alcohol	$C_9H_{10}O$	20.101	ND	ND	0.335 ± 0.033
Linalool	$C_{10}H_{18}O$	23.647	ND	2.099 ± 0.068	ND
		Aldehydes			
Octanal	$C_8H_{16}O$	5.398	0.774 ± 0.037^{b}	1.220 ± 0.250^{a}	1.014 ± 0.112^{ab}
Nonanal	$C_9H_{18}O$	23.766	4.594 ± 0.267^{a}	0.735 ± 0.034^{c}	4.082 ± 0.316^{b}
Benzaldehyde	C_7H_6O	17.741	6.893 ± 0.363^{a}	5.281 ± 0.280^{b}	3.684 ± 0.351^{c}
Decanal	$C_{10}H_{20}O$	28.084	1.107 ± 0.105^{a}	0.517 ± 0.022^{c}	0.872 ± 0.041^{b}
2-Butenal	C_4H_6O	37.733	0.506 ± 0.055^{b}	1.081 ± 0.336^{a}	0.809 ± 0.031^{ab}
Furfural	$C_5H_4O_2$	12.601	0.535 ± 0.052^{b}	1.149 ± 0.142^{a}	ND
		Esters			
1, 2-Benzenedi carboxylic acid	$C_{16}H_{22}O_4$	48.738	ND	0.326 ± 0.006^{b}	0.381 ± 0.024^{a}
Ethyl acetate	$C_4H_8O_2$	44.392	2.593 ± 0.262^{c}	16.154 ± 0.747^{a}	5.418 ± 0.398^{b}
Ethylnicotinate	$C_8H_9NO_2$	6.972	ND	0.197 ± 0.014^{b}	0.356 ± 0.035^{a}
		Ketones			
Acetophenone	C_8H_8O	22.408	3.259 ± 0.205^{b}	2.894 ± 0.269^{c}	3.860 ± 0.540^{a}
2-Heptanone	$C_7H_{14}O$	14.548	ND	ND	0.074 ± 0.018
Geranylacetone	$C_{13}H_{22}O$	5.968	1.205 ± 0.106	ND	ND
		Acids			
Hexanoic acid	$C_2H_4O_2$	5.769	37.651 ± 2.220	38.762 ± 3.393	39.438 ± 4.083
trans-3-Hexenoic acid	$C_4H_6O_2$	19.946	0.863 ± 0.102	ND	ND
Adipic acid	$C_{26}H_{46}O_4$	25.614	0.343 ± 0.061	0.360 ± 0.050	0.322 ± 0.021
Octanoic acid	$C_8H_{16}O_2$	27.248	11.353 ± 0.620^{c}	14.754 ± 1.088^{a}	13.134 ± 0.616^{b}
Tetradecanoie acid	$C_{14}H_{28}O_2$	43.717	ND	0.379 ± 0.060	ND
Nonanoic	$C_{18}H_{36}O_2$	45.511	0.425 ± 0.011	0.490 ± 0.110	0.475 ± 0.038
		Terpenoids			
Tetradecene	$C_{14}H_{28}$	30.454	ND	0.421 ± 0.038^{b}	1.401 ± 0.386^{a}
Caryophyllene	$C_{15}H_{24}$	37.011	0.675 ± 0.063^{b}	1.074 ± 0.046^{a}	0.741 ± 0.064^{b}
Copaene	$C_{15}H_{24}$	38.267	0.286 ± 0.037^{b}	0.618 ± 0.051^{a}	0.359 ± 0.040^{b}
Valerena-4.7(11)-diene	$C_{15}H_{24}$	38.925	ND	0.110 ± 0.040	ND
β- Dcimene	$C_{10}H_{16}$	39.288	1.591 ± 0.330^{b}	3.120 ± 0.330^{a}	1.896 ± 0.301^{b}
Terpinene	$C_{10}H_{16}$	39.459	0.929 ± 0.052^{b}	1.880 ± 0.306^{a}	1.114 ± 0.165^{b}
Copaene	$C_{15}H_{24}$	39.633	1.011 ± 0.200^{b}	2.025 ± 0.324^{a}	1.274 ± 0.263^{b}
β-Myrcene	$C_{10}H_{16}$	40.334	ND	0.120 ± 0.050^{a}	0.113 ± 0.012^{a}
β-Limonene	$C_{10}H_{16}$	40.475	0.476 ± 0.063^{b}	0.875 ± 0.057^{a}	0.536 ± 0.049^{b}
Squalene	$C_{30}H_{50}$	46.967	ND	ND	0.319 ± 0.033
		Others			
Dimethyl sulfide	C_2H_6S	6.965	0.377 ± 0.060^{a}	0.296 ± 0.045^{a}	0.198 ± 0.015^{b}
1,3-bis(1,1-Dimethylethyl)-Benzene	$C_{14}H_{22}$	30.192	4.699 ± 0.608^{a}	2.863 ± 0.198^{b}	2.582 ± 0.105^{b}
2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	39.905	3.297 ± 0.326	3.149 ± 0.222	2.804 ± 0.389
Total			89.532 ± 7.298^{c}	108.071 ± 7.333^{a}	92.768 ± 7.114^{b}

Note: ND: not detectable.

aldehydes and terpenes with an increase observed in the relative content percentages of alcohol (by a factor of 0.34), ester (by a factor of 1.37) and terpene (by a factor of 0.56). However, no significant changes were detected for ketones or acids.

The changes in organic acid content and types were analyzed subsequent to lactic acid bacteria fermentation, attributed them to

the utilization of precursors such as free amino acids and free fatty acids by lactic acid bacteria during fermentation. This utilization lead to the partial generation of new flavor compounds, subsequently altering the composition and levels of volatile flavor substances in the product[52]. In addition, Alcohols are produced by microorganisms through Ehrlich pathway by converting amino acids as substrates to ammonia decarboxylation and then reduced by alcohol dehydrogenase. They are also the precursors to form ester substances, making the products fragrant and fresh[16] Esters are representatives of aromatic fragrances, most of which have a floral scent. The content of esters increased after fermentation of aseptic *R. sterilis* S D Shi juice[53]; Fermentation produces new Aromatic ester compounds, such as ethyl nicotinate with a sweet honey aroma and dibutyl phthalate with a fruity aroma[54]; Pinene, ocimene, and terpinene were significantly increased after fermentation, this makes the fermentation flavor like pine smell enhanced; Moreover, after fermentation by lactic acid bacteria, the high concentration of aldehydes that cause odor will be reduced to alcohol or oxidized to acid due to instability[51]. In summary, after fermentation by BJ-1 and BJ-31, the flavor of *R. sterilis* S D Shi juice was significantly improved.

4 Conclusion

In this study, *L. plantarum* BJ-1 and *L. brevis* BJ-31, which were isolated and screened from natural fermented kimchi, were used to ferment *R. sterilis* S D Shi raw juice, and the effects of BJ-1 and BJ-31 on fermentation *R. sterilis* S D Shi raw juice were investigated by using antioxidant activity, content of organic acids, content of volatiles, electronic tongue analysis, content of amino acids, and organoleptic evaluation. The effect of BJ-1 and BJ-31 on the juice of *R. sterilis* S D Shi was investigated. The results showed that the fermentation of *R. sterilis* S D Shi juice with BJ-1 and BJ-31 could significantly enhance the antioxidant activity and effectively improve the product flavor. This research can provide experimental basis and theoretical support for the development of special resources in Guizhou Province (China) and the subsequent research and development of *Lactobacillus* fermented *R. sterilis* S D Shi beverages with high antioxidant activity and excellent flavor.

Conflicts of interest

The authors declare that there are no conflicts of interest in this work.

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